

Uncv (uncovered): a new mutation causing hairloss on mouse chromosome 11

SHAN-RU LI^{1*}, DONG-PING WANG¹, XIU-LING YU¹, BAO-SHENG GE²,
CUI-E WANG³, YI-FAN LU⁴, JI-QING LI¹, NA WU¹, RUN-FANG LI¹, SHI-EN ZHU⁵,
ZHONG-CHENG ZHANG⁵ AND YONG-FU CHEN⁵

¹Institute of Jingfeng Medical Laboratory Animal, 20 Dongdajie Road, Fengtai, Beijing, 100071, P. R. China

²Changchun University of Agriculture and Animal Sciences, 175 Xi'an Road, Changchun, 130062, P. R. China

³Beijing Institute of Microbiology and Epidemiology, 20 Dongdajie Road, Fengtai, Beijing, 100071, P. R. China

⁴Beijing Institute of Biotechnology, 20 Dongdajie Road, Fengtai, Beijing, 100071, P. R. China

⁵China Agricultural University, 2 Yuanmingyuanxi Road, Haidian, Beijing, 100094, P. R. China

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Summary

A pair of mutant mice with a first sparse coat appeared spontaneously in the production stock of BALB/c mice with a normal coat. After being sib-mated, they produced three phenotypes in their progeny: mice with normal hair, mice with a first sparse coat and then a fuzzy coat, and uncovered mice. Genetic studies revealed the mutants had inherited an autosomal monogene that was semi-dominant. By using 11 biochemical loci – *Idh*, *Car2*, *Mup1*, *Pgm1*, *Hbb*, *Es1*, *Es10*, *Gdc*, *Ce2*, *Mod1* and *Es3* – as genetic markers, two-point linkage tests were made. The results showed the gene was assigned to chromosome 11. The result of a three-point test with *Es3* and D11Mit8 (microsatellite DNA) as markers showed that the mutation was linked to *Es3* with the recombination fraction $7.89 \pm 2.19\%$, and linked to D11Mit8 with the recombination fraction $26.30 \pm 3.57\%$. The recombination fraction between *Es3* and D11Mit8 was $32.90 \pm 3.81\%$. It is suggested that the mutation is a new genetic locus that affected the skin and hair structure of the mouse. The mutation was named uncovered, with the symbol *Uncv*. Further studies showed the mutation affected not only the histology of skin and hair but also the growth and reproductive performance of the mice. The molecular characterization of the *Uncv* locus needs to be further studied.

1. Introduction

The mouse (*Mus musculus*) has the advantage of small body size, large litter size, short generation interval, resistance to infection and the ability to be bred at will (Hogan *et al.*, 1986). It has been widely used in many aspects of biomedical research. Most importantly, it is the best mammalian species for genetic manipulation because it has so many special strains available and has the same genome size as man. Since the vertebrate linkage group was first established in the mouse in 1915, completion of the mammalian genome has been a continuous effort of geneticists and other scientists

(Green, 1989). In this process, discovering, mapping, and cloning of mutations is a major task. The power and value of mouse mutants lie in: (1) the relative ease of isolating mouse mutant loci; (2) the homology of mouse and man (Witherden *et al.*, 1997) and (3) their use as animal models in related biomedical research.

More than 140 genetic loci in mouse are known to affect the skin and coat texture, over 120 of which have been mapped to all chromosomes but the twelfth and Y chromosomes (Peters *et al.*, 1997). Such mutations as hairless (*hr*), naked (*n*), alopecia (*Al*), apampischo (*ao*), bare (*ba*), bareskin (*Bsk*) and nude (*nu*) have been reported as hair loss mutations (Green, 1989). Each causes unique phenotypic and genetic characteristics and appearance. Some of them, such as *nu/nu*, *hr/hr* mice, are very good animal models, and

* Corresponding author. Tel: +86 01 63841414. e-mail: lishru@hotmail.com.

play key roles in immunology and dermatology. We report here a new mouse mutation that caused associated hair loss, had effects on mouse biological characteristics, and could be used in related areas of biomedical research. The mutation was designated uncovered (*Uncv*).

2. Materials and methods

All animals described in this paper were maintained under conventional conditions in the Institute. For examination of the mode of inheritance, four types of mating were performed as shown in Table 1. For gene mapping, the mutation in BALB/c mice was tested in crosses with C57BL/6J and CBA/N by using 11 biochemical loci – *Idh*, *Car2*, *Mup1*, *Pgm1*, *Hbb*, *Es1*, *Es10*, *Gdc*, *Ce2*, *Mod1*, *Es3* – and D11Mit8 as genetic markers. The detection methods of biochemical gene loci were as in Li *et al.* (1995). DNA extraction was according to Ausue *et al.* (1995). Primer pairs were as described in Dietrich *et al.* (1992). PCR was carried out in buffer containing 1.5 mM-MgCl₂; there were 30 cycles, each consisting of 45 s at 94 °C, 60 s at 57 °C and 60 s at 72 °C. The products of PCR were electrophoresed on 8% denatured polyacrylamide gel followed by silver staining.

3. Results

(i) The origin of the new mutant mice

A pair of mutant mice with a first sparse coat appeared spontaneously in the production stock of

BALB/c mice with a normal coat in 1990. After being sib-mated they produced three phenotypes in their progeny: mice with normal hair (H), mice with a first sparse coat and then a fuzzy coat (S), and uncovered mice (U). There were no marked differences in the appearance and body weight of the newborn mice, except that some had no normal vibrissae and would be uncovered later. The young with normal vibrissae grew hair within 10 days, some with normal coat (haired mice), others with a first sparse coat and a fuzzy coat 30 days later (sparse-haired mice).

(ii) Genetics

(a) *Mode of inheritance.* The results (Table 1) revealed: Mating of haired with uncovered mice led to sparse-haired (fuzzy) mice. Crosses of uncovered with sparse-haired mice produced uncovered and sparse-haired mice in a ratio of 77:84, mating of sparse-haired with haired mice produced sparse-haired and haired offspring in a ratio of 50:43, and mating of sparse-haired and sparse-haired mice produced haired, sparse-haired and uncovered mice in a ratio of 114:225:117. All the ratios accord with the theoretical 1:1, 1:2:1 ratios of segregation, respectively. No significant deviation (0.05 level) from the expected ratios was observed among the 152 matings and 710 progeny classified. Thus the mutants inherited an autosomal monogene that was semi-dominant.

(b) *Two-point linkage tests.* In order to identify its linkage group, the tests required two generations in all cases. The first generation (multiple heterozygotes)

Table 1. *Inheritance of the coat strain of the mutant*

Mating class	No. of matings	Phenotype of offspring			Analysis		
		H	S	U	Ratio	X ²	P
H × S	19	43	50	0	1:1	2.3	0.25–0.5
H × U	16	0	72	0	1:0	0	1.00
S × S	96	114	225	117	1:2:1	0.118	0.95
S × U	37	0	77	84	1:1	0.22	0.5–0.75

H, haired mice; S, sparse-haired mice; U, uncovered mice.

Table 2. *Result of two-point tests between Uncv and Es3*

Intercross		Backcross		Genotypes in progeny			f
BALB/c ^a	CBA/N	F1	BALB/c ^a	Nonrecombinant	Recombinant		
<i>Uncv</i> a	+ c	<i>Uncv</i> a	<i>Uncv</i> a	<i>Uncv</i> a	<i>Uncv</i> a	<i>Uncv</i> a	4/28
<i>Uncv</i> a	+ c	+ c	<i>Uncv</i> a	<i>Uncv</i> a	+ c	<i>Uncv</i> a + a	
				14	10	2	

^a BALB/c = BALB/c-*Uncv*/*Uncv*; f, fraction recombinant.

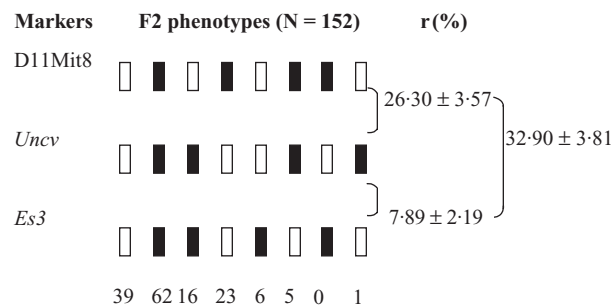


Fig. 1. Segregation of three loci in backcross progeny obtained in the backcross (BALB/c-*Uncv/Uncv* × CBA/N-+/+) F1 × BALB/c-*Uncv/Uncv*. Open rectangles represent phenotypes as parents from *Uncv/Uncv* BALB/c; filled rectangles represent phenotypes as parents from F1.

consisted of outcrossing an uncovered homozygote to an animal from C57BL/6J or CBA/N with a normal coat. The second generation was obtained by backcrossing the multiple heterozygotes of the F1 to the uncovered BALB/c mice. The offspring of this

backcross were classified and the deviation of uncovered from independent segregation with all markers was estimated. A statistically significant chi-squared value for linkage was found between the mutation and *Es3* (Table 2). It was soon evident that the mutation was linked to *Es3*, and was therefore on mouse chromosome 11.

(c) *Three-point linkage tests.* On the basis of the two-point test result, we enlarged the intercross of the uncovered BALB/c to CBA/N mice, and chose D11Mit8 (a microsatellite marker) as another genetic marker to do a three-point linkage test. The result showed that the mutation was linked to *Es3* with a recombination fraction of $7.89 \pm 2.19\%$, and linked to D11Mit8 with a recombination fraction of $26.30 \pm 3.57\%$. The recombination rate between *Es3* and D11Mit8 was $32.90 \pm 3.81\%$. According to the recombinations, the order of loci was D11Mit8 – 26.30 ± 3.57 – *Uncv* – 7.89 ± 2.19 – *Es3* (Fig. 1). From the location on the chromosome, it was concluded that the

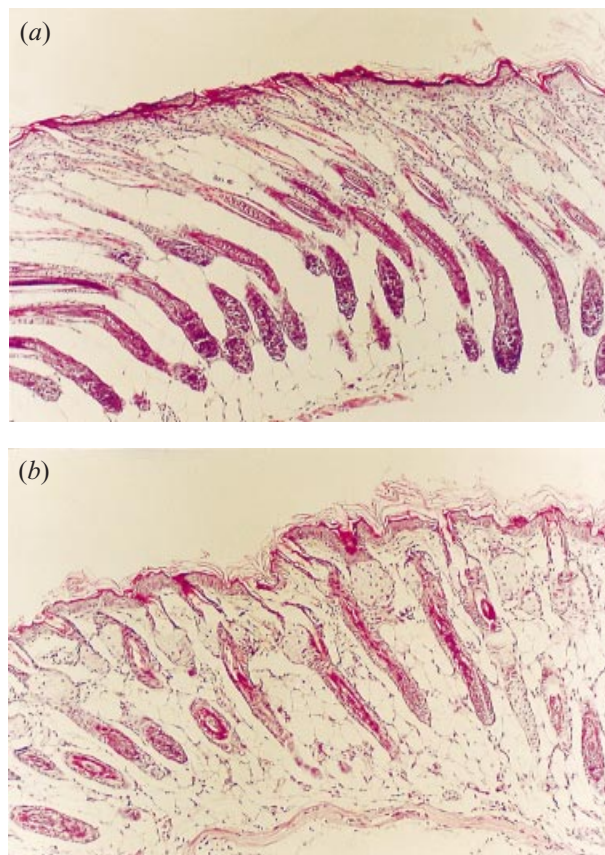


Fig. 2. (a) Histological structure of +/+ mouse skin. There are many deep and regularly positioned hair bulbs and non-hyperplasia of the sebaceous glands. (b) Histological structure of *Uncv/Uncv* mouse skin. There are a few shallow and irregularly positioned hair bulbs and hyperplasia of the sebaceous glands. H & E, × 400.

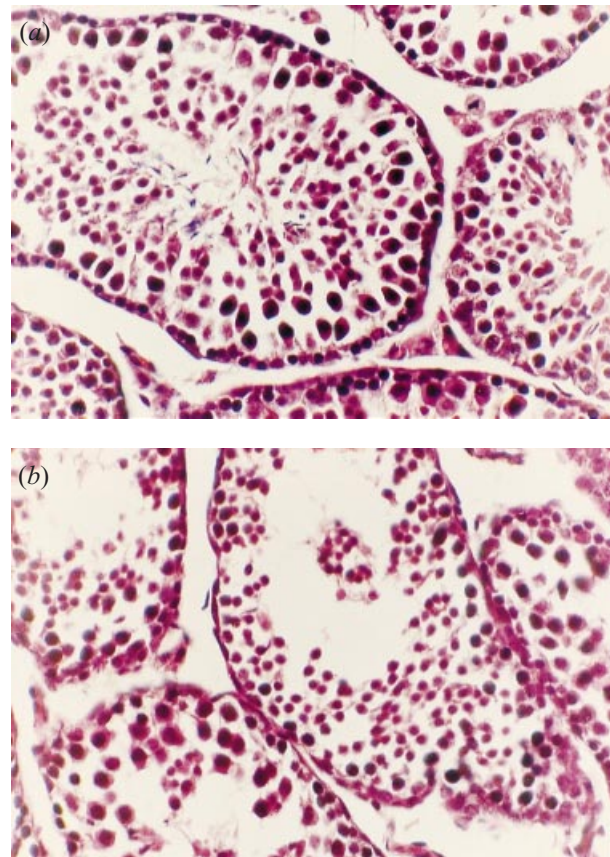


Fig. 3. (a) Histological structure of 5-week-old +/+ mouse testis. There are a large number of well-developed seminiferous cells and mature sperm in the testis. (b) Histological structure of 6-week-old *Uncv/Uncv* mouse testis. There are only a few seminiferous cells, and no sperm in the testis. H & E, × 400.

Table 3. Comparison of body weight (mean \pm SD, g) among the mutant mice at different ages

Phenotypes	Newborn M + F	2 weeks		4 weeks		8 weeks		12 weeks	
		M + F	M	F	M	F	M	F	
U	1.27 \pm 0.12 (36)	5.87 \pm 0.83 ^B (16)	9.73 \pm 1.66 ^B (30)	9.10 \pm 1.07 ^B (34)	16.59 \pm 2.34 ^{Bb} (27)	15.08 \pm 1.83 ^B (30)	19.70 \pm 1.70 ^{Bb} (18)	16.90 \pm 2.54 ^B (30)	
S	1.29 \pm 0.14 (30)	6.83 \pm 0.95 ^A (25)	12.04 \pm 1.82 ^A (39)	11.08 \pm 1.92 ^A (40)	18.00 \pm 2.77 ^{Aba} (62)	16.83 \pm 2.30 ^A (55)	21.10 \pm 1.75 ^{A,Bb} (26)	17.97 \pm 1.70 ^{Ab} (29)	
H	1.32 \pm 0.12 (38)	7.07 \pm 1.09 ^A (16)	11.52 \pm 2.22 ^A (20)	11.64 \pm 1.85 ^A (25)	18.56 \pm 2.55 ^{Aa} (25)	16.57 \pm 2.19 ^A (21)	21.37 \pm 1.71 ^{Aa} (14)	18.68 \pm 1.72 ^A (14)	

M, male; F, female; H, haired (+/+) mice; S, sparse-haired (*Uncv*/+) mice; U, uncovered (*Uncv*/*Uncv*) mice. The comparisons were made among the same sexual mutant mice at the same age.

Numbers on the same vertical row with different capital letters represent 0.01 significance level ($P < 0.01$), with different lower-case letters represent 0.05 significance level ($P < 0.05$).

mutation was a new genetic locus that affected the skin and hair structure of the mouse. According to the rules of mouse genetic nomenclature (Davisson, 1996) the mutation was named after uncovered, with the symbol *Uncv*. It has been entered into Mouse Genome Database (MGD).

(iii) Biological effects of the mutation

By comparing the differences in biological characteristics among the three phenotypes, we investigated the biological effects of the mutation.

(a) *Histological structure of skin and hair.* Histological sections of hair and skin from the abdomen and the back of mice were observed under a light microscope. Compared with the other two phenotypes, the uncovered mouse had only very short, slight fuzzy hairs that lacked a medulla. There were no significant differences between the uncovered mice and the two other phenotypes in the structures of epidermis or dermis. However, the growth cycle of the uncovered mice is defective. They had a few shallow and irregularly positioned hair bulbs and hyperplasia of the sebaceous glands (Fig. 2). It is suggested that the defective coat structure of the uncovered mice is due to the abnormal texture of the hairs themselves.

(b) *Growth.* To obtain some quantitative idea of any effects of the *Uncv* mutation on growth, affected animals and normal sibs were weighted from birth to 12 weeks. There was no significant difference among the three phenotypes at birth, but from birth onwards the weight gain of *Uncv*/*Uncv* was less than that of the other two phenotypes (Table 3).

(c) *Reproduction.* To assess the effects of the mutation on reproduction, we investigated the histological structure of testis and ovaries at 4–8 weeks of age, and litter sizes of the three phenotypes. There were no significant difference among the three phenotypes in the structure of the ovaries. There were a large number of follicles in various developmental stages and corpus lutea from 5 weeks of age. In contrast to the ovaries, there were significant differences among the three phenotypes in the structure of the testis. We found a large number of well-developed seminiferous cells and mature sperm in the testis of 5-week-old *Uncv*/+ and +/+ mice, but only a few seminiferous cells and no sperm in the testis of 6-week-old *Uncv*/*Uncv* mice (Fig. 3). It is suggested that the *Uncv*/*Uncv* male mice had a delay in sexual maturation of about 2 weeks. In addition, the *Uncv*/*Uncv* female mice had smaller litter sizes than the other two phenotypes (Table 4).

Table 4. Litter sizes (mean \pm SD) of the mutant mice

Dam	Sire			Total
	H	S	U	
H	5.19 \pm 1.94 (26)	4.67 \pm 2.07 (6)	4.44 \pm 0.97 (9)	4.95 \pm 1.78* (41)
S	4.38 \pm 1.98 (13)	4.75 \pm 1.74 (46)	4.31 \pm 1.78 (16)	4.66 \pm 1.75* (75)
U	4.57 \pm 1.62 (7)	4.20 \pm 1.26 (15)	3.64 \pm 1.22 (14)	4.06 \pm 1.31 (36)

H, haired (+/+) mice; S, sparse-haired (*Uncv*/+) mice; U, uncovered (*Uncv*/*Uncv*) mice.

* $P < 0.05$.

4. Discussion

Several mutant genes that affect skin and coat structure have been assigned to chromosome 11 of mice, such as *Al*, *Bsk*, *Bda* (bald arthritic), *nu*, *lt* (lustrous), *wa2* and *Re* (Rex) (Green, 1989), but *Uncv* has an obviously different inheritance and phenotype from these. First, although *Uncv*, like *Bda* and *Bsk*, inherits a single autosomal semi-dominant gene, it maps to a different position that is nearer to *Es3* than *Bda* and *Bsk* (Fig. 4). Secondly, *Uncv*/*Uncv* mice have no normal coat and vibrissae from birth onwards, heterozygotes are distinguishable from +/+ mice after 1 week of age by their sparse hair, and in contrast to *Bsk* and *Bda* there is no overlap between the heterozygotes and homozygotes after their coat appears. The *Uncv*/*Uncv* mice have a few shallow and

irregularly positioned hair bulbs and hyperplasia of the sebaceous glands. The defective coat structure of the uncovered mice is due to the abnormal texture of the hairs themselves. Thus, there is no candidate gene on chromosome 11 which, if mutated, would be expected to result in the *Uncv*/*Uncv* phenotype. *Uncv* is a new mutation that affects skin and coat structure mapped on mouse chromosome 11.

Many skin and coat mutations affect mouse reproductive performance, immunology and other biological characteristics. For example, both sexes of *at* and females of *mc* are sterile; hairless (*hr*) mice are fertile but most females do not nurse their young well; homozygotes of *ab* and *cr* are viable and fertile but fertility is somewhat reduced (Green, 1989). The present results show that *Uncv* also affects reproductive and growth performance. The question is why so many skin and coat mutations have effects on mouse reproductive performance. What the underlying genes could be needs to be answered in the future.

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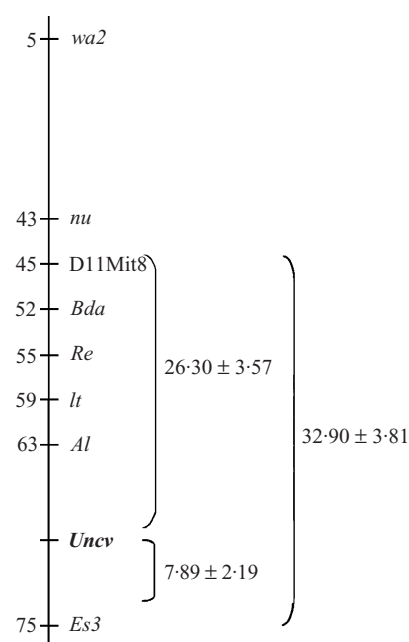


Fig. 4. Map of mutations that affect skin and coat structure on mouse chromosome 11. *Uncv* is in a different position on the chromosome from all other mutations that affect skin and coat structure.

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