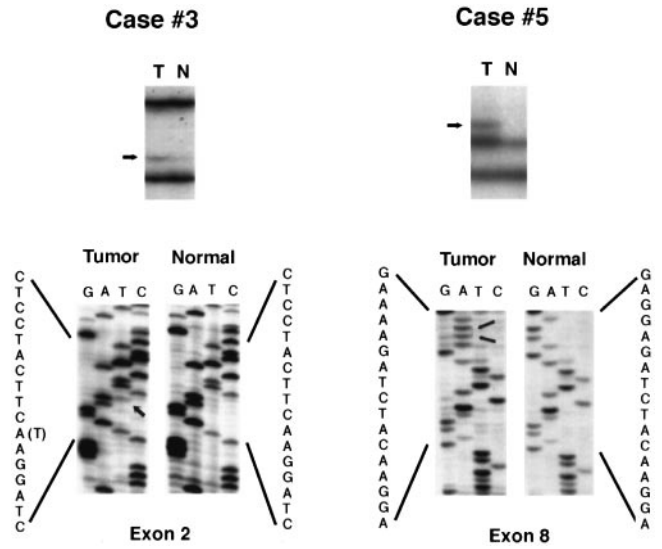

Figure 2. SSCP and sequencing analysis of angiofibromas (case 3, left; case 5, right). Both cases show aberrant bands on the SSCP gels (*arrows*; T, tumor tissue; N, normal control tissue). In addition to the aberrant bands, strong wild-type bands are present in both tumors. Sequencing analysis shows a A→T transition at nucleotide 517 (AAG to TAG; case 3) and a transversion of GG→AA at nucleotide 1184–5 (GAG GAG to GAA AAG; case 5), on exons 2 and 8.

with normal somatic cells was implicated for the negative results in a recent study that failed to detect LOH in three angiofibromas from MEN1 patients (Dong *et al*, 1997).

The identification of distinct somatic MEN1 mutations in sporadic (non-MEN1 associated) angiofibroma suggests a role for the MEN1 gene product not only in MEN1 patients, but also in the initiation and/or progression of a subset of sporadic angiofibromas.

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Erythropoietic Protoporphyria: A New Mutation Responsible for Exon Skipping in the Human Ferrochelatase Gene

To the Editor:

Erythropoietic protoporphyria is an inherited disorder associated with intense light sensitivity caused by the diminished activity of the enzyme ferrochelatase. It catalyzes the last step in haem biosynthesis by inserting ferrous ion into protoporphyrin IX. A reduction in enzyme activity of 30%–50% in different tissues is characteristic in patients and produces an accumulation of protoporphyrin IX in erythrocytes, serum, liver, and feces followed by lethal obstructive hepatic failure in some cases (Todd, 1994). The ferrochelatase gene was cloned and localized to chromosome 18q 21.3. Since then several mutations have been published with different forms of transmission, demonstrating the heterogeneity of the molecular pathology responsible for erythropoietic protoporphyria. Discovering new mutations and correlating these with the biochemical data may help in predicting the clinical course of the disease and would allow intervention to combat any possibly lethal consequences.

A blood sample was taken from a 35 y old man who has been suffering from burning sensations, pain, and iching since his early childhood, and from time to time the occurrence of small vesicles after exposure to sun light. His parents are symptomless and there is no history of photosensitivity in other family members. Physical examination revealed orange peel-like skin on his forehead, nose, and hands and his red blood cells showed red fluorescence under UVA illumination due to raised protoporphyrin levels. Its concentration in erythrocyte was 432 μg per 100 ml RBC and ferrochelatase activity in periferal lymphocytes was decreased to 35% of normal levels (Nunn *et al*, 1988). He has taken beta-carotene with slight benefical effects.

Peripheral blood samples from the probands' father, mother, sister, and 11 y old daughter were also collected and the lymphocytes isolated. Total RNA and DNA were extracted from them and complementary DNA synthetized by reverse transcriptase polymerase chain reaction using the forward primer, p1 (5′-GAGGCTGCCCAGGCAA-3′; Nakahashi *et al* 1993) and the reverse primer, p2 (5′ -TTTTCAACT-CCACACTCC-3′). The latter was designed with the help of the GCG computer program and allows amplification of most of the amino acid coding region (1105 bp). After purifying the polymerase chain reaction product, chemical cleavage of mismatch analysis was carried out to identify the site of the deletion. Asymmetric polymerase chain reactions were subsequently carried out and chain termination DNA sequencing was performed to characterize the mutation. The boundaries of exon 2 were amplified with primer sets 3 (Wang *et al*, 1994) using genomic DNA as the template.

The proband's amplified cDNA was found to be ≈100 bp shorter than that from the control (**Fig 1*A***, II.2). Polymerase chain reactions, using nested primers, showed that the site of the deletion was near the 5′-end of the cDNA (data not shown). Direct sequencing of the cDNA using the p1 forward primer subsequently revealed that exon 2 (127 bp) was missing (**Fig 1*B***). The boundaries of exon 2 in the

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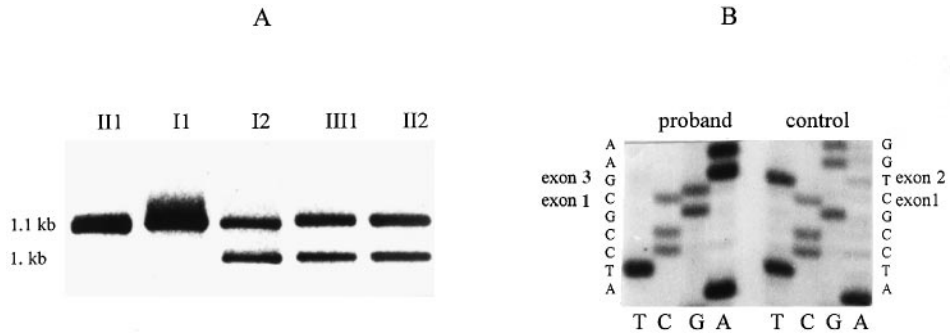

Figure 1. Exon 2 skipping in the human ferrochelatase cDNA. (*A*) Three per cent agarose gel electrophoresis of reverse transcriptase polymerase chain reaction-amplified ferrochelatase mRNA. The proband (II2), his mother (I2), and his daughter (III1) exhibit a normal polymerase chain reaction product (1.1 kb) and a short product, indicating the loss of ≈100 bp of cDNA. (*B*) Ferrochelatase cDNA sequence showing the loss of exon 2 in the patient.

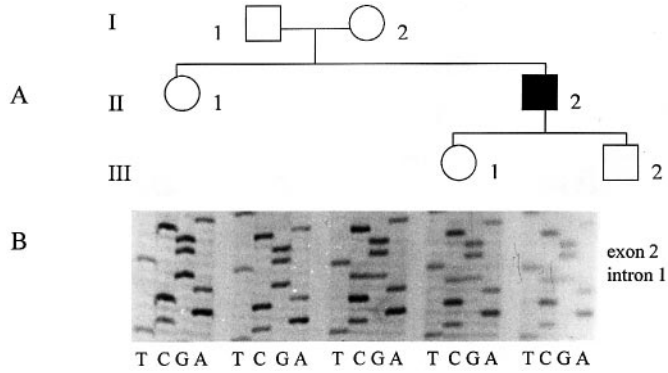

Figure 2. Identification of the mutation among the members of the family. (*A*) Pedigree. Proband with clinical symptoms is indicated by black shading. (*B*) Direct sequencing of intron 1/exon 2 boundary of ferrochelatase gene showing the G→C tranversion at IVS1–1 in the proband II2, his daughter III1, and his mother I2.

genomic DNA were therefore sequenced to determine whether or not a splice mutation was responsible for the loss of exon 2. The results showed that the mutation is indeed a G→C transversion at the acceptor site, IVS1–1, and has not been previously reported. The same mutation, but showing incomplete penetrance, was found in his mother and daughter (**Fig 2**, I2, III1).

An IVS1–23 C→T mutation was also found in the proband, his relatives (I1, II1, and III1) without the IVS1–1 G→C mutation, and two other unrelated erythropoietic protoporphyria patients (data not shown). This mutation was reported previously as a possible cause of exon 2 skipping (Nakahashi *et al*, 1992), but later refuted (Wang *et al*, 1994) when it was found in all erythropoietic protoporphyria patients and in some controls, suggesting that it may play some role in the pathogenesis of erythropoietic protoporphyria.

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Is Human Papillomavirus Type 5 the Putative Autoantigen Involved in Psoriasis?

To the Editor:

While it is well recognized that psoriasis is a T cell-mediated inflammatory disease that results in epidermal proliferation (Griffiths and Voorhees, 1996), the identity of the antigen(s) responsible for T cell activation is still a matter of debate, as recently discussed by Nickoloff and Wrone-Smith (1998). A strong correlation has been found between acute guttate psoriasis and streptococcal infections (Baker *et al*, 1993), and it has been proposed that some bacterial surperantigens or antigens might activate T cells (Telfer *et al*, 1992; Baker *et al*, 1993; Valdimarsson *et al*, 1995; Boehncke, 1996; Norris *et al*, 1997). It was also hypothesized that superantigen-activated T cells might induce abnormal expression of keratin variants that show close homology with streptococcal M protein, and might stimulate M protein-specific autoreactive T cells (Valdimarsson *et al*, 1995). It should be stressed, however, that although T cells specific for M protein have been detected in patients with psoriasis, they have as yet not been shown to cross-react with any skin components.

Based on the model of human skin grafted onto SCID mice, it was recently postulated that there are two steps in the autoimmune pathways to psoriasis (Nickoloff and Wrone-Smith, 1998). The first step is considered to be a polyclonal activation of Vβ restricted $CD4^+$ T cell subsets by some bacterial superantigens (Boehncke *et al*, 1996; Wrone-Smith and Nickoloff, 1996). These activated T cells, injected intradermally into the grafted uninvolved psoriatic skin, induced histologic changes

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