Hague (*Hag*): A New Mouse Hair Mutation With an Unstable Semidominant Allele

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ABSTRACT

A spontaneous mouse hair mutation was identified in a C3H/HeN colony. The mode of inheritance of the mutation was semidominant, with incomplete penetrance when heterozygous. The trait is controlled by a single locus hague (*Hag*), which was mapped to the telomeric region of chromosome 15. This mutation was shown to be unstable, since its transmission could be switched from semidominant to recessive. To identify the causative gene and the nature of the mutation, hague was introduced into a high-resolution and high-density molecular genetic map. Over 2000 meioses were analyzed and the mutation was mapped to the keratin 2 complex genes. A YAC and BAC physical map of the critical region was then constructed and the gene involved was located in a 600- to 800-kb-long segment. Fourteen genes were mapped to this region; of these, 11 were expressed in the skin (5 epidermic cytokeratin and 6 hard keratin genes), but none were mutated in hague mice.

HAIR follicles are relatively simple structures, with their own pool of stem cells that, once differentiated during embryonic development, undergo repeated cycles of degeneration/regeneration throughout life (Costarelis *et al.* 1990; Hardy 1992). One possible approach to the understanding of the molecular events involved in the development and cyclic life of these tiny organs might be to generate knockout mice for the genes that are known to be expressed in the hair follicle (Yamanishi 1998) and then to observe carefully the consequences, if any, of these engineered mutations. Another approach is to identify at the molecular level, by positional cloning, the genes that are affected in the many mouse hair mutations available. These two approaches are complementary and, at least in two cases, the engineered null alleles have been shown to mimic and failed to complement a spontaneous mutation [for example, fibroblast growth factor 5 (*Fgf5*) and angora (*go*) or transforming growth factor-α(*Tgfa*) and waved 1 (*wa1*; Luetteke *et al.* 1993; Mann *et al.* 1993; Hebert *et al.* 1994)]. The transgenic approach leading to the production of knockout or null alleles is powerful but, by definition, it is limited to those genes whose DNA sequence is already known and often results in unexpected and sometimes extreme consequences. The positional cloning of mutant alleles, even if it requires the breeding of a large quantity of mice, is more general and results in many instances in the identification of previously unknown genes. Using such a strategy allowed, for example, the identification of *Foxn1*, a gene encoding a transcription factor, as being altered in the nude (*nu*) mouse mutation (Nehls *et al.* 1994). Similarly, a mutation affecting the tyrosine kinase activity of the gene encoding for the receptor of the epidermal growth factor (*Egfr*) was found to be responsible for the waved 2 (*wa2*) mutation (Luetteke *et al.* 1994). Finally, a retroviral insertion helped in the molecular identification of the gene involved in the hairless (*hr*) mutation (Cachon-Gonzalez *et al.* 1994). In this article we describe a new mutation affecting the fur that was found segregating in the C3H/HeN inbred strain of mouse. This new mutation, which exhibits unusual inheritance and produces curling of the hair or balding similar to caracul (*Ca*), is genetically controlled by a single locus on chromosome 15. We gave it the provisional name of hague (bald, in Japanese), with *Hag* as a symbol.

MATERIALS AND METHODS

Mouse strains: C3H/HeN mice were purchased from Charles River Japan. BALB/c and C57BL/6J mice were purchased from CLEA Japan. The B6-*Krt2*^{SEG} strain (lab code IRCS 119), an interspecific recombinant congenic strain homozygous for the chromosomal segment spanning the interval

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TABLE 1

PCR assays for keratin genes and BAC/YAC ends

Locus	Primer 1	Primer 2	Length (bp)	GB no.
Krt1-18	TCTTCTGTCTGTGTAAATGGA	ACACAGAGAAACCTTATCTCG	75	M22832
Krt2-1	TCAGTTCATCACCAACATGACA	AGCGTTGCTGCAGGATATG	114	M10937
Krt2-4	GTCAAGCGAGTGGCCTTTAG	AACCCAGCACCGTATCCTC	194	X03491
Krt2-5	GGTGGAGCTGGAGGCCAGGG	GACGTGTGTCTGCATCTGGG	~200	AA166079
Krt2-6a	CATATTTCTGTCCCCATGGG	AAGTTGTCGGAAACACCCTG	170	AB012033
Krt2-6b	ACAGGCACTGAAATCCCAAC	GCCTCCTCAGACAGAAGCAG	18	AB0124042
Krt2-6hf	CTTGCCACTGCTCCGATGCC	CAGAGAAGGACAAGGCAGCC	378	AF343088
Krt2-7	AGATGGACAAAAGGGTATGGG	TATCTTCGGAGGAACCATGG	171	AA410039
Krt2-8	GGGTGGTCCAATCAGAGAGA	CATGCCCCACTTAGACCACT	121	D90360
Krt2-10	TTGCACAACAGGCCTAGAGG	AAGAAGGAGCCAGGAAGTGG	224	M92088
Krt2-11	CTTAGGATCTTGGAGGATCCG	TGTCAGGAAAGCAGGCAAC	121	X99143
Krt2-12	GTTCACATCCTGGCCCTG	AGACAAAGATTAGAGGGAGGGG	298	AF312018
Krt2-13	TACTGCATGCTTCAGGTGGG	GAATACCACATCTCTATGGG	447	AY028606
Krt2-16	GTCCCTCCCTCTTCCCTTC	TAGTACAAACAGGTCCCGCC	338	AY028607
Krt2-17	TTGTTTACCTGGCATCCCTC	TTATGCAGAGACCCAGACCC	167	X74784
Krt2-18	ATCTCCTTAGCTGTGCCTTCC	ACCCAGACAGAAAGTGGCC	254	AF021836
D15Kus1	CATGCTACTGAAGAAGGCTGG	ACACTGCCACACACACCCTA	113	AQ629058
D15Kus2	ACAGTGTTCATTTCTCCTGGA	CAGATGCGTACTGCCTGAAT	150	AQ629059
D15Kus3	TGGAGCCTCTGTTCGAGG	GCTTGTCTATACCCAGGGAGC	177	AQ629060
D15Kus4	ATGGCTGTAGTGGGTCGTTC	GAGGGACCAGGGAGACGT	147	AQ629061
D15Kus5	GGGTTGCTTTGTTTAGCAGC	GGGCCCTCTACAAGTTCCTC	186	AZ301627
298p5Sp6	GTCCCTTCTGGTAGGAAGTGG	TGTTACCAATGTCACTTCCTGC	126	AZ301623
298p5T7	GCAGGAATCCAGCTTTGAAG	ATTCAGTCCCCTGGTATCCC	100	AZ301624
51l7Sp6	AACCATGTCTACCAAAACCACC	ACGGACACACTGCTGAAGC	111	AZ301625
51l7T7	TGAGACAGAAGGGAGGCG	CTTCCAGTCTGCTGCCCTAG	120	AZ301626
181f4Sp6	AAAGCCTGTGCTTCGTGG	CTTGGCTAGTCCAGAGCACC	133	AZ301617
181f4T7	TGGTGCAAAAACATGCATG	TGTAAACCAACCTGACCTTGC	105	AZ301618

D15Mit41 to *D15Mit16* and containing the keratin-2 (*Krt2*) complex from strain SEG/Pas (*Mus spretus*) in a C57BL/6 genetic background was obtained from Xavier Montagutelli, Institut Pasteur (Paris). The wild-derived strains CAST/Ei (*M. musculus castaneus*) and PWK (*M. musculus musculus*) were gifts from the late V. M. Chapman, Roswell Park Memorial Institute (Buffalo). Strain MSM (*Mus musculus molossinus*) was imported from the National Institute of Genetics (Mishima, Shizuoka, Japan). C57BL/6By-*Ca, Kitl*^{Sl} mice were purchased from the Jackson Laboratory (Bar Harbor, ME). All the experiments reported in this article conformed to protocols approved by the Institutional Animal Care and Use Committee of RIKEN.

Preparation of hair samples: Hair samples were plucked from the middle area of the dorsal skin and fixed in 70% ethanol, dehydrated with 90 and 100% ethanol. Then the samples were immersed three times in xylene, mounted with Marinol (Muto-Pure Chemicals, Ltd., Tokyo) and observed under a microscope (BH-2, Olympus, Tokyo) equipped with a digital camera (Sony digital photo camera model DKC-5000, Sony, Tokyo).

Genomic DNA: Genomic DNA samples were prepared from tail clips using a classical procedure (Miller *et al.* 1988).

Molecular markers: All *D15Mit* microsatellite markers used in our experiments were from the MIT database (Dietrich *et al.* 1996). To maximize polymorphisms, PCR primers for the analysis of most keratin genes, except *Krt2-4* and *Krt2-5*, were chosen from the 3′ untranslated region. For *Krt2-4*, the primers were chosen in the first exon and for *Krt2-5* the primers were chosen in the fourth and fifth exons; all amplified products were specific of a keratin isoform. The sequences for all primers used in our experiments are listed in Table 1.

All PCR reactions were carried out in 25-μl volume under standard conditions. For microsatellites *D15Mit*s, *D15Kus2*, *D15Kus4*, *Krt2-8*, and *Krt1-18*, the parental origin of the different alleles was estimated on the basis of simple sequence length polymorphism analysis performed in 4% agarose gel; for markers *D15Kus1*, *Krt2-17*, and *Krt2-7* the allelic forms were analyzed by single-strand conformation polymorphism using the Clean DNA analysis kit (Pharmacia, Piscataway, NJ).

Yeast artificial and bacterial artificial chromosome clones: Yeast artificial chromosome (YAC) 183h3 was retrieved from the MIT database (Haldi *et al.* 1996) and purchased from Research Genetics (Birmingham, AL). YACs 70m8a9, 53m3d3 (Larin *et al.* 1991), and b22m5a1 (Haldi *et al.* 1996) were identified after screening the GENOSCOPE database (Evry, France). For each address, 10 independent clones were isolated and the sizes of the cloned DNA were determined using pulsed-field gel electrophoresis standard procedures (Carle and Olson 1987). YAC ends were isolated by three rounds of degenerated oligoprimer (DOP)-vector PCR (Wu *et al.* 1996) using DOP-JUN1 and DOP-6MW (Telenius *et al.* 1992; Wu *et al.* 1996) as degenerated primers for the insert and the primers specific to the YAC arms (Herring *et al.* 1998). Reactions were performed using 100 ng of total DNA extracted from the yeast clones as a template under the conditions described by Herring *et al.* (1998) and Wu *et al.* (1996). Bacterial artificial chromosome (BAC) ends were isolated by two rounds of DOP-vector PCR. Internal sequences from YACs were isolated by inter-B1 PCR (Hunter *et al.* 1994). The PCR products were cloned in pCR2.1 (Invitrogen, San Diego) and sequenced using an ALF sequencer (Pharmacia).

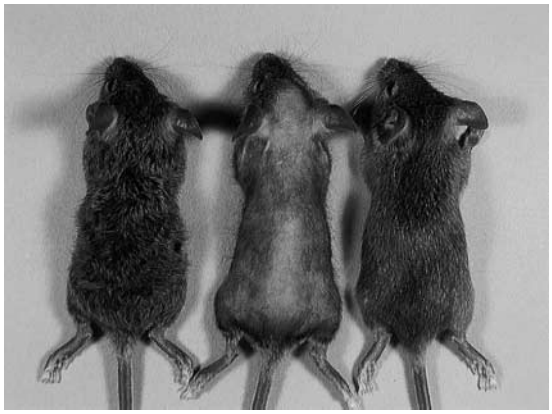


FIGURE 1.—Phenotypes segregating in the C3H/HeN colony. Four-week-old C3H/HeN mice from left to right: phenotype1, phenotype2, and wild type.

Expression of keratin genes in the skin by reverse transcriptase-PCR: Total RNA was extracted from the dorsal skin tissues of mice of each genotype at 10 days old by using the total RNA extraction kit (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, England) according to the manufacturer's instruction. Reverse transcription of messenger RNA into cDNA was performed by incubating the total RNA with SuperScript II RNase H-reverse transcriptase (GIBCO BRL, Rockville, MD) and oligo(dT) primers (GIBCO BRL). The cDNA was purified using the PCR purification kit (QIAGEN, Hilden, Germany). PCR reaction was carried out by using Advantage cDNA polymerase M (CLONTECH, Palo Alto, CA) with the oligonucleotide primers for each keratin gene. The PCR products were subjected to agarose gel electrophoresis and stained by ethidium bromide to determine the presence and size of the products. The PCR products were also sequenced to detect the mutation.

RESULTS

Description of phenotypes and inheritance of the traits: The original mutant phenotype was identified, because of extremely curly fur, in the C3H/HeN inbred strain at the RIKEN Institute and the mutation was kept in this background. When affected mice of this kind were crossed to wild-type partners of the same strain, the mutation appeared to be transmitted as a semidominant or dominant allele with both curly and normal-furred offspring. When mutant mice were intercrossed, a more extreme phenotype was observed in their progeny, with some mice being extensively depilated from 10 days of age. We refer to this severe phenotype as phenotype2 hereafter, phenotype1 being the first observed phenotype (Figure 1). As is the case for several other fur mutations (rex, caracul, etc.), curling of the hair in mice exhibiting phenotype1 was obvious in young mice and became less obvious (at least more difficult to recognize) after 2 months.

Mice with either phenotype1 or phenotype2 have the classical four types of hair: monotrich, awl, auchene, and zigzag, although each hair shaft is severely malformed and curled. In mice with phenotype2, zigzag hairs developed poorly and broken hair shafts are commonly observed under the microscope, probably indicating an increased fragility.

TABLE 2

Phenotype segregation in crosses involving C3H/HeN mice

Phenotype		Phenotype of progeny and no.		
Female	Male	Wild type	Type 1	Type 2
Type 2	Type 2	0	0	95
Wild type	Type 2	19	8	0
Type 2	Wild type	20	7	0
Wild type	Type 1	17	11	0
Type 1	Wild type	17	14	0
Type 1	Type 1	20	17	11

To study the transmission of the mutant phenotypes, different types of crosses between phenotype1, phenotype2, and wild-type (wt) mice were set as shown in Table 2. All these crosses were between mice of the same coisogenic strain, C3H/HeN.

When intercrossed, mice with phenotype2 appeared to breed true since only phenotype2 offspring were observed. Conversely, no phenotype2 was ever observed in the progeny of phenotype2 mice mated to wild type (+/+). In these crosses, no significant bias related to the sex of the partner contributing the mutant allele was observed. Surprisingly, however, in these progeny not all the offspring exhibited the same phenotype, indicating that the hague (*Hag*) mutation is not a classical *bona fide* semidominant mutation with all *Hag*/+ mice exhibiting phenotype1. In fact, in the above-mentioned cross (phenotype2 × wt), 28% of the offspring exhibited phenotype1 while the other 72% exhibited a wild-type phenotype. Similarly, when phenotype1 mice (supposed to be *Hag*/+) were mated to wild-type partners (+/+), 42% of the offspring exhibited phenotype1 while the remaining 58% had a wild phenotype. From the analysis of the different crosses, we postulated that the phenotypic heterogeneity among *Hag*/+ mice was the result of either incomplete penetrance or a polygenic control of the phenotype involving modifier genes.

Genetic localization of the trait: One way to clarify the situation reported above for the inheritance of the phenotypes was to localize precisely the locus (*Hag*) determining the hague mutation and then to perform a complete genetic analysis of the progeny born from different crosses involving the same *Hag* mutation. To achieve this, intersubspecific matings between females with phenotype2 (*Hag/Hag*) and males of the CAST/Ei strain (+/+) were set. The F_1 born from these crosses again appeared heterogeneous with curly haired (phenotype1) and wild phenotypes. To recover all three kinds of genotypes, we intercrossed F_1 animals of the two kinds (*i.e.*, wild type × wild type and curly haired × curly haired) and produced 413 F_2 mice. Among these

offspring, 120 exhibited phenotype2, 73 exhibited phenotype1, and 220 were wild type.

A complete genome scanning was then performed on a sample of 15 phenotype1 and a sample of 15 phenotype2 mice and evidence for linkage was found only with the telomeric region of mouse chromosome 15. This preliminary data being taken into account, we then typed all the 193 affected mice (120 phenotype2 and 73 phenotype1 mentioned above) for all polymorphic markers in this region and analyzed the data with MAP MANAGER (Manly 1993). The localization of the *Hag* locus on chromosome 15 was confirmed, where it appeared flanked by microsatellite markers *D15Mit173* and *D15Kus2* at the centromeric edge and by microsatellite markers *D15Mit79* and *D15Mit35* at the telomeric edge (Figure 2A).

Analysis of penetrance: Genotyping the 220 F_2 offspring with a wild phenotype (*i.e.*, noncurly hairs), we found 125 of them to be heterozygous for both *D15Kus2* and *D15Mit35*, as well as for all markers in between (Figure 2B). These mice obviously had the *Hag*/+ genotype, indicating that heterozygosity at this locus was not sufficient to generate phenotype1. In fact, in the F_2, there were two kinds of *Hag*/+ mice: some exhibiting the expected curly haired phenotype and others being wild type.

Considering this observation, we had an opportunity to test for a parental effect in the occurence of phenotype1 in genetically *Hag*/+ heterozygous mice. We compared the proportion of phenotype1 *vs.* wild type in heterozygous mice at *Krt2-17*, which is tightly linked to hague, between the F_2 populations originating from either F_1 mice with phenotype1 (cross 1) or F_1 mice with a wild-type phenotype (cross 2; Table 3). Surprisingly, we found a strong bias in the phenotype distribution, with only 3 phenotype1 mice out of 74 (4.1%) heterozygotes in cross 1, compared to 52 out of 84 (61.8%) in cross 2 ($\chi^2 = 58$, 1 d.f.).

To search for modifier genes, a second genome scan was performed on a selected sample of 63 F_2 mice born from cross 1 (phenotype1 × phenotype1) and all heterozygous for the markers between *D15Mit97* and *D15Mit16*. From these 63 mice, 36 were affected (phenotype1) while 27 had a wild phenotype. However, we did not find any evidence for a statistically significant linkage disequilibrium and accordingly concluded that the trait is controlled by a single locus only and that the phenotype of the heterozygous *Hag*/+ parents has a strong influence on the phenotype of *Hag*/+ offspring.

Loss of semidominance by inbreeding: From its origin on an inbred mouse strain and the mapping experiments above, we concluded that the Hague phenotype is controlled by a single major locus. However, in the progeny of heterozygous (*Hag*/+) wild-type mice, most of the genetically heterozygous (*Hag*/+) mice appeared with a wild-type phenotype, which suggested that the mutant allele might now behave as a recessive mutation. To test this hypothesis we intercrossed for five generations *Hag/Hag* mice exhibiting phenotype2, in their original inbred genetic background (C3H/HeN), the one where the mutation first occurred, and then we crossed some of these *Hag/Hag* coisogenic mice with wild-type (+/+) C3H/HeN mice. Surprisingly, we found no phenotype1 among 80 progeny of such a cross! Since no modifier genes were identified in the previous crosses, this suggested that the original semidominant mutation was unstable and could turn into a recessive allele. From this point on, we use the symbols *Hag* for the semidominant allele and *hag* for the recessive one.

To check this hypothesis, we decided to generate mice carrying only one mutated allele, either the semidominant original *Hag* allele or its new recessive form *hag*. For this experiment, some B6-*Krt2*^{SEG} mice were crossed with affected homozygous mice (phenotype2) from the mutant colony. At the same time, some mice homozygous for the "new recessive" allele were crossed with C57BL/6 mice. The affected F_1 (phenotype1) from the first cross were mated with the wild-type F_1 from the second cross; 181 affected F_2 mice were born from this cross, among which 88 were phenotype1 and 93 phenotype2. This allele segregation of the two markers around the *Hag* locus (*D15Mit246* and *D15Kus2*) was analyzed in the affected progeny (Figure 3). At both markers, all the phenotype2 mice were homozygous for the C3H allele and all the phenotype1 mice carried a C3H allele and a C57BL/6 allele. As expected, all phenotype1 mice carried a C3H haplotype inherited from the affected F_1 parent, and no SEG alleles were detected. This experiment demonstrated that the original semidominant allele was indeed unstable and could occasionally be turned into a recessive one, which suggested that the incomplete penetrance was caused by a change in the mode of transmission from semidominant to recessive. Using data from Table 3, we could obtain a raw estimation of the switch from semidominant to recessive, 32/84 = 38.1%, and its reversion from recessive to semidominant, 3/74 = 4.1%, after one generation on a mixed genetic background (C3H × CAST). A second mutant colony was then established with phenotype2 mice carrying the recessive allele.

We observed that the expression of the phenotype in affected heterozygous (*Hag*/+) mice varied from one animal to the next (Figure 4A), suggesting a variegated expression of the semidominant allele, *Hag*. To test this variegation hypothesis, both alleles were transferred into a genetic background increasing hair fragility. To achieve this, phenotype2 mice homozygous for either the semidominant allele (*Hag*) or the recessive allele (*hag*) were mated with mice homozygous for the caracul mutation (*Ca*) and the fur of the offspring was observed. Mice carrying the recessive allele in association with the caracul mutation *Ca* +/+ *hag* displayed a homogeneous caracul phenotype. The fur of *Hag* +/+ *Ca* mice appeared heterogeneous with hairy and bald patches, sug-

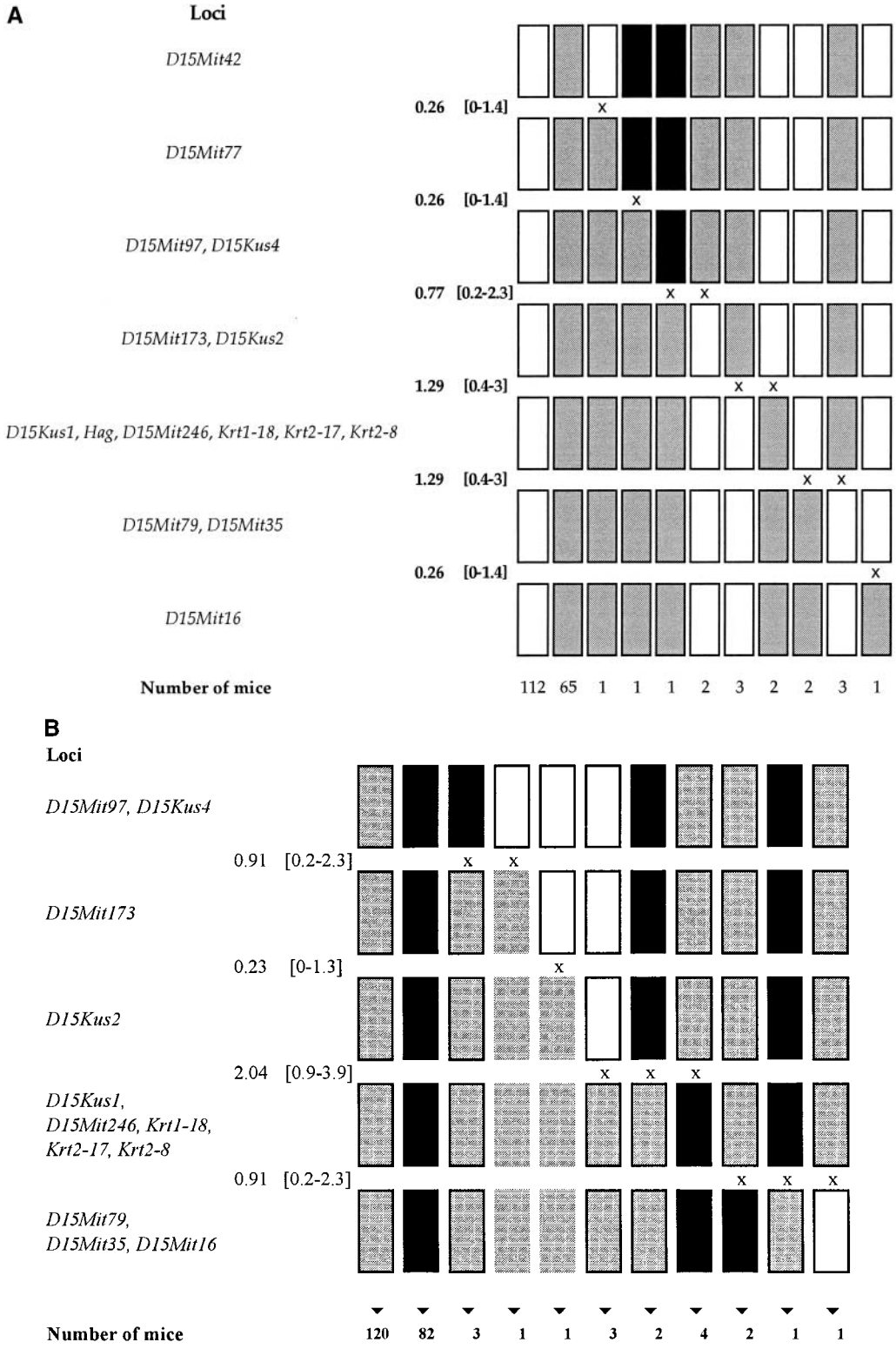


FIGURE 2.—Genotype of the F_2 progeny. (A) The 193 affected F_2 mice (phenotype1 and phenotype2). (B) The wild-type F_2 mice. Black, white, and gray boxes indicate homozygosity for the CAST allele, homozygosity for the C3H allele, and heterozygosity, respectively. The distance is indicated as centimorgans (cM) with the 95% confidence interval in brackets.

gesting that in the bald regions the *Hag* allele was expressed and interaction between *Hag* and *Ca* products was increasing the fragility of the hair shafts, while in hairy patches similar to the fur of *hag*+/+ *Ca* mice *Hag* expression was similar to *hag* expression (Figure 4B).

Physical mapping of the *Hag* locus: To refine the mapping of the mutation, a series of backcrosses and intercrosses were set between C3H/HeN mice heterozygous for the mutant allele *Hag* and wild-derived mice. Up to 2091 meioses were analyzed and three recombinant mice were identified between the *Hag* locus and the cluster *D15Mit246-Krt2-8-Krt1-18*, placing this locus centromeric to these three markers. Two YAC libraries (LARIN *et al.* 1991; HALDI *et al.* 1996) were screened by

TABLE 3

Phenotype of F_2 progeny heterozygous at *Krt2-17* in relation to their F_1 parental phenotypes

Cross	Phenotype of partners	Phenotype of F_2 progeny	
		Wild type	Phenotype1
1	Phenotype1 × phenotype1	32	52
2	Wild type × wild type	71	3

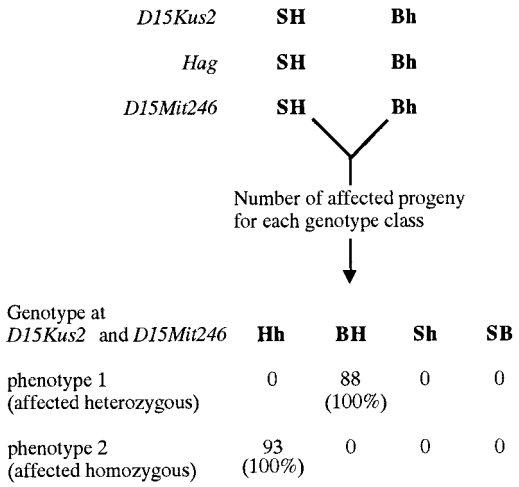


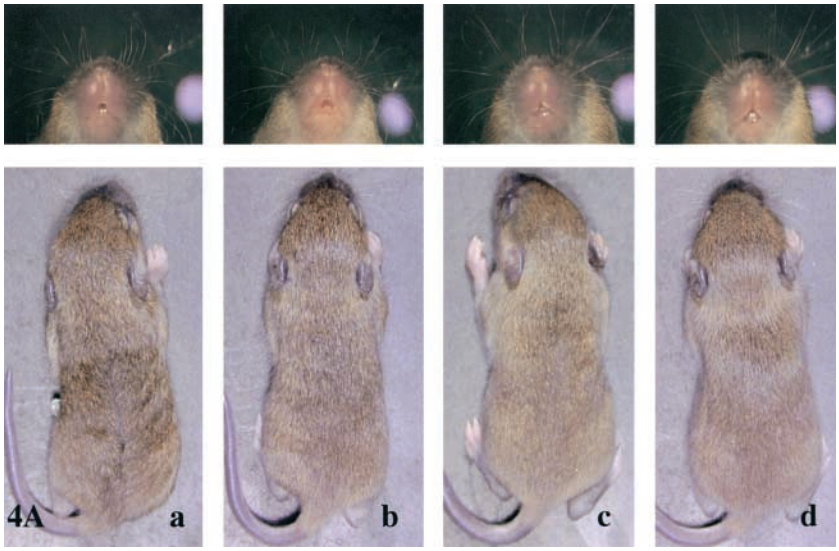
FIGURE 3.—Loss of semidominance by inbreeding. S, SEG alleles; H, C3H alleles from the mutant colony; B, C57BL/6 alleles; h, C3H alleles carried by the haplotype originating from mice that have lost the ability to produce affected heterozygous mice.

PCR for markers *D15Mit246*, *Krt2-1*, *Krt2-17*, *Krt2-16*, and *Krt2-7*. The positive clones (183h3, 70m8a9, b22m5a1, and 53m3d3) were tested by PCR for the rest of the markers mapped to the critical region (Figure 5). Two internal deletions were detected in b22m5a1 and 53m3d3. The ends of the above-mentioned YACs were cloned and four of them were reverse mapped to the contig (Figure 5): 183h3-T3 end (*D15Kus1*), 70m8a9-L end (*D15Kus2*), 70m8a9-R end (*D15Kus5*), and b22m 5a1-T7 end (*D15Kus3*). Some polymorphisms were detected for *D15Kus1* and *D15Kus2* and these two additional markers were then introduced into the genetic map, where *D15Kus2* appeared the closest centromeric landmark to *Hag* and no recombination event was found between *Hag* and *D15Kus1* (Figure 5). Among the inter-B1 sequences isolated from the YACs, only one, *D15Kus4*, was mapped back to the region. To complete a BAC contig, the MIT 129/Sv strain library and a C57BL/6 library (OSOEGAWA *et al.* 1998) were screened by PCR for *Krt2-1*, *Krt2-4*, *Krt2-6a*, *Krt2-8*, *Krt2-11*, and *Krt2-16* markers. From these screenings 13 clones were retrieved (Figure 5). The 51l7Sp6 end contained a part of the first exon and the first intron of *Krt2-6a*. Since this BAC clone was negative for the 3′ ends of both *Krt2-6* genes, *Krt2-6b* was located centromeric to *Krt2-6a*. The 254k21Sp6 end (AQ929432) contained two internal exons and the flanking intronic sequences of *Krt2-1* but did not contain the 3′ end of *Krt2-1* and *Krt2-17*; *Krt2-1* was then located telomeric to *Krt2-17* (Figure 5).

Molecular cloning of new intermediate filament genes: To find new type II hair keratin genes, we downloaded from the GenBank database all mouse expressed sequence tag (EST) sequences containing "*Mus musculus* type II hair keratin mRNA" as a criterion in the definition field. From this search, 209 EST clones were retrieved; 182 of these clones were arranged into seven contigs using the GCG package (http://www.gcg.com/). Early high-quality sequencing stop occurred for the 27 remaining EST clones, leading to poor-quality sequences and the inability to arrange these clones in contig. Three out of these seven contigs identified previously known hair keratin genes (*Krt2-10*, *Krt2-11*, and *Krt2-18*). Further analysis of the remaining four contigs was carried out by resequencing some of the EST clones and two new type II hair keratin sequences were identified (*Krt2-19* and *Krt2-20*). The first one, *Krt2-19* (AF312018), was colocalized with the previously known hard keratin genes, between *Krt2-7* and *D15Kus1*. The other one, *Krt2-20* (AY028606), was mapped between *Krt2-16* and the Sp6 end of BAC 298p5.

Candidate genes: Several genes were identified in the critical region: two nonepidermal basic cytokeratin genes, *Krt2-4* and *Krt2-7*; six epidermal cytokeratin genes, *Krt2-1*, *Krt2-5*, *Krt2-6a*, *Krt2-6b*, *Krt2-6h*, and *Krt2-17*; and six basic hard keratin genes, *Krt2-10*, *Krt2-11*, *Krt2-16*, *Krt2-18*, *Krt2-19*, and *Krt2-20*.

Because these 14 genes were potential candidates by their position, a survey of their functions was undertaken to identify the best ones. Three criteria were used: expression in the skin, phenotype of the available knockout mice, and disease associated with the human ortholog (Table 4). In a particular cell type, a type I keratin protein is always found in association with a type II keratin protein and, in human mutations, the coexpressed type I and type II keratin genes were reported to lead to the same disease (FUCHS 1995). By reverse transcriptase-PCR, no expression of *Krt2-6b* and *Krt2-7* was detected in the skin of either wild-type or hague mice. Human mutations of two hard keratin genes have been associated with the hair loss disease *monilethrix* (WINTER *et al.* 1997a,b), but no mutations were found in the hague alleles for the six hard keratin genes. Alopecia and hair anomalies are found in *pachyonychia congenita* patients, but no fur anomalies were found in mice homozygous for null alleles of *Krt2-6a* and *Krt2-6b* (WOJCIK *et al.* 2000; WONG *et al.* 2000). Moreover, no mutation was found in the coding region of *Krt2-6a* in Hague mice. *Krt2-6h*, like its human ortholog *K6HF* (WINTER *et al.* 1998), is expressed only in the companion layer



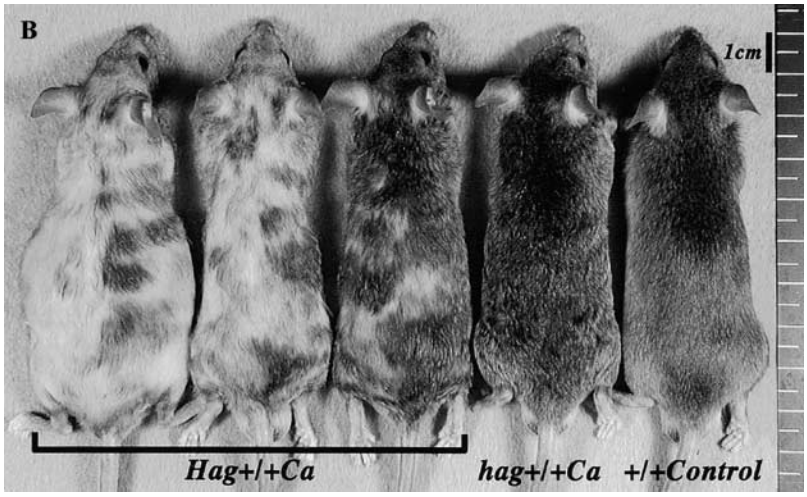


FIGURE 4.—The semidominant allele is variegated. (A) Ten-day-old mice of *Hag*/+ genotype (a, b, and c) and wild type (d). Curly whiskers and dorsal hairs are more evident in the following order: a > b > c. (B) Eight-week-old mice. From left to right, three mice heterozygous for the Caracul mutation and the semidominant allele (*Hag* +/+ *Ca*), one mouse heterozygous for both the Caracul mutation and the recessive hague (*hag* +/+ *Ca*), and one C3H wild-type mouse.

of the hair follicle but no mutation was found in the genome of *Hag/Hag* mice. Conversely, no hair anomalies have been observed in mice homozygous for a null allele of *Krt2-4* (NESS *et al.* 1998). Human mutations of *KRT1* (ortholog of *Krt2-1*), *KRT2* (ortholog of *Krt2-17*), and *KRT5* (ortholog of *Krt2-5*) lead to blistering skin diseases (reviewed by FUCHS 1995). No such skin blistering was observed in the hague mice, suggesting that these genes are not candidates.

DISCUSSION

The transmission of the trait is unusual: We have identified a semidominant mutation, Hague (*Hag*), producing hair curling. At first, the mutant allele appeared to be semidominant with incomplete penetrance (*Hag*/+), and we have shown that the semidominant allele is unstable, that it can be converted from dominant to recessive at an appreciable rate and from recessive to dominant at a lower rate, and that this instability could be due to either epigenetic modification or structural rearrangements occurring in the region. A similar situation has already been reported for several *yellow* mutant alleles (A^{hvy}, A^{iapy}, A^{iy}, and A^{vy}) at the *A* locus, chromosome 2, with an expressivity depending on the parental origin of the mutant allele in the zygote (DICKIE 1962, 1966; MICHAUD *et al.* 1994b; SIRACUSA *et al.* 1995). All these mutations occurred in a C3H background and were caused by integration of intracisternal A particle sequences in a different region of the gene: in exon 1C for A^{hvy} (ARGESON *et al.* 1996), between exon 1D and exon 2 for A^{iapy} and A^{iy} (DUHL *et al.* 1994; MICHAUD *et al.* 1994a), and in exon 1A for A^{vy} (DUHL *et al.* 1994). Transcription from the intracisternal A particle led to a deregulation of the agouti gene associated with the yellow phenotype.

Methylation of these insertions has been associated with their silencing and, in this case, despite carrying a yellow allele, the mice appear pseudo-agouti. The efficiency of this silencing process has been studied through parental germline transmission. The rate of silencing varied from one allele to another: 15.6% for A^{vy} (WOLFF 1978), 34.8% for A^{hvy} (ARGESON *et al.* 1996), and 40.6% for A^{iapy} (MICHAUD *et al.* 1994a) upon passage through

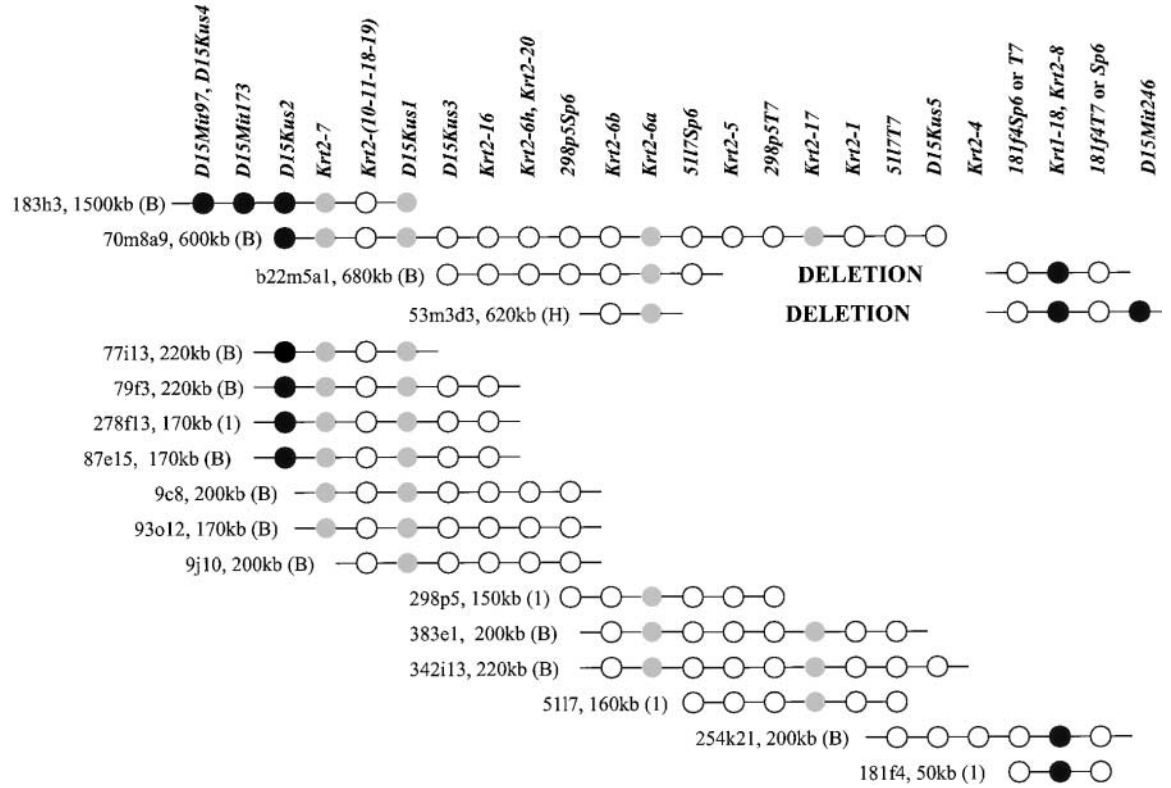


FIGURE 5.—Physical map around Hague. Clone address, insert size, and strain of origin (B, C57BL/6; 1, 129; and H, C3H) are indicated. Black denotes recombinant markers; gray, nonrecombinant markers; and white, nonpolymorphic markers that could not be typed.

the paternal germline; these rates decreased to 1.2, 1, and 2.5%, respectively, upon passage through the maternal germline. A survey of 6748 mice has shown that, at least for A^{vy}, the rate of silencing was dependent on the genetic background (WOLFF 1978). Our data showed a high switching rate of 38.2% from semidominant to recessive and a lower reverse switching rate of 4.1% on the mixed (C3H × CAST) genetic background. This data could be explained by methylation of a transposon. In this case, the integration of a mobile element containing promoter-like sequences in a keratin gene could lead to an overexpression of the gene or to the transcription of a chimeric or truncated messenger. Such a mutation would be expected to lead to a disruption of the

TABLE 4

Human diseases associated with type II keratin genes

Mouse genes	Skin expression[a]	Knockout phenotype[b]	Human homologs[c]	Human diseases associated[d]
Krt2-1	Yes		*KRT1* (*KRT10*)	*Epidermolytic hyperkeratosis*
Krt2-17	Yes		*KRT2* (*KRT9*)	*Ichthyosis bullosa of Siemens* (146800)
Krt2-4	Not tested	No fur anomalies	*KRT4* (*KRT13*)	*White sponge nevus* (193900)
Krt2-5	Yes		*KRT5* (*KRT14*)	*Epidermolysis bullosa simplex* (131900)
Krt2-6a	Yes	No fur anomalies	*KRT6A* (*KRT16*)	*Pachyonychia congenita type I* (167200)
Krt2-6b	No	No fur anomalies	*KRT6B* (*KRT17*)	*Pachyonychia congenita type II* (167210)
Krt2-6h	Yes		*K6HF*	
Krt2-7	No		*KRT7*	
Hard keratin	Yes			*Monilethrix* (158000)

[a] Expression in the skin of mutant and control mice.
[b] Phenotype of mice homozygous for a null allele.
[c] Type I keratin gene partners are in parentheses.
[d] OMIM numbers are in parentheses.

keratin network with a semidominant effect, and the subsequent silencing of the mobile element, over several generations, would then lead to a stable recessive allele as observed in the case of our hague mutation.

Fine mapping of *Hag*: By linkage analysis we have mapped the locus to the subtelomeric region of mouse chromosome 15. In this region, at least four loci associated with wavy hairs (caracul, *Ca*; crimpy, *cpy*) or hair loss (naked, *N*; shaven, *Sha*) have already been mapped (Huppi *et al.* 1998). A BAC and YAC physical map of the critical region (600–800 kb), including the mouse keratin complex genes, was constructed. We have mapped four basic hair keratin genes between *Krt2-7* and *Krt2-6b*; in humans the basic keratin genes have been recently located in a 200-kb fragment between *KRT7* and the keratin 6 gene (Rogers *et al.* 2000). In humans, the physical map of the ortholog region at 12q13 has been resolved and the cytokeratin gene order has been found to be conserved during evolution (Yoon *et al.* 1994). In both human and mouse, the basic hair keratin gene has been located between the keratin 7 gene and the keratin 6 gene. The structure of several type II keratin genes has been elucidated in different species (mouse, human, and sheep). In all cases but one, the genomic structure was conserved (<10 kb with nine exons); therefore any single type II keratin gene should be entirely within one, single BAC clone. The hague critical region being covered with only five overlapping BAC clones, genetic complementation by BAC transgenesis is now amenable to experimentation that should help in further narrowing the critical region.

Candidates genes: In humans, the cytokeratin genes have been demonstrated to be involved in several skin diseases (McLean and Lane 1995). In mice, to date, no such cytokeratin gene mutations have been found to be associated with any skin disease, but mice expressing a deleterious allele of the human keratin 14 gene displayed nearly all the symptoms of the corresponding human disease (Vassar *et al.* 1991). In addition, mice carrying mutated *Krt2-6a* transgenes exhibited severe blistering and neonatal lethality (Wojcik *et al.* 1999). These data suggested that epidermal cytokeratin gene mutations should lead to skin blistering diseases in mice, which is not observed in hague mice. It has also been reported that mutations in at least two basic hair keratin genes lead to the inherited hair disorder *monilethrix* (Winter *et al.* 1997a,b). In mice, overexpression of a sheep hard keratin gene was found to lead to hair loss similar to the phenotype of homozygous *Hag/Hag* mice (Powell and Rogers 1990). Since the *Hag/Hag* or *Hag/+* mice display a hair phenotype without any blistering of the skin, the basic hair keratin gene family may be considered top-ranked candidates in the positional cloning of *Hag*. In humans, this gene family, located in a 200-kb fragment between *KRT7* and the keratin 6 genes, contained at least six basic hair keratin genes and four expressed pseudogenes (Rogers *et al.* 2000).

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