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Letter to the Editor

Identification of an Alu-mediated 12.2-kb deletion of the complete *LPAR6* (*P2RY5*) gene in a Turkish family with hypotrichosis and woolly hair

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Abstract: Hypotrichosis is a rare form of progressive hair loss characterized by sparse and occasionally woolly hair that is curly and breaks easily. Disease-causing mutations in *LIPH*, *LPAR6* and *KRT74* have recently been identified. We describe a four-generation pedigree from Turkey following an autosomal recessive pattern, in which the four affected members had hypotrichosis and woolly hair. By sequencing *LPAR6* and the use of SNP arrays, we revealed a homozygous loss of the entire *LPAR6* gene in the affected individuals. We hypothesize that the 12-kb deletion resulted from illegitimate recombination secondary to slip-replication. The orientation of three Alu repeats around *LPAR6* may have provoked the formation of a 'triple-barrel' structure during replication, thereby allowing strand slipping. This first report of complete *LPAR6* loss expands the spectrum of known *LPAR6* mutations and suggests a novel mechanism for this gene and for the formation of DNA rearrangements in general.

Key words: hypotrichosis – woolly hair – LPAR6/P2RY5 – deletion – Alu elements

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Background

Three genes for isolated hypotrichosis with woolly hair have been identified to date. These include the lipase H (*LIPH*) gene (1) and the lysophosphatidic acid receptor 6 (*LPAR6*) gene, formerly known as *P2RY5* (2–4). Both genes cause autosomal recessive forms of this disorder. The third identified gene is encoding keratin (*KRT*) 74, which causes an autosomal dominant form of woolly hair syndrome (5).

Question addressed

We recently recruited a Turkish family with autosomal recessive isolated hypotrichosis and woolly hair. The four-generation family included four affected individuals. We obtained DNA samples of 11 individuals (Fig. 1a). The index case was a 4-year-old girl who had been referred to our clinic with hypotrichosis and woolly hair (Fig. 1c). Her parents were third-degree relatives (Fig. 1a). Clinical examination of the girl revealed curly and woolly hair, a low nuchal hairline and laterally sparse eyebrows (Fig. 1c). Her skin, nails, teeth and sweating were normal. A similar hair phenotype was observed in her mother (Fig. 1d) and her two maternal uncles (Fig. 1e, f). Ethical approval was obtained from the Institutional Review Board of the University of Bonn, and the study was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from each participant or legal guardian.

Experimental design

Venous blood samples were collected, and genomic DNA was extracted according to standard procedures. To identify the pathogenic mutation in this family, we performed Sanger sequencing of the *LIPH* and *LPAR6* genes in the index patient. In parallel, a genome-wide scan was performed in 11 family members using Illumina's HumanOmniExpress chip (Illumina Inc., San Diego, USA) to identify runs of homozygosity in the affected cases.

Results

No pathogenic mutation in *LIPH* was detected. However, we were unable to obtain any PCR product for *LPAR6* which pointed to a deletion. The analysis of the chip data by use of the homozygosity mapper (6), furthermore, revealed linkage to chromosome 13 in the region containing the *LPAR6* gene. *LPAR6* is located in intron 17 of the retinoblastoma (*RB1*) gene, and the two genes are transcribed in opposite directions (7). *RB1* is a tumor suppressor gene, and mutations cause autosomal dominant inherited retinoblastoma. As our patients showed no eye abnormalities, we assumed that the breakpoint must be located within intron 17 of *RB1*. To narrow-down the breakpoint region, we used a primer walking strategy involving several consecutive primer pairs within intron 17 in the 5′ and 3′ region of *LPAR6* (sequences obtainable on request). We detected a deletion of 12 211 basepairs (bp) between positions 47 874 769 and 47 886 981 (NCBI36/hg18; Fig. 2a). To determine whether the deletion affects splicing of *RB1*, we isolated RNA of immortalized lymphocytes of patient III:7 and performed a reverse transcriptase PCR. We used primers lying in exons 16 and 19 of *RB1* and obtained a fragment of 375 bp, which corresponds to the correctly spliced *RB1* gene (Supplementary Fig. S2).

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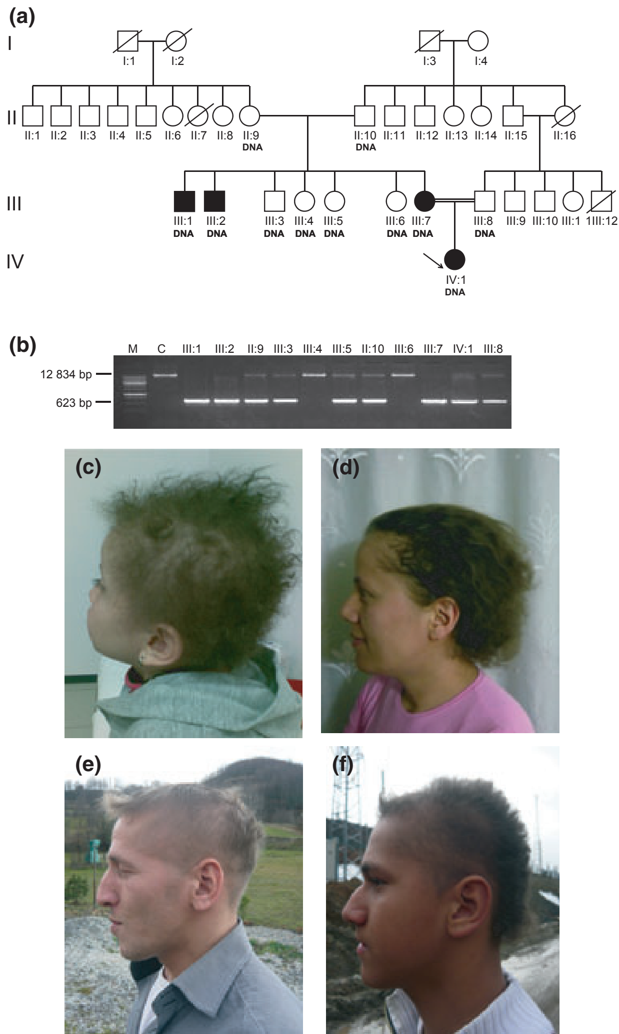

Figure 1. Pedigree structure of the Turkish family and clinical appearance of the four family members with woolly hair. The four-generation family included 11 individuals, four of whom were affected (a). The parents of the index case were third-degree relatives, as indicated by a double line. Circles denote females; squares denote males; blackened symbols indicate affected individuals; 'DNA' below symbols indicates individuals included in the mutation analysis. Deceased individuals are depicted by crossed symbols. The four-year-old index patient is indicated with an arrow. To visualize the 12-kb deletion encompassing the *LPAR6* gene, long-range PCR amplification was performed in all 11 family members using the primers 5′-atgagtctcactctgttttccc-3′ and 5′-aagtcaagtgagtaatgacaatatg-3′ (b). A 1-kb ladder was used as a size marker (M, first lane). The anticipated size of the wild-type fragment is 12 834 bp (see lane 2: healthy control (c) and individuals III:4 and III:6). A smaller fragment of 623 bp was amplified in all patients (III:1, III:2, III:7 and IV:1). Heterozygous mutation carriers showed both bands (II:9, III:3, III:5, II:10 and III:8). The index patient had a woolly hair phenotype, that is, curly and woolly hair. Her eyebrows were sparse laterally, and she had a low nuchal hairline (c). A similar hair phenotype was observed in the other affected family members. Her mother (d) and two uncles (e, f) are shown.

In the four affected cases, the deletion was in a homozygous state. In the father (III:8), the grandparents (II:9, II:10), an uncle (III:3) and an aunt (III:5) of the index patient, the deletion was heterozygous (Fig. 1b). The deletion breakpoints were detected using the primers 5-aagtcaagtgagtaatgacaatatg-3′ and 5′-atgagtctcactctgttttccc-3′ (Fig. 2a). To improve visualization of the deletion, we designed primer pairs around and within the deletion. These were used in a

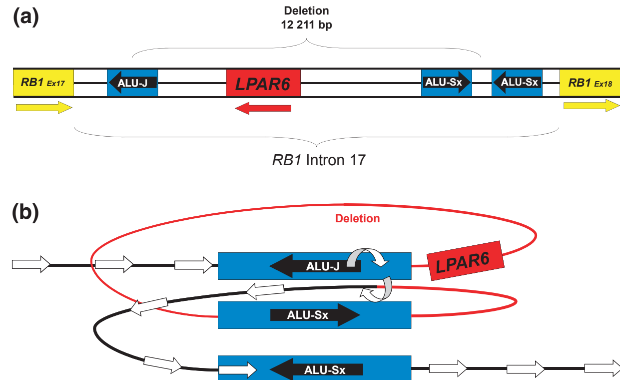

Figure 2. Schematic drawing showing the location of *LPAR6* and the Alu sequences involved in the 12-kb deletion. *LPAR6* is located in intron 17 of the *RB1* gene and is surrounded by different Alu elements (Alu-J, Alu-Sx) (a). Owing to the orientation of the three Alu repeats, a 'triple-barrel' structure involving one strand may have formed during replication. Thus, *LPAR6* and one Alu-Sx element would have been deleted following illegitimate recombination secondary to slip-replication involving one strand (b).

parallel PCR. Homozygous carriers had a 623-bp PCR product, while non-carriers had a 314-bp PCR product (Supplementary Fig. S1).

Analysis of the deleted region revealed the presence of three Alu elements. Whereas the 5′-breakpoint is located in an Alu-J repeat (GenBank entry HSU14567), the 3′-breakpoint is flanked by two Alu-Sx sequences (GenBank entry HSU14574). These two Alu-Sx sequences are orientated in opposite directions (Fig. 2b). No inverted repeats or short stretches of identical nucleotides flank the breakpoint region.

Discussion

Alu repeat sequences are a class of short retrotransposable interspersed elements (SINEs) consisting of approximately 300 bp. During evolution, the copy number of Alu elements in the human genome has increased to over one million, and Alu elements are present at around every 4 kb (8, 9). Approximately 0.27% of all human disease mutations are attributable to mobile elements, and various mechanisms have been proposed, including deletional/insertional mutagenesis, recombination, retrotransposition-mediated and gene conversion-mediated deletion, as well as 3′ transduction (10).

The presence of Alu sequences around the deletion breakpoints suggests an illegitimate recombination secondary to slip-replication involving both strands (11). This would lead to the deletion of one of these Alus, together with the nucleotides located in between these repeats. This type of deletion/formation was observed in hypotrichosis families from Russia. All of the affected cases had a homozygous deletion within the *LIPH* gene, which had been caused by uneven recombination between two Alu repeats (Alu-Y, Alu-Sc) (1). However, the deletion described in the present report involved the 3′-end of Alu-J, and its 3′-break was centred between the two Alu-Sx repeats. This suggests replication slippage involving one strand only (Fig. 2b). Owing to the orientation of the three Alu repeats, a 'triple-barrel' structure may have formed during replication, thereby allowing strand slipping. Consequently, around 12 kb comprising the complete *LPAR6* gene and its flanking sequences would have been deleted. Although a large number of mutations have been reported for patients with hypotrichosis with or without woolly hair, the uneven recombination event between Alu sequences reported by Kazantseva *et al.*

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(1) and the present Alu-mediated deletion are the first Alu-mediated events to be described for any type of isolated hair loss.

Conclusions

In conclusion, the present report is the first to describe a homozygous loss of the entire *LPAR6* gene. The presence of three Alu sequences around the deletion breakpoints suggests that these Alu elements form intrastrand hairpin loops that are easily rearranged. Formation of a 'triple-barrel' structure may promote primer–template slippage during DNA replication, thereby promoting *LPAR6* deletion if polymerase is able to bypass the loop. This expands the spectrum of *LPAR6* mutations and suggests a novel mechanism for this gene and for the formation of DNA rearrangements in general.

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Author contributions

H.M. and S.M.P. performed the experiments; E.T., A.H.P., H.I.A. and M.P. provided the DNA samples and clinical information of the patients; H.M., S.M.P. and M.L. analysed the data and wrote the paper; R.C.B. designed the research study and wrote the paper.

Conflicts of interest

The authors have no conflicts of interest to declare.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Pedigree structure of the Turkish family, sequence analysis in the index patient and confirmation of the 12.2-kb deletion by a primer pair around and within the deletion that were used in a parallel PCR.

Figure S2. Expression analysis of the *RB1* gene in patient III:7.

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Letter to the Editor

Dioxinodehydroeckol inhibits melanin synthesis through PI3K/Akt signalling pathway in α-melanocyte-stimulating hormone-treated B16F10 cells

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Abstract: Antimelanogenic activity has previously been reported in ethyl acetate fraction of *Ecklonia stolonifera*. In this study, using the isolated dioxinodehydroeckol from the fraction, we sought to investigate an antimelanogenic signalling pathway in α-melanocyte-stimulating hormone (α-MSH)–stimulated B16F10 melanoma cells. Treatment with dioxinodehydroeckol inhibited the cellular melanin contents and expression of melanogenesis-related proteins, including microphthalmia-associated transcription factor (MITF), tyrosinase and tyrosinase-related proteins TRP-1 and TRP-2. Moreover, dioxinodehydroeckol stimulated phosphorylation of Akt in a dose-dependent manner without affecting phosphorylation of ERK. These data suggest that dioxinodehydroeckol reduces melanin synthesis through the MITF regulation dependent upon PI3K/Akt signalling pathway.

Key words: dioxinodehydroeckol – *Ecklonia stolonifera* – melanin – Akt – B16F10

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Background

Melanogenesis is regulated by microphthalmia-associated transcription factor (MITF), a key transcription factor controlling the expression of melanogenesis-related enzymes including tyrosinase, tyrosinase-related proteins 1 (TRP-1) and TRP-2. Thus, expression and activity of these enzymes have been widely investigated to study for whitening function of natural compounds (1,2). Particularly, tyrosinase plays an essential role in melanogenesis. It catalyses the two rate-limiting steps in melanogenesis: the hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA) and the oxidation

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