Molecular Basis for Hair Loss in Mice Carrying a Novel Nonsense Mutation (*Hr^rh-R*) in the Hairless Gene (*Hr*)

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Abstract

Animal models carrying mutations in the hairless (*Hr*) gene provide a rich resource for study of hair follicle biology. A spontaneous mouse mutant with a phenotype strikingly similar to rhino mutants of *Hr* arose spontaneously in the mouse facility at Oak Ridge National Laboratory. Sequence analysis of *Hr* in these mutants uncovered a nonsense mutation in exon 12, designated as *Hr^rh-R* (rhino, Oak Ridge). The mutation led to significant reduction in *Hr* mRNA levels, predicted to be due to nonsense-mediated decay. Histological analysis indicated dilated hair follicle infundibula at 14 days of age that rapidly became filled with cornified material. Microarray analyses revealed that expression levels of many genes involved in keratinocyte differentiation, epidermal regeneration, and wound healing were significantly upregulated before morphological detection of the phenotype, suggesting their role in onset of the *Hr^rh-R* phenotype. Identification of this new *Hr* allele and the underlying molecular alterations allows further understanding of the role of *Hr* in hair follicle biology.

Keywords

alopecia; hair follicle; hairless mice; microarray analysis

Existing allelic mutations in the hairless (*Hr*) gene compose a set of mouse models that cause hair loss through partial or complete loss of *Hr* function across a variety of mammalian species.[1,5,10,14,45,62,73]

Hr encodes a 127-kD protein that includes a single zinc finger and a Jumonji-like C terminus domain, which is a metalloenzyme-like domain implicated in chromatin remodeling.[9,20] A predicted role for *Hr* in control of gene expression is borne out by its demonstrated ability to function as a transcriptional corepressor through heterodimerization with the thyroid hormone receptor, vitamin D receptor, and retinoic acid–like orphan receptor alpha, possibly mediated through association with histone deacetylases.[15,29,42,47,65,70] Multiple allelic mutations with various degrees of severity have arisen in *Hr*, all of which manifest in mice as an inability to regrow a normal coat of hair after the initial catagen stage, resulting in a progressive loss of hair from head-to-tail beginning in the third week of life.[18,28] Gaskoin first described a mutant mouse with hair loss and wrinkled skin as "rhinoceros" in 1856.[26] More recently, several *Hr* mutations causing a phenotype more severe than hairless (Hr^hr) were characterized as rhino alleles of *Hr* (Hr^rh), most of which were due to nonsense mutations.[3,4,20,72] The rhinocerotic

appearance and excessive skin wrinkling of rhino mutants at Hr are due to large utricles and deep dermal cysts that form from the remnants of hair follicles.[39,44,58,60] The difference in severity between Hr^hr and Hr^rh alleles is attributed to the degree to which the mutation abolishes wild-type *Hr* expression.[6] Nonsense mutations in *Hr* are also described in human patients with atrichia with papular lesions (OMIM No. 209500), but the excessive skin wrinkling is not present in affected human patients.[2,8,56]

A mouse with a hair loss phenotype similar to *Hr* mutants arose spontaneously in the Oak Ridge National Laboratory mouse colony. We report here that this new mouse model results from a novel nonsense mutation: *Hr^rh-R* (Oak Ridge rhino allele of *Hr*). We describe in detail the phenotype of *Hr^rh-R* mice, including the changes in gene expression that underlie hair loss in this model.

Materials and Methods

Animals and Tissue Collection

All mice were bred at Oak Ridge National Laboratory, and experiments were conducted under approved Institutional Animal Care and Use Committee protocols. The *Hr^rh-R* mutation was maintained on an inbred, mixed-stock genetic background by mating *Hr^rh-R/Hr^rh-R* male with *Hr^rh-R/+* females. Details of the genetic background are available from the Mouse Genome Informatics database under accession No. MGI:3580645. Coisogenic +/+ mice for sequence comparison were produced by intercross mating of *Hr^rh-R/+* mice. At various ages, mice were euthanized by cervical dislocation. Tails were harvested from littermates of each genotype for DNA isolation, and dorsal skin was collected for RNA and for histological analyses of hematoxylin and eosin–stained sections.[48]

DNA Sequencing and Genotyping

Genomic DNA was extracted from tail biopsies (0.2-cm tail snips) using the HotSHOT method.[66] All *Hr* exons including intron–exon boundaries were sequenced according to protocols described previously.[37] Sequencing results were analyzed with BLAST (Basic Local Alignment Search Tool).[7] A pair of specific primers spanning exon 12 and covering the DNA mutation was used for genotyping of the mice (forward primer: 5′-CCAAGAACCTGAGGACCAGA-3′; reverse primer: 5′-GCCAGTGTTCCTGGAAGAGA-3′).

Gene Expression Microarray Construction, Labeling, Hybridization, and Data Analysis

Total RNA was isolated from dorsal skin of 10-day old mice according to protocols standard for fibrous tissues.[37] Microarrays representing about 15,000 unique mouse genes were printed by the Center for Applied Genomics (PHRI, Newark, NJ) using the Compugen Mouse OligoLibrary 2.0. Hybridization experiments were performed as previously described.[37]

A total of 3 biological replicates (RNA from a pair of *Hr^rh-R/+* and *Hr^rh-R/Hr^rh-R* littermates at 10 days old after birth) were included in this analysis. Data were normalized using Lowess to adjust for intensity-dependent dye bias after removing spots of poor quality or low expression and subtracting local background.[54,55] The Significance Analysis of Microarrays software package was used to analyze data for statistically significant differences in gene expression, with a false discovery rate of about 3%.[67] Analysis of overrepresented Gene Ontology (GO) categories within the biological process ontology across the set of differentially expressed genes was conducted using the Database for Annotation, Visualization and Integrated Discovery 2006.[23] The detailed protocols and primary data from this study are available at the Gene Expression Omnibus database under accession Nos. GSE4055 and GSE4059. A 95% confidence interval was used for all statistical analyses. The oPOSSUM database of

transcription factor binding sites (TFBS) identified by phylogenetic footprinting was used to identify binding sites overrepresented among differentially expressed genes. When supplied with a list of genes of interest, oPOSSUM retrieves the TFBS counts for each gene in the list and computes 2 statistics (Z score, Fisher exact test) to measure overrepresentation of TFBS in the gene set relative to a background comprising all genes in the oPOSSUM database.

Northern Blot

Two probes, one corresponding to exon 12 and one to exons 5–11, were amplified from a skin cDNA pool created from wild type mice. Total RNA from dorsal skin of 1-, 7-, 14-, and 35-day old mice was extracted, electrophoresed in agarose gels, transferred to nylon membranes, and cross-linked to the membranes using ultraviolet light according to the standard protocols. Probes were randomly labeled with α-^{32}P-dCTP and hybridized at 42°C overnight. Following washing, autoradiography was used to obtain blot images.

Results

Phenotype

Compared to the well-characterized rhino mutants of Hr, homozygous Hr^{rh-R}/Hr^{rh-R} mice exhibited a similar phenotype whereas $Hr^{rh-R}/+$ mice were phenotypically normal. Similar to other Hr^{rh} alleles,[3,4,17,25,44,52,58] skin wrinkling appeared before the majority of hair was lost, and it progressed with age (Fig. 1). Hair loss in Hr^{rh-R}/Hr^{rh-R} was first detectable on the ventrum at approximately 2.5 weeks of age and proceeded in a diffuse manner across the body, a pattern of loss that differed from other Hr mutants (Fig. 2). Vibrissae were normal but became sparse with age (Fig. 2). Nail length increased, and older adult mice (about 6 months of age) displayed overgrown nails that curled under the foot pad (onychogryposis) (Fig. 3). This is a common feature in all the Hr allelic mutations.[59,61] Both sexes of Hr^{rh-R}/Hr^{rh-R} mice were fertile and viable, but females had difficulty nursing owing to a propensity for skin lesions. Thymus weight was significantly reduced in Hr^{rh-R}/Hr^{rh-R} mice compared to $Hr^{rh-R}/+$ ($P < .05$).

Utricle and Dermal Cysts Formation

Histological examinations in skin from the dorsal interscapular region suggested grossly evident changes. Hair follicles completed postpartum development normally, and at 5 days of age mutants (Hr^{rh-R}/Hr^{rh-R}) could not be differentiated from littermate controls ($Hr^{rh-R}/+$ or +/+). Dorsal interscapular skin was examined histologically from replicate mice at 7, 10, 14, 18, 21, and 35 days of age. All mice were in late anagen stage from 7 to 14 days, at which point all entered catagen regardless of genotype. Early changes were observed in Hr^{rh-R}/Hr^{rh-R} mice at 14 days of age (Fig. 4). Infundibula were mildly ectatic and filled with laminated cornified cells. The stratum granulosum extended from the epidermis to the entrance of the sebaceous gland duct. The stratum granulosum was mildly hyperplastic (hypergranulosis) with prominent large basophilic keratohyalin granules. This finding was restricted to the layers producing the cornified material that filled the dilated infundibulum. By contrast, the influndibulae of the normal mice was lined by tight, compact squames that covered a thin-stratum granulosum with fine, dark basophilic granules (Fig. 5). By 18 days of age, the influndibular changes were similar, but the dilation was larger in the mutant mouse (Fig. 6) compared to the normal mice (Fig. 7).

As the mutant hair follicle entered catagen, a rim of keratinocytes surrounded the dermal papilla (Fig. 8). At 21 days of age, when hair follicles were in telogen or early anagen, the remaining club hair was still present in many follicles in which the infundibulum was ectatic and filled with laminated cornified material (Fig. 9). No club hairs remained at 5 weeks of age. As follicles entered catagen at 5 weeks of age, a thin serpentine-shaped band of cells, surrounded by a thick

hyalin membrane (glassy membrane), extended from the abnormal ectatic infundibulum to the dilated deep dermal cyst, which developed from keratinocyte remnants that surrounded the dermal papilla (Figs. 10, 11). Some of the cells surrounding these cysts differentiated into sebocytes.[63]

Novel Nonsense Mutation

Because of the marked similarity between Hr^{rh-R}/Hr^{rh-R} mice and rhino mutants of Hr, we identified the underlying Hr^{rh-R} mutation by sequencing the exons of Hr in Hr^{rh-R}/Hr^{rh-R} mice. A single nucleotide change (from C to T) was identified in position 3134 of Hr mRNA (NM_021877) in Hr^{rh-R}/Hr^{rh-R} mice (Fig. 12) whereas only C is identified at this position in the wild-type mice (Fig. 13). $Hr^{rh-R}/+$ mice were found to have both C and T at position 3134 (Fig. 14), consistent with the heterozygosity. This change in exon 12 was predicted to cause a premature stop codon at arginine 814 (R814X). Samples from a total of 100 Hr^{rh-R}/Hr^{rh-R}, $Hr^{rh-R}/+$, and +/+ mice were sequenced to confirm the mutation, and there were no deviations from the expected genotypes.

Owing to its distance from the terminal exon, the mutant Hr^{rh-R} mRNA should be degraded through nonsense-mediated decay.[68] We used Northern blotting to test this prediction, hybridizing with a probe covering exon 12. Exon 12 is a consensus exon present in the majority of mRNAs reported for Hr and is not known to be alternatively spliced in skin. As shown in Fig. 15, one major band was present in all samples, corresponding to the known size of the Hr transcript. Hr expression was dramatically reduced in Hr^{rh-R}/Hr^{rh-R} mice at all ages examined (1, 7, 14, and 35 days), compared to $Hr^{rh-R}/+$ littermates. Similar results were obtained with a probe spanning exons 5-11 (data not shown). Therefore, the Hr^{rh-R} mutation is expected to result in hair loss and skin wrinkling through marked loss of HR protein, similar to other rhino alleles of Hr.

Gene Expression Profiling

Microarrays were used to identify changes in gene expression that preceded onset of the hair follicle phenotype, as a means to profile early molecular events consequent to reduced expression of Hr. At 10 days of age, a total of 120 genes were differentially expressed in skin of Hr^{rh-R}/Hr^{rh-R} mice compared to $Hr^{rh-R}/+$ littermates ($P < .05$). Remarkably, only 1 gene exhibited decreased expression; 119 of 120 genes were significantly upregulated. These results are consistent with loss of the transcriptional corepressor function of Hr.[71] Results are shown in Table S1.

Within the set of differentially expressed genes, 63 of 120 were annotated with a descriptive gene symbol; 13 of the 63 have established roles in some aspect of skin and hair follicle function. This subset includes epidermal keratins 1 and 10 (*Krt1* and *Krt10*), both of which show increased expression in Hr^{rh-R}/Hr^{rh-R} mutants. *Krt 1* and *Krt10* are coexpressed in the suprabasal layers of differentiating epidermis and are widely used as markers of keratinocyte differentiation.[24,60] Several additional genes, either involved in keratinocyte differentiation or expressed specifically in the differentiated layer of the epidermis, were upregulated, including neuroblastoma myc-related oncogene 1 (*Mycn*), homeo box D13 (*Hoxd13*), fos-related antigen 1 (*Fos11*), and ephrin A3 (*Efna3*).[19,32,40,51,53] Six genes activated in wound healing and epidermal regeneration—stefins A1 and A3 (*Stfa1* and *Stfa3*), integrin beta 4 (*Itgb4*), inhibin beta E (*Inhbe*), neuropsin (*Prss19*), and trefoil factor 1 (*Tff1*)—also exhibited significantly increased expression in skin from Hr^{rh-R}/Hr^{rh-R} versus $Hr^{rh-R}/+$ controls.[11,22,31,33,34,38,43] In addition, 57 of the 120 differentially expressed genes (47%) encode proteins that play some role in the immune response, including 36 genes or expressed sequence tags that encode components of T-cell receptors and 10 that encode segments of immunoglobulins.

Differentially expressed genes annotated with Entrez GeneIDs (76 of 120) were analyzed for functional enrichment based on GO representation within the biological process ontology using DAVID 2006, with $P < .05$ for significance.[23] The most highly enriched GO category was that of organelle organization and biogenesis ($P = .0012$); other significantly enriched categories included those of skeletal development, regulation of metabolism, and nucleobase, nucleoside, nucleotide, and nucleic acid metabolism (Table 1).

Hr acts as a transcriptional regulator that in turn interacts with other transcription factors to control hair follicle development and cycling.[12] In silico promoter analysis was used to determine if genes differentially expressed as a result of reduced *Hr* expression shared one or more regulatory elements, which might further illuminate the molecular pathways through which *Hr* acts in skin. The oPOSSUM database identifies statistically overrepresented TFBS in a gene's putative promoter regions.[27] Like other TFBS identification programs, oPOSSUM is not yet comprehensive across the entire genome; however, it uses phylogenetic footprinting to restrict the search space to regions conserved between mouse and human, making it conservative and markedly reducing the number of false-positive detections.[27] As shown in Table 2, a total of 12 TFBS were significantly enriched among the set of differentially expressed genes (Fisher exact test, $P < .05$). The most significant was the binding site for MAX ($P = .00026$), a transcription factor that heterodimerizes with members of the Myc superfamily of transcription factors, such as MYCN. The binding site for MYCN was also overrepresented, and the *Mycn* gene was upregulated in Hr^{rh-R}/Hr^{rh-R} skin. Other enriched TFBS include those for aryl-hydrocarbon receptor nuclear translocator protein (ARNT) and upstream stimulatory factor 1 (USF1). ARNT executes nuclear translocation of the Ah receptor, which has been hypothesized to interact directly with HR.[35,64] USF1 is a transcriptional regulator of genes involved in wound repair, a function shared by several genes differentially expressed in Hr^{rh-R}/Hr^{rh-R} skin.[6,49]

Discussion

Mutations in *Hr* represent the richest set of allelic mutations known to cause hair loss in multiple species. Despite this wealth of models, the mechanisms through which these mutations lead to hair loss remain poorly defined at the molecular level. We identified a novel nonsense mutation in *Hr* gene that leads to reduced expression, presumably through nonsense-mediated decay. Hr^{rh-R} represents the 15th cloned or putative allele of *Hr* reported to cause hair loss in mice. One explanation for this abundance of mutant alleles is that *Hr* represents a gene for which mutations cause an obvious phenotype but not lethal or decreased fecundity, facilitating their recognition and propagation in mouse colonies worldwide.

The phenotype of Hr^{rh-R}/Hr^{rh-R} mice resembles that of other rhino alleles of *Hr* with respect to lesions within the hair follicle and the eventual wrinkling of the skin as hair follicle remnants become filled with cornified material.[45] The main difference between Hr^{rh-R} and other *Hr* mutants is that hair loss develops in a diffuse manner across the body, first noticeable on the ventrum, rather than in a rostral-caudal pattern coincident with hair cycle progression.[61] The reason for this difference remains unknown but might result from modifier alleles that affect retention of club hairs and are unique to the mixed-stock genetic background on which Hr^{rh-R} is maintained. It is worth noting that the pattern of hair follicle lesions in histological sections appeared uniform, as opposed to the diffuse pattern in hair loss of this mouse mutant.

Northern blots indicate that little *Hr* mRNA remains in the skin of Hr^{rh-R}/Hr^{rh-R} mice. Therefore, Hr^{rh-R} represents an additional member of the allelic series of *Hr* mutations that impair *Hr* expression and cause hair loss owing to dramatic reduction in HR protein.

Loss of *Hr* expression led to upregulation of a number of genes involved in epidermal regeneration and differentiation, before the point at which obvious morphological lesions were detected in sections of dorsal skin. These changes suggest that when HR is lost or significantly reduced, as predicted in Hr^{rh-R}/Hr^{rh-R} mice owing to the marked reduction in *Hr* mRNA, the coordinated pattern of gene expression necessary for progression of hair follicle differentiation through the hair cycle is significantly altered. Genes upregulated relative to heterozygous controls include several early markers of keratinocyte differentiation such as keratins 1 and 10 and *Fos11*.[24,50,51] Also in this set are genes linked more specifically to the hair follicle, including *Mycn, Hoxd13, Efna3,* and *Bmi1*.[32,41] *Bmi1* has been implicated as a self-renewal gene expressed in hair follicle stem cells,[21,46] a population proposed to be controlled in part by *Hr*.[71] Six genes upregulated in Hr^{rh-R}/Hr^{rh-R} skin are also activated during wound healing (*Stfa1, Stfa3, Itgb4, Inhbe, Prss19,* and *Tff1*).[*] The single gene (RIKEN cDNA 1500031M22) that was downregulated in Hr^{rh-R}/Hr^{rh-R} skin is a putative mouse homologue of a wound-healing gene found in *Xenopus laevis*.[57,69]

At the same time, about 47% of upregulated genes are involved in some component of immune function. These changes may be reflective of *Hr* involvement in the immune system, which has been suggested by premature thymic atrophy in both Hr^{rh-R}/Hr^{rh-R} mice and other *Hr* mutants.[45,59]

In silico promoter analysis of differentially expressed genes suggests that HR interacts with pathways utilizing the Myc/Max superfamily of transcriptional regulators. *Max* and *Mycn* are coexpressed in all epithelial layers of the epidermis and hair follicle and in the follicle bulb, bulge, outer root sheath, and sebaceous gland. Together they play key roles in directing the fate of hair follicle stem cells, a process proposed to be controlled in part by *HR*.[19,71] It cannot be determined from these results if enrichment of *Myc* and *Max* binding sites among genes differentially expressed in Hr^{rh-R}/Hr^{rh-R} skin is a primary event of HR loss or secondary to changes in the hair follicles. However, that this enrichment is present in genes differentially expressed before dramatic structural alterations suggests cross-talk between HR and Myc/Max signaling as a potential molecular mechanism for HR regulation of cell populations and differentiation in the hair follicle. Further experiments will be necessary to explore this possibility.

In summary, we have cloned and characterized a new allelic mutation of *Hr* that produces a rhino phenotype through loss of *Hr* expression. Upregulation of a number of genes involved in keratinocyte differentiation precedes manifestation of the phenotype and supports a proposed role for Hr in control of keratinocyte fate. Additional study will be necessary to identify the genes that are direct targets of HR and that initiate the cascade of events that lead to hair loss in *Hr* mutants.

Acknowledgments

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[*]References 13, 16, 22, 30, 31, 34, 36, 38, 43, 50

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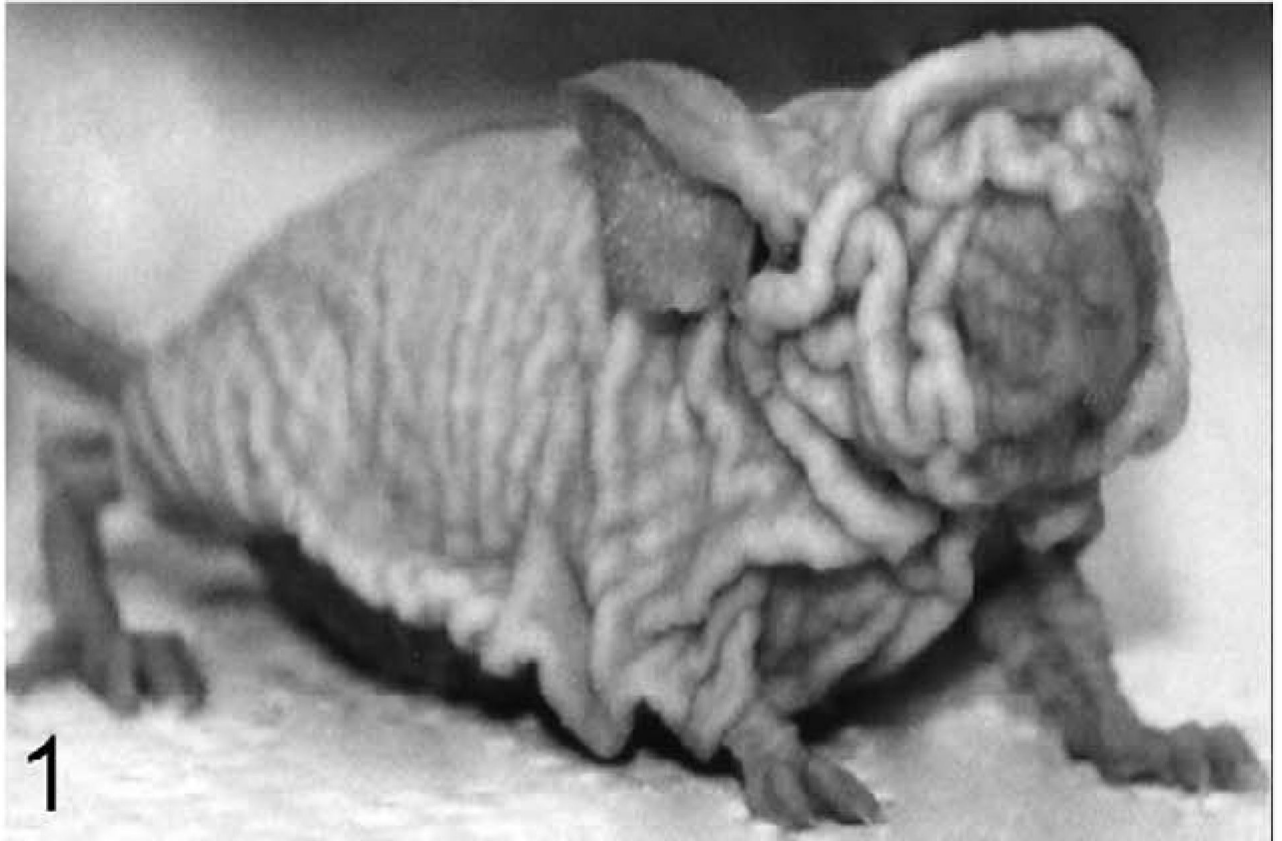
Figure 1.
Skin; *Hr^{rh-R}/Hr^{rh-R}* mouse, 5 months old. Skin wrinkling is characteristic of rhino mutants of *Hr*.

Figure 2.
Skin; Hr^{rh-R}/Hr^{rh-R} mouse, 4 weeks old. Scattered hair loss is readily visible at 4 weeks of age, occurring in a diffuse manner across the trunk.

Figure 3.
Nail; Hr^{rh-R}/Hr^{rh-R} mouse, 6 months old. Nails are increasingly long and curved in Hr^{rh-R}/Hr^{rh-R} mice.
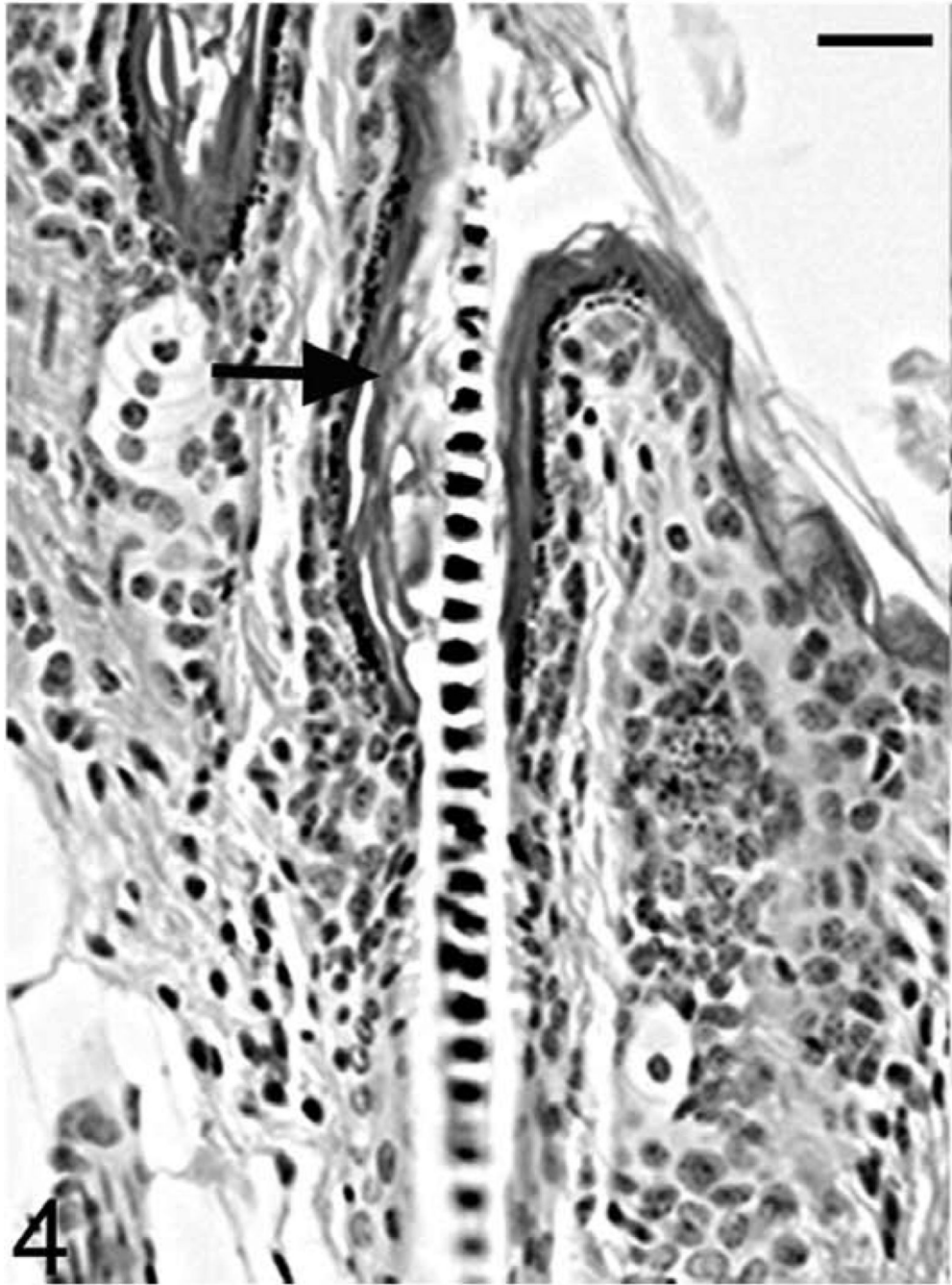
Figure 4.
Skin; Hr^{rh-R}/Hr^{rh-R} mouse, 14 days old. Mouse dorsal skin from the interscapular region was sectioned and stained with HE. Infundibula were mildly ectatic and filled with laminated cornified cells (black arrow). The stratum granulosum extended from the epidermis to the entrance of the sebaceous gland duct. Bar: 20 μm.
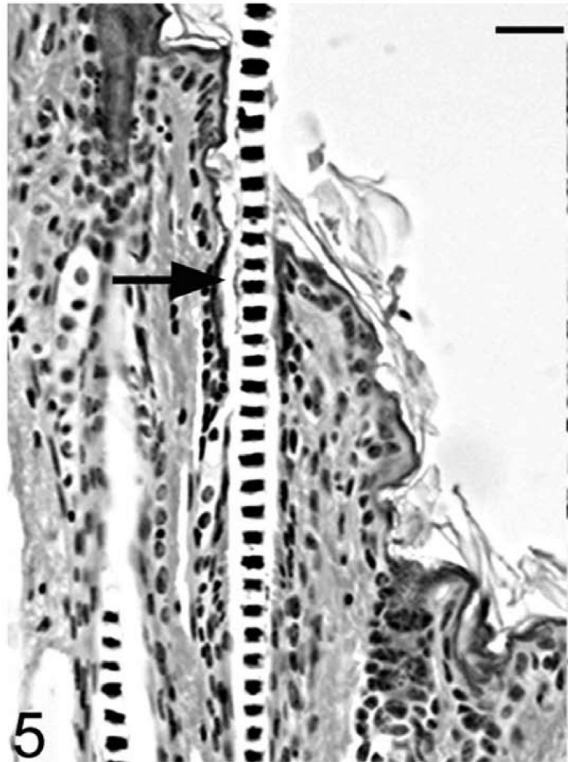
Figure 5.
Skin; Hr^{rh-R}/+ mouse, 14 days old. Dorsal skin from the interscapular region was sectioned and stained with HE. The influndibulae (black arrow) were normal and lined by tight, compact squames that covered a thin-stratum granulosum with fine, dark basophilic granules. Bar: 20 μm.
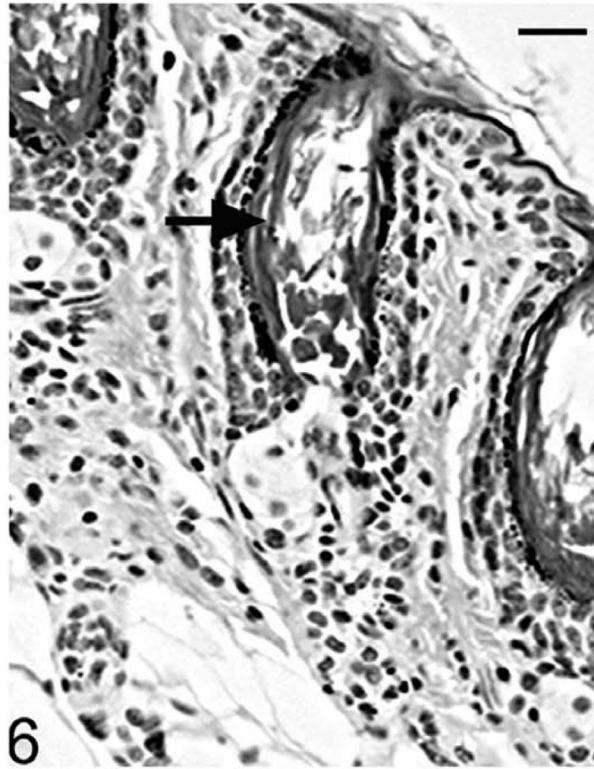
Figure 6.
Skin; Hr^{rh-R}/Hr^{rh-R} mouse, 18 days old. Dorsal skin from the interscapular region was sectioned and stained with HE. The influndibulae were ectatic and filled with more laminated cornified cells (black arrow) when compared to 14-day-old Hr^{rh-R}/Hr^{rh-R} mouse. The dilation also became larger. Bar: 20 μm.
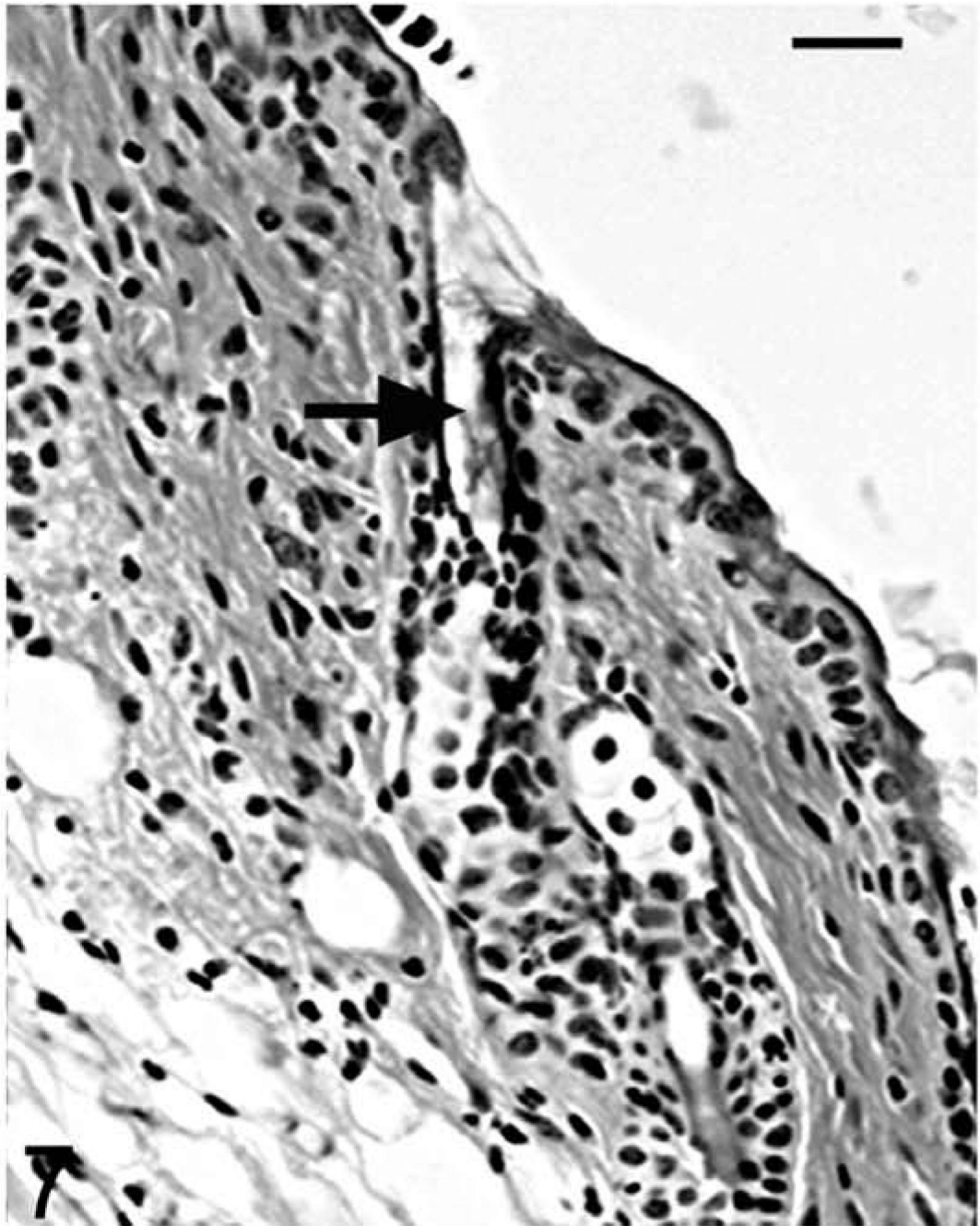
Figure 7.
Skin; *Hr^rh-R*/+ mouse, 18 days old. Dorsal skin from the interscapular region was sectioned and stained with HE. The influndibulae appeared normal (black arrow). Bar: 20 μm.

Figure 8.
Skin; Hr^{rh-R}/Hr^{rh-R} mouse, 21 days old. HE-stained section indicates a rim of keratinocytes surrounding the stranded dermal papilla (black arrow). Bar: 20 μm.
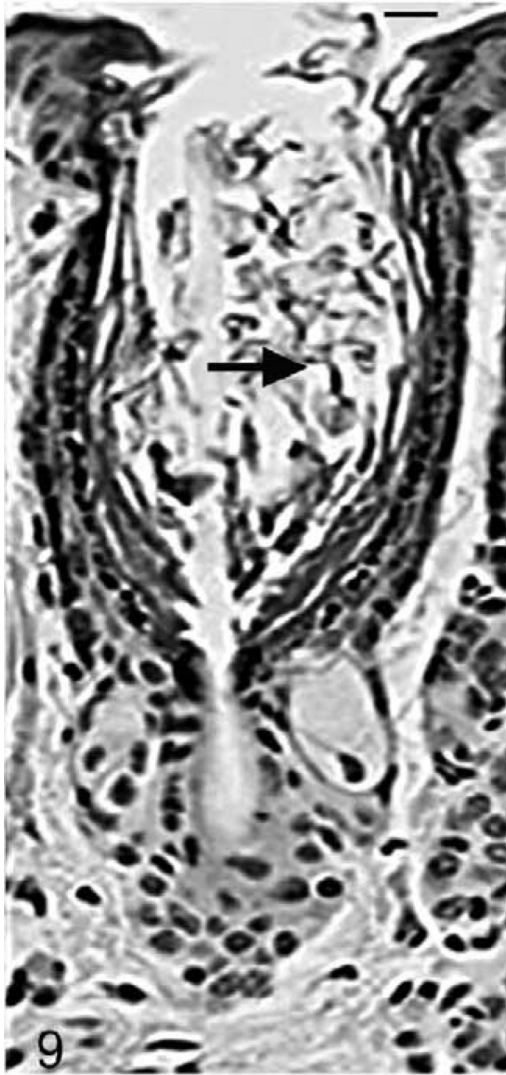
Figure 9.
Skin; *Hr^{rh-R}/Hr^{rh-R}* mouse, 21 days old. HE-stained section indicates the ectatic infundibula filled with laminated cornified material (black arrow). Bar: 20 μm.
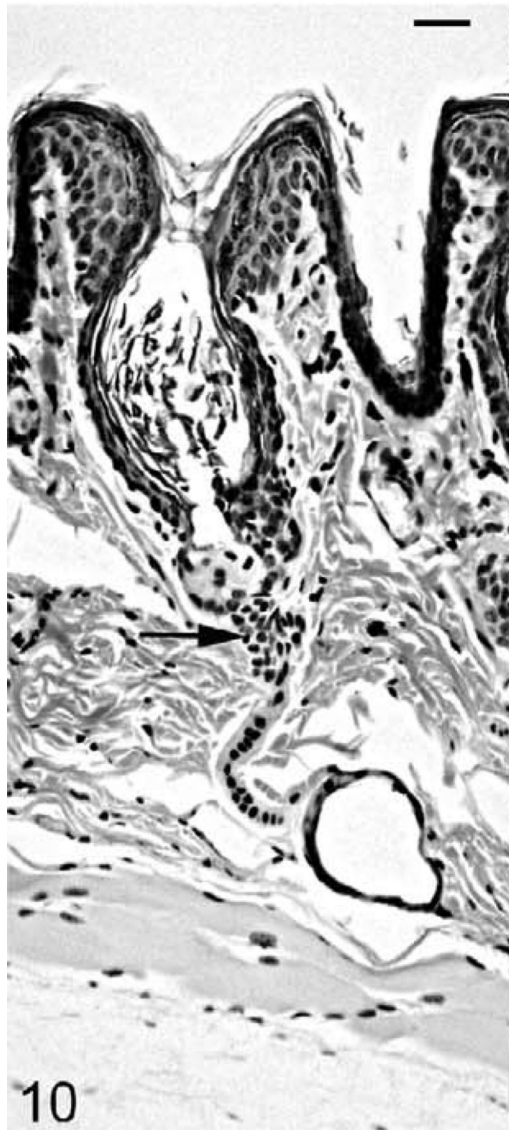
Figure 10.
Skin; Hr^{rh-R}/Hr^{rh-R} mouse, 5 weeks old. HE-stained section indicates the epithelial connection (black arrow) between abnormal infundibulum and deep dermal cyst. Bar: 80 μm.
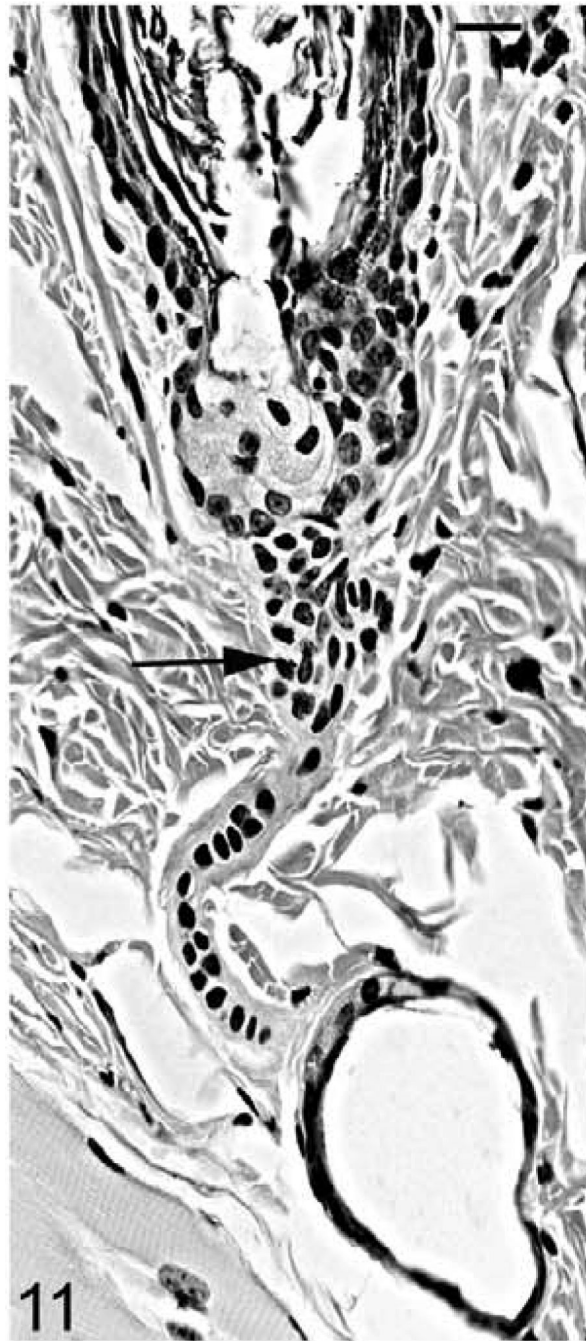
Figure 11.
Skin; Hr^{rh-R}/Hr^{rh-R} mouse, 5 weeks old. Magnified HE-stained section indicates the epithelial connection (black arrow) between abnormal infundibulum and deep dermal cyst. Bar: 20 μm.
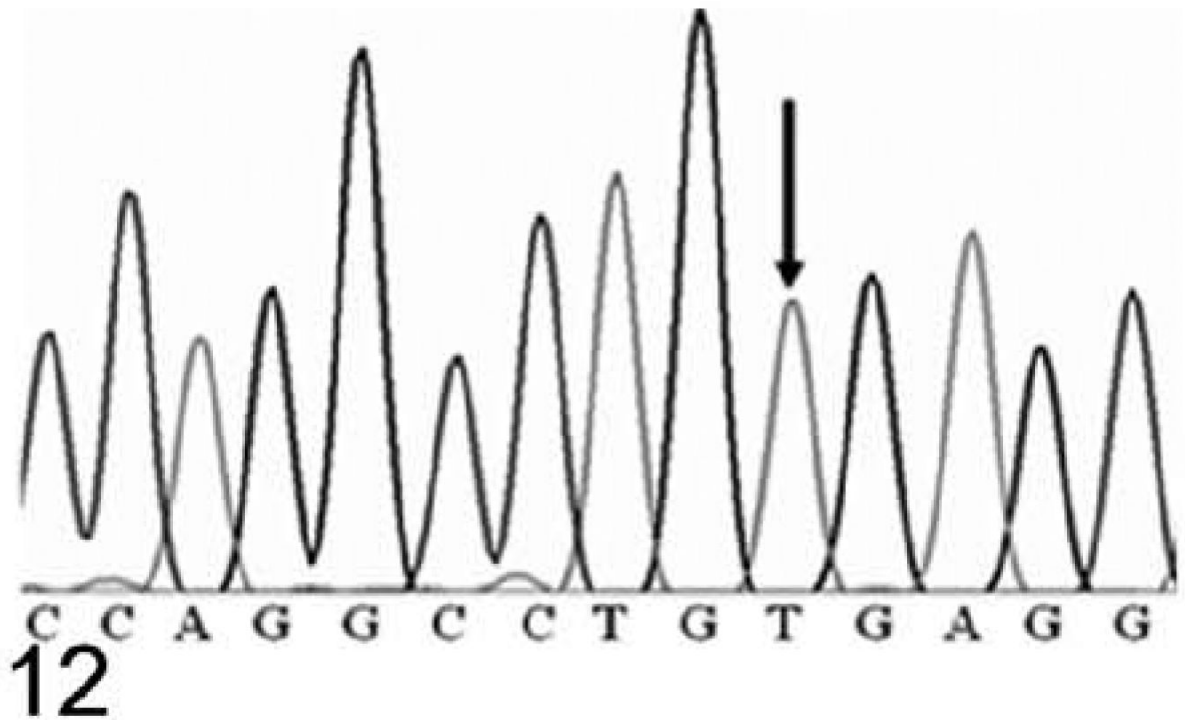
Figure 12.
DNA sequence; Hr^{rh-R}/Hr^{rh-R} mouse, 4 weeks old. The electropherogram of DNA sequence around Hr^{rh-R} mutation site in a homozygous mutant mouse (Hr^{rh-R}/Hr^{rh-R}). The arrow indicates the base mutanted in the mutant mice from C to T (3134C to T of NM_021877).
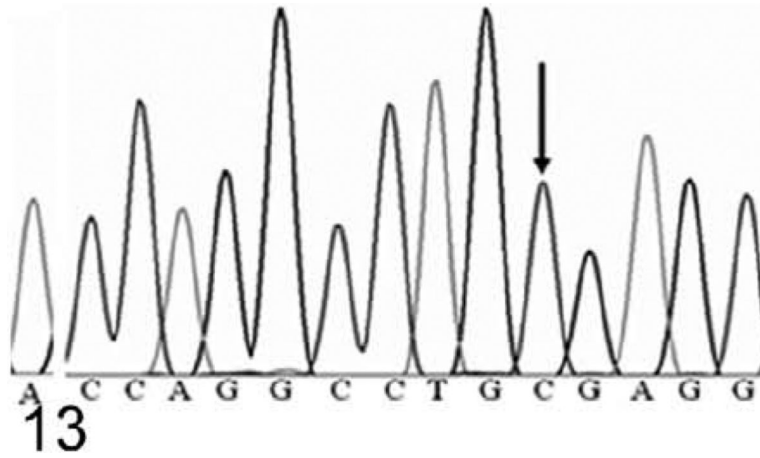
Figure 13.
DNA sequence; wild-type mouse, 4 weeks old. The electropherogram of DNA sequence around *Hr^rh-R* mutation site in a wild-type mouse (+/+). Arrow indicates that the base here is C (3134C in NM_021877).

Figure 14.
DNA sequence; Hr^{rh-R}/+ mouse, 4 weeks old. The electropherogram of DNA sequence around Hr^{rh-R} mutation site in a heterozygous mouse (Hr^{rh-R}/+). Arrow indicates that the base is changed from C to C/T in the heterozygotes (3134C in NM_021877).
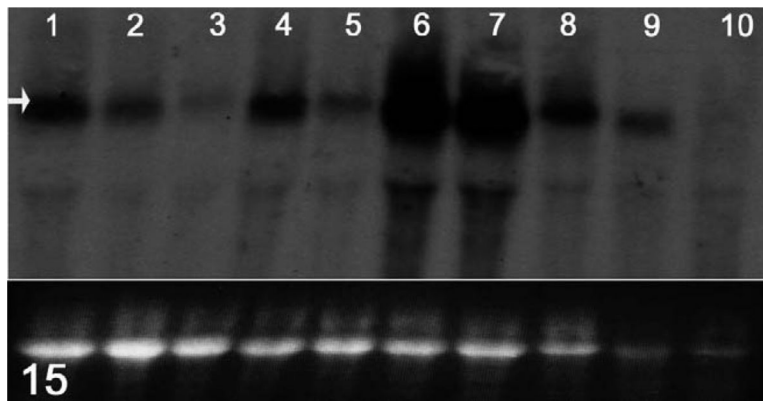
Figure 15.
RNA expression; effects of the mutation on *Hr* mRNA levels. Total RNA was extracted from dorsal skin of each genotype at different ages (1, 7, 14, 35 days after birth). A probe designed to span exon 12 of the *Hr* cDNA was amplified from wild-type skin cDNA using polymerase chain reaction. From the left side, lanes and samples are as follows: lane 1, 1 day, +/+; lane 2, 1 day, Hr^{rh-R}/+; lane 3, 1 day, Hr^{rh-R}/Hr^{rh-R}; lane 4, 7 days, Hr^{rh-R}/+; lane 5, 7 days, Hr^{rh-R}/Hr^{rh-R}; lane 6, 14 days, +/+; lane 7, 14 days, Hr^{rh-R}/+; lane 8, 14 days, Hr^{rh-R}/Hr^{rh-R}; lane 9, 35 days, Hr^{rh-R}/+; lane 10, 35 days, Hr^{rh-R}/Hr^{rh-R}. Arrow indicates the position of *Hr* transcript. The bottom panel represents the intensity of 28S rRNA from the ethidium bromide–stained gel as a loading control.

Table 1

Significantly Gene Ontology Categories

Biological Process Gene Ontology Term	No.	*P*
Organelle organization and biogenesis	11	.0012
Skeletal development	4	.0105
Cytoskeleton organization and biogenesis	6	.0166
Cortical actin cytoskeleton organization and biogenesis	2	.0268
Cortical cytoskeleton organization and biogenesis	2	.0301
Regulation of metabolism	14	.0427
Nucleobase, nucleoside, nucleotide and nucleic acid metabolism	17	.0439

Table 2

Transcription Factor Binding Sites Significantly Overrepresented Among Genes Differentially Expressed in $Hr^{rh-R}/Hr^{rh,-R}$

Transcription Factor	No. Genes	Fisher *P*
Max	13	.00026
USF	14	.0025
Mzf1-4	29	.0028
Spz1	6	.0086
Arnt	10	.014
NF-kappaB	10	.019
N-Myc	19	.026
bZIP910	20	.027
Irf-1	6	.029
Sox-9	13	.042
Yin-Yang	26	.049
Aml-1	14	.049