

Planar Cell Polarity Cadherin Celsr1 Regulates Skin Hair Patterning in the Mouse

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TO THE EDITOR

Skin hairs develop in a highly coordinated manner, and a complex genetic program controls their size, texture, density, and patterns (Schmidt-Ullrich and Paus, 2005; Sick *et al.*, 2006; Schlake, 2007). In addition to their physical features, hairs are distributed in macroscopic patterns. In rodents, all body hairs are oriented caudally and those on the feet are pointed distally. Here, we report that hair patterning on the body and legs in mice are disturbed by targeted inactivation of *Celsr1*, a seven-pass cadherin (*Celsr*) that is homologous to the *Drosophila* planar cell polarity (PCP) protein Flamingo/Starry night (*Fmi/Stan*; Strutt and Strutt, 2007).

Celsr1 conditional mutant mice were produced by introduction of loxP sites in introns 25 and 29 of the *Celsr1* gene, which contains 35 exons. They were crossed with PGK-Cre mice to generate a mutant allele with deletion of exons 26–29 (Figure 1a). Analysis of mutant RNA by reverse transcriptase-PCR and sequencing confirmed that exons 26–29 (nucleotides 7812–8257, GI:115648152), which encode transmembrane segments 5–7, were deleted. Translation of the mutant mRNA is predicted to produce a truncated protein with a scrambled sequence of 15 amino acids and a premature stop downstream of T2603 (GI:115648153). Quantitative RT-PCR showed that the concentration of the *Celsr1* transcript was fivefold lower in the mutant than in the wild type (Figure 1b), pointing to mRNA instability and suggesting that our allele (*Celsr1^{ko}*) is a null.

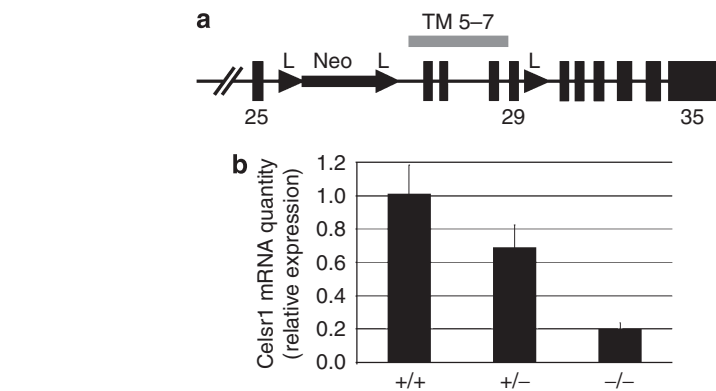


Figure 1. *Celsr1* targeting strategy. (a) Exons 26–29, which encode transmembrane segments 5 to 7 (TM 5–7), were flanked by LoxP sites (L) to allow their deletion by the Cre recombinase. Neo, neomycin cassette. (b) qRT-PCR analysis using primers located in a region unaffected by the deletion (nucleotides 7260–7365 of *Celsr1* cDNA, GI: 115648152) shows that mRNA levels are decreased by 80% in homozygous mice ($n = 4$). Data are corrected using the Reelin signal as internal control. +/+, wildtype mice; +/-, heterozygous mice; -/-, homozygous mice. Data are expressed as mean ± SEM.

Homozygous animals had a kinky or looping tail, and around 20% died *in utero* with various degrees of neural tube opening (Figure S1). Some animals displayed a striking hair-patterning defect that developed independently of the looptail trait. In wildtype and heterozygous *Celsr1^{ko}* mice, guard and pelage hairs were normally oriented, pointing towards the caudal part of the body and the distal extremity of limbs. In homozygous *Celsr1^{ko/ko}* mice, with the exception of vibrissae, hairs formed abnormal, whorl-like patterns that spiraled around a few centers on the body and the head (Figure 2a, arrowheads). On the limbs, hairs did not point distally like in control animals, but were oriented in a rosette-like manner (Figure 2a, arrowhead). In *Celsr1/Emx1* mice, where *Celsr1* was

inactivated following Cre expression in the embryonic limb ectoderm from E11.5 (see Figure S1b in Zhou *et al.*, 2008), the rosette-like pattern was seen on limbs, but no whorls developed on the body. Inasmuch as *Emx1* is not expressed in the mesoderm and neural crest, this observation indicates that *Celsr1* acts autonomously in the epidermis and not in other lineages.

Mutant hairs were straight and appeared to have a normal physical structure (not shown), indicating that abnormal patterns did not result from hair curvature or twisting, but rather from aberrant disposition and orientation. Examination of histological sections in the dorsal skin of 10- and 21-days-old mice confirmed that hair follicles were regularly orientated in wildtype animals (Figure 2b and c left, respectively). In contrast, in regions of abnormal patterning in mutants,

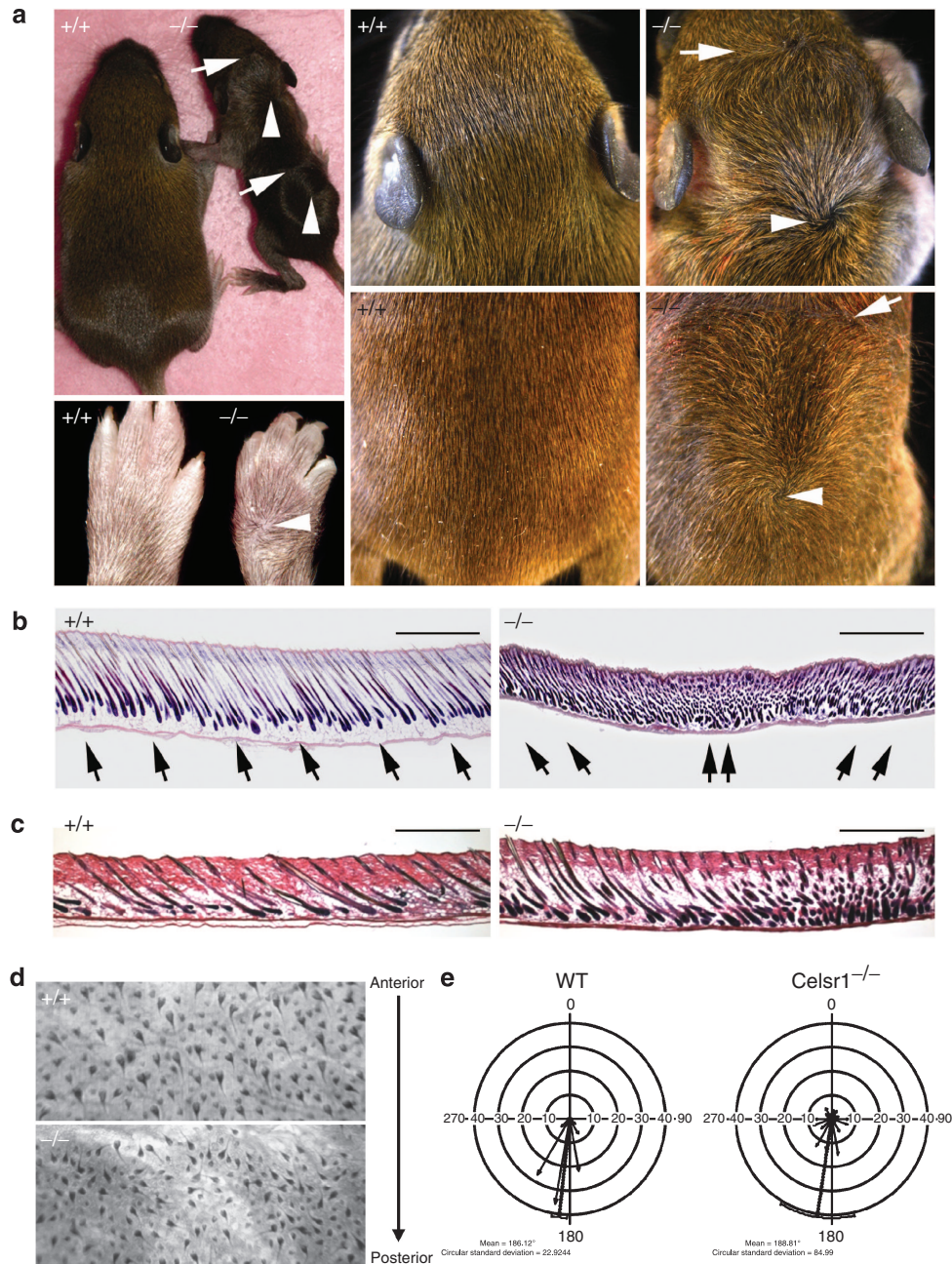


Figure 2. Abnormalities in *Celsr1*-deficient mice. (a) *Celsr1* mutation disrupts skin hair patterning. Whorls (arrowheads) and crests (arrows) are observed on the body and feet in mutant (–/–) but not wild-type (+/+) mice. (b, c) Skin sections in P10 (b) and P21 (c) mice stained with hematoxylin and eosin. Hair follicle orientation (arrows) is uniform in wildtype, but disorganized in mutant (–/–) skin. Bar = 1 mm. (d) Flat-mount preparations of skin of wildtype (+/+) and *Celsr1*^{ko/ko} (–/–) mice at postnatal day one. Bar = 500 μ m. (e) Quantification of hair follicle orientation angles using circular statistics. The mean vector was 186.131° for wildtype and 188.808° for *Celsr1*^{ko/ko} (–/–). However, circular standard deviation was 22.924° for wildtype and 84.99° for *Celsr1*^{ko/ko} ($P < 0.01$).

follicles and hairs in areas located within a few millimeters from each other assumed very different orientations (Figure 2b and c right, respectively). Flat-mount preparations from the dorsal skin of wildtype and *Celsr1* mutant mice at postnatal day 1 disclosed the abnormal angular

distribution of growing hairs, which was confirmed using circular statistics. The circular standard deviation was 22.924° for wildtype and 84.99° for *Celsr1*^{ko/ko} ($P < 0.01$; Figure 2d and e). Mutant hair bulbs were histologically unremarkable, and both the density and distribution of mitotic figures,

detected using phosphohistone-H3 immunohistochemistry, were normal (Figure S2).

A recent study of the embryonic epidermis of a mouse with mutations in *Celsr1* (*Celsr1*^{Crsh} allele) showed that mutant hair bulbs fail to acquire a normal oblique orientation between

the hair placode and the late germ (peg) stage, but instead remain oriented radially to the skin at all stages (Devenport and Fuchs, 2008). Our observation that *Celsr1* mutant follicles are obliquely orientated suggests that a secondary orientation occurs during the early postnatal period.

Body and leg hair-patterning defects similar to those described here are mimicked by inactivation of *frizzled6*, an ortholog of *Drosophila Frizzled* gene (Guo et al., 2004; Wang et al., 2006). In addition, mice deficient in *Celsr3* and *Frizzled3* have similar brain-wiring anomalies. This suggests that *Celsr1-Frizzled6* and *Celsr3-Frizzled3* act in similar pathways (Tissir et al., 2005). In flies, *Frizzled* controls patterning of skin appendages, together with *Fmi/Stan* and other core PCP genes such as *Van Gogh*, *Disheveled*, and *Prickle* (Wang and Nathans, 2007; Simons and Mlodzik, 2008). The hair-patterning phenotypes in *Celsr1* and *Frizzled6* mutant mice resemble the defects seen in *Fmi/Stan* and *Frizzled* *Drosophila* mutants, thus providing further evidence that *Frizzled* and *Celsr* (*Fmi/Stan*) are key elements of PCP-related pathways that pattern ectodermal derivatives and are evolutionary conserved. In the *Drosophila* wing, *Frizzled* localizes to the distal side of cells, *Van Gogh* to the proximal side, and *Fmi/Stan* to both.

This polarized distribution is thought to affect the hair orientation by controlling the cytoskeletal machinery responsible for hair assembly. Similarly, in the embryonic skin, polarization of the *Celsr1*, *van gogh-like 2*, and *Frizzled6* is found in wildtype, but not in corresponding mutant mice (Devenport and Fuchs, 2008). It will be interesting to study the protein-protein interactions that generate this polarized distribution.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Aurélia Ravni¹, Yibo Qu¹, André M. Goffinet¹ and Fadel Tissir¹

¹Institute of Neuroscience, Université Catholique de Louvain, Brussels, Belgium
E-mail: fadel.tissir@uclouvain.be

SUPPLEMENTARY MATERIAL

Figure S1. *Celsr1^{ko}/Celsr1^{ko}* mutant mice have a looping tail (arrowhead) and open neural tube.

Figure S2. Immunocytochemical staining of skin cryostat sections using phosphohistone-H3 antibody.

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Using a Structured Image Database, How Well Can Novices Assign Skin Lesion Images to the Correct Diagnostic Grouping?

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TO THE EDITOR

The cognitive basis of expertise in dermatology has received little formal attention (Jackson, 1975). Crucial to any such account is how experts recognize dermatological lesions—that is, how they attach semantics to

images. Insights into these processes might allow improved rates of skill acquisition and may be relevant to attempts to use computers to diagnose solitary skin lesions such as skin cancers. Although not designed to answer fundamental cognitive questions, a

number of very different techniques are suggested as tools or heuristics to facilitate diagnosis. In melanoma for instance, diagnostic strategies range from the use of rule-based systems, such as the ABCD system (Friedman et al., 1985), to approaches that rely on what might be termed a gestalt approach, such as the use of the ‘ugly duckling’ sign (Grob and Bonerandi,