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Case Report

Hairless Gene Nonsense Mutations in Alopecia Universalis: A Case Report

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Abstract

Alopecia universalis (AU) congenital, known as generalized atrichia, is a severe form of autosomal recessive alopecia that results in complete hair loss of scalp and body. Mutations in the human hairless gene (HR) are associated with the phenotype of the disease. A consanguineous couple who had a child with the generalized atrichia sign referred to us for genetic counseling. According to the patient's symptoms and after thorough examination and history taking, the HR gene was the candidate gene to be assessed and analyzed. For this purpose targeted primers were designed for all exons of the HR gene followed by running PCR for exons amplification. Finally, the PCR products were sequenced. Whole-gene sequence analysis revealed a nonsense homozygous mutation in exon 6 that, according to the ACMG guide, is a pathogenic variant. Sequence analysis of the exon in parents reveals that they are heterozygout for the non-sense mutation, as well.

Keywords: Alopecia; Human hairless gene; Alopecia universalis

Introduction

Hereditary human hair loss encompasses diverse types in which androgenic alopecia (male pattern baldness) and alopecia areata are the two most common forms, respectively (1). These kinds of alopecia have unclear inheritance of pattern with unknown precise etiology. For example, there is a tendency to relate alopecia areata to an autoimmune mechanism as immune infiltrates surround the hair follicles in the disorder (2). Contrary to these, congenital alopecia universalis (MIM 203655) or congenital atrichia (MIM 209500) follows autosomal recessive inheritance pattern with known molecular basis (alopecia3). Congenital hairlessness could be seen in families with autosomal dominant or X-linked inheritance (MIM 300042) with different gene or genes involvement (3). In autosomal recessive congenital atrichia, the initial hair growth is normal, but after birth, when the hair starts to shrink, the follicles are no longer able to regenerate leading to permanent hair loss (4). Mutations in the human hairless gene (HR) are associated with the phenotype of the disease

(5). Studies in RODENT and the human HR gene have revealed molecular mechanisms that indicate HR gene is involved in hair growth and development (6). The HR gene product is a nuclear transcription factor that is essential for the proper functioning of the skin and hair follicles (7).

In this study, we report a consanguineous couple with a child afflicted with alopecia universalis in whom molecular analysis of HR gene reveals c.1753C> T (Q585 *) homozygous mutation.

Case Report

Clinical data of the patient

The patient being in our study is a 6-year old boy whose parents are first cousins (as seen in the pedigree, the grandparents of both paternal and maternal sides are first cousins as well, increasing apparently the consanguineous coefficient of the patient) (Fig. 1).

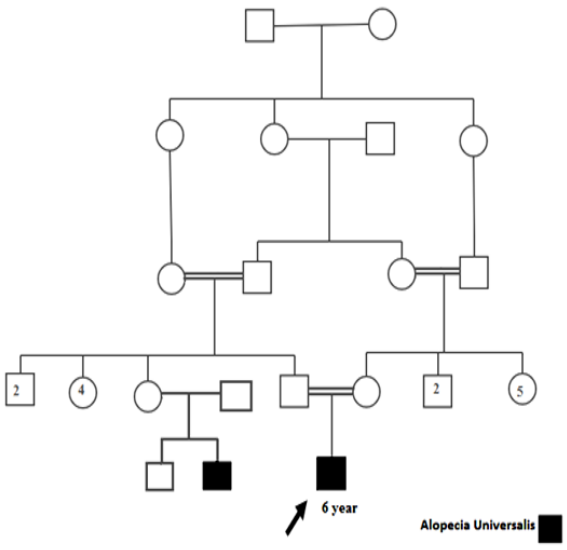

Fig. 1: Family pedigree

His birth weight was 2.9 kg and there has been no abnormality in his physical and mental growth and development thus far. In physical examination there is no abnormality in the nails, teeth, sweating, height and head circumference. In accordance with normal physical and mental situation there is no report in family history in terms of deafness, seizure or any other defects, though a very similar phenotypic finding of alopecia universalis resembling our reported patient is seen in his first cousin who is also a male person.

The patient was born with abnormally long and scanty hair which fell off gradually in his first 6-month and never regrew. Moreover, the complete hairlessness of the scalp, eyebrow and eyelashes was apparent after his first birthday with no improvement to treatment.

DNA extraction PCR

Genomic DNA was extracted from peripheral blood by salting-out method. Primers were designed for all HR gene exons (NM_005144) (Table 1). Then to run PCR, 300 nmol of Forward and Reverse primer was combined with 10 µg of Master Mix (Pishgam Biotech Co., Tehran, Iran), 100 ng DNA and lastly 6 µg of distilled water to reach a final volume of 20 µg. Finally, PCR was performed according to the following procedure (Sensoquest, Germany).

The initial denaturation temperature was 95 °C for 5 min, then 95 °C for 1 min, 62 °1 min, 72 °40 sec and finally 72 °C as the final extension temperature for 10 minutes. All the amplification products were confirmed by setting up them on 2% agarose gel before sequencing them by Sanger method.

Sanger Sequencing

Whole-gene sequence analysis with Mutationsurveyor Software revealed a nonsense homozygous mutation in exon 6. Parental Sequence Analysis shows that they are heterozygous (Fig. 2, 3). According to ACMG guideline, this variant is considered to be pathogen.

Null variant (nonsense) affecting gene HR, which is a known mechanism of disease (PVS1), extremely low frequency in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium (PM2), Multiple lines of computational evidence support a deleterious effect on the gene or gene product (PP3).

Table 1: Primers of HR gene

		Primer 5---3	*Product size*
EX2	Forward	TGTAAGGTGCTTGGGACATG	900
	Reverse	TAGAGAGATGGAGCTGCTCAGG	
EX3	Forward	TCAGTTCTGCCCATCCATTTAG	961
	Reverse	TCCACTCATAAAGCCTACAGACC	
EX4,5	Forward	TGGCTCTGAGTGTGGATGG	678
EX6	Reverse	TGTACCCAGTTTGACACCTTCC	
EX7,8	Forward	AGGCTCTCGACAGGGCTGTG	441
EX9-10	Reverse	AGAGGCAGCCAACGAATGAC	
EX11-12	Forward	AGAAAGCACGAGTTTTGTGG	768
EX13-14	Reverse	AGCGCTGAACAAGAATATGC	874
EX15-16	Forward	GAGTCCATTGTTCATTCTTGTG	881

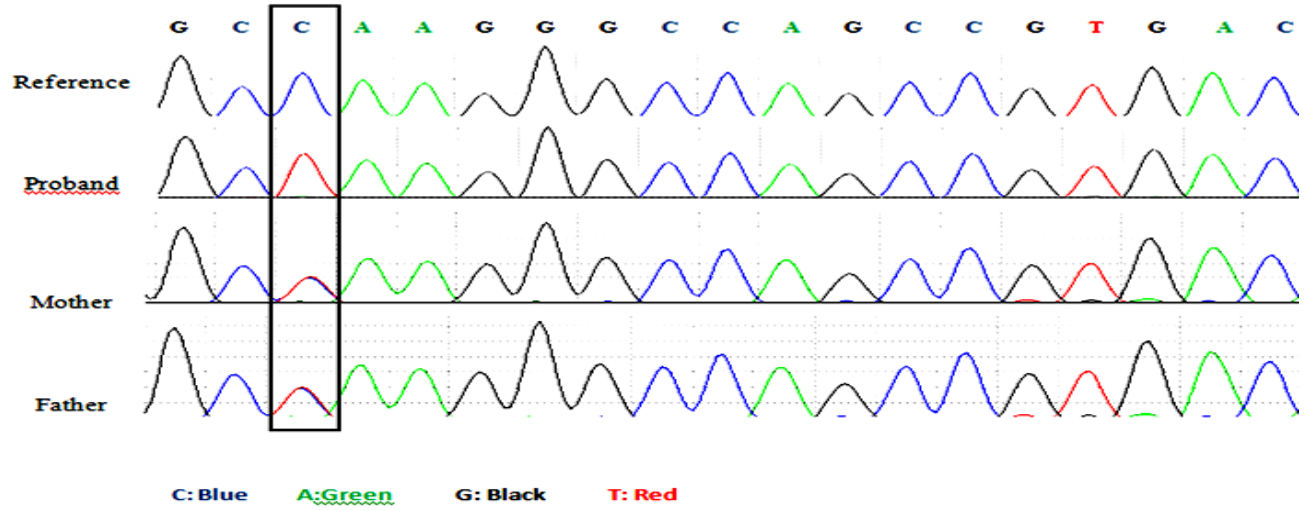

Fig. 2: A nonsense homozygous mutation in exon 6 in proband and parents

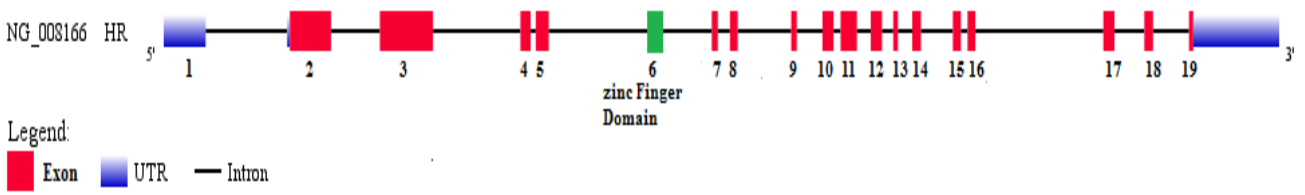

Fig. 3: Genetic structure of the HR gene containing 19 exons and 14 kb long. The size of the exons is proportional to the length of the rectangles. Exon 1 is non-coding. The second Zinger Finger is in exon 6, shown in green

Discussion

HR, the first gene shown to be involved in hair cycle regulation, has an essential role for the onset of follicular hair after birth (8). The whole gene with 19 exons and the corresponding introns spans over 14 kb long located on chromosome 8p12. The Zink Finger domain which is crucial for DNA binding is located in exon 6. According to (9), any mutation in the Zink Finger domain of the gene will negate the activity of the DNA binding activity resulting in disturbance of

hair-cycle events (10). It seems to play a role in the cellular transition to the first cycle of the hair phase, and in its absence the hair follicle is destroyed and never new hair is grown (11-13). The HR protein interacts with transcription factors and also affects a number of nuclear factors, including the thyroid hormone receptor and the vitamin D receptor. Because HR is a transcriptional regulator, degradation of this gene's product results in defects in hair follicle regeneration (6, 14). During the hair development cycle HR protein regulates hair follicle remodeling by stimulating Wnt signaling. In mutant HR, overexpression of Wnt signaling inhibits Wnt pathway inhibition and hair follicle remodeling occurs (15). In the patient we examined, a nonsense homozygous mutation was found in exon 6 of the gene. This mutation results in the creation of a PTC (premature termination codon) that converts the amino acid 585 of glutamine to stop codon. It eventually leads to the phenomenon of Nonsense-Mediated mRNA Decay (NMD), as nonsense mutation or any stop codon in all exons except for last or penultimate exons will lead to NSMD (16). In this phenomenon, mRNA with a PTC is selectively destroyed with no functional translated protein product (17). Considering the explanations given for the role of the HR gene in hair follicle remodeling, this mutation causes damage to the HR gene product. Due to the absence of this protein product, phenotypic symptoms of the disease, most notably severe hair loss has been occurred in the patient.

Conclusion

Whole-gene sequence analysis revealed a nonsense homozygous mutation in exon 6 that, according to the ACMG guide, is a pathogenic variant. Sequence analysis of the exon in parents reveals that they are heterozygout for the nonsense mutation, as well. We report a consanguineous couple with a child afflicted with alopecia universalis in whom molecular analysis of HR gene reveals c.1753C> T (Q585 *) homozygous mutation.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

There is no conflict of interest.

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