

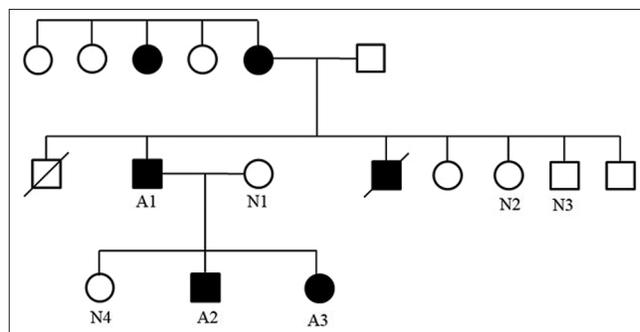
## A novel loci of the HR gene in Marie - Unna hereditary hypotrichosis using whole-exome sequencing

Sir,

Marie - Unna hereditary hypotrichosis is a rare and distinctive form of congenital hypotrichosis. It is a rare autosomal dominant hair disorder with the notable absence of non-dermatological signs that are typical components of syndromic hereditary hypotrichosis, such as retinal degeneration, hearing impairment or intellectual disability. Patients with Marie - Unna hereditary hypotrichosis are usually born with sparse hair but later regrowth of coarse hair occurs. As they age, hair loss gradually occurs on the scalp, eyebrows and eyelashes and progresses to complete baldness.

The human hairless [*HR*] gene has been reported to cause autosomal dominant Marie - Unna hereditary hypotrichosis (MIM 146550), autosomal recessive atrichia with papular lesions (MIM 209500) and alopecia universalis congenita (MIM 203655). The *HR* gene spans over 14kb on chromosome 8p21 and is composed of 19 exons. The protein product of the *HR* gene has a zinc-finger domain and is a putative transcription co-repressor for multiple nuclear receptors that are highly expressed in the brain, epidermis and hair follicles.<sup>1</sup> In Marie - Unna hereditary hypotrichosis, genetic mutations have been reported in upstream open reading frames in the 5' untranslated region of *HR* in several studies.<sup>2-4</sup> Mutations in *U2HR* (second open reading frame in the 5' untranslated region) increase expression of the *HR* gene and lead to disruption in hair follicle morphogenesis and cycling.<sup>2</sup> Until now, there have been no reports of genetic variants located in the protein-coding regions of *HR*.

A 21-year-old male presented with progressive alopecia on the scalp that began in childhood. He had patches of alopecia on the scalp, particularly the occipital margins, with coarse hair on the frontal area. Eyebrows and eyelashes were absent but there were no ectodermal abnormalities. Laboratory findings were normal. Histological examination revealed a reduced number of hair follicles; however, inflammation was not present around the hair follicles. Microscopic examination of the hair shafts did not reveal the abnormalities typical of fragile hair shaft disorders, including trichorrhexis nodosa, trichorrhexis invaginata, pili torti or monilethrix. The patient reported that his father (49 years old) also had complete baldness that began in childhood and his 9-year-old sister had been exhibiting progressive alopecia for the past several years [Figure 1]. After obtaining approval from the



**Figure 1a:** Pedigree of a family with Marie-Unna hereditary hypotrichosis. Squares indicate males; circles, females; black symbols, affected individuals (A1, A2, and A3) and white boxes and circles nonaffected individuals (N1, N2, N3, and N4)

Institutional Review Board at Uijeongbu St. Mary’s Hospital, Catholic University of Korea (UC16TISI0167) and informed consent from the patient and family members, blood samples were collected and kept in Uijeongbu St. Mary’s Hospital Human Biobank. The blood of the three affected patients and family members without hair loss ( $n = 4$ ) were provided for whole-exome sequencing. Whole exome sequencing was performed on an Illumina HiSeq 2500 system (Illumina, San Diego, CA, USA). Sequenced reads were aligned to a reference genome using the Burrows–Wheeler Aligner. The

average mapping depth for each sample was larger than 110-fold. Raw variants were called using GATK4, and low quality ( $Q$ -score  $< 20$ ) and low depth ( $< 10$ -fold) variants were filtered out. In addition, variants with global minor allele frequencies greater than 0.001 in either all populations or the East Asian population of the Genome Aggregation Database were excluded. Finally, we identified six single nucleotide variants satisfying an autosomal dominant pattern in exonic regions. Of the six single nucleotide variants, three were nonsynonymous and the other three were synonymous

**Table 1: Novel autosomal dominant alleles in the Marie-Unna hereditary hypotrichosis family**

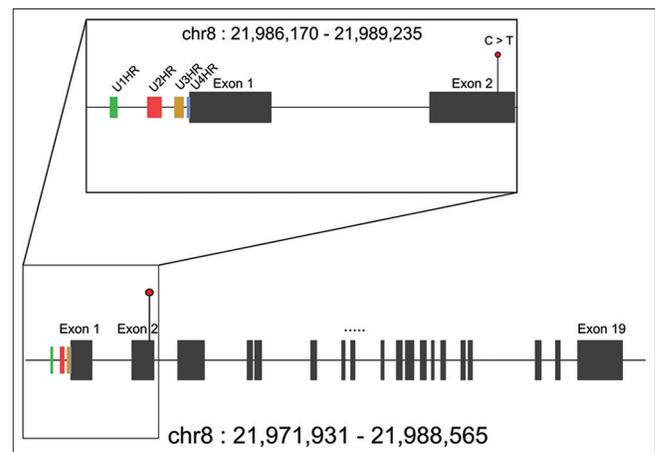
Chromosome	Position	Reference	Gene	Type	AA change	Predicted pathogenicity		MAF in genome aggregation database		Genotype	
						SIFT	PolyPhen	All	East Asian	Normal family members ( $n=4$ )	Affected ( $n=3$ )
1	1460657	C	ATAD3A	Nonsynonymous	p.R418W	Deleterious	Probably damaging	0.0002	0.0002	CC	CT
17	3627626	C	HASPIN	Nonsynonymous	p.L133F	Deleterious	Probably damaging	0	0	CC	CT
19	56467088	T	NLRP8	Nonsynonymous	p.M555K	Deleterious	Probably damaging	$2.0 \times 10^5$	0.0002	TT	TA
8	21986201	G	HR	Synonymous	-	NA	NA	$4.1 \times 10^5$	0.0006	GG	GA
8	22277067	C	SLC39A14	Synonymous	-	NA	NA	0	0	CC	CT
19	57840444	T	ZNF543	Synonymous	-	NA	NA	0	0	TT	TC

Identified six SNVs satisfying an autosomal dominant pattern in exonic regions are shown in the table. Of the six SNVs, three were nonsynonymous and the other three were synonymous SNVs. SNVs: Single nucleotide variants, MAF: Minor allele frequency, AA: Amino acid; Genomic coordinates were based on Human genome assembly GRCh37 (hg19), NA: Not available, SIFT: Sorting intolerant from tolerant

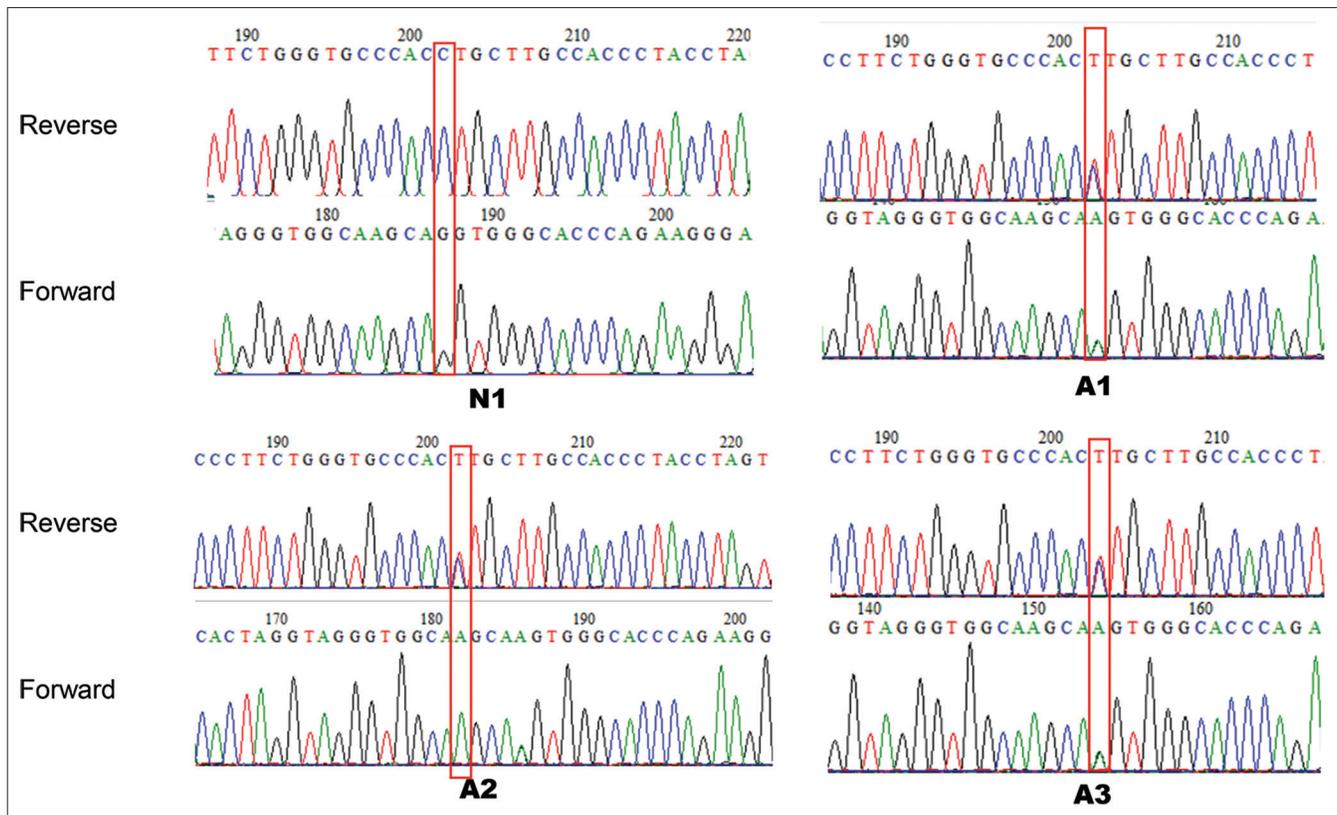


**Figure 1b:** A family with Marie-Unna hereditary hypotrichosis

single nucleotide variants [Table 1]. No insertions, deletions or frameshift variants were identified. For the three nonsynonymous single nucleotide variants (*HASPIN*, *NLRP8*, and *ATAD3*), the pathogenic effect was predicted using Sorting Intolerant from Tolerant (SIFT) and PolyPhen. However, we could not find any evidence for



**Figure 2a:** Genomic locations of the novel synonymous variant found in this study. Human *HR* consists of 19 exons. Four open reading frames (U1HR–U4HR) are located in the 5’ untranslated regions in exon 1. Before our study, all Marie-Unna hereditary hypotrichosis-associated alleles were found in untranslated regions. The novel variant we found is located in exon 2. Each black box represents an exon



**Figure 2b:** Results of Sanger sequencing of novel synonymous variants in *HR*. We analyzed the DNA sequences of both strands (forward and reverse) of the *HR* gene of the family members via Sanger methods. The results of Sanger sequencing and whole-exome sequencing of the variant were matched

the relationship between the single nucleotide variants/their genes and hair loss or related symptoms via literature search and “Open Targets Platform”<sup>5</sup> (is a web application that provides an interface to the data that is being aggregated and reprocessed on a regular basis from an increasing list of valuable public data sources). Among the synonymous single nucleotide variants, one variant was located in exon 2 of *HR* [Figure 2a]. We carried out Sanger sequencing for the six single nucleotide variants identified on whole-exome sequencing and all genotypes matched in the results from both methods [Figure 2b].

Until now, heterozygous autosomal dominant mutations in the *U2HR* frame of the *HR* gene have been reported in families with Marie - Unna hereditary hypotrichosis worldwide.<sup>2-4</sup> However, we could not find mutations in the *U2HR* in the affected individuals. Although whole-exome sequencing was performed to find a disease-causing mutation within the coding regions with a high sequencing depth (110×), we could detect a disease-causing mutation on the untranslated regions that are sequenced in the process of exome target enrichment.<sup>6</sup> Previously reported mutations in *U2HR* caused a change in RNA expression of *HR* without changing the amino acid sequence of *HR*.<sup>2</sup> The possible molecular mechanism of Marie - Unna hereditary hypotrichosis is gain-of-function of the *HR*.<sup>2</sup> Recent studies have shown that synonymous variants can change protein folding and function

as well as mRNA structure and splicing. If the synonymous single nucleotide variant leads to altered mRNA and protein expression of *HR*, a novel molecular cis-regulatory mechanism for *HR* can be established. Although it was a synonymous single nucleotide variant, determining its role in Marie - Unna hereditary hypotrichosis will require further validation. But based on the previous functional studies of the *HR* gene, we suggest that this variant in *HR* may be a genetic factor in this affected family. Further mouse model studies to validate this association and establish molecular mechanisms on how synonymous mutations could cause hair loss have to be done.

#### Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has given his consent for his images and other clinical information to be reported in the journal. The patient understands that name and initials will not be published and due efforts will be made to conceal identity, but anonymity cannot be guaranteed.

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**Conflicts of interest**

There are no conflicts of interest.

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