Sparse and Wavy Hair: A New Model for Hypoplasia of Hair Follicle and Mammary Glands on Rat Chromosome 17


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Abstract
Mutant animals in the skin and hair have been used to identify important genes in biomedical research. We describe a new mutant rat, sparse and wavy hair (swh), that spontaneously arose in a colony of inbred WTC rats. The mutant phenotype was characterized by sparse and wavy hair, which was most prominent at age 3–4 weeks, and was inherited in an autosomal recessive manner. The swh/swh rats showed impaired gain of body weight, and their hair follicles were reduced both in number and size, associated with hypoplasia of the sebaceous glands and the subcutaneous fat tissue. Female swh/swh rats were unable to suckle their offspring. Their mammary glands were hypoplastic, and differentiation of mammary epithelial and myoepithelial cells was impaired. Linkage analysis of 579 backcross rats localized the swh locus to a 0.35-cM region between D17Rat131 and D17Rat50 in the distal end of rat Chr 17. The swh locus spanned the 3.7-Mb genomic region where 24 genes have been mapped and corresponded to the centromere region of the mouse Chr 2 or the region of the human Chr 10p11.1–p14. None of the genes or loci described in mouse or human hair and skin diseases mapped to these regions. These findings suggest that the rat swh is a novel mutation associated with impaired development of the skin appendages, such as hair follicles, sebaceous glands, and mammary glands, and will provide an experimental model to clarify a gene and mechanisms for development of skin appendages.

Introduction
Mutations involving the hair abnormalities have provided valuable models to understand not only hair development but also other important mechanisms for tissue differentiation and growth regulation (Ahmad et al. 1998; Cachon-Gonzalez et al. 1994; Nakamura et al. 2001; Sundberg 1994). For example, major manifestation of gene disruption of transforming growth factor alpha (Tgfa) was waviness of the whiskers and fur, which had been known as waved-1 mutation (Luetteke et al. 1993; Mann et al. 1993). A spontaneous mutation, waved-2, which mimicked waved-1, turned out to be a mutation of epidermal growth factor receptor (Egfr) (Luetteke et al. 1994). Although recent technologies of gene targeting and random mutagenesis are producing many mutations involving the hair, detailed analysis of a mutant with an interesting phenotype is necessary to identify another gene involved in important biological processes.

Some mutations involving the hair are accompanied by defects of the skin and its appendages, such as nails, teeth, sebaceous glands, and mammary glands (Pispa and Thesleff 2003). These organs develop from the skin under close interactions between the epithelium and the mesenchyme. The hair follicles are formed during embryogenesis owing to a series of signals between dermal cells and overlying surface epithelial cells (Hardy 1992). The mammary glands also develop under sequential reciprocal interactions between the epithelium and adjacent mesenchyme (Veltmaat et al. 2003). Both embryonic mammary gland development and hair follicle development require the expression of the transcriptional mediator of the Wnt pathway, Lef-1 (van Genderen et al. 1994).


Therefore, it has been suggested that development of these two closely related organs could share more signalling molecules.

We describe a new mutation causing abnormal hair texture and mammary gland hypoplasia, which spontaneously occurred in the WTC inbred rat strain colony at the National Cancer Center Research Institute in 1998. The mutation, sparse and wavy hair (swh), is inherited in an autosomal recessive manner and was mapped by linkage study of 579 backcross rats.

### Materials and Methods

#### Rats

WTC-swh was maintained by brother–sister mating of swh/+ heterozygous female and swh/swh homozygous male. Two to three rats were housed in a cage, and were fed CE-2 food pellets (CLEA Japan, Tokyo) ad libitum. For body weight analysis, 12 swh/swh rats (5 males and 7 females) and 7 swh/+ animals (7 males and 7 females) were weighed every 8 days. For linkage analysis, ACI/Njcl females (CLEA Japan) were mated to swh/+ males. F1 hybrid females from this initial cross were then backcrossed to swh/+ males to produce the N2 progeny. A total of 579 N2 progeny was produced in this study.

#### Histopathology

For general histopathological examination, tissues were prepared from swh/swh and swh/+ animals at 30 weeks of age. Skins were prepared from head, dorsal, and ventral areas of the animals. Samples from other organs that including brain, spinal cord, eye, liver, kidney, spleen, pancreas, esophagus, stomach, small intestine, colon, ecem, heart, lung, thymus, lymph node, pituitary gland, thyroid, adrenal gland, testis, prostate, seminal vesicle, ovary, and uterus. Mammary glands during lactation were excised from two swh/swh and two swh/+ females on the day of their delivery. Sections from all tissues were embedded in paraffin, sliced to 3 μm, and stained with hematoxylin and eosin (H&E). Brain and spinal cord sections also were stained with Luxol fast blue MBS.

#### Immunohistochemistry

Expression of α-smooth muscle actin (α-SMA) (Di Tommaso et al. 2003) in the mammary gland during lactation was immunohistochemically demonstrated using avidin-biotin complex method. Sections in sodium citrate buffer (pH 6.0) were treated with microwave and then were treated with 3% hydrogen peroxidase, normal horse serum, anti-α-SMA antibody (M0851, Dako, Glostrup, Denmark) at 1:50 dilution, followed by ABC-peroxidase procedures (ABC kit, Vector Laboratories, Burlingame, CA). Immunocomplexes were visualized with 3,3′-diaminobenzidine tetrahydrochloride as a chromogen. As a negative control, normal serum was used instead of primary antibodies. The sections were counterstained with Mayer’s hematoxylin to facilitate examination under a light microscope.

#### DNA Extraction

Tail biopsies were digested with lysis buffer (100 mM Tris-HCl [pH 7.5], 12.5 mM EDTA, 150 mM NaCl, 1% sodium dodecyl sulfate [SDS], .8 mg/ml proteinase K) at 50°C overnight. Genomic DNA was extracted by using the automatic DNA purification system GENEXTRACTOR TA-100 (Takara, Kyoto, Japan).

#### Genotype Analysis

Forty-four SSLP markers showing size differences between ACI/N and WTC/Kyo were selected for genome scan and the subsequent haplotype analysis (http://waldo.wi.mit.edu/rat/public). For fine mapping of the swh, primer pairs were designed to amplify the dinucleotide repeats within the telomeric region of the rat chromosome 17 (Table 1). Polymerase chain reaction (PCR) analyses were carried out in 15 μl total volume reactions containing 20 ng genomic DNA as described previously (Serikawa et al. 1992). Fifty arbitrarily-primed representational difference analysis (APRDA) markers were also used for genome scan. Genotyping of AP-RDA markers were performed by previously described methods (Yamashita et al. 2000).

#### Linkage Analysis

Initial genome scan was carried out with the random selected 48 backcross progeny. Gene order and recombination frequencies were calculated with the Map Manager computer program (Manly 1993). Gene order of loci around swh was determined by haplotype analysis of 579 backcross rats.

### Table 1. Primers for fine mapping of swh

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</table>

Results

Skin and Hair Phenotypes of the WTC-swh Mutant Rat

Homozygous swh/swh mutants were easily distinguishable from their normal siblings by their sparse fur and small body size at 14 days postnatal development. Sparse fur of swh/swh mutants was most prominent at 3–4 weeks of age (Figure 1A). With increasing age, sparse fur became milder in swh/swh rats, but the hair around their eyelids and tails remained to be defective. Homozygous swh/swh rats showed impaired gain of body weight after the age of 2 weeks (Figure 1B). Both male and female of swh/swh rats were fertile. However, swh/swh females failed to suckle her offspring throughout the period of lactation, and most of their offspring died before weaning. We therefore bred swh/swh rats by brother–sister mating of swh/+ female and swh/swh male rats.

Histopathological examination of the skin revealed the decrease of the hair follicles in both number and size and the reduction of the subcutaneous fatty tissues in swh/swh rats, compared with swh/+ rats (Figure 1C and 1D). Collagen fibers were dominant in the dermis, filling the spaces among the immature hair follicles and sebaceous glands. Keratinization of the epidermis was stronger. These abnormalities of the skin were most predominantly observed in ventral area rather than head or dorsal areas. No organs, except for the skin and mammary glands in lactating period (see later discussion), showed histological differences between swh/swh and swh/+ rats. Fat tissues in the abdominal cavity were normal.

Figure 1. External features and body weights of swh/swh and swh/+ rats. (A) External features of swh/swh (left) and swh/+ (right) rats at 4 weeks of age. Sparse and wavy hair can be seen in the swh/swh mutant rat. (B) Body weights of swh/swh and swh/+ rats. Twelve swh/swh rats (5 males, 7 females) and 14 swh/+ rats (7 males, 7 females) were used. Each value is presented as mean ± SD. Significant differences (P < .01; Student t-test) were found between swh/swh and swh/+ rats in both sexes throughout the experimental period. (C, D) Histology of the ventral skin of swh/swh and swh/+ rats. Normal hair development was found in the swh/+ rat (C), and atrophies of hair follicles, sebaceous gland, and subcutaneous fat were prominent in the swh/swh rat (D). In the swh/swh rat, the high density of the collagen fibers in the dermis and the remarkable keratinization of the epidermis were also observed.

Impaired Differentiation of Mammary Epithelial and Myoepithelial Cells

At postparturition, mammary glands of the swh/+ rats demonstrated that alveolar duct epithelium proliferated to form numerous secretory alveoli replacing interlobular connective tissue and intralobular adipose tissue (Figure 2A). In contrast, the alveoli growth of the swh/swh rats was still poor with evidently remaining adipose tissue (Figure 2B). On the other hand, in periods other than the postparturition period, the difference was unclear possibly because the mammary glands are physiologically hypoplastic.
In the 
\(swh/\) rats, the expression of \(\alpha\)-SMA, which is known to be specifically expressed in smooth muscles (Skalli et al. 1986), was detected only in the myoepithelial cells, not in the alveolar epithelial cells (Figure 2C). In contrast, in the \(swh/\) rats, \(\alpha\)-SMA was strongly detected in the alveolar epithelial cells, as well as myoepithelial cells (Figure 2D). This indicated that differentiation of stem cells into mammary epithelial cells was impaired in the \(swh/\) rats.

**Genetic Analysis**

\(S\) mutation was inherited in a recessive manner as proved by the traditional intercross and backcross analyses. Data from inheritance tests using intercross progeny showed that ratio of affected to normal animals was not different from the expected ratio (1:3) based on autosomal recessive inheritance (12:30, chi square = .14, \(P = .71\)). Data from all pooled inheritance and linkage tests using backcross progeny also showed no significant difference between observed and expected ratio (1:1) based on autosomal recessive inheritance (298:334, chi square = 1.03, \(P = .31\)).

To map \(swh\), 579 backcross progeny were produced from the \((ACI \times WTC-\) \(swh\)\()\(F_1 \times WTC-\) \(swh\) cross. A genome scan was performed by typing DNA samples from 48 randomly selected animals for 3 visible coat colors, 50 AP-RDA, and 35 microsatellite markers. Linkage of \(swh\) was first detected on distal Chr 17 with \(D17Rat51\). DNA samples of the 48 progeny were then typed for the additional Chr 17 markers, including \(D17Kyo3-Gad2\) and 10 microsatellite markers. \(S\) showed significant linkage with all of the markers and was localized to the region between \(D17Rat\ 62\) and \(D17Rat140\) (Figure 3).

**Figure 2.** Mammary glands at postparturition. H&E staining of representative mammary glands in \(swh/+\) (A) and \(swh/swh\) (B) rats (bar, 150 \(\mu m\)). In \(swh/swh\) mutant rats, poor growth of the alveoli was clear, and the adipose tissue filled the space among the imperfect lactating mammary glands. Immunohistochemical staining with the smooth muscle marker, \(\alpha\)-SMA, revealed that mammary alveolar epithelial cells were still positive for \(\alpha\)-SMA in \(swh/swh\) rats (D) while not in \(swh/+\) rats (C) (bar, 50 \(\mu m\)).

**Figure 3.** Genetic mapping of \(swh\). Right, distribution of haplotypes observed among 28 backcross progeny carrying recombinant chromosome between \(D17Rat62\) and \(D17Rat140\). Black boxes, homozygote for the WTC-\(swh\) allele. White boxes, heterozygote for the ACI allele. Left, the high-resolution map obtained by using 579 progeny. \(S\) was narrowed down to the 0.34-cM interval between \(D17Rat131\) and \(D17Rat50\), and showed no recombination with \(D17Rat51\), \(D17rat65\), \(D17Kyo1\), \(D17Kyo2\), \(D17Kyo3\), \(D17Kyo4\), and \(D17Kyo5\) in 579 informative meioses. Distances are given in centimorgans (cM) to the left of the map.
To obtain a high-resolution genetic map around \textit{swb}, all the 579 backcross progeny were genotyped for the markers located between \textit{D17Rat131} and \textit{D17Rat50}. \textit{Swb} showed no recombination with \textit{D17Rat13}, \textit{D17Rat65}, \textit{D17Kyo1}, \textit{D17Kyo2}, \textit{D17Kyo3}, \textit{D17Kyo4}, and \textit{D17Kyo5} in 579 informative meioses (Figure 3).

### Table 2.

List of genes located in the \textit{swb} locus and their mouse or human orthologs

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Physical Position of the swh Locus

Physical positions of the SSLP markers around the swh locus were determined by BLAST search program (www.ncbi.nlm.nih.gov/genome/seq/rnblast.html) against the rat genome sequences. D17Rat131, which defined the proximal side of the swh locus, was located at the position of 93.4 Mb (Table 2). On the other hand, D17Rat50 and D17Rat140, which defined the distal side, did not have significant homologies to the published rat genome sequences. However, the telomere of the rat Chr 17 was mapped to the position of the 97.1-Mb of the rat genome sequence, and the swh locus was concluded to be located in a 3.7-Mb region between D17Rat131 and telomere. This region contained 24 genes and ESTs.

HomoloGene database at the NCBI revealed that the swh critical region corresponded to centromeric region of the mouse Chr 2 or p11.1–p14 of the human Chr 10 (Table 2).

Discussion

A unique mutation, swh, was identified. Swh/swh mutant rats displayed hypoplasia of the hair follicles, and swh/swh female rats at a postparturition period displayed hypoplasia of the mammary glands. The hypoplasia of hair follicles was associated with hypoplasia of the sebaceous glands and subcutaneous fat tissue. The hypoplasia of the mammary glands was associated with incomplete differentiation of the mammary epithelial cells, which was associated with positive α-SMA staining. All the abnormalities were observed in the skin-derived tissues, and were caused by a single mutation on Chr 17. These data indicated that the swh mutation involved a gene that is critical in differentiation from the skin to its appendages and suggested that a fundamental developmental pathway, such as Wnt and sonic hedgehog signaling pathways, could be involved (St-Jacques et al. 1998; van Genderen et al. 1994). The most prominent phenotype of the swh/swh mutants, abnormal hair texture, was considered to be due to hypoplasia of hair follicles, as observed in other hair texture mutants (Trigg 1972). Pelage hair follicles are formed between the 13th day of embryogenesis and the first few days after birth, and no more hair follicles are newly formed thereafter (Millar 2002; Trigg 1972). Therefore it can be speculated that the swh gene is functional in the embryo and that its mutation causes the decrease in the number of hair follicles. The sebaceous glands differentiate from the upper part of the outer root sheath of the hair follicle (Lavker et al. 1999), and their hypoplasia could be caused by hypoplasia of the hair follicles. Subcutaneous fat tissue, whose formation needs interaction with hair follicles (Hausman et al. 1981), was also markedly hypoplasic, whereas fat tissues in the abdominal cavity were not affected. This indicated that hypoplasia of the sebaceous glands and subcutaneous fat tissues resulted from that of the hair follicles.

Fine linkage mapping of swh using 571 backcross rats and analysis of the draft rat genome sequence localized that the swh-critical region to a 3.7-Mb genomic region of the rat Chr 7 telomere. Comparative mapping showed that no genes involved in mouse or human hair and skin diseases were mapped to this region. In the swh-critical region, 24 genes (6 known genes and 18 putative genes) have been mapped, and Shb3lp1 and LOC291345 were especially intriguing. Shb3lp1 is involved in EGFR signaling (Scita et al. 1999), and it has been shown that a point mutation of the Egfr gene causes the abnormal hair texture and impaired maternal lactation in the waved-2 mouse (Fowler et al. 1995; Luetteke et al. 1994). The similarity of phenotypes between the swh/swh rats and the waved-2 mouse suggested Shb3lp1 as a good candidate for the swh gene. A putative gene (LOC291345) shows similarity to MAP/microtubule affinity-regulating kinase 4 (MARK4). Human MARK4 is expressed downstream in the Wnt signaling pathway under the regulation of Tcf/LEF1 (Kato et al. 2001). Considering the important role of the Wnt signaling pathway in the hair follicle development, LOC291345 was also considered as a good candidate. Online gene expression data was available for 14 of the 24 genes. Among them, 3 genes, LOC291353 (similar to 40S ribosomal protein S8), LOC207171 (similar to Aphp1ip), and Shb3lp1, were expressed in 13-dpc embryo, in which pelage hair follicles begin to form. Therefore, these three genes were also considered as candidates.

In summary, a single mutation on Chr 17 was shown to cause abnormalities in hair follicle formation and mammary epithelial differentiation, and a list of 24 candidate genes was produced. Future cloning of the responsible gene will contribute to clarification of the mechanisms involved in the development of skin appendages.

Acknowledgments

This work was supported by the grants-in-aid for scientific research from the Japan Society for the Promotion of Science and for the 2nd and 3rd-term Cancer Control Strategy from the Ministry of Health, Labor and Welfare. We are thankful to Ms. M. Yokoe for her excellent technical assistance. WTC-swh rat strain will be deposited in the National Bio Resource Project-Rat (www.anim.med.kyoto-u.ac.jp/rbtr) and can be distributed through the project.

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Received July 29, 2004
Accepted January 5, 2005

Corresponding Editor: Roger Reeves