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Research Letter

Mutational analysis of 29 patients with autosomal recessive woolly hair and hypotrichosis: *LIPH* mutations are extremely predominant in autosomal recessive woolly hair and hypotrichosis in Japan

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Supplementary file

Autosomal recessive woolly hair and hypotrichosis (ARWH) is a rare form of congenital alopecia characterized by sparse hair on the scalp, and it sometimes expands to all the body hair. ARWH is known to be caused by mutations in *LIPH* or *LPAR6*.[1,2] Recently, Zernov *et al.* reported that the *KRT25* gene causes autosomal-recessive hypotrichosis with woolly hair.[3]

LIPH encodes a membrane-bound member of the mammalian triglyceride lipase family, lipase H.[1] LPAR6 encoded by *LPAR6* and LIPH are both involved in the same pathway of regulation of hair differentiation and growth.[1,2] To date, 24 pathogenic mutations in *LIPH* have been reported in ARWH (www.hgmd.cf.ac.uk, as of HGMD professional, 2016.1). They comprise 9 missense/nonsense, 2 splice-site, 5 small-deletion, 2 small-insertion, 3 small-indel, 2 gross-deletion and 1 gross-insertion mutations. Of these, the 2 mutations c.736T>A and c.742C>A in *LIPH* have been reported as extremely prevalent causative mutations for ARWH in the Japanese population. We confirmed the high frequencies of the 2 founder mutations, especially of c.736T>A, in 819 Japanese controls.[4] The *LIPH* mutations c.736T>A (p.Cys246Ser) and c.742C>A (p.His248Asn) were proven to be dysfunctional by *in vitro* studies.[5] Precisely how often these two founder mutations in *LIPH* are seen in Japanese ARWH patients remains uncertain.

In the present study, we analyzed the entire coding regions of *LIPH* in a cohort of 29 cases of ARWH from 29 independent families. The clinical characteristics of the patients are summarized in Table 1. Following approval by the Medical Ethics Committee of the Nagoya University Graduate School of Medicine and informed consent, screening for *LIPH* mutations in genomic DNA was performed, as previously described,[6] with all studies conducted according to the *Declaration of Helsinki* principles.

Sanger sequencing of *LIPH* revealed pathogenic mutations in 27 (93%) individuals, all of them with mild or severe hypotrichosis (Table 1). Of the 27 ARWH patients with *LIPH* mutations, homozygous c.736T>A mutations were found in 16 cases, and compound heterozygous c.736T>A and c.742C>A mutations were found in 10 patients. Case 1 had compound heterozygous mutations, including one novel mutation, c.558_559insT (p.Lys187Ter) (Fig. S1). No mutation in *LIPH* was identified in the other 2 patients. We performed Sanger sequencing of all the exons and flanking introns of *LPAR6* in the two individuals, Cases 28 and 29, in whom we detected no mutations in *LIPH*. No pathogenic mutations in *LPAR6* were found in these 2 cases.

The c.558_559insT (p.Lys187Ter) mutation in Case 1 causes a truncated LIPH protein which lacks the β9 loop, the lid domain and one of three catalytic residues,

248His (Fig. S1c and d). In addition, the mutation occurs in exon 4 of *LIPH*; thus, it most likely leads to nonsense-mediated RNA decay. In the literature, several nonsense and frameshift mutations of exon 4 had been described in ARWH patients.[7]

Very recently, Kinoshita-Ise *et al.* reported that the difference in the frequency of underdeveloped hairs might be a major factor contributing to the clinical diversity of hair sparseness in *LIPH* c.736T>A homozygotes of ARWH.[8] We previously reported that ARWH cases homozygous for c.742C>A showed a severe phenotype, and in contrast, patients with mild hypotrichosis were seen among the ARWH patients homozygous for c.736T>A. Our hypothesis was that c.742C>A (p.His248Asn) might affect the enzyme function more seriously than c.736T>A (p.Cys246Ser) does, because p.248His is known as one of three catalytic residues (Fig. S1c and d).[6] The present data suggest a similar tendency for this genotype/phenotype correlation, although no individual among the present 29 cases had a homozygous c.742C>A mutation (Table. 1). The two cases of ARWH, Cases 28 and 29, had neither *LIPH* nor *LPAR6* mutation. We were unable to exclude the possibilities of a large deletion, an intronic mutation or a promoter-site mutation of *LIPH* or *LPAR6*. Additionally, other genes causative of congenital hypotrichosis and woolly hair including *KRT25* are possible candidates.[3]

Kahn *et al.* previously investigated the genotypes of *LPAR6* and *LIPH* in 17 consanguineous Pakistani families with hypotrichosis/woolly hair, revealing that eight of the families had *LPAR6* mutations and the other nine families harbored *LIPH* mutations.[9] In contrast, the present study demonstrated that 27 out of 29 Japanese ARWH patients (93%) had *LIPH* mutations as the cause of their ARWH. We previously reported a total of 15 ARWH patients having *LIPH* mutations.[4,6,10] Altogether, among the 44 ARWH reported patients with *LIPH* mutations, the c.736T>A mutation clearly predominates as the ARWH-causing *LIPH* mutation (allele frequency, 76%), followed by c.742C>A (allele frequency, 18%) and others (Table S1). These results suggest that, for Japanese ARWH patients, an initial mutation search for clinical genetic diagnosis should focus on exon 6 of the *LIPH* gene. From the present study, 93% of ARWH patients had at least one of the two founder mutations in exon 6 of *LIPH*. Highly prevalent founder mutations in *LIPH*, as seen in the Japanese population, might exist in other ethnic populations.

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Table 1. Summary of the ARWH patients analyzed in the present study

Case	Age (yrs.)/Gender	Severity of hypotrichosis	*LIPH* mutations	Additional clinical information
1	6/F	mild	c.558_559insT /c.736T>A compound hetero	
2	3/F	mild	c.736T>A homo	
3	4/F	mild	c.736T>A homo	
4	17/F	mild	c.736T>A homo	
5	25/F	mild	c.736T>A homo	
6	1/F	severe	c.736T>A homo	
7	2/M	severe	c.736T>A homo	
8	2/F	severe	c.736T>A homo	Her younger sister also had ARWH with the identical genotype in *LIPH*.
9	2/F	severe	c.736T>A homo	
10	3/M	severe	c.736T>A homo	
11	3/F	severe	c.736T>A homo	
12	4/F	severe	c.736T>A homo	
13	5/F	severe	c.736T>A homo	
14	5/F	severe	c.736T>A homo	
15	6/M	severe	c.736T>A homo	
16	6/F	severe	c.736T>A homo	Her younger sister also had ARWH with the identical genotype in *LIPH*.
17	26/F	severe	c.736T>A homo	She showed hypotrichosis of the eyelash, eyebrow, and underarm and pubic hair.
18	0/M	severe	c.736T>A/c.742C>A compound hetero	

19	**1/F**	**severe**	**c.736T>A/c.742C>A compound hetero**	
20	**2/M**	**severe**	**c.736T>A/c.742C>A compound hetero**	
21	**2/M**	**severe**	**c.736T>A/c.742C>A compound hetero**	
22	**6/F**	**severe**	**c.736T>A/c.742C>A compound hetero**	
23	**8/M**	**severe**	**c.736T>A/c.742C>A compound hetero**	
24	**8/F**	**severe**	**c.736T>A/c.742C>A compound hetero**	
25	**18/F**	**severe**	**c.736T>A/c.742C>A compound hetero**	
26	**20/F**	**severe**	**c.736T>A/c.742C>A compound hetero**	
27	**28/M**	**severe**	**c.736T>A/c.742C>A compound hetero**	**He had hypertrophic cardiomyopathy and chronic kidney disease.**
28	**33/F**	**mild**	**undetected**	
29	**1/F**	**severe**	**undetected**	
Abbreviations: ARWH, autosomal recessive woolly hair; LIPH, lipase, member H; yrs., years; M, male; F, female				