Exp. Anim. 60(2), 161–167, 2011

—Original—

Retrotransposon-Mediated *Fgf5*$^{go\text{-}Utr}$ Mutant Mice with Long Pelage Hair

Seiya MIZUNO, Saori IIJIMA, Tomoko OKANO, Noriko KAJIWARA, Satoshi KUNITA, Fumihiro SUGIYAMA, and Ken-ichi YAGAMI

Laboratory Animal Resource Center, University of Tsukuba, 1–1–1 Tennodai, Tsukuba, Ibaraki 305-8575, Japan

Abstract: We found 6 spontaneous mutant mice with long pelage hair in our ICR breeding colony. The abnormal trait was restricted to long hair in these mice, which we named *moja*. They were fertile and showed the same growth and behavior as wild-type mice. To investigate the manner of the genetic inheritance of the *moja* allele, offspring were bred by mating the *moja* mice; all offspring had long pelage hair. Furthermore, we performed a reciprocal cross between *moja* mice and wild-type ICR mice with normal hair. All offspring exhibited normal hair suggesting an autosomal recessive inheritance of the trait. The *moja/moja* hair phenotype was maintained in skin grafted onto nude mice, suggesting that circulating or diffusible humoral factors regulating the hair cycle are not involved in the abnormal trait. The phenotype of *moja/moja* mice is similar to that of *Fgf5*-deficient mice. Therefore, we examined the expression of *Fgf5* by RT-PCR in *moja/moja* mice. As expected, no *Fgf5* expression was found in *moja/moja* mouse skin. PCR and DNA sequence analyses were performed to investigate the structure of the *Fgf5* gene. We found a deletion of a 9.3-kb region in the *Fgf5* gene including exon 3 and its 5' and 3' flanking sequences. Interestingly, the genomic deletion site showed insertion of a 498-bp early transposon element long terminal repeat. Taken together, these results suggest that the long hair mutation of *moja/moja* mice is caused by disruption of *Fgf5* mediated by insertion of a retrotransposon.

Key words: *Fgf5*, long pelage hair, mice, spontaneous mutant, retrotransposon

Introduction

There are many mutations in the visible phenotypes of the hair of mice. Mouse Genome Informatics, the database resource for the laboratory mouse (http://www.informatics.jax.org/), reports 619 genotypes of abnormal hair growth comprising: abnormal hair cycle, alopecia, delayed hair appearance, ectopic hair growth, excessive hair, focal hair loss, hairless, long hair, loss of cilia, premature hair loss, premature hair regrowth, progressive hair loss, and retarded hair growth. Of the genotypes for abnormal hair growth, 12 have been annotated as long hair phenotypes. It seems that 5 different genes are involved in the phenotypic expression of long hair growth: fibroblast growth factor 5 (*Fgf5*), gasdermin A3 (*Gsdma3*), high mobility group AT-hook (*Hmga2*), skin/coat color 6 (*Skc6*), and skin/coat color 8 (*Skc8*).

The hair growth cycle of mammals is composed of

(Received 5 October 2010 / Accepted 3 December 2010)

Address corresponding: F. Sugiyama, Laboratory Animal Resource Center, University of Tsukuba, 1–1–1 Tennodai, Tsukuba, Ibaraki 305-8575, Japan

three periods: anagen (follicle generation and hair production), catagen (follicle regression), and telogen (resting phase) [5]. FGF5 functions as an important factor in the inhibition of hair growth and in inducing of progression to catagen in hair follicles [6]. Deficiency of FGF5 facilitates hair elongation, resulting in long pelage hair.

We found spontaneous mutant mice with long pelage hair in our ICR breeding colony. We tentatively called the mutation *moja*, which means long and shaggy coat appearance in Japanese. In the present study, test crosses indicated that the *moja* phenotype was inherited in an autosomal recessive manner. Skin grafting experiments revealed that the *moja* phenotype was not influenced by endocrine factor(s). Based on these results, we used the candidate gene approach to elucidate the molecular basis of the novel *moja* mutation for long hair growth. Genomic and cDNA analyses clearly indicated that the *moja* mutation involved genomic deletion of exon 3 of *Fgf5*. Furthermore, we identified an early transposon element long terminal repeat (LTR) at the site of the deletion. Here, we propose that the *moja* phenotype is caused by retrotransposon-mediated *Fgf5* deletion ($Fgf5^{go\text{-}Utr}$).

Materials and Methods

Animals

ICR mice were purchased from CLEA Japan Co., Ltd. (Tokyo, Japan) and used as wild-type mice. Nude mice (CD1-Foxn1^{nu}) were obtained from Charles River Laboratory Japan Co., Ltd. (Yokohama, Japan). Animals were kept in plastic cages under pathogen-free conditions (sentinel mice were examined periodically throughout the study) in a room maintained at 23.5 ± 2.5°C and 52.5 ± 12.5% relative humidity under a 14-h light:10-h dark cycle. Mice had free access to commercial chow (MF for ICR mice and sterilized NMF for nude mice; Oriental Co., Ltd., Tokyo, Japan) and filtered water (for ICR mice) or sterilized water (for nude mice). Animal experiments were carried out in a humane manner with approval from the Institutional Animal Experiment Committee of the University of Tsukuba and in accordance with the Regulations for Animal Experiments of our university and Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions under the Jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Skin graft

Adult *moja* and wild-type mice were sacrificed by cervical dislocation. Circular skin plugs about 10 mm in diameter were harvested by incision from the backs of donors after shaving, soaked in PBS, and stored on ice before grafting. Three adult nude mice were anesthetized by intraperitoneal injection of pentobarbital solution. For the double graft procedure, the graft beds were prepared on the right and left back of nude mice. The grafts were fixed with Aron Alpha (Daiichi Sankyo, Co., Ltd., Tokyo, Japan), covered with sterilized cotton, and bandaged with elastic vinyl tape for 1 week. The coat appearance of nude mice was evaluated 25 weeks after skin grafting.

RT-PCR

Total RNA was prepared from the skin using an Isogen RNA extraction kit (Nippon Gene, Tokyo, Japan). Aliquots of 5 μg of total RNA were used for cDNA synthesis using the SuperScript II first-strand synthesis system with oligo(dT) (Invitrogen, Carlsbad, CA, USA). cDNAs were amplified with ExTaq DNA polymerase (Takara, Kyoto, Japan). PCR was performed for 35 cycles. The sequences of the forward and reverse primers and PCR product lengths (bp) for each gene were as follows: *Fgf5* (GTC CTT GCT CTT CCT CAT CTT CT, GGA CGC ATA GGT ATT ATA GCT GTT T, long *Fgf5*: 514 bp, short *Fgf5*: 409 bp), *Fgf5* exon 1 to exon 2 (GAG ATC ACT GGC GTT ATA AAT ATC C, CTG AAA ACT CCT CGT ATT CCT ACA A, 615 bp), and GAPDH (ACC ACA GTC CAT GCC ATC AC, TCC ACC ACC CTG TTG CTG TA, 452 bp).

Genomic PCR

To determine whether *Fgf5* exons 1, 2, and 3 are present in *moja* mice, genomic DNA purified from tail tips was amplified using primer sets for exon 1 (AAT AAC AGC GAG AAA CCA GTC TT, CTC CAC TGG AAA CTG CTA TGT TC, 802 bp), exon 2 (GGT CAC AAA AGG TCA TTA TAT GTT C, GTT TAC AAG TGC ATT TAC CCT TTAC, 502 bp), and exon 3 (AAA TGG TGT TGC AGA GGT AAC TTA G, GAT GAC GCC TGT ATA GAG AGT TGT T, 640 bp). To investigate the site of

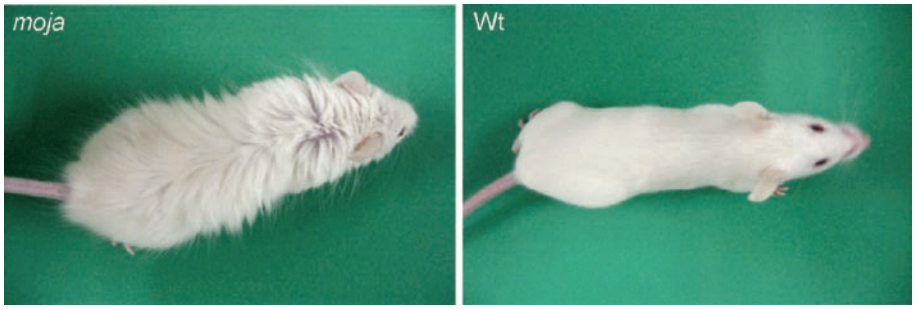

Fig. 1. *Moja* mice with long pelage hair. *Moja* mice had long pelage hair compared with wild-type (Wt) controls.

deletion in detail, genomic DNA from *moja* mice was amplified using a primer set corresponding to intron 2 and the 3' flanking region of *Fgf5* (CAT CAG TCT CAT AAA TCA GCC CAC AAA GTA, ATT GAT CCA GTC TTC ATC TTA GCC AGT TGT). The PCR products were subcloned and sequenced with Big Dye® Terminator V3.1 Cycle Sequencing Kit and Applied Biosystems 3130 Genetic Analyzer (Applied Biosystems Japan Ltd., Tokyo, Japan).

Results

Inheritance of the moja phenotype

ICR mice were obtained from CLEA Japan Co., Ltd. in 2007, and have been maintained at our laboratory by random mating. Six *moja* mice with abnormal hair growth were found in 2009. *Moja* mice are fertile and do not exhibit any gross anomalies other than the long and shaggy hair over the whole body from the head to the tail root (Fig. 1). To examine whether the abnormal hair is heritable, we carried out test crosses between *moja* mice. Of 25 progeny, consisting of 8 males and 17 females, all the adult mice showed long and shaggy hair identical in appearance to their parents. *Moja* males were crossed with wild-type ICR females with a normal coat appearance. Of 29 progeny, consisting of 16 males and 13 females, all the adult mice showed a normal coat appearance. Furthermore, all progeny (8 males, 6 females) had the normal hair phenotype on crosses between *moja* females and wild-type ICR males. These results suggest that the *moja* phenotype is inherited in an autosomal recessive manner.

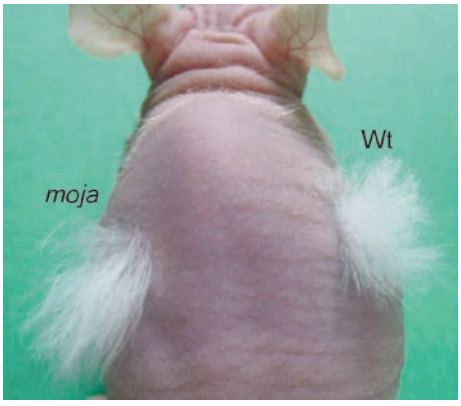

Fig. 2. Skin grafts on a nude mouse. A 12-week-old nude mouse developed abnormally long pelage hair derived from *moja/moja* (*moja*) mouse skin and normal pelage hair derived from wild-type mouse skin.

Skin grafting using nude mouse hosts and moja/moja skin

To investigate whether the development of abnormal long hair is ameliorated by endocrine factor(s), we grafted *moja/moja* mouse skin onto nude mouse hosts. Donor skin was shaved of hair immediately before transplantation. Three nude mice received grafts of *moja/moja* mouse skin and wild-type ICR mouse skin on the left and right back, respectively. One nude mouse was successfully grafted and the coat appearance was evaluated 25 weeks after skin grafting. The hair derived from *moja/moja* mouse skin was markedly longer than that from the wild-type mouse skin (Fig. 2). These observations suggest that *moja/moja* hair is the result of a functional defect in a gene expressed in the skin.

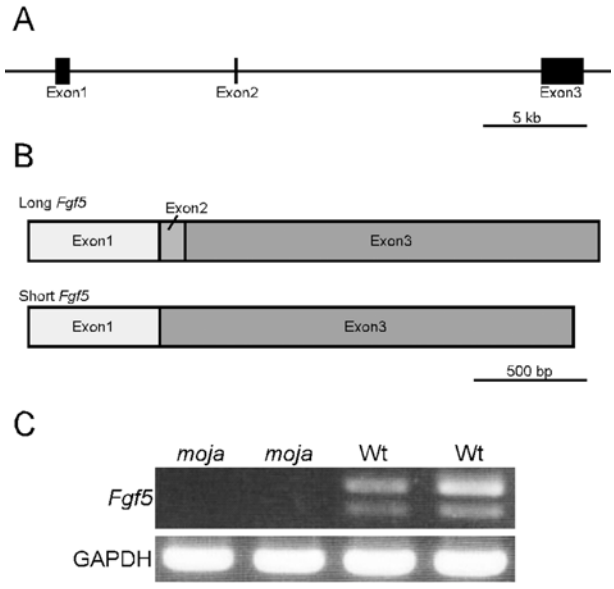

Fig. 3. Expression of *Fgf5* in *moja/moja* mice. (A) A schematic diagram indicating the genomic structure of *Fgf5*. (B) Two schematic diagrams indicating long and short *Fgf5* transcripts. (C) RT-PCR of *Fgf5* from exon 1 through exon 3 demonstrated the absence of both long and short *Fgf5* products in *moja/moja* (*moja*) mouse preparations.

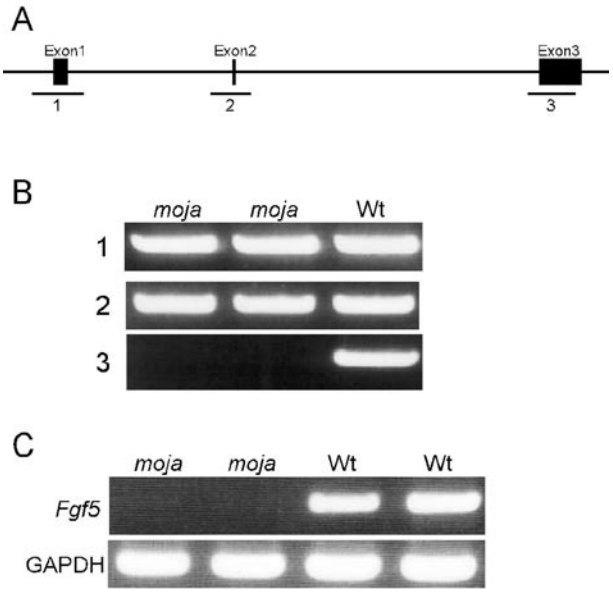

Fig. 4. Deletion of *Fgf5* exon 3 in *moja/moja* mice. (A) The genomic structure of *Fgf5* was investigated using three PCR primer sets. Three amplicons are shown as bars labeled 1, 2, and 3 below the schematic diagram of the mouse *Fgf5* genome. (B) Genome PCR for (1) exon 1, (2) exon 2, and (3) exon 3 of *Fgf5* in *moja/moja* (*moja*) and wild-type (Wt) mice DNA indicated deletion of exon 3 in *moja* mice. Amplicons for exons 1, 2, and 3 were 802, 520, and 640 bp respectively. (C) RT-PCR of *Fgf5* exon 1 to exon 2 showed the absence of *Fgf5* product in *moja/moja* mouse preparations.

Expression of Fgf5 in moja/moja mice

The *Fgf5* gene consists of 3 exons (Fig. 3A), and alternative splicing results in two transcripts, long and short *Fgf5* (Fig. 3B). The short transcript lacks the region corresponding to exon 2. *Fgf5* is expressed in a variety of tissues, including the skin, and it has been reported that homozygous *Fgf5* mutant mice have significantly longer pelage hair than wild-type mice [6]. Therefore, *Fgf5* is a candidate gene for the *moja* phenotype. We examined *Fgf5* expression in mouse skin. In wild-type mouse skin, RT-PCR using primers derived from exons 1 and 3 revealed amplicons of 514 and 409 bp for long and short *Fgf5*, respectively. As expected, no amplification of long or short *Fgf5* was observed in *moja/moja* mouse skin (Fig. 3C).

Deletion mutation of Fgf5 in moja/moja mice

Next, we performed PCR analysis using genomic DNA from *moja/moja* and wild-type mice to determine the presence or absence of exons 1, 2, and 3 of *Fgf5* (Fig. 4A). PCR using primers derived from the 5' and 3' flanking regions of exons 1 and 2 yielded amplicons of 802 and 520 bp, respectively, in both *moja/moja* and wild-type mice. In contrast, although PCR using primers derived from intron 2 and exon 3 yielded an amplicon of 640 bp in wild-type controls, no amplification product was obtained in *moja/moja* mice (Fig. 4B). These results suggest the genomic deletion of exon 3 of *Fgf5* in *moja/moja* mice.

To examine whether *moja/moja* mice express incomplete *Fgf5* transcripts, RT-PCR was performed using primers derived from exons 1 and 2 with RNA from wild-type and *moja/moja* mouse skin as templates. The expected amplicon of 640 bp was observed in wild-type controls, but not in *moja/moja* mice (Fig. 4C). These observations suggest that the *moja* phenotype is caused by a loss-of-function mutation of *Fgf5*.

Insertion of an early transposon element in Fgf5$^{go\text{-}Utr}$

PCR primers based on intron 2 and the 3' flanking

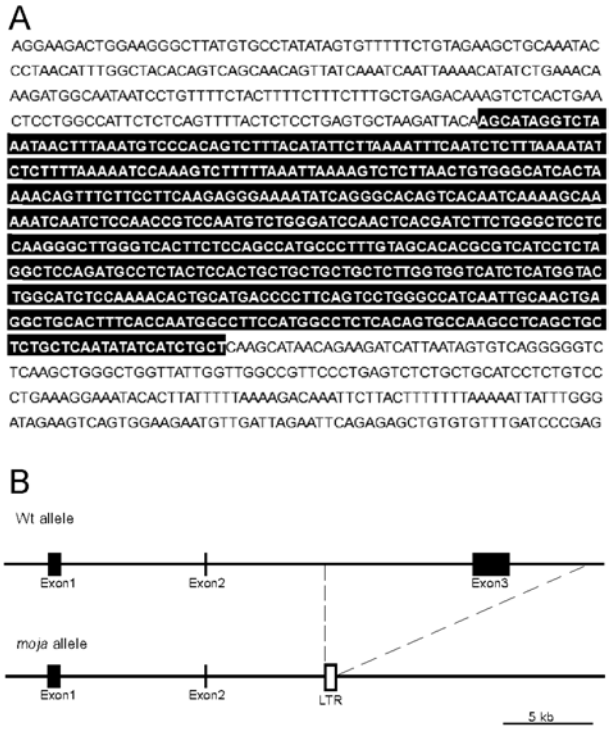

Fig. 5. Insertion of LTR at the deletion site of *Fgf5*. (A) Analysis of nucleotide sequences was performed using subcloned DNA amplified using primers derived from intron 2 and the 3' flanking region of *Fgf5*. Insertional DNA of 498 bp is shown on a black background. (B) The schematic diagram shows the comparison of *Fgf5* genomic structure between wild-type and *moja* alleles.

sequences of *Fgf5* amplified a product of approximately 2.5 kb from the *moja* allele, while an expected PCR product of 11.3 kb was detected on the wild-type *Fgf5* allele derived from ICR mice in our breeding colony (data not shown). The amplicon of 2.5 kb was sequenced and the results indicate that the deleted region of 9.3 kb, containing the whole sequence of exon 3, was replaced by a 498-bp sequence in the *moja* allele. Interestingly, a sequence comparison of the insertion 498-bp sequences in the *moja* allele showed more than 98% identity to an early transposon (ETn) LTR. The insertional sequence of 498 bp and the junctional sequences of normal intron 2 and the 3' flanking sequence of *Fgf5* are shown in Fig. 5A. The results suggest that the genomic deletion in *Fgf5* in *moja* mice may involve a retrotransposon insertion. The gene symbol for the *moja* mutation was named $Fgf5^{go\text{-}Utr}$ in accordance with Guidelines for Nomenclature of Genes, Genetic Markers, Alleles, and Mutations in Mouse and Rat (http://www.informatics.jax.org/mgihome/nomen/gene.shtml).

Discussion

In this study, we examined spontaneous mutant mice with long pelage hair. Molecular analyses revealed that the *moja* mutation was caused by a genomic deletion of exon 3 in *Fgf5*. Hebert *et al*. [6] achieved targeted disruption of *Fgf5* ($Fgf5^{tm1Mrt}$) by replacing the first exon with the neomycin resistance gene, creating a nonfunctional allele of *Fgf5*. The homozygous $Fgf5^{tm1Mrt}$ mice showed noticeably longer hair than their heterozygous and wild-type littermates, and their coat appearance was long and shaggy. The gene-targeted *Fgf5* (–/–) mutants showed no obvious phenotypic abnormalities other than abnormal hair growth. The phenotype of homozygous $Fgf5^{go\text{-}Utr}$ mice is similar to that of homozygous $Fgf5^{tm1Mrt}$ mice.

Five *Fgf5* mutant alleles, $Fgf5^{tm1Mrt}$, $Fgf5^{tm1Tzi}$, $Fgf5^{m1Btlr}$, $Fgf5^{go}$, and $Fgf5^{go\text{-}Y}$, have been registered at the Mammalian Phenotype Ontology Annotations of Mouse Genome Informatics database (http://www.informatics.jax.org/). In addition to $Fgf5^{tm1Mrt}$, a targeted mutation using a cassette containing lacZ ($Fgf5^{tm1Tzi}$) was performed on the endogenous *Fgf5* gene in ES cells [13], and mutant mice homozygous for $Fgf5^{tm1Tzi}$ showed increased hair follicle activity. Furthermore, $Fgf5^{tm1Tzi}$ homozygotes were reported to have even longer hair in the *Fgf6*- and *Fgf7*-deficient genetic backgrounds [9]. $Fgf5^{m1Btlr}$ was produced through random germline mutagenesis with *N*-ethyl-*N*-nitrosourea (ENU) (http://mutagenetix.scripps.edu/home.cfm). This mutation is a G-to-T transversion at position 562 of the *Fgf5* transcript, which results in replacement of codon 112 for glutamic acid with a stop codon. The *porcupine* phenotype of abnormally long pelage hair was observed in G3 mice homozygous for $Fgf5^{m1Btlr}$. Similar to these induced mutant mice, $Fgf5^{tm1Mrt}$, $Fgf5^{tm1Tzi}$, and $Fgf5^{m1Btlr}$, abnormal long hair growth was also identified in spontaneous *Fgf5*-mutant mice. The autosomal recessive gene angora (*go*) was found in the BALB/c strain at the Jackson Laboratory [4]. A similar mutant gene associated with an abnormal long hair phenotype was also

identified in the C57BL/Lac strain at Stolbovaya Farm in Moscow [1]. The latter gene is known as angora-Y, *go-Y*, and was shown to be allelic with *go* [2]. Hebert *et al*. [6] also clearly demonstrated that the *go* phenotype is caused by deletion of most of exon 1 and a 2-kb sequence immediately upstream of the translational initiation site of *Fgf5*, resulting in a null allele, $Fgf5^{go}$. To our knowledge, however, the molecular basis of $Fgf5^{go\text{-}Y}$ has not yet been reported.

The autosomal recessive mutation $Fgf5^{go\text{-}Utr}$ presented in this study appeared spontaneously in outbred mice of our ICR colony. Sequences encoding exon 3 of *Fgf5* were deleted in $Fgf5^{go\text{-}Utr}$. The deletion extended over a total of 9.3 kb encompassing the whole region of exon 3 along with its 5' and 3' flanking regions. Interestingly, the deletion site in *Fgf5* showed the LTR of ETn, which is a mobile genetic element (Fig. 5B). It has been reported that approximately 10% of spontaneous mutations may be due to endogenous retroviral elements in laboratory mice [11, 14]. Recombination between the LTRs results in deletion of the internal region leaving only a single LTR, known as a solo LTR, in the host chromosome [3, 7]. The deletion of the 9.3-kb region of the genomic DNA containing exon 3 of *Fgf5* in $Fgf5^{go\text{-}Utr}$ mutants may have been caused by ETn insertion, although the exact relation between insertion of LTR and the deletion of the 9.3-kb region is unclear. Therefore, the $Fgf5^{go\text{-}Utr}$ allele is a novel mutation of *Fgf5* mediated by a retrotransposon.

Although previous studies have demonstrated that FGF5 is an important factor in regulation of the hair follicle cycle, it is not clear whether or not FGF5 is involved in human diseases. Recently, a genome-wide association study, using very large study samples of European ancestry from the Global Blood Pressure Genetics consortium, identified *FGF5* as being associated with blood pressure [10]. Moreover, two different genome-wide association studies indicated that blood pressure and hypertension are strongly associated with *FGF5* in Japanese [12] and Han Chinese populations [8]. These results suggest that *Fgf5*-deficient mouse strains, including $Fgf5^{go\text{-}Utr}/Fgf5^{go\text{-}Utr}$ mice, may be useful models for studying the relationship between *FGF5* and hypertension.

In conclusion, we successfully identified a novel mutation, $Fgf5^{go\text{-}Utr}$, in *moja* mice. The long pelage hair phenotype of these mice is due to the deletion of a 9.3-kb genomic sequence consisting of exon 3 of *Fgf5* and its flanking regions. The presence of a solo LTR at the deletion site suggests that the disruption of *Fgf5* may have been caused by an ETn insertion in these mice.

Acknowledgment

This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan (19300143).

References

1. Blandova, Z.K. 1967. *Mouse News Lett*. 36: 56–57.
2. Blandova, Z.K., Dushkin, V.A., Malashenko, A.M., and Shmidt, E.F. 1983. Laboratory Strains of Mice for Medical and Biological Research, Moscow, Nauka (in Russian).
3. Boeke, J. and Stoye, J.P. 1997. Retrotransposons, endogenous retrovirues, and the evolution of retroelements. pp. 345–435. *In*: Retrovirues (Coffin, J.M., Hughes, S.H., and Varmus, H.E. eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
4. Dickie, M.M. 1963. New mutations: angora. *Mouse News Lett*. 29: 39.
5. Hardy, M.H. 1993. The secret life of the hair follicle. *Trends Genet*. 8: 55–61.
6. Hebert, J.M., Rosenqist, T., Gotz, J., and Martin, G.R. 1994. FGF5 as regulator hair growth cycle: Evidence from targeted and spontaneous mutation. *Cell* 78: 1017–1025.
7. Katzourakis, A., Rambaut, A., and Pybus, G. 2005. The evolutionary dynamics of endogenous retroviruses. *Trends Miceobiol*. 13: 463–468.
8. Liu, C., Li, H., Qi, Q., Lu, L., Gan, W., Loos, R.J., and Lin, X. 2010. Common variants in ornear FGF5, CYP17A1 and MTHFR genes are associated with blood pressure and hypertension in Chinese Hans. *J. Hypertens*. (in press).
9. Neuhaus, P., Oustanina, S., Loch, T., Kruger, M., Bober, E., Dono, R., Zeller, R., and Braun, T. 2003. Reduced mobility of fibroblast growth factor (FGF)-deficient myoblasts might contribute to dystrophic changes in the musculature of FGF2/FGF6/mdx triple-mutant mice. *Mol. Cell. Biol*. 23: 6037–6048.
10. Newton-Cheh, C., Johnson, T., Gateva, V., Tobin, M.D., Bochud, M., Coin, L., Najjar, S.S., Zhao, J.H., Heath, S.C., Eyheramendy, S., Papadakis, K., Voight, B.F., Scott, L.J., Zhang, F., Farrall, M., Tanaka, T., Wallace, C., Chambers, J.C., Khaw, K.T., Nilsson, P., van der Harst, P., Polidoro, S., Grobbee, D.E., Onland-Moret, N.C., Bots, M.L., Wain, L.V., Elliott, K.S., Teumer, A., Luan, J., Lucas, G., Kuusisto, J., Burton, P.R., Hadley, D., McArdle, W.L., Wellcome Trust Case Control Consortium, Brown, M., Dominiczak, A., Newhouse, S.J., Samani, N.J., Webster, J., Zeggini, E.,

Beckmann, J.S., Bergmann, S., Lim, N., Song, K., Vollenweider, P., Waeber, G., Waterworth, D.M., Yuan, X., Groop, L., Orho-Melander, M., Allione, A., Di Gregorio, A., Guarrera, S., Panico, S., Ricceri, F., Romanazzi, V., Sacerdote, C., Vineis, P., Barroso, I., Sandhu, M.S., Luben, R.N., Crawford, G.J., Jousilahti, P., Perola, M., Boehnke, M., Bonnycastle, L.L., Collins, F.S., Jackson, A.U., Mohlke, K.L., Stringham, H.M., Valle, T.T., Willer, C.J., Bergman, R.N., Morken, M.A., Döring, A., Gieger, C., Illig, T., Meitinger, T., Org, E., Pfeufer, A., Wichmann, H.E., Kathiresan, S., Marrugat, J., O'Donnell, C.J., Schwartz, S.M., Siscovick, D.S., Subirana, I., Freimer, N.B., Hartikainen, A.L., McCarthy, M.I., O'Reilly, P.F., Peltonen, L., Pouta, A., de Jong, P.E., Snieder, H., van Gilst, W.H., Clarke, R., Goel, A., Hamsten, A., Peden, J.F., Seedorf, U., Syvänen, A.C., Tognoni, G., Lakatta, E.G., Sanna, S., Scheet, P., Schlessinger, D., Scuteri, A., Dörr, M., Ernst, F., Felix, S.B., Homuth, G., Lorbeer, R., Reffelmann, T., Rettig, R., Völker, U., Galan, P., Gut, I.G., Hercberg, S., Lathrop, G.M., Zelenika, D., Deloukas, P., Soranzo, N., Williams, F.M., Zhai, G., Salomaa, V., Laakso, M., Elosua, R., Forouhi, N.G., Völzke, H., Uiterwaal, C.S., van der Schouw, Y.T., Numans, M.E., Matullo, G., Navis, G., Berglund, G., Bingham, S.A., Kooner, J.S., Connell, J.M., Bandinelli, S., Ferrucci, L., Watkins, H., Spector, T.D., Tuomilehto, J., Altshuler, D., Strachan, D.P., Laan, M., Meneton, P., Wareham, N.J., Uda, M., Jarvelin, M.R., Mooser, V., Melander, O., Loos, R.J., Elliott, P., Abecasis, G.R., Caulfield, M., and Munroe, P.B. 2009. Genome-wide association study identifies eight loci associated with blood pressure. *Nat. Genet*. 41: 666–676.

11. Takabatake, T., Ishihara, H., Ohmachi, Y., Tanaka, I., Nakamura, M.M., Fujikawa, K., Hirouchi, T., Kakinuma, S., Shimada, Y., Oghiso, Y., and Tanaka, K. 2008. Microarray-based global mapping of integration sites for the retrotransposon, intracisternal A-particle, in the mouse genome. *Nucleic Acids Res*. 36: e59
12. Takeuchi, F., Isono, M., Katsuya, T., Yamamoto, K., Yokota, M., Sugiyama, T., Nabika, T., Fujioka, A., Ohnaka, K., Asano, H., Yamori, Y., Yamaguchi, S., Kobayashi, S., Takayanagi, R., Ogihara, T., and Kato, N. 2010. Blood pressure and hypertension are associated with 7 loci in the Japanese population. *Circulation* 121: 2302–2309.
13. Tzimagiorgis, G., Michaelidis, T.M., Lindholm, D., and Thoenen, H. 1996. Introducyion of negative selection marker into replacement vectors by a single ligation step. *Nucleic Acids Res*. 24: 3476–3477.
14. Zhang, Y., Maksakova, I.A., Gagnier, L., van de Lagmaat, L.N., and Mager, D.L. 2008. Genome-wide assessments reveal extremely high levels of polymorphism of two active families of mouse endogenous retroviral element. *PloS Genet*. 4: e1000007.