Cotsarelis G, Sun TT, Lavker RM (1990) Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. *Cell* 61:1329–37

Lin MH, Kopan R (2003) Long-range nonautonomous effects of activated Notch1 on tissue homeostasis in the nail. *Dev Biol* 263: 343–359

Nam JS, Turcotte TJ, Smith PF, Choi S, Yoon JK (2006) Mouse cristin/R-spondin family proteins are novel ligands for the Frizzled 8 and LRP6 receptors and activate β-catenin-dependent gene expression. *J Biol Chem* 281:13247–57

Oshima A, Rochat C, Kedzia K, Kobayashi K, Barrandon Y (2001) Morphogenesis and renewal of hair follicles from adult multipotent stem cells. *Cell* 104: 233–245

Paus R, Cotsarelis G (1999) The biology of hair follicles. *N Engl J Med* 341:491–7

Rhee H, Polak L, Fuchs E (2006) Lhx2 maintains stem cell character in hair follicles. *Science* 312:1946–9

Togo T, Utani A, Naitoh M, Ohta M, Tsuji Y, Morikawa N *et al.* (2006) Identification of cartilage progenitor cells in the adult ear perichondrium: utilization for cartilage reconstruction. *Lab Invest* 86:445–57

Zaias N, Alvarez J (1968) The formation of the primate nail plate. An autoradiographic study in squirrel monkey. *J Invest Dermatol* 51:120–36

Mutations in the Serum/Glucocorticoid Regulated Kinase 3 (*Sgk3*) Are Responsible for the Mouse Fuzzy (*fz*) Hair Phenotype

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TO THE EDITOR

Mouse mutants have provided substantial insight into genes controlling cutaneous pigmentation, hair follicle development, and trichogenesis. Because of the ease of ascertaining these phenotypes, more than 200 different skin, hair, and pigmentation loci have been defined by spontaneous mouse mutants, many of which are represented by multiple alleles (www.informatics.jax.org). Among these mutants is fuzzy (*fz*), for which there are at least five previously described alleles (Dickie and Woolley, 1950; www.informatics.jax.org). Homozygous *fz* mice have sparse fur, abnormal hair morphology (Mann, 1964; Mayer *et al.*, 1974), and an overall acceleration of hair follicle cycling due to abnormalities of catagen and anagen phase initiation (Mecklenburg *et al.*, 2005). Here, we report a new allele of *fz* and describe the molecular basis of the phenotype.

In a 129S6/SvEvTac × C57BL/6J (129 × B6) F_2 intercross, we found a sibling pair of animals with sparse curly coats, and curved vibrissae, closely resembling the *fz* phenotype (Figure 1a and b). We mapped the new recessive phenotype in a genome-wide screen of DNA from 108 affected ((129 × B6) × 129 (N_5F_1) × CAST/Ei)F_2 animals to the extreme proximal portion of chromosome 1 in the vicinity of the *fz* locus (Dickie and Woolley, 1950). All animal procedures were approved by the Animal Care and Use Committee at Children's Hospital Boston. Fine mapping with novel microsatellite repeat markers localized the mutation to a 193 kb interval between 9.902 and 10.095 Mb (www.ensembl.org), which contains the genes for the serum/glucocorticoid regulated kinase 3 (*Sgk3*) as well as two novel expressed sequence tags. Sequencing of the predicted exons and intron/exon boundaries of the expressed sequence tags yielded no differences between the mutant and wild-type alleles. However, genomic PCR analysis of *Sgk3* in homozygous mutant DNA revealed a deletion of 2.4 kb encompassing exon 18, a portion of intron 17 as well as sequences encoding the 3′ untranslated portion of the mRNA (Figure 1c). Southern blotting of mutant and wild-type DNA with an exon 18 probe confirmed the germline deletion (Figure 1d). This mutation is predicted to truncate the Sgk3 protein at valine 440, deleting the C-terminal 55 amino acids (V439X), which include the entire C-terminal protein kinase C-like domain (Figure 1j). On the basis of these data, we concluded that our mutant was an allele of *Sgk3* (*Sgk3*$^{fz\text{-}Mdf}$).

To determine if *Sgk3* was also mutated in genetically confirmed *fz* alleles, we sequenced the *Sgk3* gene in B6.Cg-*fz H54 Mlph*^{ln}/+ *H54*/+/J and found a single base-pair insertion in exon 10 (ins579A of the cDNA) predicted to result in a frameshift and premature termination of the protein after leucine 192 (L192X) within the serine/threonine kinase domain (Figure 1e). In *fz*^{ica}, we demonstrated by RT-PCR an abnormally long RNA transcript resulting from aberrant splicing induced by a class II retrotransposon insertion in intron 6 (Figure 1f and g). Genomic PCR and Southern blotting revealed a deletion of exon 16 of a radiation-induced *fz* allele, frowzy (*fy*; Figure 1h and i), predicted to result in an in-frame deletion of 52 amino acids (P359–F410).

Sgk3 is one of three homologous Sgk proteins in mammalian genomes that have been implicated in a wide variety of homeostatic processes and stress responses (Tessier and Woodgett, 2006). During the course of this work, the *Sgk3* targeted deletion phenotype was described independently by two groups (McCormick *et al.*, 2004; Alonso *et al.*, 2005), and a third reported the positional cloning of an N-ethyl-N-nitrosourea-induced mutant allele of *Sgk3* (Masujin *et al.*, 2004; Okada *et al.*, 2006). In each case, the investigators found a phenotype virtually identical to *fz,* and collectively implicated *Sgk3* in the proliferation, differentiation, and

Abbreviation: EST, expressed sequence tag

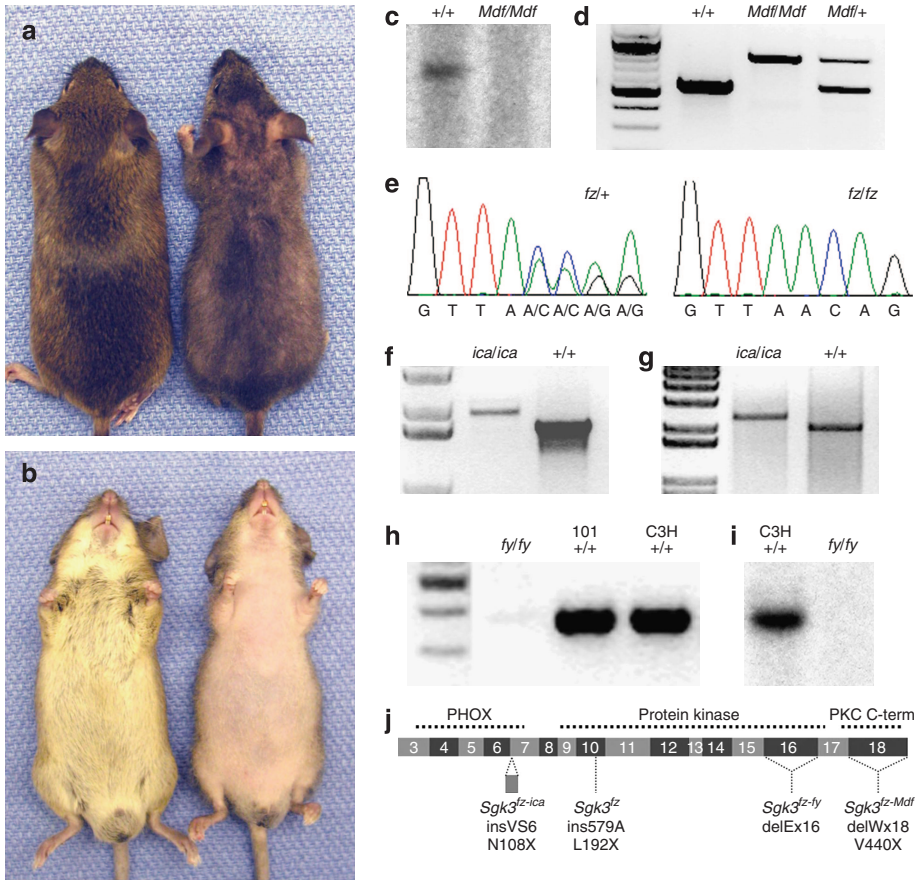

Figure 1. Identification of *Sgk3* mutations in four fuzzy (*fz*) alleles. (**a**) Dorsal and (**b**) ventral views of +/+ (left) and –$Sgk3^{fz\text{-}Mdf/fz\text{-}Mdf}$ (right) animals. Note the sparse, somewhat curly fur in the mutant. (**c**) Southern blot of *Eco*RI-digested +/+ and $Sgk3^{fz\text{-}Mdf/fz\text{-}Mdf}$ DNA with an *Sgk3* exon 18 probe demonstrating the absence of exon 18 in the mutant DNA. (**d**) PCR of genomic DNA using $Sgk3^{fz\text{-}Mdf}$-deletion-specific primer cocktail amplifying the 3′ end of the deletion in wild-type animals and across the deletion in mutants. (**e**) Sequence of an exon 10 genomic amplicon in B6.Cg–*fz*/+ and –*fz*/*fz* animals demonstrating the ins579A mutation resulting in a premature stop codon in *Sgk3*. (**f**) RT-PCR of STOCK-$fz^{ica/ica}$ total spleen RNA demonstrating a 183 bp insertion of sequences derived from a class II retrotransposon insertion in exon 6. (**g**) PCR of genomic DNA for the 3′ junction of the retrotransposon insertion and the wild-type allele. (**h**) Amplification of exon 16 of genomic DNA from frowzy (fz^{fy}) and the two wild-type strains, 101 and C3H, from which it is derived. (**i**) Southern blot of *Eco*RI-digested C3H/HeJ and $fz^{fy/fy}$ DNA with an *Sgk3* exon 16 probe demonstrating the absence of exon 16 in the mutant DNA. (**j**) Diagram of the location and nature of mutations in *Sgk3* reported here. Numbered boxes in alternating colors represent segments of the Sgk3 protein encoded by individual exons.

migration of hair follicle cells as a result of abnormalities in other signaling pathways important for keratinocyte development. Nonetheless, despite the physical resemblance of the mutant alleles and the chromosomal proximity of *Sgk3* to *fz*, their relationship was overlooked. Here, we make that link by demonstrating that our mutant, *fz*, Iasi congenital atrichia, and *fy* are alleles of *Sgk3*: $Sgk3^{fz\text{-}Mdf}$, $Sgk3^{fz}$, $Sgk3^{fz\text{-}ica}$, and $Sgk3^{fz\text{-}fy}$, respectively. In fact, the original *fz* allele is a near-perfect genocopy of one of the two reported targeted mutant alleles, which truncates the protein in exon 10 (Alonso *et al*., 2005).

Ironically, the molecular basis of a phenotype known for more than 50 years has been unraveled nearly simultaneously by both forward and reverse genetics. In so doing, it highlights the ongoing potential value of the numerous spontaneous mutant mice with cutaneous phenotypes for which the mutated gene has not yet been positionally cloned.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary Materials and Methods.

REFERENCES

Alonso L, Okada H, Pasolli HA, Wakeham A, You-Ten AI, Mak TW *et al*. (2005) Sgk3 links growth factor signaling to maintenance of progenitor cells in the hair follicle. *J Cell Biol* 170:559–70

Dickie MM, Woolley GW (1950) Fuzzy mice. *J Hered* 4:193–6

Mann SJ (1964) The hair of the fuzzy mouse. *J Hered* 55:121–3

Masujin K, Okada T, Tsuji T, Ishii Y, Takano K, Matsuda J *et al*. (2004) A mutation in the serum and glucocorticoid-inducible kinase-like kinase (*Sgkl*) gene is associated with defective hair growth in mice. *DNA Res* 11:371–9

Mayer TC, Mittelberger JA, Green MC (1974) The site of action of the fuzzy locus (*fz*) in the

mouse, as determined by dermal–epidermal recombinations. *J Embryol Exp Morphol* 32:707–13

McCormick JA, Feng Y, Dawson K, Behne MJ, Yu B, Wang J *et al.* (2004) Targeted disruption of the protein kinase SGK3/CISK impairs postnatal hair follicle development. *Mol Biol Cell* 15:4278–88

Mecklenburg L, Tobin DJ, Cirlan MV, Craciun C, Paus R (2005) Premature termination of hair follicle morphogenesis and accelerated hair follicle cycling in Iasi congenital atrichia (fzica) mice points to fuzzy as a key element of hair cycle control. *Exp Dermatol* 14:561–70

Okada T, Ishii Y, Masujin K, Yasoshima A, Matsuda J, Ogura A *et al.* (2006) The critical roles of Serum/Glucocorticoid-regulated Kinase 3 (SGK3) in the hair follicle morphogenesis and homeostasis: the allelic difference provides novel insights into hair follicle biology. *Am J Pathol* 168:1119–33

Tessier M, Woodgett JR (2006) Serum and glucocorticoid-regulated protein kinases: variations on a theme. *J Cell Biochem* 98: 1391–407

Identification of Defective Fas Function and Variation of the Perforin Gene in an Epidermodysplasia Verruciformis Patient Lacking EVER1 and EVER2 Mutations

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TO THE EDITOR

Epidermodysplasia verruciformis (EV), an infrequently reported lifelong clinical entity, is characterized by abnormal susceptibility to human papillomaviruses (HPVs). The natural course of the disease may at times be punctuated by the transformation of EV into squamous cell carcinoma; the lesions are preferentially located on sun-exposed sites. Nonsense mutations in two adjacent novel genes, named *EVER1* and *EVER2*, have recently been associated with the disease in some consanguineous families and sporadic cases (Ramoz *et al.*, 2002; Orth, 2006). Despite these findings, we have recently described an EV case with a lack of EVER gene mutations and a remarkable $CD8^+$ T-cell lymphocytopenia (Azzimonti *et al.*, 2005). Although EV has recently been classified as a primary deficiency in innate immunity to specific HPV genotypes (Notarangelo *et al.*, 2004), with the central role assigned to keratinocytes, the molecular mechanisms underlying abnormal susceptibility to a single type of weakly pathogenic infectious agent are still unclear.

A 59-year-old woman was admitted to our hospital with a diagnosis of EV. Physical examination revealed a limited number of multiple, flat, whitish and reddish papular lesions on the hands and forearms (Figure 1a and b) and a few pityriasis versicolor-like lesions on the trunk (Figure 1c). Surprisingly, the patient had never developed either cutaneous premalignant or malignant lesions, even in sun-exposed areas; thus, her forehead did not present any erythematous lesions. As reported in Figure 1d and e, biopsy specimens from lesions of the forearm and hand showed hyperkeratosis, acanthosis, and numerous large cells with pale staining of the cytoplasm and perinuclear vacuolization in the spinous and granular layers, resembling the typical histological features of EV (de Oliveira *et al.*, 2003).

This study was approved by the Research Ethics Committee ''Maggiore Hospital'' Novara and conducted according to the Declaration of Helsinki Principles. Written informed consent was obtained from the patient.

HPV DNA analysis was performed on samples collected with prewetted cotton-tipped swabs from different sites of the skin and on a formalin-fixed, paraffin-embedded papular lesion from

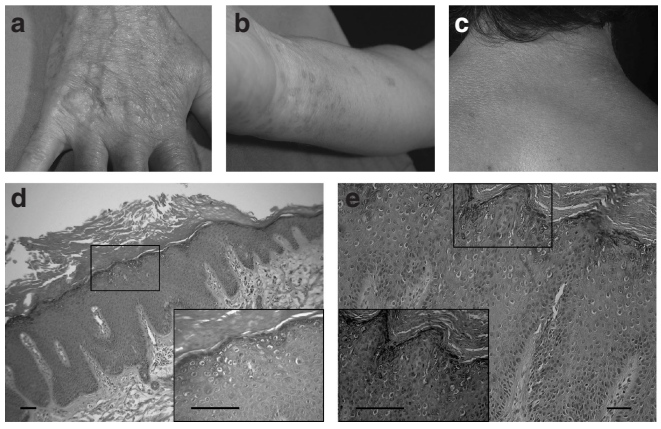

Figure 1. Clinical and histological findings from the study patient. Flat whitish and reddish papular lesions on the (**a**) hand and (**b**) forearm, (**c**) pityriasis versicolor-like lesions on the trunk. (**d**) A biopsy specimen from a papular lesion on the right forearm shows hyperkeratosis and acanthosis. The inset shows numerous large cells with pale staining of the cytoplasm and perinuclear vacuolization in the spinous and granular layers. (**e**) A biopsy specimen from a flat wartlike papule on the back of the hand, performed when she was 39 years old (1987), shows the same histopathological findings. Bar = 10 μm.

Abbreviations: ALPS, autoimmune lymphoproliferative syndrome; EV, epidermodysplasia verruciformis; HPV, human papillomavirus; IL, interleukin; PRF1, perforin gene