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

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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, Cc2, 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 ± 2.19%, and linked to D11Mit8 with the recombination fraction 26.30 ± 3.57%. The recombination fraction between Es3 and D11Mit8 was 32.90 ± 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 (nu), alopecia (Al), apampisco (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

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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

<table>
<thead>
<tr>
<th>Mating class</th>
<th>No. of matings</th>
<th>Phenotype of offspring</th>
<th>Ratio</th>
<th>X²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>H × S</td>
<td>19</td>
<td>H:43; S:50; U:0</td>
<td>1:1</td>
<td>2.3</td>
<td>0.25-0.5</td>
</tr>
<tr>
<td>H × U</td>
<td>16</td>
<td>H:0; S:72; U:0</td>
<td>1:0</td>
<td></td>
<td>1.00-1.00</td>
</tr>
<tr>
<td>S × S</td>
<td>96</td>
<td>H:114; S:225; U:117</td>
<td>1:2:1</td>
<td>0.118</td>
<td>0.95</td>
</tr>
<tr>
<td>S × U</td>
<td>37</td>
<td>H:0; S:77; U:84</td>
<td>1:1</td>
<td>0.22</td>
<td>0.5-0.75</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Intercross</th>
<th>Backcross</th>
<th>Genotypes in progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c × CBA/N</td>
<td>BALB/c × CBA/N</td>
<td>BALB/c × CBA/N</td>
</tr>
<tr>
<td>Uncv a + c</td>
<td>Uncv a + c</td>
<td>Uncv a + c</td>
</tr>
<tr>
<td>Uncv a + c</td>
<td>Uncv a + c</td>
<td>Uncv a + c</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Nonrecombinant</th>
<th>Recombinant</th>
<th>f</th>
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<tbody>
<tr>
<td>Uncv a</td>
<td>Uncv a</td>
<td>14</td>
</tr>
<tr>
<td>Uncv a</td>
<td>Uncv a</td>
<td>10</td>
</tr>
<tr>
<td>Uncv a</td>
<td>Uncv a</td>
<td>2</td>
</tr>
<tr>
<td>Uncv a</td>
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<td>2</td>
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<td>2</td>
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<tr>
<td>Uncv a</td>
<td>Uncv a</td>
<td>4/28</td>
</tr>
</tbody>
</table>

* BALB/c = BALB/c–Uncv/Uncv; f, fraction recombinant.
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Uncv markers F2 phenotypes (N = 152) r(%) D11Mit8 26.30 ± 3.57 Uncv 7.89 ± 2.19 Es3 32.90 ± 3.81

Fig. 1. Segregation of three loci in backcross progeny obtained in the backcross (BALB/c–Unc/Unce×CBA/N–+/+) F1×BALB/c–Unce/Unce. Open rectangles represent phenotypes as parents from Unce/Unce 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 ± 2.19%, and linked to D11Mit8 with a recombination fraction of 26.30 ± 3.57%. The recombination rate between Es3 and D11Mit8 was 32.90 ± 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/Unce 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/Unce mouse testis. There are only a few seminiferous cells, and no sperm in the testis. H&E, ×400.
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 Unc. It has been entered into Mouse Genome Database (MGD).

(ii) 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 Unc 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 Unc/Unc 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 Unc/+ and +/+ mice, but only a few seminiferous cells and no sperm in the testis of 6-week-old Unc/Unc mice (Fig. 3). It is suggested that the Unc/Unc male mice had a delay in sexual maturation of about 2 weeks. In addition, the Unc/Unc female mice had smaller litter sizes than the other two phenotypes (Table 4).
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, It (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 automosal 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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References


Green, M. C. (1989). Catalog of mutant genes polymorphic


