A novel large deletion mutation of *FERMT1* gene in a Chinese patient with Kindler syndrome

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Abstract: Kindler syndrome (KS; OMIM 173650) is a rare autosomal recessive skin disorder, which results in symptoms including blistering, epidermal atrophy, increased risk of cancer, and poor wound healing. The majority of mutations of the disease-determining gene (*FERMT1* gene) are single nucleotide substitutions, including missense mutations, nonsense mutations, etc. Large deletion mutations are seldom reported. To determine the mutation in the *FERMT1* gene associated with a 7-year-old Chinese patient who presented clinical manifestation of KS, we performed direct sequencing of all the exons of *FERMT1* gene. For the exons 2–6 without amplicons, we analyzed the copy numbers using quantitative real-time polymerase chain reaction (qRT-PCR) with specific primers. The deletion breakpoints were sublocalized and the range of deletion was confirmed by PCR and direct sequencing. In this study, we identified a new 17-kb deletion mutation spanning the introns 1–6 of *FERMT1* gene in a Chinese patient with severe KS phenotypes. Her parents were carriers of the same mutation. Our study reported a newly identified large deletion mutation of *FERMT1* gene involved in KS, which further enriched the mutation spectrum of the *FERMT1* gene.

Key words: Kindler syndrome, *FERMT1* gene, Mutation
doi:10.1631/jzus.B1500080   **Document code:** A   **CLC number:** R758.66

1 Introduction

Kindler syndrome (KS; OMIM 173650) is a rare autosomal recessive skin disorder characterized by blistering, extensive epidermal atrophy, photosensitivity, increased risk of cancer, and poor wound healing. The mucosal involvement included early and severe periodontitis, and/or esophageal, gastrointestinal, and genital involvement (Jobard *et al.*, 2003; Siegel *et al.*, 2003; Has and Bruckner-Tuderman, 2006; Kern *et al.*, 2007). It was first described by Theresa Kindler (1954) in a 14-year-old girl who had suffered from acral blistering since childhood and subsequently developed poikiloderma and photosensitivity.

The KS disease-determining gene, termed *FERMT1* gene (OMIM 607900), was mapped to chromosome 20p12.3 (Jobard *et al.*, 2003; Siegel *et al.*, 2003). This gene contains 15 exons and spans 48.5 kb of genomic DNA. The coding sequences extend from exon 2 to exon 15. The encoded protein (kindling-1, 677 amino acid (aa), molecular weight of 77.3 kDa) is implicated in linking the actin cytoskeleton to the extracellular matrix (Jobard *et al.*, 2003; Siegel *et al.*, 2003). Lack of kindling-1 in keratinocytes would result in decreased cell adhesion, reduced proliferation, increased apoptosis, and loss of cell polarity (Herz *et al.*, 2006).

In our present study, genetic analysis, including the direct sequencing of all exons and quantitative

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real-time polymerase chain reaction (qRT-PCR) were performed in order to identify a new, larger deletion mutation spanning about 17 kb from the intron 1 to intron 6 of *FERMT1* gene in a Chinese KS family.

2 Materials and methods

2.1 Patient and DNA samples

Genomic DNA was extracted from the peripheral blood leukocytes of the patient and her parents, using the phenol-chloroform method. Informed consent was obtained from the patient's parents. The project was approved by the Ethics Committee of the Capital Institute of Pediatrics (Beijing, China).

2.2 Molecular genetic analysis

2.2.1 DNA sequence analysis

For the patient with clinically confirmed KS, we screened all exons (exons 1–15) and flanking sequences of the *FERMT1* gene using Sanger sequencing. The primers for the PCR and the annealing temperatures were as described by Siegel *et al.* (2003). The PCR products were purified and direct sequencing of fragments was carried out with an ABI 3730 automatic sequencer (Applied Biosystems, USA).

2.2.2 Copy number analysis of *FERMT1* gene

For the exons without amplification products by Sanger sequencing, we performed qRT-PCR in order to detect their copy numbers on an ABI Prism™ 7500 Sequence Detection System (Applied Biosystems, USA) using SYBR Green Master Mix (Applied Biosystems, Foster, USA). All data analysis was performed using the $2^{-\Delta\Delta C_T}$ method in Microsoft Excel Version 2007. Primers were designed using the Primer Express 3.0 software (Applied Biosystems, USA) for the 9 exons of the *FERMT1* gene and were evaluated using the National Center for Biotechnology Information's BLAST program. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was selected as endogenous control. All samples were run in triplicate. We used the $\Delta\Delta C_T$ method to analyze the copy numbers and compare them with those of 20 healthy samples. Experiments were replicated at least twice if a deletion/duplication was suspected to have occurred.

2.2.3 Breakpoint analysis of *FERMT1* gene

In order to sublocalize the deletion breakpoints, we designed 9 pairs of primers to amplify the non-overlapping fragments in introns 1 and 6. Subsequently, the junction fragment was amplified with the template DNA of the patient using a pair of primers, P10F in intron 1 (forward primer: 5'-AGGCAA GCAGTTAGGCCTAAT-3') and P10R in intron 6 (reverse primer: 5'-ATGACAGAGCCCATTTCCTG-3') to confirm the 5' and 3' breakpoints of the *FERMT1* gene in the patient.

3 Results

3.1 Clinical features of the patient with Kindler syndrome

The patient, a 7-year-old female born to consanguineous parents, presented with recurrent blistering over the hands and feet (Fig. 1a). Her parents stated that blister generation might be associated with local friction. When given topical mupirocin ointment the sites of lesions were consequently healed without scarring, and it has been so since the early neonatal period. When she was eight months old, her teeth came through but dental caries were also observed. Subsequently, constipation, including dry stool and defecation difficulties, began at about the age of one. When exposed to sunlight she was found to be prone to photosensitivity. When she was seven years old, she was admitted to our department of dermatology. Upon physical examination, poikiloderma was noted on her face, neck, and upper chest, and erythematous lesions and skin atrophy were observed on the trunk and four extremities (Figs. 1b and 1c). In addition, pseudoainhum was obviously apparent. Examination of the oral cavity showed poor preservation of teeth and severe periodontitis, with easy bleeding (Figs. 1d–1f). She also stated that slight dysphagia existed. Electron microscopy showed a fracture of the lamina densa (Fig. 1g). Histopathological examinations of the skin biopsy with the abdominal involved area showed hyperkeratosis, atrophy of the epidermis, fracturing of the basal layer, and melanophages in the papillary dermis. A biopsy of the skin lesion showed the features of poikiloderma (Fig. 1h). The results of routine blood examinations, cranial computed tomography, and electroencephalogram were all normal.
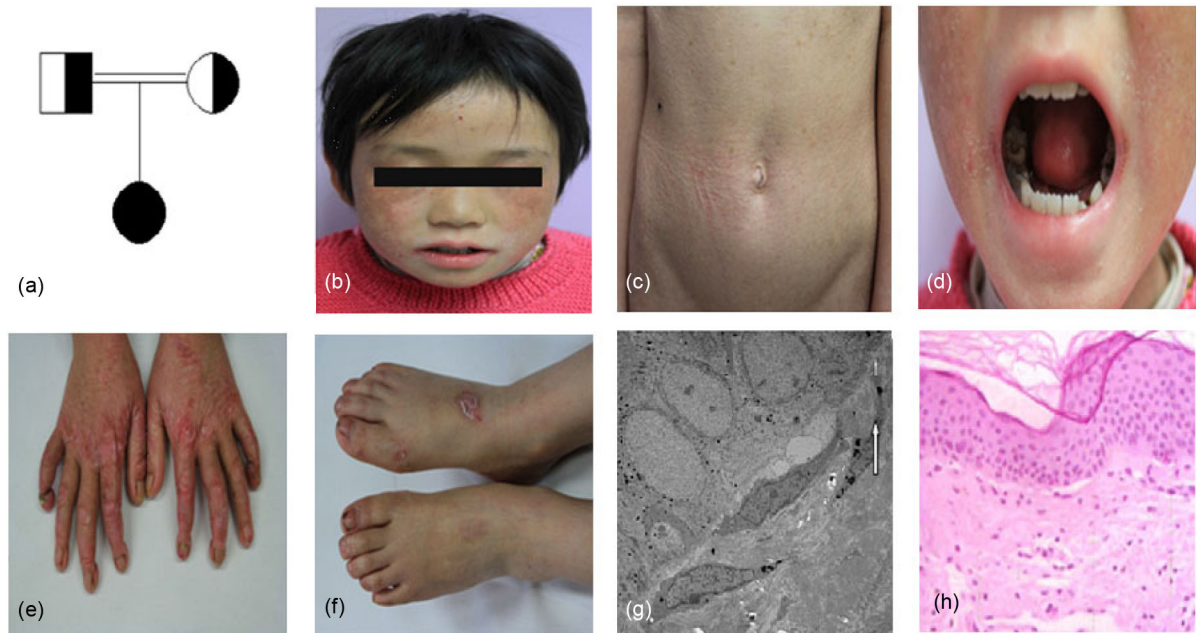
Fig. 1 Clinical features of the patient with Kindler syndrome
(a) Pedigree figure of the family (circle=female, square=male, black=carrier of the *FERMT1* gene mutation; blank=normal);
(b) Poikiloderma on her face; (c) Cigarette-paper-like atrophy on the skin of abdomen; (d) Erosion on the gingivitis;
(e) Pseudoainhum and marked skin atrophy on hands; (f) Erosions and scars on the dorsal aspects of the feet; (g) Electron microscopic showed the fissure in lamina lucida (arrows); (h) Histopathological examinations of skin biopsy of the abdominal involved area showed hyperkeratosis and melanophages in the papillary dermis

3.2 Molecular genetic analysis

3.2.1 DNA sequence analysis

After the Sanger sequencing of the *FERMT1* gene, no mutation was identified within 10 exons, including exons 1 and 7–15 in the patient. However, DNA sample from the patient failed to yield PCR amplification products for exon 2 to exon 6 (5 exons), whilst they could be amplified in the normal controls and her parents, which revealed that the amplification condition was optimal.

3.2.2 Gene copy number changes and breakpoint confirmation

Since no amplification products for exon 2 to exon 6 were observed with the patient, we were able to precisely identify the copy numbers of this gene (exons 1, 2–7, 14, and 15) using qRT-PCR on genomic DNA. This result further showed that, for exons 2–6, both of the patient's parents carried one copy and the patient had no copy. However, for exons 1, 7, 14, and 15, the patient and her parents carried two copies (Fig. 2).
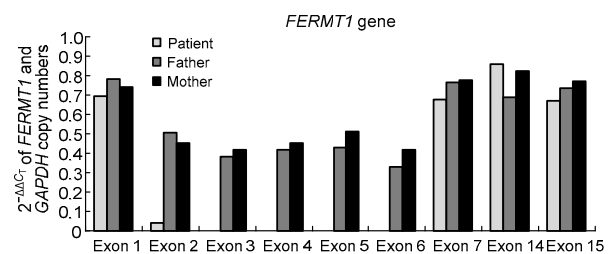

Fig. 2 Gene copy number changes using qRT-PCR
qRT-PCR results showed zero copy at exon 2 to exon 6 for the patient

The results of DNA sequencing and copy numbers analysis revealed that the 5' and 3' breakpoints may be closed to exons 1 and 6, respectively. Several amplification results from intron 1 and intron 6 confirmed that 5' and 3' breakpoints were located in introns 1 and 6, respectively. With primers P10F and P10R, the truncated PCR product (763 bp) was obtained from the patient, which revealed that a homozygous large deletion mutation of 17 252 kb was observed in the patient. Meanwhile, her parents both carried the same heterozygous deletion mutation. However, no truncated fragments were observed within the normal controls (Fig. 3).
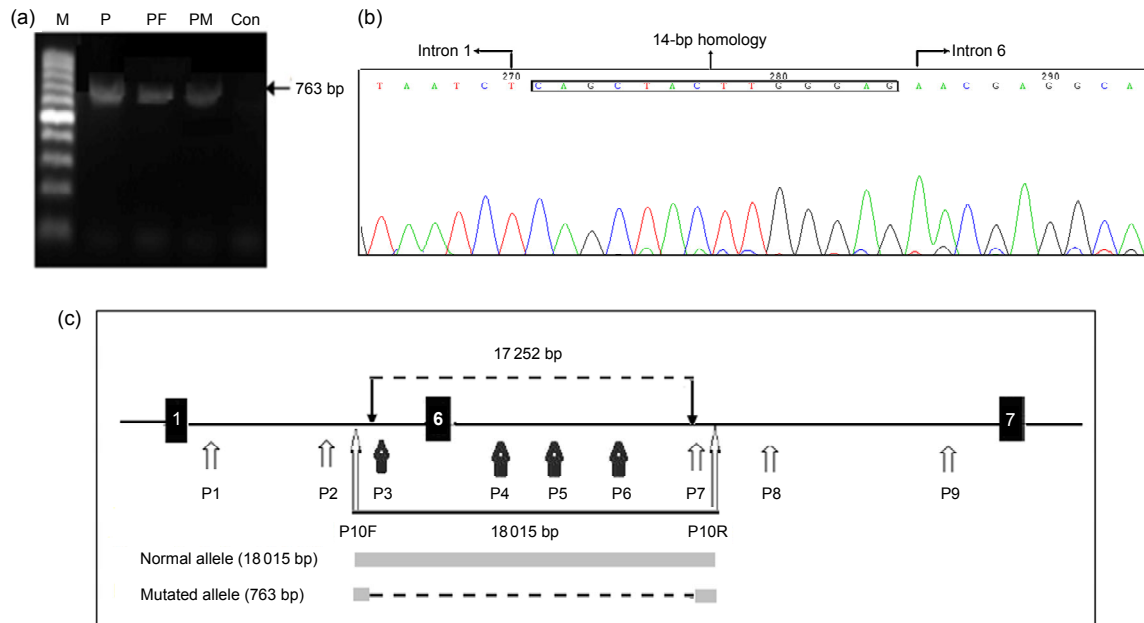
Fig. 3 Breakpoint confirmation by genetic methods

(a) 763-bp product was obtained from the patient and her parents, while no amplicons were detected for the normal control since the 18 015-bp product from the normal allele might be too large to amplify in this experiment. M: DNA marker I; P: patient; PF: patient's father; PM: patient's mother; Con: control. (b) 763-bp fragment from the patient revealed the 5' and 3' breakpoints within introns 1 and 6 and the 14-bp short homology across the breakpoints is shown in the box. (c) *FERMT1* genomic region spans exons 1–7, and the 17 252-bp deletion is delineated with a dashed line. P: primer

4 Discussion

In this paper, we have reported a Chinese patient with KS, who had been molecularly confirmed to carry a large homozygous deletion of the *FERMT1* gene. To date, over 70 *FERMT1* gene mutations have been reported (Lai-Cheong *et al.*, 2007; Mansur *et al.*, 2007; Has *et al.*, 2008; Zhou *et al.*, 2009; Mas-Vidal *et al.*, 2010; Kartal *et al.*, 2015; Youssefian *et al.*, 2015). Many mutations are single nucleotide substitutions, including missense mutations, nonsense mutations, etc.

The predicted protein structure of kindlin-1 encoded by *FERMT1* gene reveals several domains of interest, in particular the FERM domain which is interrupted by a pleckstrin homology (PH) domain (Jobard *et al.*, 2003; Yates *et al.*, 2012). The protein is expressed at high levels in epithelia, in particular in the epidermis and the gastro-intestinal tract (Sadler *et al.*, 2006). Loss of kindlin-1 would interrupt the organization and anchorage of the actin cytoskeleton to integrin-associated platforms. To our knowledge, our patient carried the largest genomic deletion of the *FERMT1* gene, spanning 17 kb from intron 1 to intron 6.

We suspected that it was possible that this deletion might lead to a complete loss of exon 1 to exon 7 (1–319 aa). Moreover, it is noteworthy that the deletion range contained the translation initiation site (TIS) ATG located in exon 2. We used ORF (open reading frame) Finder (http://www.ncbi.nlm.nih.gov/gorf/orfig.cgi) to predict the complementary DNA (cDNA) sequences lacking in the original TIS for potential protein encoding segments. The predicted TIS might be the new start codon, which was 690 bp in length and encoded a 230-aa protein. The predicted protein starting at ATG in exon 7 was 447 aa shorter than normal kindlin-1. It is therefore presumed that the truncated kindlin-1 fragment might lead to elimination of the entire N-terminal FERM domain and most of the PH domain of kindlin-1. It is also possible that no kindlin-1 is expressed at all. It is a pity that we cannot perform a mutant protein expression analysis since no further samples of the patient's skin were available.

During the repair of double strand breaks (DSBs) in DNA, several different mechanisms are involved, including non-homologous end-joining (NHEJ),

microhomology-mediated replication-dependent recombination (MMRDR), and homologous recombination (HR) (including non-allelic homologous recombination (NAHR) and single strand annealing (SSA)) (Chen *et al.*, 2010; Schipler and Iliakis, 2013). Large genomic deletions of the *FERMT1* gene can often occur through erroneous recombination of non-allelic homologous repetitive elements, such as Alu sequences (Has *et al.*, 2006). By blasting these with the wide sequences, we found that the 5' and 3' breakpoints had 14 bp identical sequences. Meanwhile, analysis of the intron sequence repeat elements by the RepeatMasker program revealed that the 5' and 3' breakpoints were located in the AluSg and AluSp elements, respectively. Thus, we suspected that the deletion breakpoints embedded in Alu repeats might be mediated by Alu-mediated HR. Zhou *et al.* (2009) assumed that 3017-bp deletion in the *FERMT1* gene may be caused by matrix attachment regions (MARs) (the short homologous sequence GA and the sequence TTTAAA), rather than by the Alu/Alu HR.

To date, the genomic Sanger sequencing has been the main detection method to screen the *FERMT1* mutation for KS patients, since the majority of *FERMT1* mutations are single nucleotide substitutions, including missense mutations, nonsense mutations, etc. However, in some cases, *FERMT1* mutations could not be disclosed using the methods outlined above. In view of the presence of many repeat sequences in introns of the *FERMT1* gene, we suspected that a certain proportion of cases might be due to large scale deletion of this gene. As more KS patients were confirmed with large deletion of the *FERMT1* gene (Has *et al.*, 2008; Zhou *et al.*, 2009), many researches revealed that a rational mutation detection strategy of *FERMT1* mutations would be necessary for the genetic diagnosis of KS patients (Takeichi *et al.*, 2015). Thus, we proposed that a copy number analysis of *FERMT1* gene might be an optimal procedure for the patients not fully confirmed by genomic Sanger sequencing. This could help us to preliminarily determine the deletion range. Subsequently, the 5' and 3' breakpoints would be sublocalized by PCR and direct sequencing. Primers designed near the two breakpoints would help us to confirm the deleted sequences.

Our study identified a new large deletion mutation of *FERMT1* gene, which might expand the mutation database. Moreover, based on the experience of detecting the mutation, we would emphasize that a rational diagnostic procedure of *FERMT1* gene mutations screening would be advisable.

Compliance with ethics guidelines

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中文概要

题　目：Kindler 综合征患儿一例：*FERMT1* 基因新大片段纯合缺失突变

目　的： Kindler 综合征（Kindler syndrome，KS；OMIM 173650），又称伴大疱的先天性皮肤异色症，是一种罕见的常染色体隐性遗传性皮肤病，其致病基因为编码 Fermitin 家族同源物 1 蛋白的 *FERMT1* 基因。本研究旨在对一例临床诊断为 KS 的患儿及其父母进行 *FERMT1* 基因突变分析。

创新点： 本研究中的 KS 病例为 *FERMT1* 基因新纯合缺失突变所致，为目前证实缺失范围最大的病例。

方　法： 经知情同意后，采集一例患儿及父母外周血。首先利用 Sanger 测序检测患儿 *FERMT1* 基因所有外显子（外显子1~15）及两侧内含子区域；随后利用实时定量聚合酶链式反应（qRT-PCR）测定部分外显子（外显子 1、2~7、14 和 15）的拷贝数，最终通过 PCR 和测序方法确定患儿 *FERMT1* 基因的断裂位点。

结　论： 本研究报告了一例 *FERMT1* 基因新大片段纯合缺失（17 252 bp 纯合缺失）所致的 KS（图 1~3），扩展了该基因突变数据库。同时，本研究提示该疾病的基因诊断方法不应局限于常规 DNA 外显子测序，应综合多种方法进行突变分析，以防止大片段缺失病例的漏检。

关键词： Kindler 综合征；*FERMT1* 基因；突变