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Extensive size polymorphism of the human keratin 10 chain resides in the C-terminal V2 subdomain due to variable numbers and sizes of glycine loops

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ABSTRACT **Existing data suggest that the human keratin 10 intermediate filament protein is polymorphic in amino acid sequence and in size. To precisely define the nature of the polymorphism, we have used PCR amplification and sequence analyses on DNA from several individuals including five with documented size variations of the keratin 10 protein. We found no variation in the N-terminal or rod domain sequences. However, we observed many variations in the V2 subdomain near the C terminus in glycine-rich sequences with a variation of as much as 114 base pairs (38 amino acids), but all individuals had either one or two variants. Our results show that (*i*) the keratin 10 system is far more polymorphic than previously realized, (*ii*) the polymorphism is restricted to insertions and deletions of the glycine-rich quasipeptide repeats that form the glycine-loop motif in the C-terminal domain, (*iii*) the polymorphism can be accounted for by simple allelic variations that segregate by normal Mendelian mechanisms, and (*iv*) the differently sized PCR products most likely represent different alleles of a single-copy gene per haploid genome.**

Keratins form a large family of cytoplasmic intermediate filaments (IFs). The more than 30 known keratin chains are differentially expressed along the different pathways of epithelial cell development and differentiation. They are subdivided into acidic type I and neural-basic type II chains. Most epithelial cells express a particular pair of type I and type II chains that assemble into keratin IFs characteristic of that cell type (1–6). For example, terminally differentiating epidermal keratinocytes express keratins 1 (type II) and 10 (K10; type I).

Keratins, like all other IF proteins, have a central α-helical rod domain of conserved secondary structure. The rod domain is flanked on both sides by end domains that display particular patterns that permit their classification into subdomains. These subdomains are the high homology or H subdomains, regions with sequence variation or V subdomains, and highly charged ends or E subdomains (1, 2, 7). For K10, the rod domain is flanked on the N-terminal end by E1, V1, and H1 subdomains and on the C-terminal side by V2 and E2 subdomains (Fig. 1). Although the principles of the structural organization of the rod domain and its possible function during IF assembly are largely known (9–11), little is known about the structure and function of the end domains of the IFs. In comparison to most other keratins, the E and V subdomains of epidermal keratins 1 and 10 are unusually glycine-rich (8, 12–14) and, therefore, likely to adopt a distinct configuration and conceivably possess a unique function (15). Notably, solid-state NMR experiments have

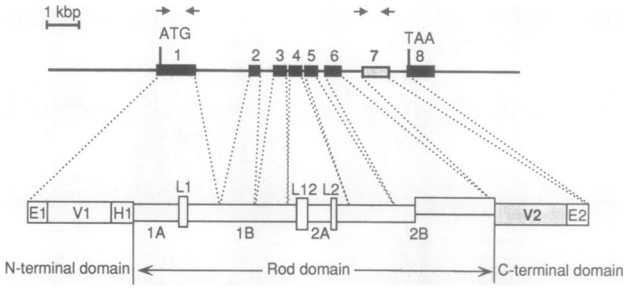

FIG. 1. Gene and protein structure of human K10. The upper line shows the gene structure of the human K10 gene (ghK10BK) characterized in this work. The exons are drawn as solid or stippled boxes and numbered 1–8. The locations of the ATG site and the TAA stop codon are indicated. The lower line shows the subdomain organization of the K10 protein. The central α-helical rod domain with its subdomains 1A, 1B, 2A, and 2B and linkers L1, L12, and L2 is flanked on both sides by end domains. The N-terminal domain consists of the E1, V1, and H1 subdomains; the C-terminal domain contains the V2 and E2 subdomains (for details see refs. 1, 7, and 8). All of the allelic variants we have found are restricted to exon 7 encoding the V2 subdomain (stippled boxes). The dotted lines denote the protein subdomain encoded by the exons. The small arrows above the upper line show the positions of the two sets of primers used for the PCR amplification of the DNA regions encoding the N and C termini of human K10.

shown that these glycine-rich regions are highly flexible (2, 16). These glycine-rich sequences follow the form $X(Y)_n$ where X is usually an aromatic or rarely a long-chain aliphatic residue, Y is usually glycine but may also include polar residues, and n is highly variable representing 1–35 residues (13). They are arranged as quasirepetitive peptides and we have proposed that these glycine-rich sequences form a structural protein motif that we have termed the glycine loop (17).

Two versions of the human K10 sequence have been published (8, 14) that show significant sequence variations only in the glycine-rich quasipeptide repeats of the V2 subdomain. Furthermore, comparisons with the mouse (18, 19) and bovine (20, 21) equivalents of K10 showed similar variations in this region. Interestingly, size polymorphism of the human K10 has also been reported (22). Indeed, four polypeptide chains of different apparent molecular mass have been described for K10 (22). However, it is not yet clear whether these K10 variants are due to multiple genes or different alleles within the human population or are caused by differential posttranscriptional RNA processing. In this paper we address the following three major questions: (*i*) how many K10 genes are there per haploid genome, (*ii*) are there

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Abbreviations: IF, intermediate filament; K10, keratin 10.
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different alleles for the human K10 gene in the human population, and if so, (*iii*) where do the differences reside.

MATERIALS AND METHODS

Keratin-enriched protein fractions were prepared, solubilized in sample buffer [0.5 M Tris·HCl, pH 6.8/2.1% (wt/vol) SDS/10% (vol/vol) 2-mercaptoethanol/10% (vol/vol) glycerol/0.05% bromophenol blue], and separated by one-dimensional SDS/PAGE according to Laemmli (23) as described (6, 22). Coomassie blue-stained epidermal keratins were quantitated with an LKB laser densitometer. For immunoblot analysis, proteins from unstained gels were transferred by semidry blotting onto nitrocellulose sheets (24) and incubated with the polyclonal rabbit anti-keratin antiserum 6-2/1 preferentially reactive to acidic keratins as described (6, 22).

High molecular weight genomic DNA was isolated according to Maniatis *et al.* (25). Briefly, tissue samples obtained from squamous cell carcinomas of the head and neck, normal interfollicular epidermis, and 12 whole foreskins from normal individuals were either ground to a fine powder under liquid nitrogen or cut into small pieces, digested with proteinase K (200 μg/ml), and phenol-extracted. Finally, the precipitated DNA was dissolved in water.

Using an EMBL-3 library and our specific K10 cDNA clone (8), we isolated a 13.6-kilobase-pair (kbp) genomic fragment (ghK10BK) containing the human K10 gene. This genomic clone was used as a control in the PCR experiments.

PCR amplification of the N- and C-terminal end domains of the human K10 gene was performed according to the manufacturer's protocol (Perkin–Elmer) using specific primers (for location of primers see Fig. 1) located in conserved sequences as detailed in Figs. 3 and 4. In addition, because of the high G+C content (>70%) of these sequences, we had to use conditions of very high stringency for the annealing and elongation to obtain reproducible and reliable results with our control K10 clone. This was accomplished by adding 10% glycerol and 3.5% (vol/vol) formamide to the reaction mixture. The reactions were performed for 33 cycles, each cycle consisting of 1 min at 95°C and 1.5 min at 72°C. PCR products were either separated on a 3% NuSieve 3:1 agarose gel (FMC) or an 8% polyacrylamide gel according to Maniatis *et al.* (25). Some of the PCR products were subcloned into the pGEM-3Z vector (Promega) for DNA sequencing.

RESULTS AND DISCUSSION

The Published K10 DNA Sequences Are at Variance. Comparison of the two published versions of the human K10 sequence (8, 14) showed significant variations with respect to insertions and deletions of glycine-rich sequences in the C-terminal domain. There were only minor nucleotide replacements in sequences encoding other portions of the K10 chain that did not result in net insertions or deletions. To clarify these variations, we isolated a genomic clone containing 4.2 kbp of the entire K10 gene as well as 5.6 kbp of 5′ upstream and 3.8 kbp of 3′ flanking sequences (Fig. 1). Surprisingly, our genomic clone displayed yet another sequence organization within the C-terminal V2 subdomain with respect to numerous insertions and deletions of glycine-rich quasipeptide repeats (17), apparently conveying a polymorphic situation to the K10 gene.

Polymorphism of K10 Polypeptides. Analyses of epidermal keratin proteins have revealed pronounced interindividual variations for keratins 1, 5, and 10 that are manifested by related protein chains showing slight differences in electrophoretic mobility (22, 26, 27). With respect to K10, a total of four variants were detected and designated as variants K10a (Fig. 2 *a* and *d*), K10b (Fig. 2 *b* and *c*), K10c (Fig. 2*c*), and

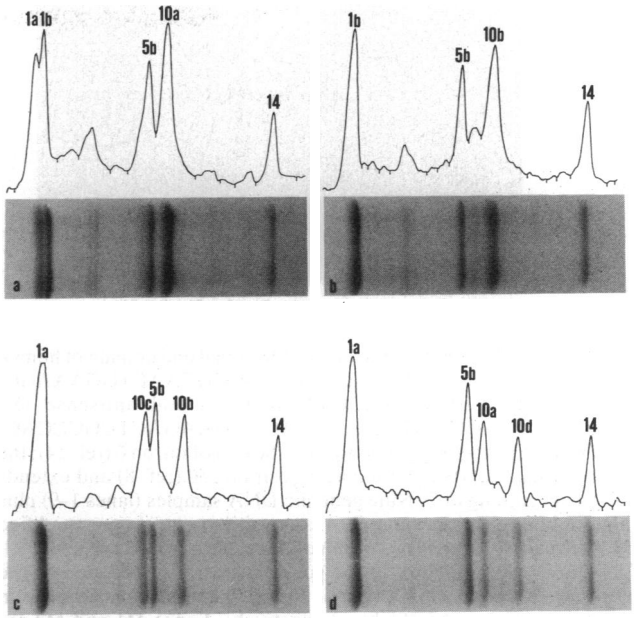

FIG. 2. Densitometry of Coomassie blue-stained epidermal keratins separated by SDS/PAGE. Each gel track was scanned at different positions using an LKB laser densitometer equipped with an HP 3390 integrating printer plotter. Mean integrals for the K10 alleles were 28.4% (K10a) (*a*), 26.3% (K10b) (*b*), 14.5% (K10c) and 16.4% (K10b) (*c*), and 12.6% (K10a) and 13.4% (K10d) (*d*). The various keratin alleles are named as suggested (22). Note that the heterozygous samples contain approximately equal amounts of the two alleles, which are approximately half of the amount of the homozygous allele.

K10d (Fig. 2*d*) due to the immunorecognition pattern with anti-keratin antibodies (compare Fig. 5) and their similar charge characteristics on two-dimensional gels (data not shown and ref. 22).

Laser densitometric quantitation of the K10 variants indicated a 1:1 ratio of the doublet polypeptides (Fig. 2 *c* and *d*), whereas the single bands were grossly double in intensity (Fig. 2 *a* and *b*). These data likewise suggested that the K10 gene is polymorphic and that at least four codominantly expressed alleles exist within the human population. Indi-

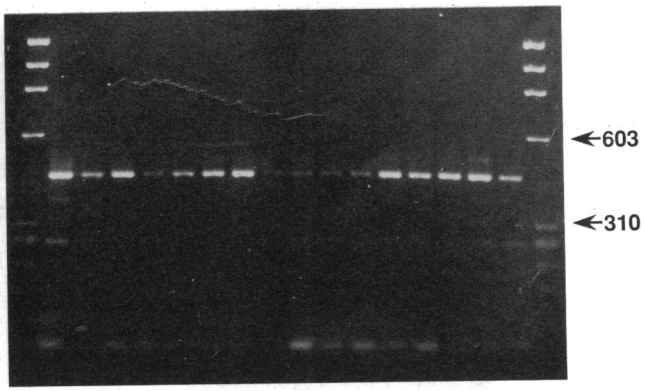

FIG. 3. PCR amplification of the N-terminal end domain of human K10. The primers used were sense (5′-CCCGCAGTGGAGGAGGAGGAGGAGG-3′) and antisense (5′-CCAAGTAGGAAGCCAGGCGGTCATT-3′) located between amino acid position 14 of the E1 subdomain and amino acid position 145 of the beginning of the rod domain segment 1A (8). Sixteen genomic DNA samples were amplified and electrophoretically separated on a 3% agarose gel. The first lane to the left of the marker lane on the right is the positive control of the 13.6-kbp K10 gene clone (ghK10BK). Bands at 603 and 310 bp are indicated.

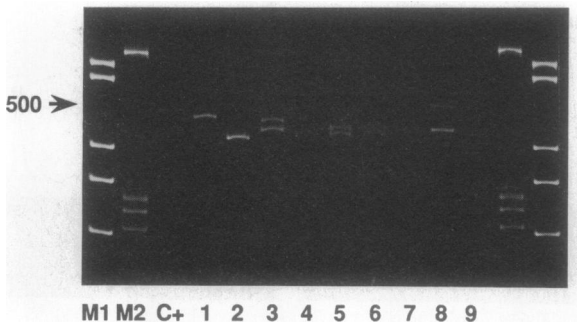

FIG. 4. PCR amplification of the C-terminal end domain of human K10. The primers used were sense (5′-CGGCAACTGGAAAGC-TACCCACCGTTTTCCAACGTTAGAG-3′) and antisense (5′-TTTCTGCTGACCTTGGTCCCTTAGATGAAGACTCGCCCAC-3′). The first primer is located toward the end of intron 6 (ref. 14); the second primer starts at amino acid position 550 (ref. 8) and extends into the beginning of 7. Nine genomic DNA samples (lanes 1–9) plus the 13.6-kbp K10 clone (lane C+) as a positive control were amplified by PCR and separated on a 8% nondenaturing polyacrylamide gel. The known size (500 bp) for the positive control is indicated on the left side. By sequencing, the band in lane 4 and the smaller band in lane 8 are 443 bp and 455 bp, respectively. Lanes M1 and M2 are molecular size markers, *Hin*fI-digested ϕX174 and a 1-kilobase DNA ladder (GIBCO/BRL), respectively.

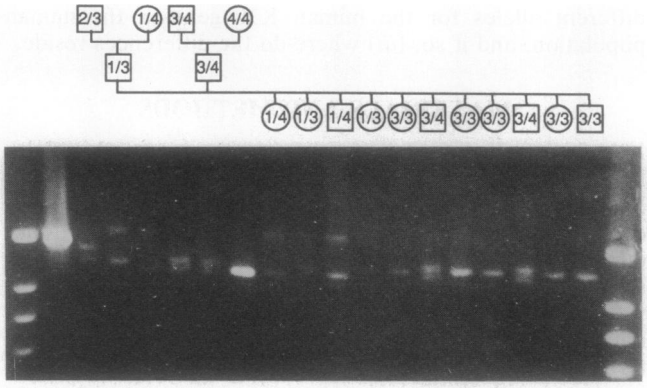

FIG. 6. Mendelian segregation of human K10 size alleles. DNA from transformed lymphocytes of a three-generation kindred family (Centre d'Etude du Polymorphisme Humain cell lines; ref. 28) was used for PCR amplification of the C-terminal end domain of K10 as above. Both the gel data (3% agarose gel) and pedigree distribution are shown. The pedigree is aligned to the gel lanes. The four alleles present in this family are numbered 1–4 according to decreasing size. The two outer lanes represent the molecular size markers (1-kilobase ladder). The second lane from the left shows the 13.6-kbp K10 clone (ghK10BK) as a positive control.

viduals displaying K10 doublets may, therefore, be considered heterozygous and those with one keratin variant may be considered homozygous for the respective alleles.

All this has then prompted us to undertake a systematic analysis of the human K10 gene to determine whether its polymorphism is restricted to the glycine-rich C-terminal V2 subdomain or whether it might also include the N-terminal glycine-rich V1 subdomain of the K10 chain.

PCR Amplification Shows Extensive Size Polymorphism in the V2 Subdomain Rather than the V1 Subdomain of the K10 Gene. Using specific primers, we have analyzed the N and C termini of K10 by PCR amplification. Within the N-terminal domain spanned by the primers, no size variations were detected for the 16 genomic DNA samples tested (Fig. 3). In contrast, the PCR products obtained for the entire C-terminal domain of the K10 gene with these samples manifested a complex picture and displayed considerable size variations (Fig. 4). In addition to four individuals displaying only one, albeit differently sized bands, several others had two bands of equal intensity. Because in no case were more than two bands seen this strongly, these differently sized PCR products seem to represent different alleles of one single-copy gene per haploid genome.

To test this conclusion, we analyzed genomic DNA from those individuals with established K10 protein chain patterns. Individual 1 had protein isoform K10a (Fig. 5 *a* and *b*), but at the DNA level two closely spaced bands could be distinguished for the amplified DNA (Fig. 5*c*). Hence, this individual is heterozygous for two allelic variants that are too small to resolve at the protein level. Individuals 2 and 5 had protein isoform K10b, which appears as a single DNA allele that is slightly less than K10a. Individual 3 has protein isoforms K10a and K10b and has the two DNA bands that correlate to the former individuals. Finally, individual 4 has protein isoforms K10a and K10d and has the two DNA bands of expected size. These data establish a direct correlation between our earlier data (22) of K10 isoform sizes at the protein level with the allelic size variants of amplified DNA in this study.

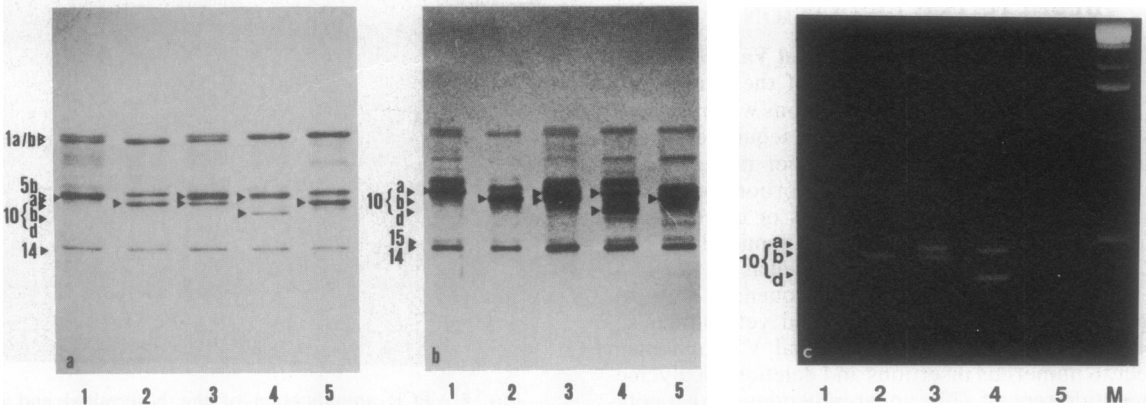

FIG. 5. Electrophoretic separation of K10 alleles at the protein and gene levels. (*a*) Coomassie blue-stained gel (SDS/PAGE) of epidermal keratins from various individuals. (*b*) Corresponding immunorecognition pattern using a polyclonal rabbit anti-keratin antiserum preferentially reactive to acidic keratins (22). (*c*) PCR amplification of the C-terminal end domain by using genomic DNA from the same individuals. The patterns shown in *a* and *b* display the keratins (1a plus 1b), 5b, 10a, and 14 (lanes 1); 1b, 5b, 10b, and 14 (lanes 2); (1a plus 1b), 5b, (10a plus 10b), and 14 (lanes 3); 1a, 5b, (10a plus 10d), and 14 (lanes 4); and 1a, 5b, 10b, 14 (lanes 5). The antiserum also detects keratins 14 and 15 and the differentiation-related processing products of K10. Note that keratins 5b and 10a comigrate under these conditions. The PCR products in *c* show that the number of bands and the size variations found correspond with changes found for the K10 proteins in *a* and *b*. The molecular size markers (lane M) used in *c* are the 1-kilobase DNA ladder (GIBCO/BRL).

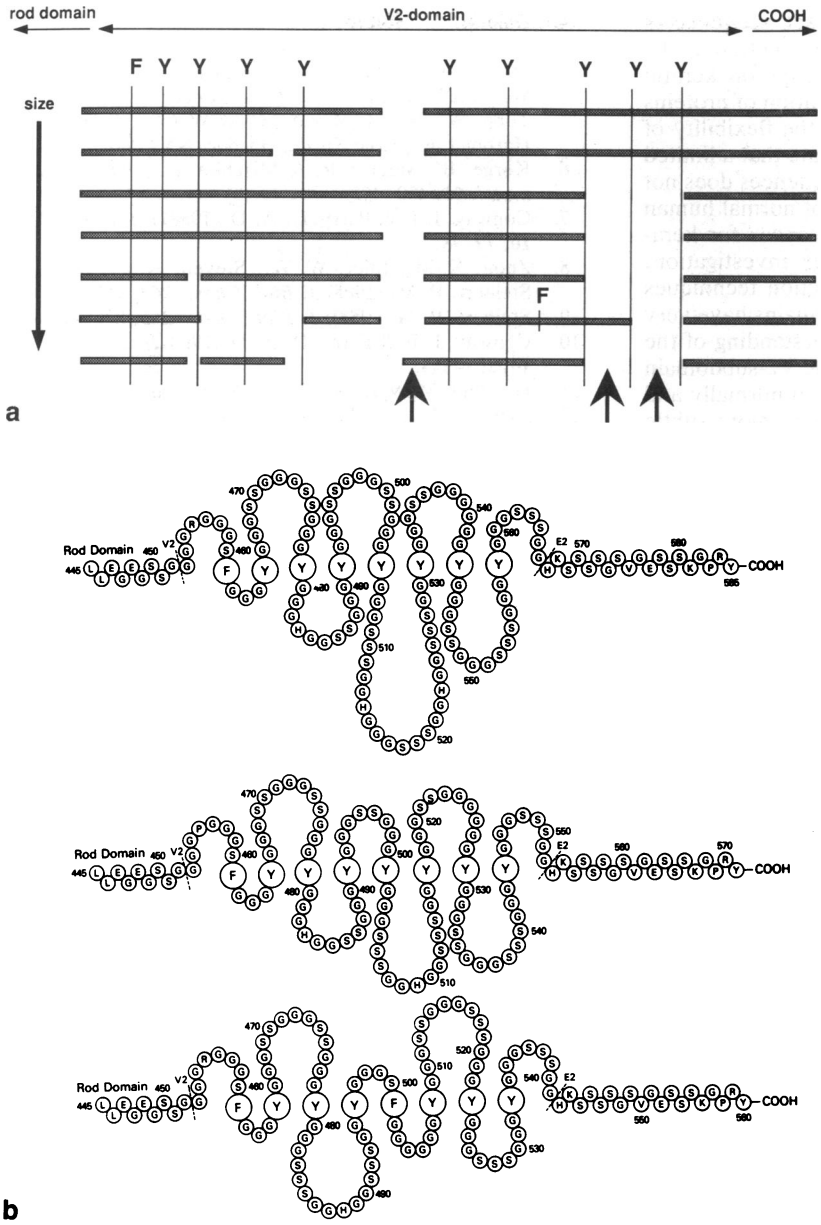

FIG. 7. Insertions and deletions in the V2 domain of human K10. (*a*) This scheme summarizes the insertions and deletions of seven allelic variants so far characterized. The positions of the aromatic residues (F, phenylalanine; Y, tyrosine) are shown. From the present sequencing data, the beginning and the end of the V2 domain are conserved, and the deletions found most often occur at the three regions marked by the arrows. (*b*) This drawing shows a two-dimensional representation of the glycine-loop motif present in the V2 subdomain of three of the known human K10 alleles based on our recent model for such sequences (17). Although the beginning and the end of the V2 subdomain do not show alterations, in the middle part, insertions and deletions of G+C-rich sequences cause striking variations of the size of glycine loops among different variants.

To characterize these size variations in detail, we subcloned some of the PCR products from heterozygotic individuals for sequencing. All the sequence variations were encountered only in the V2 subdomain of the C terminus. Seven alleles could be identified that had V2 subdomains ranging in size from 401 to 515 base pairs (bp). This range of 114 bp corresponds to 38 amino acids or ≈3 kDa at the protein level, a result very much in accordance with the estimated molecular mass of the respective K10 chains (K10a = 56.0 kDa, K10b = 55.0 kDa, K10c = 58.2 kDa, and K10d = 54.0 kDa; Fig. 2).

By use of PCR analysis of genomic DNA samples, which inherently has greater resolution than protein gels, it is, therefore, clear that the K10 gene system is far more polymorphic than previously realized.

Inheritance of the K10 Sequence Polymorphism. To elucidate the inheritance characteristics of the K10 polymorphism, we used the same PCR technique to amplify the V2 subdomain of the 17 members from one Centre d'Etude du Polymorphisme Humain family (28). A total of four differently sized alleles (labeled 1–4 according to decreasing size) could be distinguished within the family, each individual being either homozygous for one or heterozygous for a pair of alleles present in the parents (Fig. 6). Consequently, these results demonstrate unambiguously that the size polymorphism of the K10 gene is inherited as a normal Mendelian trait and thus provide additional evidence that there are different alleles rather than multiple genes present in the human genome.

Location of Insertions and Deletions. Fig. 7*a* summarizes the location of the insertions and deletions found in the V2 subdomain of the human K10 gene. Apparently, the beginning and the end of the V2 subdomain do not show sequence variations, whereas the deletions occurred most often at the three regions marked by arrows. These alterations either change the size of the glycine loop between two aromatic residues or delete one or two entire glycine loops including an aromatic residue. As an example, Fig. 7*b* illustrates the size variations for three K10 alleles in a two-dimensional representation of the glycine-loop motif. For the two smallest alleles detected so far, there have been multiple sites of deletions in the middle part with respect to the larger alleles.

Our data thus establish that the K10 polymorphism is due to insertions and deletions of glycine-loop quasipeptide repeats in the V2 subdomain. This finding provides strong evidence for the opinion that the general structural feature of

the glycine-loop motif rather than its exact sequence is essential for the maintenance of the proper function of the K10 protein (17). We think that glycine loops on keratin chains are involved in the structural organization of proteins within epidermal cells and maintenance of the flexibility of the entire epidermis (17, 29). Our data indicate that a limited degree of variation in these flexible-loop sequences does not alter the physiology or structural integrity of normal human epidermis. The pathophysiological consequences for keratinizing disorders, however, merit further investigation. Moreover, as recent work using microinjection techniques has shown that IF chains of different end domains have very distinct functions in cells (30, 31), the understanding of the structural and functional consequences of the V2-subdomain polymorphism on cytoskeletal organization in normally and abnormally keratinizing epidermis will require more subtle approaches than the simple deletion experiments performed so far (32, 33).

Genetic Implications of the K10 Polymorphism. These data demonstrate the presence of a trinucleotide repeat polymorphism [that is, $(GGC)_n$, encoding the frequent consecutive glycines] within the coding sequence of a gene unlike the usual intergenic trinucleotide repeats (34). This DNA polymorphism involving the K10 gene also provides a method to explore the potential involvement of the gene in familial skin disorders by genetic linkage analysis. This is a particularly attractive possibility since there are already many different size alleles within the few individual samples analyzed in Figs. 4–6, therefore suggesting a relatively low frequency of each allele in the population. Since the family of type I keratins is located at chromosome region 17q21, as shown by the use of specific cDNA clones in conjunction with somatic cell hybrid analysis and *in situ* hybridization (35), the presence of any recombinants between a skin disease susceptibility and this marker will be diagnostic in excluding K10 and other closely linked type I keratins within this cluster as the cause of the disease. Hence, definitive information can be obtained from linkage analyses of even small families in which the disease gene is segregating. Demonstration of involvement of a defect in the K10 gene in a skin disease by linkage analysis will be particularly important prior to molecular analysis of the defective gene since most molecular variations in K10 genes will presumably simply represent the common polymorphism described here.

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1. Steinert, P. M. & Roop, D. R. (1988) *Annu. Rev. Biochem.* **57**, 593–625.
2. Steinert, P. M., Torchia, D. R. & Mack, J. W. (1988) in *The Biology of Wool and Hair*, eds. Rogers, G. E., Reis, P. J., Ward, K. A. & Marshall, R. C. (Chapman & Hall, London), pp. 157–167.
3. Moll, R., Franke, W. W., Schiller, D. L., Geiger, B. & Krepler, R. (1982) *Cell* **31**, 1–24.
4. Dale, B. A., Holbrook, K. A., Kimball, J. R., Hoff, M. & Sun, T.-T. (1985) *J. Cell Biol.* **101**, 1257–1269.
5. Sun, T.-T., Eichner, R., Schermer, A., Cooper, D., Nelson, W. G. & Weiss, R. A. (1984) in *Cancer Cells*, eds. Levine, A., Topp, W., Van de Woude, G. & Watson, J. W. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), Vol. 1, pp. 169–176.
6. Korge, B., Stadler, R. & Mischke, D. (1990) *J. Invest. Dermatol.* **95**, 450–455.
7. Conway, J. F. & Parry, D. A. D. (1988) *Int. J. Biol. Macromol.* **10**, 79–98.
8. Zhou, X.-M., Idler, W. W., Steven, A. C., Roop, D. R. & Steinert, P. M. (1988) *J. Biol. Chem.* **263**, 15584–15589.
9. Steinert, P. M. (1990) *J. Biol. Chem.* **265**, 8766–8774.
10. Conway, J. F. & Parry, D. A. D. (1990) *Int. J. Biol. Macromol.* **10**, 328–334.
11. Hatzfeld, M. & Weber, K. (1990) *J. Cell Biol.* **110**, 1199–1210.
12. Johnson, L. D., Idler, W. W., Zhou, X.-M., Roop, D. R. & Steinert, P. M. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 1896–1900.
13. Steinert, P. M., Parry, D. A. D., Idler, W. W., Johnson, L. D., Steven, A. C. & Roop, D. R. (1985) *J. Biol. Chem.* **260**, 7142–7149.
14. Rieger, M. & Franke, W. W. (1988) *J. Mol. Biol.* **204**, 841–856.
15. Steinert, P. M., Steven, A. C. & Roop, D. R. (1985) *Cell* **42**, 411–419.
16. Mack, J. W., Torchia, D. A. & Steinert, P. M. (1988) *Biochemistry* **27**, 5418–5426.
17. Steinert, P. M., Mack, J. W., Korge, B., Gan, S.-Q., Haynes, S. R. & Steven, A. C. (1991) *Int. J. Biol. Macromol.* **13**, 130–139.
18. Steinert, P. M., Rice, R. H., Roop, D. R., Trus, B. L. & Steven, A. C. (1983) *Nature (London)* **302**, 794–800.
19. Krieg, T. M., Schafer, M. P., Cheng, C. K., Filpula, D., Flaherty, P., Steinert, P. M. & Roop, D. R. (1985) *J. Biol. Chem.* **260**, 5867–5870.
20. Jorcano, J. L., Rieger, M., Franz, J. K., Schiller, D. L., Moll, R. & Franke, W. W. (1984) *J. Mol. Biol.* **179**, 257–281.
21. Rieger, M., Jorcano, J. L. & Franke, W. W. (1985) *EMBO J.* **4**, 2261–2267.
22. Mischke, D. & Wild, G. (1987) *J. Invest. Dermatol.* **88**, 191–197.
23. Laemmli, U. K. (1970) *Nature (London)* **277**, 680–685.
24. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354.
25. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
26. Wild, G.-A. & Mischke, D. (1986) *Exp. Cell Res.* **162**, 114–126.
27. Mischke, D., Wille, G. & Wild, G. A. (1990) *Am. J. Hum. Genet.* **46**, 548–552.
28. White, R. L., Lalouel, J.-M., Nakamura, Y., Daus-Keller, H., Green, P., Bowden, D. W., Mathew, C. G. P., Easton, D. F., Robson, E. B., Morton, N. E., Gusella, J. F., Haines, J. L., Retief, A. E., Kidd, K. K., Murray, J. C., Lathrop, G. M. & Cann, H. M. (1990) *Genomics* **6**, 393–412.
29. Hohl, D., Mehrel, T., Lichti, U., Turner, M. L., Roop, D. R. & Steinert, P. M. (1991) *J. Biol. Chem.* **266**, 6626–6636.
30. Vikstrom, K. L., Borisy, G. G. & Goldman, R. D. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 549–553.
31. Miller, R. K., Vikstrom, K. & Goldman, R. D. (1991) *J. Cell Biol.* **113**, 843–855.
32. Albers, K. & Fuchs, E. (1987) *J. Cell Biol.* **105**, 791–806.
33. Lu, X. & Lane, E. B. (1990) *Cell* **62**, 681–696.
34. Kremer, E. J., Pritchard, M., Lynch, M., Yu, S., Holman, K., Baker, E., Warren, S. T., Schlessinger, D., Sutherland, G. R. & Richards, R. I. (1991) *Science* **252**, 1711–1714.
35. Lessin, S. R., Huebner, K., Isobe, M., Croce, C. M. & Steinert, P. M. (1988) *J. Invest. Dermatol.* **91**, 572–578.