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The Charles River "Hairless" Rat Mutation is Distinct from the *Hairless* Mouse Alleles

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The Charles River (CR) "hairless" rat is one of the autosomal recessive hypotrichotic animal models actively studied in pharmacologic and dermatologic research. Despite its widespread use, the molecular basis of this monogenic mutation remains unknown, and the skin histologic features of this phenotype have never been described. However, the designation "hairless" has been used as an extension of the hairless mouse (*hr*) nomenclature on the basis of the clinical absence of hairs in both phenotypes. We present a description of the histopathologic changes in heterozygous and homozygous CR hairless rat mutants during the first month of life. The postnatal homozygous rat skin was characterized by abnormal keratinization of the hair shaft and formation of a thick and dense layer of corneocytes in the lower portion of the epidermal stratum corneum. This layer prevented the improperly keratinized hair shaft from penetrating the skin surface. Starting from the latest stages of hair follicle (HF) development, obvious signs of HF degeneration were observed in homozygous skin. This process was extremely rapid, and by day 12, mainly atrophic HFs with abnormal or broken hairs were present in the skin. Therefore, the mutation in the CR rat abrogates cell proliferation in the hair matrix and affects keratinocyte differentiation in the HF and interfollicular epidermis, a phenotype that is completely distinct from *hr/hr*. To test whether the CR rat harbored a mutation in the *hr* gene, we analyzed the coding region of this gene and consensus intron splice site sequences in mutant rats and found no mutation, further supporting phenotypic evidence that the hairless phenotype in CR rats is not allelic with *hairless*. Finally, using intragenic polymorphisms, we were able to exclude homozygosity at the hairless locus by use of genotypic analysis. Thus, morphologic analysis of successive stages of phenotype development in the CR hairless rat, together with definitive molecular studies, indicate that this mutation may be unique among the other hypotrichotic rat mutations.

Laboratory rat models that lack a normal coat offer a distinct advantage in studies of percutaneous drug absorption (1, 2), wound healing (3), and skin pharmacology and photobiology (4). The Charles River (CR) hairless rat is one of the most widely used among these models. This outbred rat line is commercially available (Charles River Co, Wilmington, Mass.) and is actively used in experimental dermatologic and skin pharmacologic studies. At the same time, the pathomorphology of this phenotype has not been described in literature, and the gene underlying this mutation remains unknown. This clearly impedes more extensive use of this hypotrichotic rat model in experimental dermatologic studies and in particular, in studies of hair follicle biology.

In addition to the CR "hairless" rat, several mutations are known that demonstrate hypotrichosis in rats. These include naked (*n*) (5), fuzzy (*fz*) (6), "hairless" (*hr*) (7), Rowett nude (*rnu*) (8), shorn (*shn*) (9), and "hairless" Wistar (*hW*) (10) among others. With the exception of the nude mutation (11), the molecular basis for these hypotrichotic rat mutations is still unknown, thus markedly limiting the use of these models in dermatologic studies. Some of the hypotrichotic rat mutants have a similar macroscopic appearance, yet only a few have been studied histologically, and the possible relationships between these mutations are still unclear. It is likely that some of them are allelic or even identical mutations with only minor differences in phenotype that are probably determined by differences in genetic background.

Recently, it was reported that different allelic mutations in the mouse hairless (*hr*) gene result in complete hair loss in mice, and consequent development of a highly specific hairless phenotype (12, 13). The macroscopic appearance and skin histologic features of mouse *hr* alleles are different to some extent depending on genetic background and type of underlying mutation. Nevertheless, all of these mouse mutants are characterized by extensive hair shedding after entry into first HF catagen regression due to complete loss of HF integrity (14). Despite the fact that the rat hairless gene has been cloned and characterized (15), no mutation in this gene has been reported in a rat model as yet. The data presented by Roberts and co-workers (7), and David (16), contain a detailed description of hypotrichotic rats with a phenotype similar if not identical to that of hairless mice, and strongly support the possibility that hairless gene mutations can underlie hypotrichosis not only in humans (17), nonhuman primates (18), and mice (19-21), but also in rats. Unfortunately, in the 50 years since the initial description, further information has not come to light on the rats described by David (16), thus suggesting the possible extinction of this mutation.

The gross appearance of CR hairless rats is similar to the appearance of adult hairless (*hr/hr*) mice and to that of the hairless rats described by David (16). This may be one possible reason why the CR hairless rat mutation is designated in literature as *hr/hr* and is assumed to be allelic to *hairless* (9). The identification of hairless gene mutations in a new rodent genus with larger hair follicles than those in mice would provide an attractive model for further studies of hairless gene biology and the pathogenesis of hairlessness. Therefore, we sought to test the possible involvement of the hairless gene in the development of hairlessness in CR mutant rats.

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We studied skin morphologic features of mutant CR rats during the first month of postnatal life, including HF morphogenesis, and the first catagen regression. The major defect in CR hairless rat skin is abnormal keratinization of the hair shaft, but not the follicular disintegration and dermal cyst formation observed in the hairless mouse phenotype. This basic defect of CR rat skin is the reason for the failure of normal hair shaft formation as early as postnatal HF morphogenesis. Thus, CR mutant rats are hypotrichotic from birth, whereas in contrast, in *hr*/*hr* mice, the first postnatal hair pelage is apparently normal. Furthermore, to explore the possible association of CR "hairless" rat phenotype with a hairless gene mutation, we performed direct sequencing of all exons and splice sites of the hairless gene in the CR rats. Unexpectedly, the obtained morphologic and molecular data disprove the long-standing assumption that the CR "hairless" rat is an allele of hairless. Thus, CR hypotrichotic rats represent a unique and potentially valuable model for the study of HF biology.

Materials and Methods

Animals and sample collection: A breeding stock of CR hairless rats (Crl:CD(SD)-*hr*BR), including homozygous and heterozygous mutant animals, as well as wild-type CR rats was obtained from Charles River Co (Wilmington, Mass.; www.criver.com). Rats were housed in community cages under standard conditions (12 h light periods, water and rat chow available ad libitum), following all relevant guidelines for laboratory animal care, and were free of cutaneous lesions. The studies were approved by the animal care and use committee of Institute of Comparative Medicine (Columbia University).

Gross observations and weighing of pups (15 litters in total) were performed daily to document general development and skin and hair condition. The adult animals and pups at days 1 to 21 of postnatal development were euthanized by use of CO_2 asphyxiation. Skin specimens were harvested from the cranial region of the dorsum and fixed in buffered 4% paraformaldehyde. Specimens were sectioned (4μm) and processed for H&E staining. Freshly obtained blood samples were supplemented with EDTA solution and processed for genomic DNA isolation, using the Puregene DNA isolation Kit (Gentra System, Minneapolis, Minn.) according to the manufacturer's protocol.

Polymerase chain reaction and DNA sequencing: Genomic DNA was isolated from 4 homozygous mutant CR female rats. Polymerase chain reaction (PCR) amplification of DNA fragments encompassing exon sequences of the rat hairless gene (GenBank accession No. U71293) was carried out, using 50 ng of each (forward and reverse) intron primer, 1X reaction buffer, 0.2 m*M* dNTPs, and 1.25 U of Platinum *Taq* DNA polymerase (Gibco BRL, Gaithersburg, Md.) were used in each 30-μl reaction to amplify 100 ng of genomic DNA. The PCR analysis was carried out for 35 cycles: 95°C for 1 min, annealing temperature between 58 and 66°C for 1 min, and 72°C for 1 min, in an OmniGene Thermal Cycler (Marsh Scientific, Rochester, N.Y.). The PCR products were electrophoresed in a 1 to 2% agarose gel in 1XTBE (Tris-Borate-EDTA) buffer, then eluted from the agarose gel by use of the QIAquick gel extraction kit (Qiagen Inc., Santa Clarita, Calif.). After elution, PCR products were purified, using the High Pure PCR product purification kit (Boehringer, Mannheim, Germany), and were sequenced directly, using the ABI Prism Rhodamine Terminator Cycle Sequencing Ready Reaction Sequencing Kit and the ABI Model 310 DNA Sequencer (PE Applied Biosystems, Foster City, Calif.). The intron primers were placed 60 to 110 base pairs into the introns flanking the splice junctions. Thus, we analyzed the entire coding region of the hairless gene, consensus splice site sequences, and part of the 5' UTR, 370 base pairs upstream from the translation initiation codon.

Results

Gross appearance of CR hairless rats: The heterozygous CR hairless rats were indistinguishable from wild-type age-matched animals at all stages of development. The homozygous mutants could be distinguished from phenotypically normal heterozygous siblings at birth by complete absence of vibrissae and by the appearance of shiny skin. At postnatal days 3 to 4, the difference became more prominent and the mutant skin appeared glossy, with more prominent dermal ridges, whereas the skin of heterozygous littermates was smooth, matte, and pale pink (Fig. 1A). At that age, the heterozygous pups had dense vibrissae up to 0.5 cm long, and the first pelage hairs had penetrated the skin surface on the back. Homozygous mutants remained completely naked, with a few curved, thin vibrissae not longer than 0.2 cm. At 12 days of age, whereas heterozygous littermates developed normal hair pelage, homozygous mutants remained nearly bare with some sparse, curved, short hairs that were rather thick and overall resembled bristle (Fig. 1B). At 3 weeks, homozygous animals developed sparse and abnormal hair cover that was spread over the body unevenly, mostly on the hind limbs, the ventral aspect, on the head, around the eyes, and on the caudal region of the dorsum (Fig. 1C). This hair cover became increasingly sparse with age, especially in fe-

Figure 1. Heterozygous and homozygous Charles River (CR) rat pups at various stages of postnatal development. (A) Postpartum day 2. The skin of a homozygous pup (above) is shiny and dry, compared with pink, pale skin of heterozygous animal. (B) Postpartum day 12. Whereas the heterozygous pup has a normal hair cover, its homozygous littermate remains naked. Notice growth retardation in the homozygous animal. (C) 25-day-old siblings. The homozygous rat developed some sparse, short hairs, but remained hairless in general appearance.

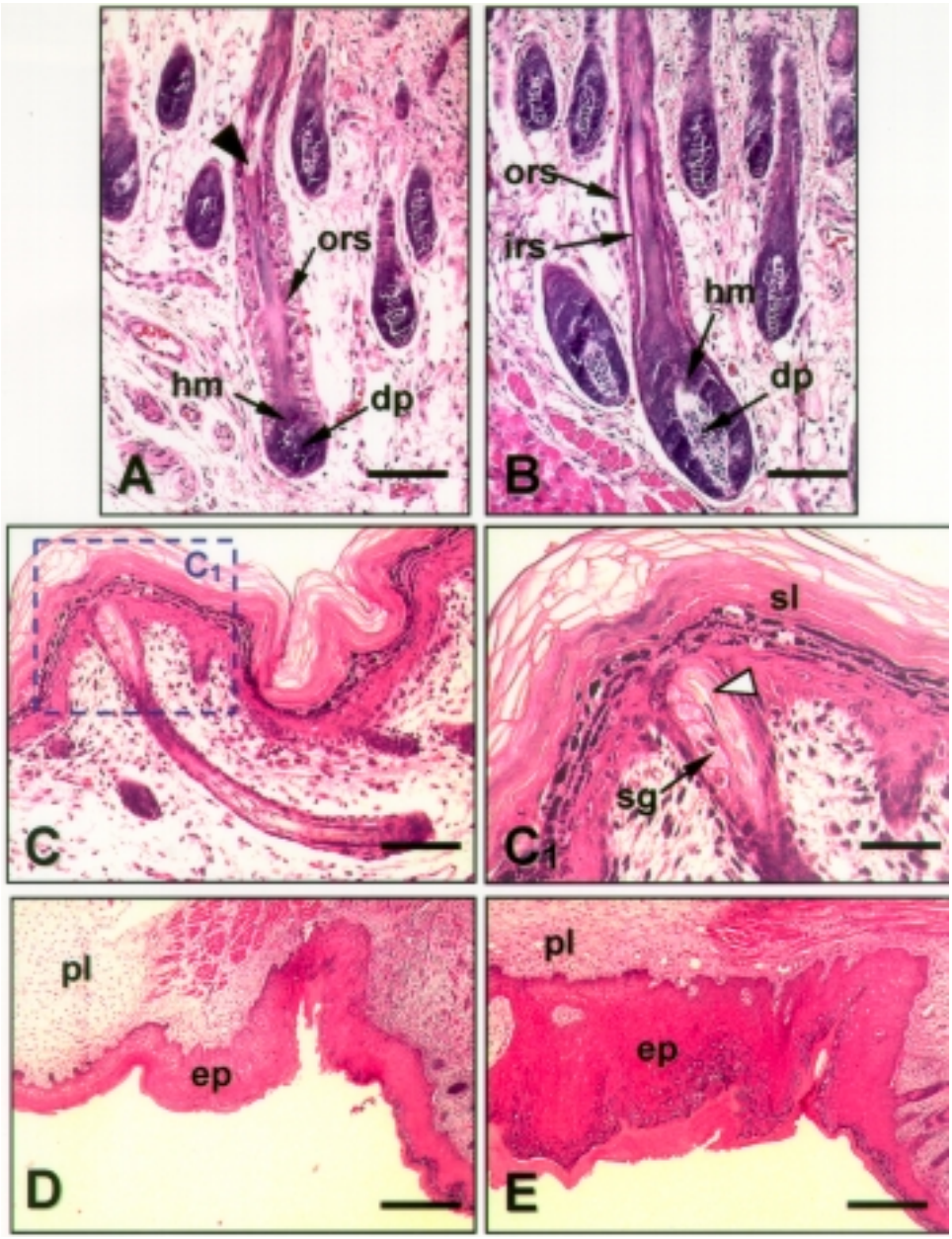

Figure 2. Photomicrographs of sections of skin from 4-day-old (heterozygous) and (homozygous) CR rat pups. (A) Homozygous (-/-) skin has prominent signs of atrophy. The hair matrix (hm) and dermal papilla (dp) has no normal structure and are underdeveloped. The outer root sheath (ORS) is thickened and vacuolated, and the normal inner root sheath (IRS) is not visible. (B) Normal hair follicles in heterozygous skin. (C) The skin of a homozygous animal. Notice the thickened portion of stratum corneum. (C_1) Close up of framed area in C. The hair shaft is abnormal with irregular shape. The tip of the hair shaft is unable to penetrate the granular, abnormally thick, compact cornified layer and is coiled at the level of the sebaceous gland (sg). (D) Palate (pl) histologic features in heterozygous and homozygous (E) 2-day-old pups. The palatine epithelium (ep) in homozygous animals is hyperplastic with a thickened granular layer, in comparison with that in heterozygous animals. H&E stain; bars: A and B, 80μm; C, 60μm; C_1, 30μm; D and E, 140μm.

males, which appeared completely naked at the age of 3 months. Males retained some curved and sparse hairs mainly, on the head and the caudal region of the dorsum.

Starting from the second week of postnatal life, yellowish pigmented spots appeared on the dorsal skin of homozygous mutants. This pattern later disappeared in females, but persisted in males. Thus, adult males had a yellow, greasy appearance of the skin, whereas adult female back skin remained smooth and pink.

Crosses of heterozygous females with homozygous mutant males during the study period resulted in 15 litters (147 pups in total). Only 39% of offspring were homozygous, thus suggesting reduced prenatal viability of homozygous mutants.

At birth, the difference in weight between homozygous and heterozygous littermates was insignificant. Interestingly, however, after the first week of postnatal life, homozygous pups underwent a brief arrest in weight gain, and during days 15 to 18, they weighed approximately half the weight of their normal littermates. After this short period of growth retardation, body weight gradually increased again, but homozygous animals remained approximately 10% smaller than heterozygous age-matched animals. Homozygous males and females were fertile; however, homozygous hairless females did not nurse or raise their pups well.

Skin histologic features: At birth (postpartum day 1), histologic examination of the skin revealed no prominent differences between homozygous and heterozygous littermates in morphology of the developing HFs; however, a difference was observed in stratum corneum structure. In contrast to heterozygous mouse skin, the lowermost corneocytes in homozygous skin were densely compacted, with no space between them, thus forming a tightly packed corneocyte layer. We also noted that the vibrissae follicles contained thinner, curved hair shafts in homozygous mutants, compared with normal heterozygous animals.

By day 4, HFs in homozygous skin showed prominent signs of atrophy. The hair matrix and dermal papilla did not have normal structure and were underdeveloped. As a result, the total hair bulb volume was significantly lower, compared with that of heterozygous HFs (Figs. 2A and 2B). Although the HF outer root sheath (ORS) remained intact or even hypertrophic in many HFs, the inner root sheath (IRS) and, often, the hair shaft started to disintegrate, mainly just above the hair matrix zone, thus leaving the hair canal empty. The hair shaft appeared thinner and improperly keratinized, especially in the upper portion (Fig. 2A, arrowhead). The difference in the

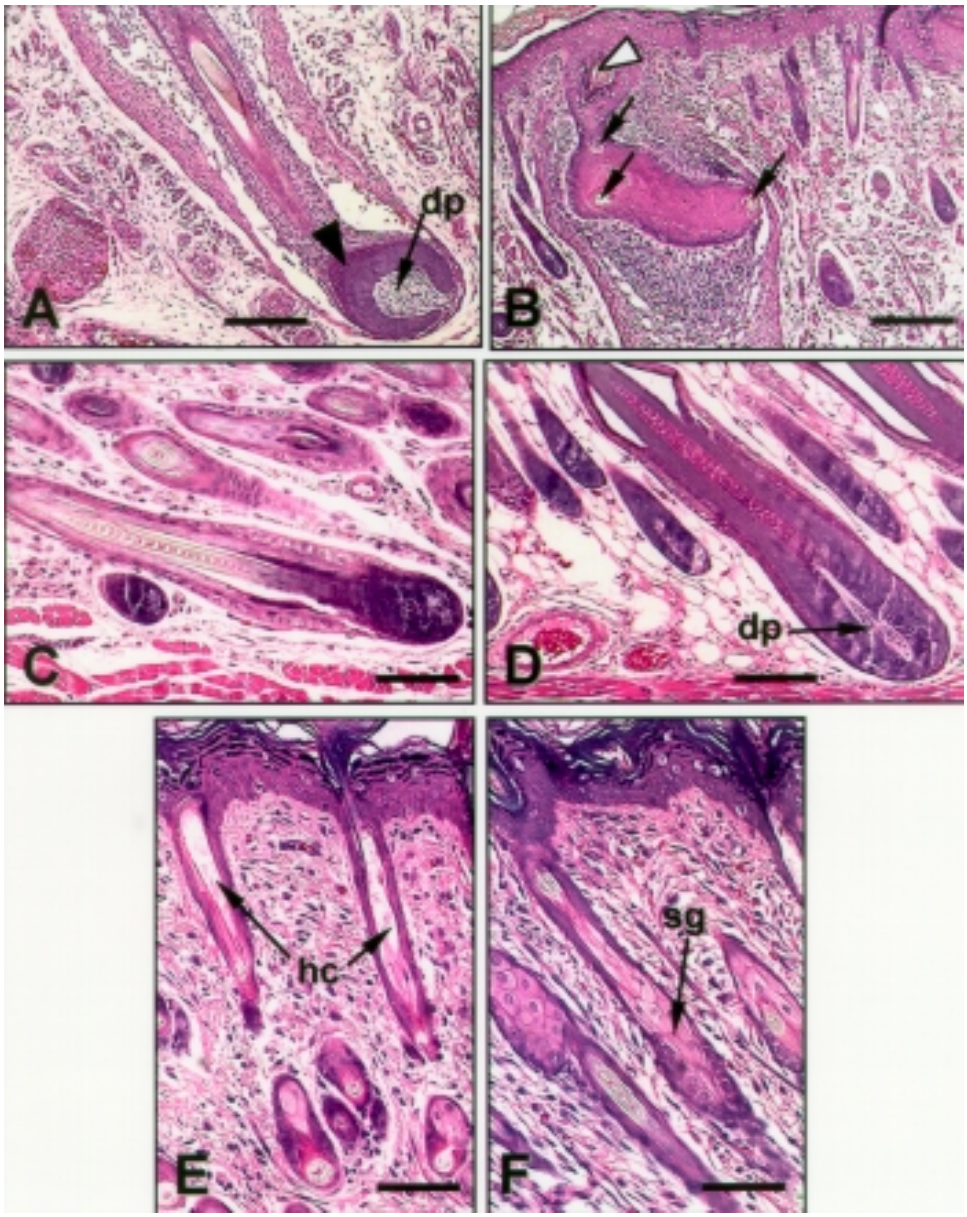

Figure 3. Photomicrographs of sections of skin from 12-day-old heterozygous (+/-) and homozygous (-/-) CR rat pups (H&E). (A) In the lower portion of homozygous vibrissa follicle the hair matrix (arrowhead) and precortex are significantly underdeveloped, while DP has an apparently normal architecture. (B) The vibrissae hair shaft is abnormally thin and curved (arrows indicate multiple sections of one shaft) with an amorphous coiled end (white arrowhead). (C) The HFs in homozygous skin are quite atrophic with the most prominent abnormalities in the bulb region. The lower ORS cells are enlarged and vacuolated. The hair matrix, precortex, and dermal papilla are largely missing or abnormal in contrast to anagen hair follicles in heterozygous skin (D). (E) The upper portion of the hair shaft has disintegrated and the hair canal (hc) is usually empty. (F) The sebaceous glands (sg) have lost the connection with the skin surface and occupy the empty portion of the hair canal. H&E stain; bars: A, 120 μm; B, 70 μm; C and D, 80 μm; and E and F, 60 μm.

stratum corneum structure became more marked. By day 4, mean thickness of the stratum corneum in homozygous and heterozygous animals was approximately the same (56 and 52μm, respectively), but the compact lowermost layer of corneocytes in homozygous animals (Fig. 2C) was thicker than that in heterozygous littermates (23μm versus 5μm on average). These corneocytes were so tightly packed that, in some instances, they formed an amorphous keratinous mass. The largely abnormal hair shafts did not reach the epidermis at all. The few hair shafts that penetrated the spinous layer of the epidermis were not able to penetrate the thick, compact layer of the lowermost corneocytes. These hairs coiled in on themselves at the level of the granular layer (Fig. $2C_1$), forming a conglomeration of keratinous material and sebum. The hair canal did not form and the sebaceous glands had no connection with the skin surface.

The lower portion of vibrissae follicles showed apparently normal architecture of the DP and hair matrix, but the precortex was substantially underdeveloped (Fig. 3A), indicating severe impairment of keratinocyte proliferation and differentiation. In the upper portion of the vibrissa follicle, the normal IRS and ORS were replaced by a disorganized epithelial structure that contained thin, coiled hair shafts (Fig. 3B).

In addition to the vibrissae abnormalities, examination of a cross-section of the muzzle region revealed prominent hyperplasia of the palatine epithelium in young homozygous CR rats. The palatine epidermis in mutants was five to six times thicker, compared with that of heterozygous littermates (128μm versus 24μm on average), and the palate architecture was markedly abnormal. The granular layer in the palate of mutant pups was abnormally thick and diffuse, whereas in the palatine epithelium of heterozygous rats it was less prominent, compared with the granular layer in the epidermis. Of interest, no differences in the structure of the associated cheek epithelium were observed (Figs. 2D and 2E).

Day 12 was characterized by further atrophy of the hair matrix and precortex in homozygous HFs (Fig. 3C). Dermal papilla (DP) cells also were disorganized and did not have the specific architecture (Fig. 3C) characteristic for a normal anagen HF (Fig. 3D). The upper portion of the hair shaft disintegrated, and the hair canal usually remained empty (Fig. 3E). The sebaceous glands retained their volume, but in most instances, lost the connection with the skin surface due to atrophy of the upper portion of the hair canal. Therefore, the sebaceous glands (SG) occupied the empty downward portion of the hair canal (Fig. 3F). Thus, at day 12, the HFs in homozygous skin were characterized by enlarged SG located unusually deep in the dermis and atrophic epithelial sheaths containing thin and abnormal hair shafts lacking any medullary structure.

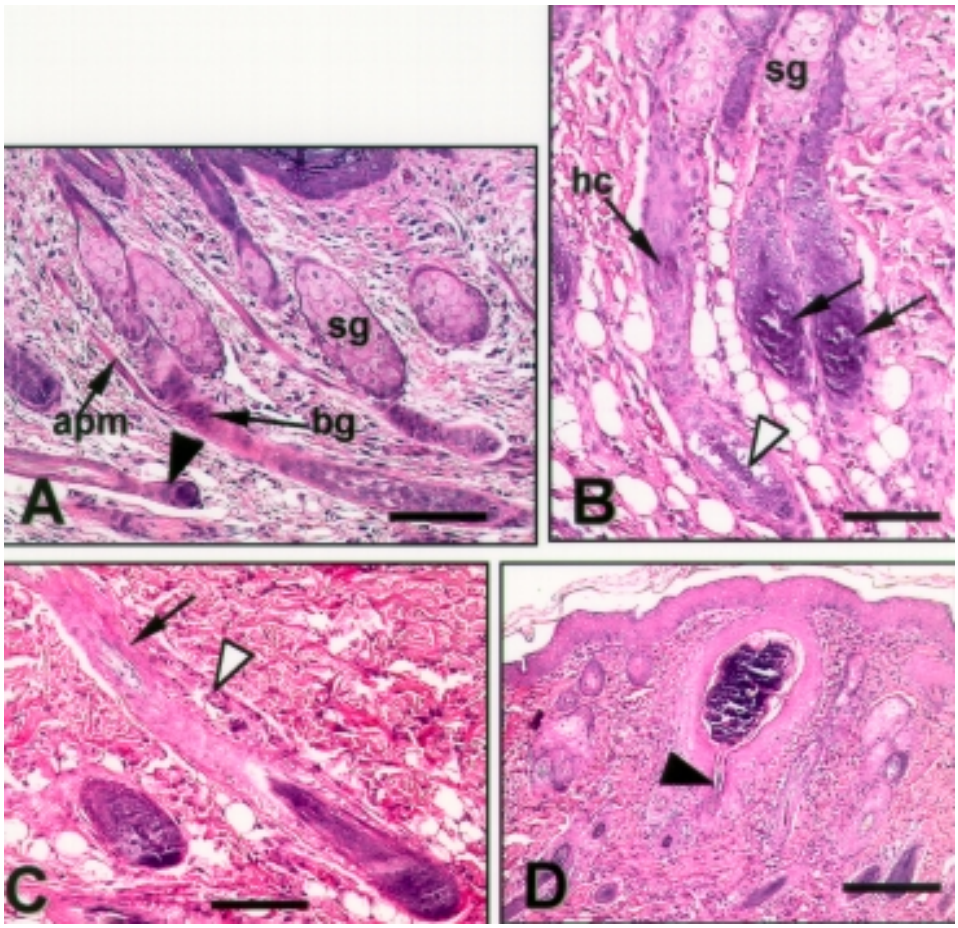

Figure 4. Photomicrographs of sections of skin from 21-day-old homozygous (-/-) pups. (A) Note the absence of DP structures, failure of hair club formation, and upward movement of the lower HF portion (arrowhead) while heterozygous follicles undergo normal catagen transformation; sebaceous glands (sg) are prominently enlarged. A few HFs attempted a catagen-like contraction; however, they did not contain normal club hair structures. (B) In the skin of adult homozygous rats at the stage that corresponds to anagen in heterozygous skin, some follicles underwent limited proliferation in the lower portion (arrows), but never produced a normal matrix (white arrowhead), precortex, IRS, or hair shaft. (C) In HFs of the new generation, normal DP structures were never observed. Notice that the hair shaft of the preceding generation is abnormally thin (arrow), does not form normal club hair, and is embedded into vacuolated epithelium (white arrowhead). (D) In a few instances, cystic structures were seen in the skin of homozygous animals that were associated with the upper portion of the HFs, whereas the lower HF portion remains atrophic and contains an abnormal hair shaft (black arrowhead). am = arrester pili muscle; bg = bulge region. H&E stain; bars: A, B, and C, 80 μm; and D, 160 μm.

At three weeks of age, when normal-haired heterozygotes enter the catagen stage of the HF cycle, the skin of homozygous mutants was characterized by the predominant absence of DP structures, failure of club hair formation, and upward movement of the lower HF portion (Fig. 4A). A few follicles attempted a catagen-like contraction; however, normal club hair structures or DP remnants were not apparent. Interestingly, the keratinocyte clusters localized in the bulge region often retained their integrity (Fig. 4A).

In the skin of adult homozygous rats at the stage corresponding to anagen in the normal-haired heterozygous animals, some follicles underwent limited proliferation in the lower portion, but never produced a normal matrix, precortex, IRS, and hair shaft (Fig. 4B). Normal DP and club hair structures also were never observed (Figs. 4B and 4C). In a few instances, cystic structures were seen in the skin of homozygous animals that were associated with the upper portions of the HFs (Fig. 4D).

Sequence analysis of hr gene structure in CR hairless rats: Sequencing of all exons and consensus exon-intron splice sites as well as a portion of the 5' UTR revealed no mutation in the hairless gene. Some polymorphic changes have been found in comparison with the sequence deposited into the GenBank (accession No. U71293). In the 5'UTR, we found a G to A substitution at position 243. Then we found four polymorphic changes in exons 1, 3, and 5 that did not affect the amino acid sequence, and two A-to-G transitions in exons 4 and 5 (positions 2,014 and 2,104, respectively) that resulted in the substitution of a lysine with an arginine residue. These substitutions were found in the heterozygous state in homo- and heterozygous rat mutants, thus indicating their polymorphic nature (Table 1).

Discussion

The first sign of a morphologic difference between homozygous and heterozygous skin in mutant CR rats was the formation of a thick, dense layer of corneocytes in lower stratum corneum. This layer resembled the so-called "stratum lucidum" that is found principally in the thick epidermis of palmar and plantar human skin (22) and of the footpads in mammals (23). This abnormally thick, dense layer of corneocytes and the probable prevention of sebum output to the skin surface were the likely reasons for the dry, shiny appearance of the skin in newborn homozygous pups. The stratum lucidum plays an important role in functioning of the epidermal permeability barrier in mammals (24, 25). Therefore, this layer may not only affect hair eruption in these rats, but most likely disturbs the barrier function of homozygous skin as well. This peculiarity of CR mutant rat skin should be taken into account when performing any pharmacologic experiment using this animal model.

The prominent hyperplasia of the palatine epithelium in young homozygous rats suggests that some other part of the digestive tract may be affected in mutant animals as well. This potential alteration of digestive tract epithelia may be the reason for the growth arrest in 2- to 3-week-old pups and consequent growth retardation in young animals.

Impaired cornification of the hair shaft, failure of club hair formation, and abnormal structure of the stratum corneum suggest that the putative gene responsible for the CR mutant rat phenotype is essential for the regulation of keratinocyte differentiation. In particular, the gene is most likely involved in regulation of the latest steps of this process, involving cornification, since

Table 1. Polymorphic changes in the Charles River (CR) rat hairless gene, compared with the sequence deposited to the GenBank (accession No. U71293)

Nucleotide:Exon	Nucleotide	Amino acid	U71293	Charles River
-243:5'UTR	G to A		-	+
500:1/2	C to A	Thr	-	+
761:1/2	T to C	Arg	-	+
2,014:4	A to G	Lys to Arg	-	+/-
2,104:5	A to G	Lys to Arg	-	+/-
2,108:5	A to C	Gly	-	+

structural abnormalities in the hair shaft were seen only at advanced stages of HF morphogenesis when the hair shaft forms its normal corticomedullary structure. This hypothesis is supported by specific changes in the interfollicular epidermis, reminiscent of a "stratum lucidum"-like layer of corneocytes.

Starting from the latest stages of HF development (stages 6 to 8), several obvious signs of HF degeneration were seen in homozygous skin. This process was extremely rapid, and by day 12, mainly atrophic HFs with abnormal or broken hairs were present in the skin (Fig. 3C). The SGs in homozygous skin were prominently enlarged, perhaps resulting from sebocyte hyperproliferation, or from the absence of SG contact with the skin surface. Lacking contact with the skin surface, most SG occupied the empty upper portion of the HFs and in many cases even spread downward occupying the middle portion of the dermis. Therefore, the autosomal recessive mutation in CR rats abrogates cell proliferation in the hair matrix and markedly affects keratinocyte differentiation in the HF and interfollicular epidermis.

Up to now, at least 17 hypotrichotic phenotypes in rats have been described. Therefore, it is likely that the CR hairless mutation in rats is allelic to some already known hypotrichotic rat mutation. Unfortunately, the histologic data on some of the previously described hypotrichotic rats are limited or absent, and in many instances, the clinical and macroscopic description is poor. Therefore, we cannot easily compare the CR hairless rat with the hypotrichotic rats reported to date. Nevertheless, one can exclude from the list of possible allelic candidates the Rowett and New Zealand nude rats that have appearance similar to that of CR rats, but are characterized by impaired thymic development and have no abnormalities in the interfollicular epidermis (8, 26). Furthermore, these rats have been documented to have mutation in the *whn* gene (11). The "hairless" Wistar is an incomplete dominant trait that has no epidermal abnormalities and is quite different from the CR mutant rat with regard to HF histopathologic features (27).

Hirosaki hairless (*hhr*) (28) rats have a gross appearance similar to that of CR rats (curved, short vibrissae at birth and absence of normal first hair pelage), but their skin contains large keratin-filled dermal cysts that are absent in CR mutants. The gross appearance and some clinical features of CR hairless rats are somewhat similar to the *shn* (shorn) mutants (9), but unfortunately histologic data on this mutation are not available in the literature. The other mutation that is reminiscent of CR hypotrichotic rat phenotype is fuzzy (*fz*) (6). These mutants have curved vibrissae at birth, and an abnormal first pelage that is sparsely distributed and is characterized by short, broken hairs. Adult animals are entirely naked. With age, the males can be differentiated from the females by a yellow greasy characteristic of the skin similar to the skin of CR rat mutants. Histologically, fuzzy skin is characterized by the presence of coiled abnormal hair shafts just beneath the epidermis. Dermal cysts are never seen in fuzzy mutant skin, however, HF atrophy is not prominent in fuzzy skin, thus casting doubt on the allelic relationship between fuzzy and CR hairless.

Table 2. Comparison of CR hypotrichotic rat phenotype with rhino (*hrrh/hrrh*) mice, which represent the prototype of hairless gene mutations

	CR "hairless" rat	Rhino (hr^{rh}/hr^{rh}) mice
Epidermis	Slight acanthosis. Abnormal corny layer.	Slight acanthosis. Normal corny layer.
First hair pelage	Sparse and abnormal hairs. General appearance is naked.	Normal first hair pelage.
Hair shaft defects	Thin, fragile, abnormally keratinized hairs that lack medullary structure.	Normal hair shafts.
Stage of hair cycle affected	Anagen.	Catagen.
Hair loss	Diffuse loss of abnormal hairs during first 2–3 months of life.	Rapid wave-like hair loss during postpartum days 14–20.
Hair follicle defect	Hair matrix underdevelopment. Abnormal keratinization.	IRS mispositioning. HF disintegration and cyst formation.
Dermal papilla defect	Abnormal DP structure in anagen and its consequent disintegration.	Normal DP structure in anagen. Failure of DP upward movement during catagen.
Sebaceous gland	Hypertrophic.	Hypotrophic.
Hair canal	Normal.	Significantly dilated with resultant formation of utriculi.
Hair regrowth	Some abnormal and sparse hairs occur due to the second and third waves of hair growth.	Never occurs.
Nails	Normal.	Excessively long and curved.
Reproduction	Females are fertile.	Females are mostly infertile.

The CR "hairless" rat also differs from the *hairless* rat mutants described by Davis (16; see also 5, 7), which have a normal first hair pelage and are indistinguishable from normal littermates until 15 to 18 days, have normal vibrissae, and most likely are allelic to the *hr/hr* mouse mutation. In addition, the skin of these *hr/hr* mutant rats contained numerous dermal cysts and utriculi (7) that are absent in CR mutants, in which the main histopathologic feature is not HF disintegration and cyst formation, but abnormal differentiation of hair shaft and epidermal keratinocytes. The hairless phenotype in mice bearing allelic mutations at the *hr* locus has been extensively studied (12, 14, 19, 20). Comparison of the CR hypotrichotic rat phenotype with specific features of hairless phenotype in mice reveals prominent differences (Table 2). The absence of a mutation in the hairless (*hr*) gene in CR mutant rats underscores the observed morphologic differences and provides the second line of evidence that argues against any association between CR rat phenotype and hairless gene. Finally, the identification of heterozygous intragenic *hr* polymorphisms in homozygous affected rats (Table 1) provides a third line of genetic linkage evidence against the hairless gene being involved in the CR hypotrichotic rat phenotype.

Collectively, clinical and morphologic analysis of successive stages of specific phenotype formation in the CR "hairless" rat, together with DNA sequence and genetic linkage studies, provides

substantial evidence that this mutation is, in fact, not "hairless," and may be unique among the other hypotrichotic rat mutations.

Acknowledgments

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