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한국인 가족에 발생한 백색 해면상 모반 증례

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〈Abstract〉

A Familial Case of White Sponge Nevus in a Korean Family

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White sponge nevus (WSN) is a rare autosomal dominant disorder characterised by rough thickening, fissure formation, and a whitish colour change in the oral mucosa. This disorder predominantly affects the nonkeratinized stratified squamous epithelium of the mucosa. We experienced a familial case of WSN (i.e., a mother and her two daughters) and performed keratin gene analysis and immunohistochemical staining. The results of a mutation analysis revealed the presence of a heterozygous missense mutation 344T to G in KRT13, predicting an amino acid change leucine (L) to arginine (R), in the 1A domain of the KRT13 polypeptide. Immunohistochemically, the loss of keratin 4 expression was found.

Key words: White lesion, White sponge nevus, Keratin 13, Keratin 4, Mutation

Ⅰ. INTRODUCTION

White sponge nevus (WSN) is a rare autosomal dominant disorder characterised by benign, painless, white plaques of the mucous membranes. It mainly affects the stratified squamous epithelium of the oral mucosa. This disease was first described by Hyde in 1909, but the term "white sponge nevus" was coined by Cannon in 1935. The buccal mucosa is the most commonly affected site, followed by the tongue, labial mucosa, floor of the mouth, and the palate[1]. Extraoral sites, including the mucosa of the nose, pharynx, esophagus, vagina, and rectum can also be affected. The symptoms are

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typically present during childhood or adolescence. Most lesions are painless and do not require treatment. Differential diagnosis of this lesion is important, because some whitish lesions are premalignant or are manifestations of specific systemic disease. Histologically, WSN shows epithelial thickening, parakeratosis, and extensive vacuolization of the suprabasal keratinocytes.

We examined a familial case of WSN in a Korean family and identified the clinical and histological characteristics. We also analysed the keratin gene mutations to determine the familial pattern.

II. Methods

1. Description of patients

The proband in this family was 19-year-old female Korean patient (Figs. 1-A and 2-II:3). She and her older sister (23 years, Figs. 1-B and 2-II:1) presented complaining about the esthetic problems associated with white, asymptomatic, corrugated, spongy mucosal plaques with thick, fissured surfaces. The lesions were distributed bilaterally in the buccal mucosa, labial mucosa, gingiva, palate, tongue, and floor of the mouth (Figs. 1-A, B). The patients' histories revealed that disease onset occurred several years previously. A review of the family history revealed that the proband's mother (45 years, Figs. 1-C and 2-I:2) had similar oral lesions. The patients were healthy non-smokers with no relevant previous medical history. The patients stated that no other mucosal lesions were present. Incisional biopsies of the buccal mucosa were obtained from the proband (II:3) and her sister (II:1). Histological

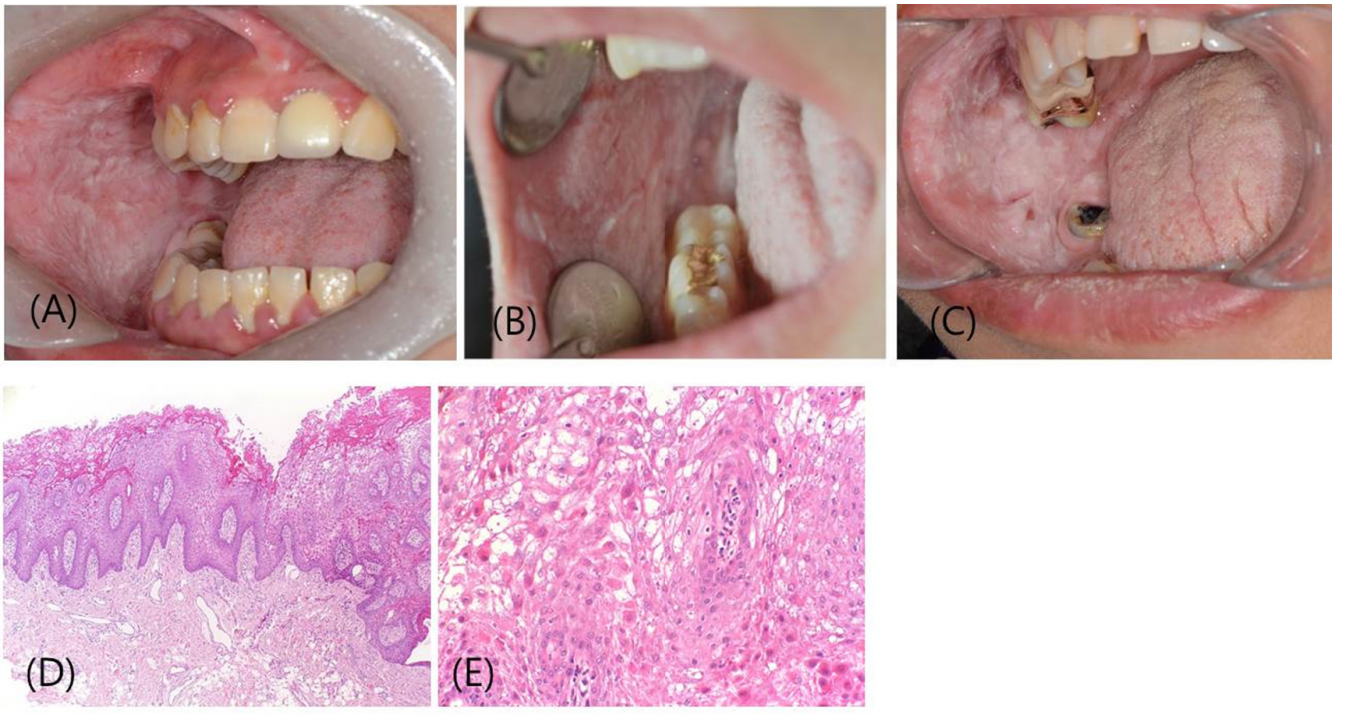

Fig. 1. Clinical presentation and histopathology of white sponge nevus. (A) The proband, a 19-year-old female; (B) the proband's sister, a 23-year-old female; (C) the proband's mother, a 45-year-old female; (D) prominent hyperparakeratosis and marked acanthosis with clearing of the spinous cell layer are present in the histologic image; (E) perinuclear keratin condensation without evidence of epithelial dysplasia is also present (original magnification (A, B, C), x40 (D), and x200 (E); haematoxylin and eosin stain (D,E)).

analysis revealed the presence of hyperparakeratosis and acanthosis with vacuolization in the spinous cell layer. Intracellular edema and perinuclear keratin condensation were present in both cases (Fig. 1-D, E).

2. Genetic analysis

Ethical approval for the genetic studies was obtained from the Review Board at the Yongin Severance Hospital, Yongin, Korea (IRB number: 8-2013-0020). Written consent was obtained from the members of the family. Genomic DNA was extracted from oral mucosal samples from the two patients and from one unaffected family member (Fig. 2-I:1, I:2, II:3). The samples were taken and processed using the swab protocol of the QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany).

The KRT4 and KRT13 genes were amplified using the polymerase chain reaction (PCR) method and primers described in previous studies[2]. The primers were added to a mixture of Gold ST*R 10X Buffer (Promega, Madison, WI, USA), 1.0 U of AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA), and template DNA. The PCR conditions were: (95°C for 7 min) × 1; (94°C for 1 min, 62°C for 1 min, 72°C for 1 min) × 35; and (72°C for 7 min) × 1. The products were stored at 4°C until direct sequencing (Macrogen, Seoul, Korea).

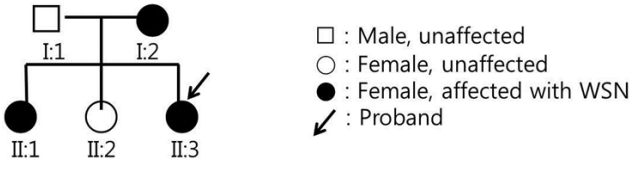

Fig. 2. Pedigree of proband's family.

3. Immunohistochemical staining

Cytokeratin 4 (mouse monoclonal IgG, working dilution: 1/100; abcam, USA) and Cytokeratin 13 (mouse monoclonal IgG, working dilution: 1/100, abcam) were used as primary antibody in this study. Formalin fixed, paraffin embedded 2 normal oral mucosa and 2 white sponge nevus tissue samples were cut into 4uM tissue sections and deparaffinised with xylene. After hydrated with graded ethanol, the endogenous peroxidise activity was blocked with endogenous block solution (Dako, USA). Antigen retrieval was then performed by pressure-cooking for 3min at full pressure in antigen retrieval buffer (Dako). Tissue sections were incubated with primary antibody at room temperature for 1h and were then sequentially incubated with the Real Envision HRP Rabbit/Mouse detection system (Dako) at room temperature for 30 min. The sections were developed with 3,3'-diaminobenzidin chromogen and were then counterstained with haematoxylin.

Ⅲ. RESULTS

Direct sequencing of the PCR products derived from the patients revealed the presence of two new mutations. One was heterozygous missense mutation 344 T to G in exon 1A of the KRT13 gene from the proband (II:3) and her mother (I:2). This mutation is predicted to change codon 115 of the KRT13 coding sequence from leucine to arginine. This mutation was not found in the unaffected father (I:1) (Fig. 3). The other mutation was 1583 A to G, which was located in the non-coding region of KRT4 of the proband (II:3), her mother(I:2) and her father (I:1). Neither the N160del, 153-154insQ mutation in the KRT4-1A region nor the E449K, E520K mutations in the KRT4-2B region were present in any of the three family members.

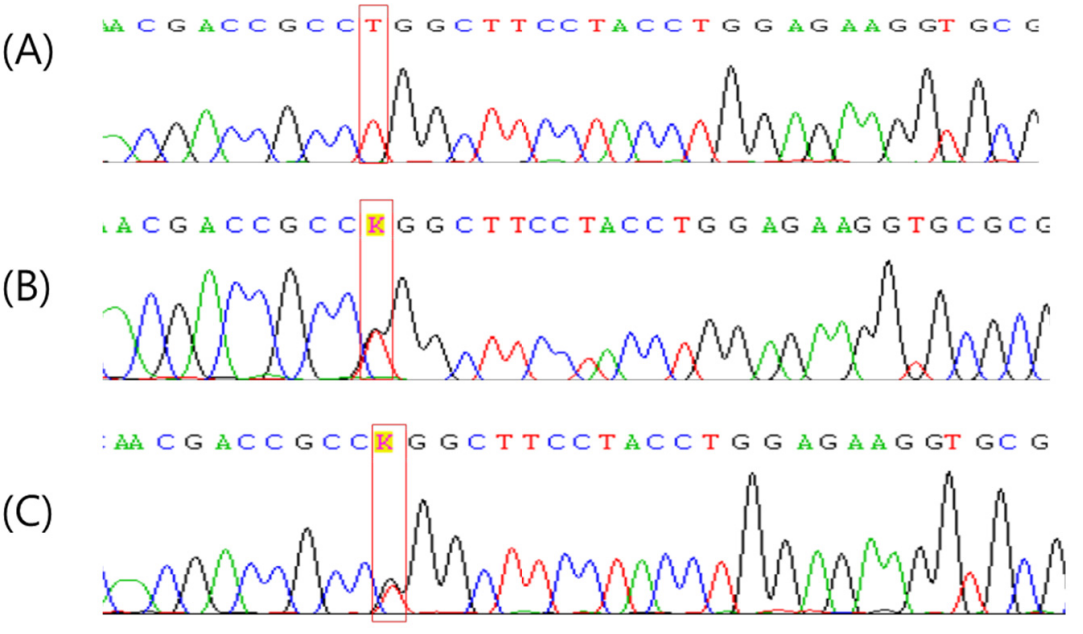

Fig. 3. Partial DNA sequence of exon 1A of the keratin 13 gene. The red box indicates the position of the mutation 344T to G. The mutation predicts the amino acid change L115R in the keratin 13 polypeptide. (A) DNA sequence of the proband's father (I:1). (B) DNA segment from the proband's mother (I:2). (C) DNA segment from the proband (II:3).

1. Immunohistochemical staining

Keratin 4 and keratin 13 expression were comparatively investigated in 2 normal oral mucosa and 2 white sponge nevus tissue samples. No difference was found between normal oral mucosa (Fig.4 a) and white sponge nevus tissues (Fig.4 b,c) in expression pattern of keratin 13. Diffuse positive for keratin 13 was found in suprabasal layer of 2 normal oral mucosa and 2 white sponge nevus tissues. By contrast, keratin 4 expression was remarkably decreased in white sponge nevus tissues than normal oral mucosa tissues. Diffuse positive pattern for keratin 4 expression was found in the suprabasal layer of 2 normal oral mucosa tissues (Fig.4 d). In contrast, keratin 4 showed no expression in one white sponge nevus patient (Fig.4 e) and other one showed focal positive reaction for keratin 4 in parakeratotic layer (Fig.4 f).

Ⅳ. DISCUSSION

The KRT4 and KRT13 gene mutations were found to be associated with WSN. Keratins are structural proteins of epithelial cells that can be divided into two subgroups, type 1 and type 2, based on biochemical properties. They polymerize to form hetero-polymeric intermediate filaments (IF). IF are abundant in stratified epithelia, in particular in the suprabasal layers of the epidermis and key components of the cytoskeleton in cells. Keratin polypeptides consist of four central α-helical rod domains (1A, 1B, 2A, and 2B). These domains show a remarkable degree of conservation. Keratin missense mutations lead to a collapse of the IF network that causes cytolysis in response to even minor mechanical stress and induce excessive basal cell proliferation. The suprabasal keratinocytes of oral mucosa express KRT4 and KRT13, and the mutation leads to reactive change manifesting as mucosal hyperkeratosis[3-6].

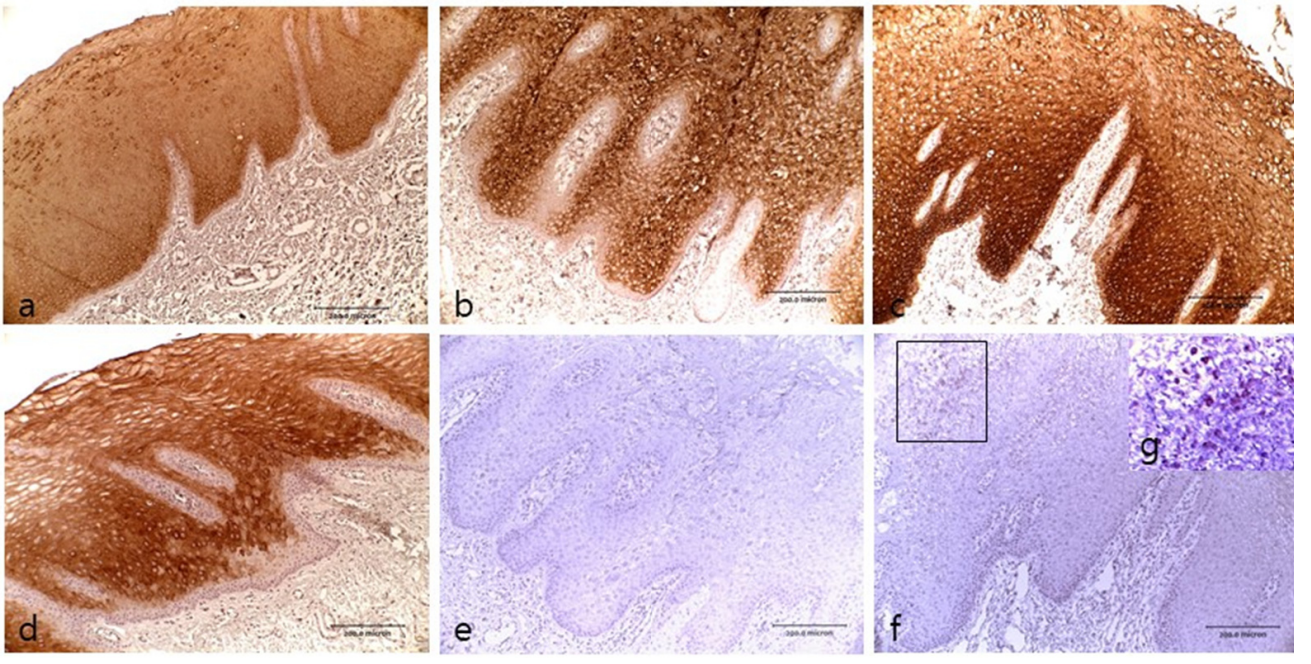

Fig. 4. Immunohistochemical staining of keratin 4 and 13. Diffuse positive for keratin 13 was found in suprabasal layer of normal oral mucosa (a) and WSN tissues (b, c); diffuse expression of keratin 4 was showed in suprabasal layer of normal oral mucosa (d), whereas negative response (e) or focal positive staining (f) was found in WSN tissues. (original magnification, x100, x400, respectively).

To date, four pathogenic mutations in the KRT4 gene[2,5-10,] and seven pathogenic mutations in the KRT13 gene[9,11-15] have been identified. To the best of our knowledge, our findings have revealed the presence of a novel KRT13 gene mutation[2,5-15]. We confirmed the presence of a heterozygous missense mutation 344 T to G in the KRT13-1A region, and the predicted amino acid change was leucine to arginine. Many researchers have reported T to C changes in the same or a similar region of KRT13, which results in the replacement of leucine by proline[9,11-13]. Our results revealed a T to G change that predicted the amino acid change of leucine to arginine, instead of proline. However, because leucine changed to a different amino acid, it was considered that leucine might have an important role in WSN. Since leucine is the non-polar hydrophobic amino acid, and arginine is a polar hydrophilic alkali amino acid, the changes in polarity could affect the structure of the protein that causes damage to the cytokeratin frame work. A 1583 A to G mutation in non-coding area of KRT4 gene was observed in the proband and her mother, but it seemed that this single nucleotide phenomenon (SNP) was not related to WSN, because the same mutation was observed in the unaffected father. Zhang et al. reported a 2324 A to G mutation in non-coding region of the KRT4 gene, which might be a neutral polymorphism.[2] Even when compared to our results, it is possible that this SNP might not be associated with the disease. Shimizu el al.[2,5,10] reported that mutation of the KRT4-2B gene might be a characteristic of populations in East Asian countries, but this mutation was not found in the present study.

In contrast to genetic analysis, the patients expressed keratin 13 and loss of keratin 4 immunohistochemically. We can extrapolate that genetic mutation of keratin 13 does not seem to affect its expression, Instead, the loss of keratin 4 may be due to unproven genetic mutation or epigenetic dysregulation. Further study to investigate genetic and

epigenetic mechanism of the loss of keratin expression should be required.

Conflict of interest: No funding sources or no interest are declared.

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