Keratin 1 Gene Mutation Detected in Epidermal Nevus with Epidermolytic Hyperkeratosis

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Since 1994, four cases of epidermal nevus with epidermolytic hyperkeratosis (EH) caused by keratin 10 gene mutations have been reported, although no keratin 1 (K1) gene mutation has yet been reported. We detected a K1 gene (*KRT1*) mutation in epidermal nevus with EH in a 10-year-old Japanese male. The patient showed well-demarcated verrucous, hyperkeratotic plaques mainly on the trunk, covering 15% of the entire body surface. No hyperkeratosis was seen on the palms or soles. He had no family history of skin disorders. His lesional skin showed typical granular degeneration and, ultrastructurally, clumped keratin filaments were observed in the upper epidermis. Direct sequence analysis of genomic DNA extracted from lesional skin revealed a heterozygous 5′ donor splice site mutation c.591 + 2T > A in *KRT1*. This mutation was not detected in genomic DNA samples from the patient's peripheral blood leukocytes or those of other family members. The identical splice mutation was previously reported in a family with palmoplantar keratoderma and mild ichthyosis, and was demonstrated to result in a 22 amino-acid deletion p.Val175_Lys196del in the H1 and 1A domains of K1. To our knowledge, the present patient is the first reported case of epidermal nevus associated with EH caused by a K1 gene mutation in a mosaic pattern.

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INTRODUCTION

Epidermal nevi are skin hamartomatous lesions comprised of keratinocytes. Their clinical features include circumscribed verrucous lesions of any size, single or multiple in nature, and they can occur at any site, frequently following Blaschko's lines. Epidermal nevi are thought to reflect a genetic mosaicism, and it has been often hypothesized that epidermal nevi with epidermolytic hyperkeratosis (EH) reflect differentiation specific, suprabasal keratin gene mutations. The term ''EH'' in this case means pathological changes seen in bullous congenital ichthyosiform erythroderma, a keratin 1 (K1)/keratin 10 (K10) disease, although EH is a histological feature of more than one disease. Indeed, K10 gene mutations were reported in four cases of epidermal nevi with EH (Paller *et al.*, 1994; Moss *et al.*, 1995), although no K1 gene mutation has yet been reported. A mosaic mutation in the V1 domain of keratin 16 was reported to underlie unilateral palmoplantar verrucous nevus with vacuolar degeneration of keratinocytes in the upper epidermis (Terrinoni *et al.*, 2000).

A diverse range of subtly different phenotypes including classical bullous congenital ichthyosiform erythroderma has been described with mutations in K1 (reviewed by Lane and McLean, 2004). Splice site mutations affecting the K1 peptide 1A or 2B domain caused epidermolytic palmoplantar keratoderma (PPK) (reviewed by Terron-Kwiatkowski *et al.*, 2002). Now a total of 47 different mutations have been reported in K1 (The Human Intermediate Filament Database). Here we report that a splice site mutation in the K1 gene, previously reported in a case of PPK and mild ichthyosis, was associated with the epidermal nevus with EH disease phentoype. As far as we know, the present case is the first reported patient of epidermal nevus with EH associated with a K1 gene mutation.

RESULTS

Clinical features

A 10-year-old Japanese male showed well-demarcated verrucous, hyperkeratotic plaques mainly on the trunk, covering 15% of his entire body surface. They distributed following the Blaschko's lines (Figure 1). No hyperkeratosis was seen on the palms and soles. The other family members including his parents and elder brother had neither bullous congenital ichthyosiform erythroderma nor epidermal nevus.

Typical granular degeneration and clumped keratin filaments were seen in the epidermal nevus

Light microscopy of the skin samples from the nevus on the trunk revealed typical granular degeneration with large keratohyalin granules in the upper epidermis (data not

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Abbreviations: EH, epidermolytic hyperkeratosis; k2e, keratin 2e; K1, keratin 1; K10, keratin 10; PPK, palmoplantar keratoderma

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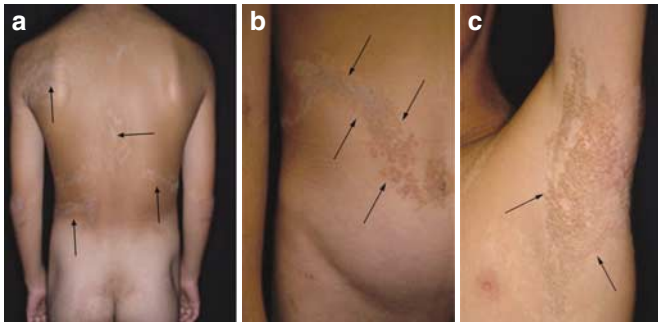

Figure 1. Clinical features of epidermal nevi at the age of 10. Well-demarcated verrucous, hyperkeratotic plaques, light to dark brown in color, were seen on the (**a**) back, (**b**) axilla, and (**c**) abdomen.

shown). Electron microscopy showed clumped keratin filaments in the upper epidermal keratinocytes (data not shown). Some of those keratinocytes with abnormal keratin clumps were undergoing degeneration.

A splice site mutation in K1 gene (*KRT1*) was identified in lesional skin but not in peripheral blood

Mutation analysis of the entire 1–9 exons including the intron–exon boundaries of the K1 gene (*KRT1*) revealed a heterozygous T>A substitution at base position 591+2, in intron 1 (c.591+2T>A). (Figure 2). This mutation disrupts the *KRT1* exon 1 donor splice site. This mutation was not detected in genomic DNA samples from patient's peripheral blood leukocytes (Figure 2a) or those of his family members. No other mutation was found in the entire exon and intron/exon borders of the K1 and K10 genes. The mutation was not found in 100 normal, unrelated Japanese alleles (50 healthy unrelated Japanese individuals) by sequence analysis, and was unlikely to be a polymorphism (data not shown).

By mutant allele-specific amplification analysis (Hasegawa *et al.*, 1995; Xu *et al.*, 2003), a 102-bp fragment derived from the mutant allele was amplified from the genomic DNA sample extracted from the lesional skin (Figure 2b). The 102-bp fragment was sequenced and it was confirmed that the fragment was derived from the targeted region of K1 gene, *KRT1*. The mutant allele-specific amplification showed no PCR product bands from the peripheral blood cell DNA samples from the patient, any other family members or controls.

K1 expression was weak and keratin 2e (K2e) expression was upregulated in the epidermal nevus lesion

Immunofluorescence studies revealed that K1 and K10 were present in the lesional epidermal suprabasal layers, although K1 expression was weaker than that in the normal control skin (Figure S1a–d). In the regions showing granular degeneration, abnormal, large granules in the degenerated keratinocytes were positive for K1 and K10. K2e was expressed only in the uppermost spinous and the granular layers of epidermis in normal control skin (Figure S1f). In the patient's lesional skin, K2e expression was seen in the almost all suprabasal epidermal layers, suggesting an upregulated expression of K2e in the lesional epidermis (Figure S1e).

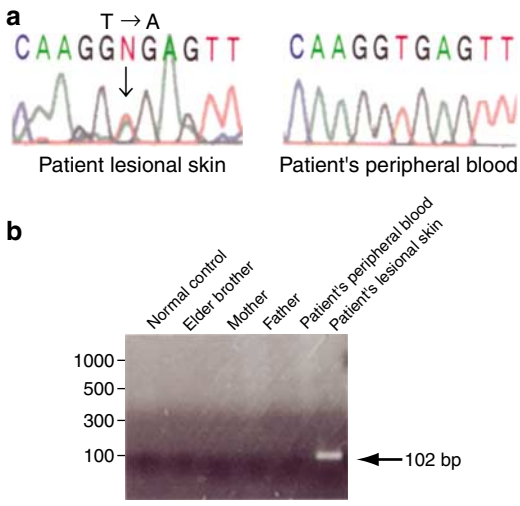

Figure 2. A splice site mutation c.591+2T>A was detected in the lesional skin. (**a**) Direct sequencing of *KRT1* exon 1 PCR products derived from patient's lesional skin revealed heterozygous donor splice site mutation c.591+2T>A. This mutation was not detected in genomic DNA samples from the patient's peripheral blood leukocytes. (**b**) Mutant allele-specific amplification analysis showed the amplification band from the mutant allele as a 102-bp fragment only from the DNA sample from the patient's lesional skin, confirming the presence of the mutation c.591+2T>A in the patient's epidermal nevus.

DISCUSSION

All the reported causative mutations underlying epidermal nevus with EH affected the K10 gene (Paller *et al.*, 1994; Moss *et al.*, 1995). As far as we know, the present case is the first reported case of epidermal nevus with EH caused by a K1 gene mutation.

K2e expression increased in the lesional epidermis of the present case. Although we do not have any direct evidence, K2e expression might be upregulated compensatively in the epidermis with disrupted keratin network. Indeed, increased K2e expression was also observed in the lesional epidermis with disturbed keratin network of ichthyosis bullosa of Siemens patients (Akiyama *et al.*, 2005).

In our case, the causative K1 mutation was detected only in the lesional skin, but not in the peripheral blood cells, as previously reported in K10 mutations in epidermal nevus with EH (Paller *et al.*, 1994; Moss *et al.*, 1995). Our findings further support that the mutation detection of K1 as well as K10 in epidermal nevus can be reliably performed only from direct examination of lesional skin, not from analysis of other tissue or peripheral blood cells.

As epidermal nevus is a disease caused by somatic mosaicism, widespread skin lesions increase the risk of germ-line transmission (Paller *et al.*, 1994). In case causative K1 or K10 mutations are transmitted in germ-line, a half of the children from patients with epidermal nevus with EH are expected to be affected with ichthyosis on the whole body. Mutation analysis using a patient's sperm gives us information on germ-line transmission (Zlotogora, 1998; Rantamaki *et al.*, 1999). If the germ-line transmission is confirmed, prenatal genetic screening may be applied for the offspring of