melanocytes is unclear, but it may be that they are immature and not fully differentiated, and/or, some are still located in the epidermis in newborn skin whereas in adult dorsal skin they are invariably located in the dermis (reviewed in Hirobe, 1995).

In conclusion, our work has demonstrated that neonatal UVR treatments are probably as effective at inducing MM in pigmented mice as albino strains. Furthermore, we have shown that RAS activation alone is sufficient to predispose melanocytes to UVR-induced transformation, and, although the precise mechanism is yet to be determined, it does not always involve loss of *Ink4a* or *Arf*. It may be that activated Ras simply promotes melanocyte proliferation, or alternatively, that it may interfere with the DNA damage response and apoptotic pathways. This mouse model further consolidates the mounting evidence that *NRAS* or *BRAF* mutations co-operate with solar UVR in the development of melanoma.

Elke Hacker,* Nicole Irwin,* H. Konrad Muller,† Marianne Broome Powell,‡ Graham Kay,* Nicholas Hayward,* and Graeme Walker*
*Queensland Institute of Medical Research, Brisbane, Queensland, Australia; †School of Medicine, University of Tasmania, Hobart, Tasmania, Australia; ‡Division of Radiation and Cancer Biology, Stanford University, Stanford, California, USA

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Address correspondence to: Graeme Walker, Queensland Institute of Medical Research, Brisbane, Queensland, Australia. Email: graew@qimr.edu.au

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A Missense Mutation in the Cadherin Interaction Site of The Desmoglein 4 Gene Underlies Localized Autosomal Recessive Hypotrichosis

To the Editor:

We recently described a novel form of hair loss, termed localized autosomal recessive hypotrichosis (LAH, OMIM 607903), which is a rare, autosomal recessive disorder affecting the scalp, trunk and extremities, and largely sparing the facial, pubic and axillary hair (Kljuic *et al*, 2003). Typical hairs are fragile and break easily, leaving short sparse scalp hairs with a characteristic appearance. We and others reported linkage of LAH to chromosome 18, in the region of the desmosomal cadherin gene cluster (Kljuic *et al*, 2003; Rafiq *et al*, 2003), in which we discovered a novel member of this gene family, known as desmoglein 4 (Kljuic *et al*, 2003). We first identified a large, intragenic deletion of exons 5–8 in the desmoglein 4 gene as the underlying mutation in two unrelated families of Pakistani origin (Kljuic *et al*, 2003), which was then subsequently reported in a total of five additional Pakistani families by our group (Moss *et al*, 2004)

Abbreviations: *DSG4*/DSG4, human desmoglein 4 gene/protein; LAH, localized autosomal recessive hypotrichosis

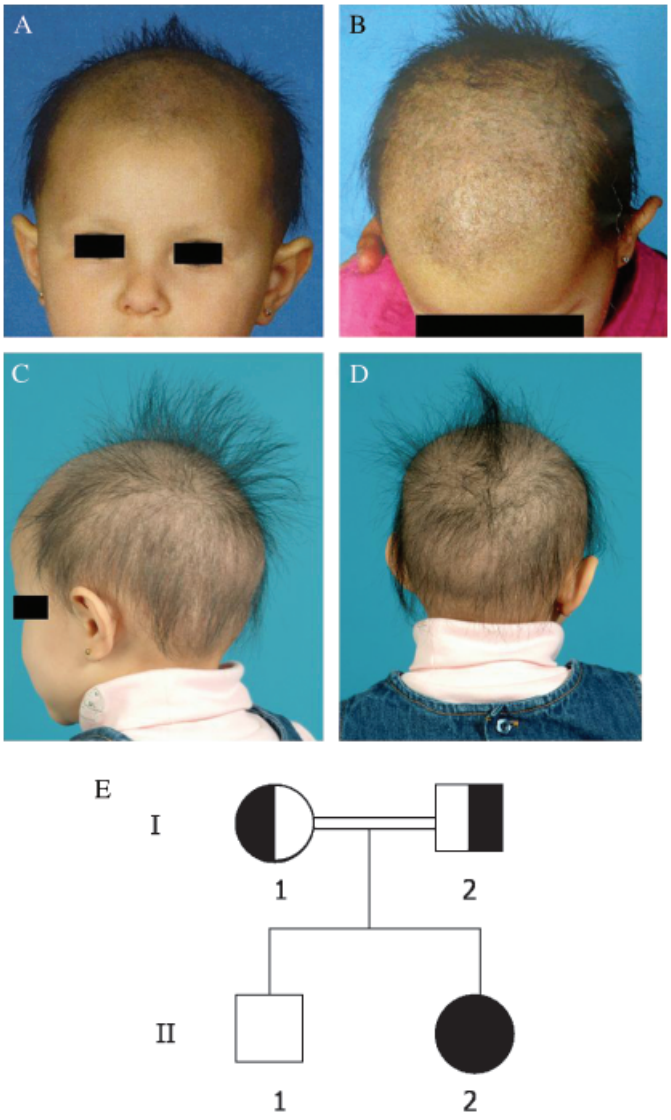

Figure 1
Clinical findings and pedigree of LAH family. (*A*, *B*) Clinical presentation of localized autosomal recessive hypotrichosis (LAH) in individual II-2, age 3 y. Note the sparse hair and follicular hyperkeratosis on the scalp and the eyebrows. (*C*, *D*) The same proband (II-2) at age 5 y. (*E*) The affected child (II-2; arrow) was born to first-cousin parents of Iraqi origin (I-1 and I-2), and she has an unaffected older brother (II-1).

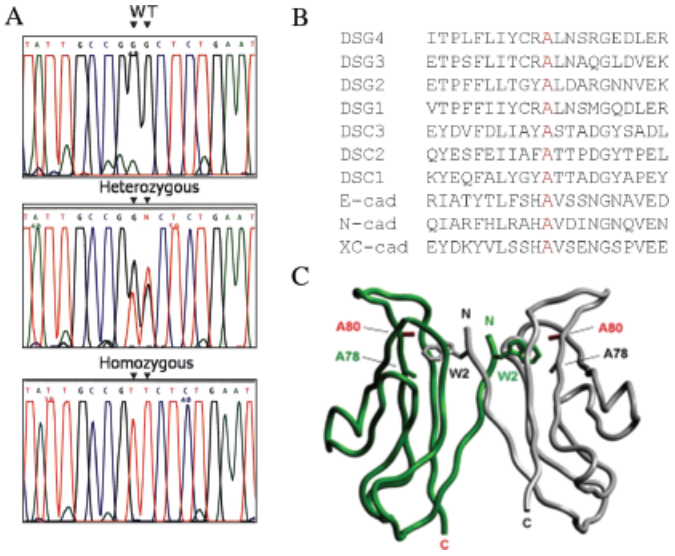

Figure 2
Molecular analysis of the human desmoglein 4 gene (*DSG4*) gene in the family. (*A*) Sequence analysis of the PCR products revealed a homozygous tandem dinucleotide transversion of nucleotides 384 and 385 in the affected individual. Both parents are heterozygous for a wild-type and a missense mutant allele. (*B*) Amino acid sequence alignment of the region harboring the mutation in human desmogleins (DSG1-4), Desmocollins (DSC1-3), classical cadherins (N- and E-cad), and *Xenopus* C-cadherin (XC-cad) whose extracellular domain crystal structure has been solved (Boggon *et al*, 2002). The highly conserved alanine residue (*A*) is highlighted in red. (*C*) A ribbon diagram depicting the adhesive dimer of N-cadherin, which is closely related to DSG4 (the sequence identity in the EC1 domain is $\sim$ 34%, while the sequence homology is $\sim$ 58%). The position of the mutation A129S, which corresponds to position A80S in the mature DSG4 protein lacking the signal sequence and pre-domain, are shown in red in each monomer. A78 and A80 are conserved as small hydrophobic residues in classical and desmosomal cadherins to facilitate binding of the Trp 2 side chain from the dimer partner. Disruption of this binding pocket by the mutation A129S is predicted to abrogate DSG4 adhesion.

and others (Rafiq *et al*, 2004), suggesting widespread dispersion of this chromosome. Using comparative genomics, we also demonstrated that human LAH is allelic with both *lanceolate hair* (*lah* and *lahJ*) mouse mutations (Kljuic *et al*, 2003). More recently, we identified three independent rat mutations with the *lanceolate hair* (*lah*) phenotype (Bazzi *et al*, 2004; Jahoda *et al*, 2004; Meyer *et al*, 2004). In order to expand the allelic series of mutations in the desmoglein 4 gene underlying LAH in humans, we have undertaken a molecular analysis of the *DSG4* gene in suspected LAH affected families from around the world.

Here, we report a family of Iraqi origin with one child affected with LAH (Fig 1). The affected 5-y-old girl has one 6-y-old brother with normal hair (Fig 1*E*). Their parents are first cousins of Iraqi origin, are unaffected (Fig 1*E*) and have no family history of other hair disorders. The affected child was born without hair and was not ritually shaved. Subsequently, sparse coarse hair growth was accompanied by itching, redness and roughness of the scalp, which showed prominent follicular hyperkeratosis (Fig 1*A*, *B*). The hair shafts were marked by terminal fractures and trichorrhexis nodosa but no other specific abnormalities, and hair amino acid analysis was normal.

At the age of 2 mo, the proband showed complete alopecia with follicular prominence on the scalp. By 15 mo, there was sparse, coarse, brittle hair with follicular hyperkeratosis, erythema and scaling affecting particularly the scalp, but also eyebrows and eyelashes. Now aged 5, the girl's scalp hair remains sparse and is clearly brittle, less than 1cm long at sites of friction and up to 8 cm in other areas. She now has marked follicular hyperkeratosis on the extensor aspects of the limbs. The skin is otherwise normal with no papular lesions on the limbs, and no palmoplantar keratoderma. Sweating, teeth and nails appear normal. Overall, the clinical findings are most consistent with a diagnosis of LAH.

We obtained blood samples from the two children and both parents. Genomic DNA was isolated from peripheral blood collected in EDTA-containing tubes according to standard techniques (Sambrook *et al*, 1989). All samples were collected following informed written consent of the subjects and the study was conducted in accordance with

protocols approved by the Institutional Review Board (IRB) of Columbia University and the Declaration of Helsinki Guidelines. To screen for a mutation in the human *DSG4* gene, all exons and splice junctions were PCR amplified from genomic DNA and sequenced directly in an ABI Prism 310 Automated Sequencer, using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Foster City, California), following purification in Centriflex Gel Filtration Cartridges (Edge Biosystems, Gaithersburg, Maryland) as we described earlier (Kljuic *et al*, 2003). The mutation was identified by visual inspection and comparison with control sequences generated from unrelated, unaffected individuals (Fig 2*A*).

We identified a homozygous tandem dinucleotide transversion mutation in the affected proband, which changed the GG at nucleotide positions 384–385 to a TT dinucleotide (Fig 2*A*). Both parents were heterozygous carriers of this mutation, and the unaffected brother was genetically normal. To exclude the possibility that this mutation represents a polymorphism, we screened a panel of seventy unrelated, unaffected individuals. Since the mutation destroys an *Nci*I restriction site, we digested the PCR products of exon 5 with *Nci*I and confirmed the absence of the variant from this representative sample of 140 chromosomes (data not shown). The first G at nucleotide 384 occurs in the third base of arginine codon 128, and is therefore a silent mutation (CGG to CGT). The second G at nucleotide 385, however, occurs in the first base of the neighboring alanine codon 129, resulting in the substitution of alanine by serine (GCT to TCT). This missense mutation, designated A129S, occurs within the cadherin interaction sequence R-A-L, and converts it to R-S-L. This region of DSG4 is thought to be critical for cadherin–cadherin dimerization interactions between cells based on data from classical cadherins (Boggon *et al*, 2002; Patel *et al*, 2003) and is thus predicted to be essential for cell–cell adhesion.

Cadherins present on the surface of opposing cells must dimerize in order to mediate adhesion. X-ray crystallography experiments have revealed the atomic-level mechanism of adhesion for type I cadherins, which interact by swapping the N-terminal β-strand between partner EC1 domains. Of note, all critical residues for these interactions, including A129 of DSG4 (Fig 2*B*), are conserved between the classical and desmosomal cadherins (Patel *et al*, 2003). The side chain of Trp 2, within the swapped β-strand, serves as a key anchoring residue which becomes buried in a core pocket of the partner that is lined by residues A78 and A80 (numbering for the mature N-cadherin; Fig 2*C*) where the latter corresponds to the position of the A129S mutation in DSG4. The small size of the alanine side chain, and its hydrophobic character, are important for interactions with the Trp 2 side chain from its adhesive partner. Thus, we predict that the mutation A129S, which introduces a hydrophilic serine side chain might abrogate or negatively impact adhesive function. Indeed, site-directed mutagenesis of the corresponding alanine residue of N-cadherin (Fig 2*B*) to methionine has been shown to completely abrogate adhesion (Tamura *et al*, 1998). Moreover, missense mutations in this same region have recently been demonstrated in the *lah*/*lah* mouse (Kljuic *et al*, 2003) and the *lah*/*lah* rat (Jahoda *et al*, 2004), which are believed to disrupt the DSG4 interaction interface and lead to a similar ultrastructural defect observed in the torn desmosomes of the epidermis of the *Dsg4* null *lah*^{J} mouse (Kljuic *et al*, 2003). The identification of A129S in this family represents the first mutation in human LAH that is distinct from the common Pakistani deletion allele of exons 5–8. Since the patients identified to date present with very similar clinical features, our findings suggest that raising the awareness of LAH as a differential diagnosis for hypotrichosis will reveal additional cases and further define an allelic series of mutations in human *DSG4* in addition to the existing mouse and rat models (Meyer *et al*, 2004).

Andrew G. Messenger,* Hisham Bazzi,† Richard Parslew,‡ Lawrence Shapiro,§ and Angela M. Christiano†
*Department of Dermatology, Royal Hallamshire Hospital, Sheffield, UK; †Departments of Dermatology and Genetics & Development, Columbia University, New York, New York, USA; ‡Department of Dermatology, Royal Liverpool Children's NHS Trust, Liverpool, UK; §Departments of Biochemistry & Molecular Biophysics and Ophthalmology, Columbia University, New York, New York, USA

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Address correspondence to: Angela M. Christiano, PhD, Department of Dermatology, Columbia University, College of Physicians & Surgeons, 630 West 168th Street VC-1526, New York, NY 10032, USA. Email: amc65@columbia.edu

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