Study on hair follicle in Yuyi curly hair rats by histology and electron microscopy

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Abstract：A spontaneous semi-dominant mutation arose in a breeding colony of Sprague-Dawley (SD) rats. Mutant strains named "Yuyi curly hair rats (YCHR)" showed a distinctive curled pelage at 3 days of age after birth. The morphology of hair follicle from YCHR was studied and compared with SD Littermates to explore the mechanisms of the bending of the hair shaft. The study was designed to analyse the structure of hair follicle by histology and ultrastructure. Histological analysis demonstrated that all hair follicles were bent in the mutant rats. Higher magnification showed that the vavying cuticula layer of the inner root sheath （IRS） but not in SD rats. Scanning electron microscopy showed that the mutant hair shaft was curved and uneven thickness in cross sections. Compound of trichohyalin granula and intermediate filaments（IF）was observed in the IRS of the mutant. The results showed that inconsistent keratinization and irregulary IF in the cell of the IRS were associated with curliness of hair follicles in mutant strains.

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Key words：Curly rats；hair follicle；morphology；ultrastructure

1.Induction

There are many mutations in the rat that cause hair abnormalities, which of them are valuable models for similar human disorders and provide experimental approaches to study the underlying pathophysiological mechanisms affecting development and maintenance of the hair (Sibilia et al, 2003；Johnson et al，2003；Kuramoto et al,2005).A new rat mutation arose spontaneously in a closed colony of Sprague-Dawley (SD) Rat in our lab， which was isolated and established a mutant rat strain termed "Yuyi curly hair rat"（Zhang et al,2007）.Its curly appearance was a characteristic trait. Published reports mainly focused on some genes discovered as spontaneous rat mutations that then led to a wavy-coated phenotype appeared. For example, waved-2, wa2, was shown to be mutation of the epidermal growth factor receptor（EGFR）affecting skin and hair.Whereas the interesting question of various aspects of curly hair follicle morphogenesis has not yet been adequately addressed（Thibaut at al, 2005; Max-Planck Institute of Immunobiology,2007; Luetteke et al, 1993; Fowler et al,1995）.

In the present study, we observe the hair follicle structure using the histopathology, scanning electron microscopy and transmission electron microscopy to gain a detailed understanding of the cellular milieu of the hair follicle in which these molecular systems operate. Our purpose is to investigate further the structure of hair follicle and the potential factor involved in the morphological development of curly hair.

2.Materials and methods

2.1. Animals and tissues

The skin of YCHR homozygous and hemizygous rats aged 4（n=2 and n=2，respectively）weeks，and of age-matched wild-type rats(n=2) were used in this study. Mutant strains and normal littermates were obtained from the colony of Labotatory Animal Center of Zhengzhou University. All rats were cared for according to the Guide for the Care and Use of Laboratory Animals，and the study was approved by the Medical Ethical Committee of Zhengzhou University. The animals were killed by decapitation after deep ether anaesthesia，and the skin from the upper back quickly removed.

2.2. Histology and ultrastructure

The dorsal skin were removed immediately and biopsies were taken both for histology , Scanning electron microscopy（SEM）and transmission electron microscopy（TEM）.For histology，the skin was fixed in 10% neutral buffered formalin overnight，embedded in paraffin， sectioned at 5 microns, and stained with hematoxylin and eosin. For SEM, samples were fixed in 3% paraformaldehyde in 0.2 M phosphate buffer at pH 7.4. After dehydration in a graded series of acetone, the specimens were ritical-point dried using CO2, coated with cromum and observed under a S-3500 scanning electron microscope. For TEM，samples were washed in 5% sucrose cacodylate buffer， postfixed

with 1% osmium tetroxide，dehydrated and embedded in epoxy resin after 4h in Karnovsky·s fixative. Ultrathin sections were cut， collected on formvar coated grids and examined under a H-7500 transmission electron microscope.

3. Result

3.1. The phenotype of mutant rats

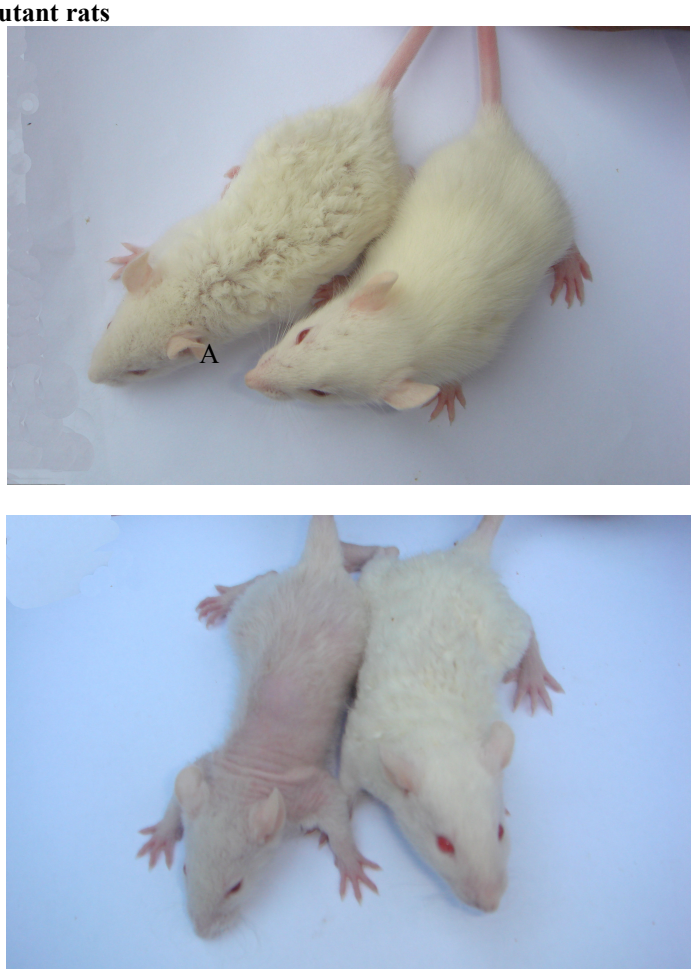

Fig 1. Phenotype of mutant rats. (A) The dorsal view of heterozygous rat（left） shows a curly pelage compared with that of a wild-type littermate（right）. (B) The homozygote（left）displays a transient hair loss in contrast to the heterozygote（right）.

3.2. Histology

Histological examination showed striking alterations in the distribution and morphology of hair follicles, and in the progression of the follicle through the stages of the hair cycle in mutant rats.Hair follicles were straight and a well-developed hair shaft was detectable within the hair canal in wild-type rats, whereas hair follicles of mutant rats exhibited a curved shape. At this stage of development, the average length of hair follicles in mutant stains was shorter than that seen in wild-type rats. The homozygote was more severe in comparison to the heterozygote. Higher magnification showed that the vavying cuticula layer of the inner root sheath（IRS）but not in wild-type rats, which may be associated with abnormalities of the IRS （Fig 2）.

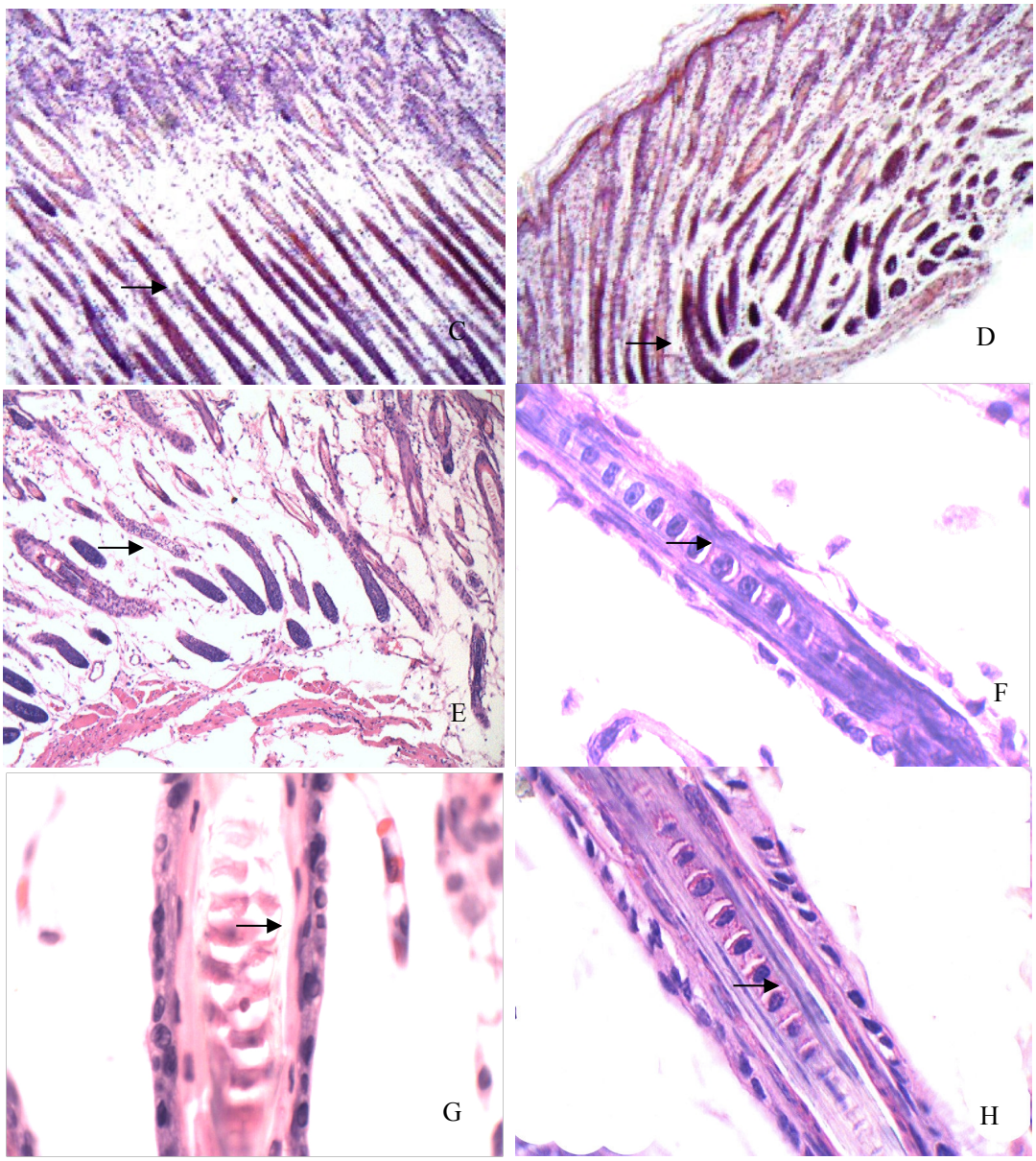

Fig 2. Hematoxylin/eosin staining of dorsal skin section from wild-type rats（C）and mutant rats at 4 weeks of age demonstrated that all hair follicles were bent and hair shafts were fragile in the homozygote（D）and the heterozygote （E）（arrows）. Higher magnification showed the curve cuticula layer of the IRS in the homozygote（F） and the heterozygote（G）, which was straight and continuous in wild-type rat（H）（arrows）.Scale bars（C,D,E）:100μm and（F,G,H）:40μm.

3.3. Ultrastructure

3.3.1. Scanning electron microscopy

To study the observed phenotype in greater detail,scanning electron microscopy was performed on pelage hairs of mutants and wild-type.The hair shaft was severely curved and irregular and deformed cuticle cells with ragged borders were observed in mutants compared with wild-type. The homozygote was more severely curved in contrast to the heterozygote.The cross section of the wild-type follicles was elliptic, whereas that of the mutant follicles was circular（Fig 3,arrows）.

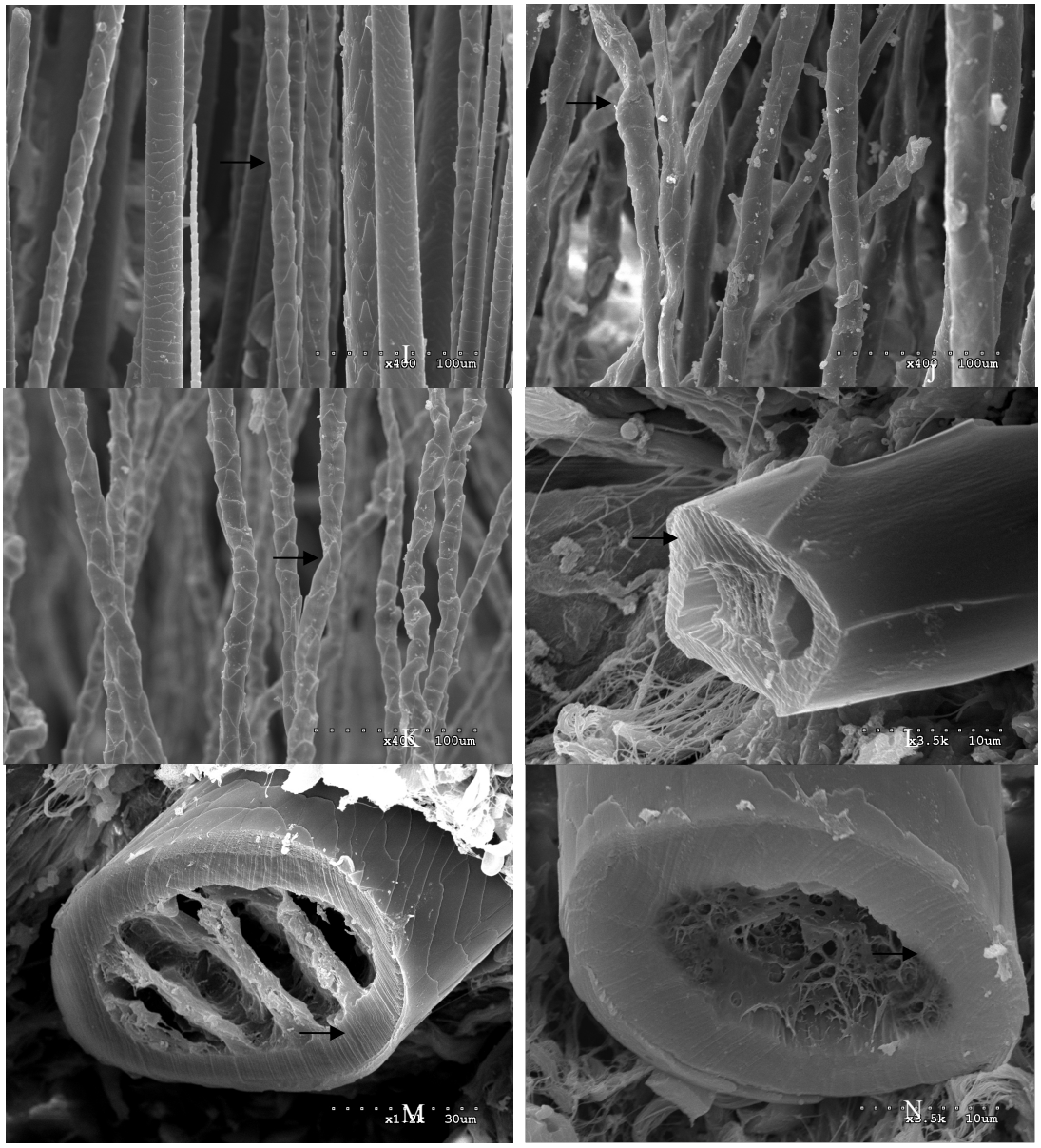

Fig 3. SEM images of plucked dorsal pelage hairs of wild-type（I）and mutants.（J）The hair shaft of the homozygote.（K）The hair shaft of the heterozygote.The cross section of the homozygote（L）and the heterozygote was circular（M）,whereas that of the wild-type follicle was elliptic（N）（arrows）.Scale bar is given in Fig 3 respectively.

3.3.2. Transmission electron microscopy

Transmission electron microscopic analysis demonstrated abnormal differentiation of Henle's layers， Huxley's layers and the IRS cuticle not recognized at the light microscopy level for mutations.In cuticle layer of the IRS in mutant rats,we found compound of intermediate filaments that was curve together with keratohyalin granules. The Huxley's layer, containing vacuoles filled with trichohyalin, was often torn or distorted. Vacuoles most likely derived from swelling of mitochondria and suggested a disrupted osmotic balance and thus early signs of cell degeneration. Vacuoles deriving from fixation artifacts were excluded, as many mitochondria still showed a normal, well-preserved morphology.In the mutant rats, the vavying Henle·s layer with keratohyalin granules was observed（arrows）（Fig 4）.

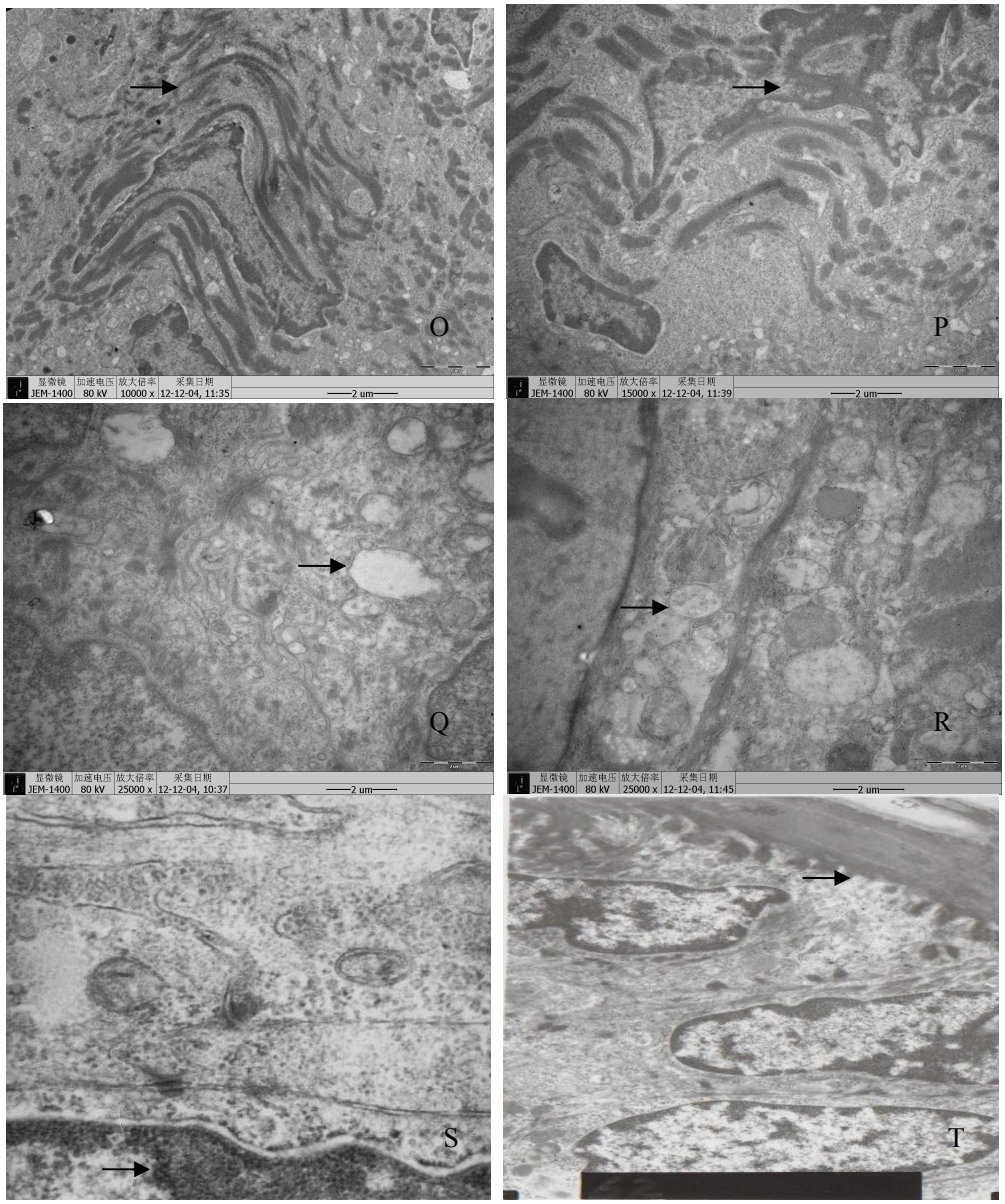

Fig 4.Transmission electron microscopy of hair follicle from mutants and wild-type .（O）The cuticle layer of the IRS in the homozygote.（P）The cuticle layer of the IRS in the heterozygote.（Q）The Huxley's layer of the homozygote.（R）The Huxley's layer of the heterozygote.（S）The Henle's layer of the homozygote.（T）The inner root sheath（IRS）of wild-type rats was ordered（arrows）.Scale bars:2μm.

4. Discussion

Inbred laboratory rats have proved to be useful model systems for studying hair biology（Sundberg et al, 1996）. Yuyi curly hair rats was a spontaneous semi-dominant mutation in SD Rats that resulted in hair abnormalities. Though this mutant has long been known, its cutaneous abnormalities are poorly defined. Here we provide a systematic analysis of hair phenotype in mutated rats. The murine hair shaft consists of the medulla and cortex covered by a single layer of cuticle cells. It is enveloped by the IRS, consisting of its own cuticle, which interdigitates with the hair, the Huxley's and the Henle's layer. The IRS stretches from the bulb to the mid-isthmus where it degenerates. It is surrounded by the outer root sheath（ORS）, which is continuous with the interfollicular epidermis and encases the entire follicle（Steinert et al, 2006; Alonso et al, 2006; Muller-Rover, 2001）. Several genetic alterations with different follicular localizations of the primary aberration give rise to curly or wavy hair in rats. An involvement of EGFR-mediated signals in the determination of hair

shape is stressed by various rat strains. Both a mutation of TGF-α in waved-1 rats and its genetic ablation cause a pronounced waviness of the hair coat （Luetteke et al, 1994; Mann et al, 1993） .In the study ， We have demonstrated morphological abnormalities that occur in the IRS of hairs. Hair shafts of mutated rats were thin, curly, short, irregular，and have an abnormal sheen appearance and abnormal hair cuticle. Histological and ultrastructural analysis demonstrated abnormalities in all three layers of the IRS of hairs in mutated rats. The analysis of SEM has confirmed previous reports that hair form resulted from the cross-sectional shape of the hair shaft. A circular cross section would give rise to straight hair, whereas a pronounced elliptic cross section would give rise to curly hair（Dawber et al, 1997）.Our researchers suggested that the IRS played a major role in hair shaft moulding and confirmed previous reports that waviness of hairs in rats can be caused by alterations of the IRS. It has been demonstrated that the IRS was thicker on the concave side of hair shaft in the HF of mutated rats than that of wild-type rats. It could be the differences in differentiation on opposite sides of the developing hair shaft that affected cell size or shape, which would inevitably give rise to hair curvature （Kikkawa et al, 2003; Porter et al, 2003）.Because the morphological investigations of mutant strains in this report suggested hair curling in mutants was a consequence of different proliferation rates within the IRS of hairs that led to inconsistent keratinization and irregulary IF in the cell of the IRS, it appeared to be reflected by the shape of the follicle. We believe that the mutants are suitable models to study this process further.

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