Am. J. Hum. Genet. 46:548–552, 1990

Allele Frequencies and Segregation of Human Polymorphic Keratins K4 and K5

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Summary

Two electrophoretic variants for each of the human keratins K4 and K5 that are expressed in squamous nonkeratinizing epithelia lining the upper digestive tract could be distinguished on SDS-PAGE. Based on a sampling size of 1,299 unrelated individuals, calculation of allele frequencies showed the alleles to be in Hardy-Weinberg equilibrium. The genetic basis of this variation was confirmed by both quantitative gene dosage dependence and the transmission of the variants as Mendelian traits in two families. Thus the human keratin genes K4 and K5 are polymorphic, and each presents with two codominant alleles (a and b).

Introduction

Intermediate filaments are protein polymers which, together with actin filaments and microtubules, form the cytoskeleton of animal cells. In epithelial cells the intermediate filaments are made up by keratins, a large family of related polypeptides, whose patterns of expression vary not only with the cell type but also with the route and stage of epithelial differentiation.

In humans there are more than 20 different keratins encoded by at least as many differentially expressed genes (for reviews, see Sun et al. 1984; Franke 1987; Fuchs 1988). They can be subdivided into two distinct classes on the basis of their relatedness (Moll et al. 1982): type I keratins (K10–K19) are small (40–56.5 kD) and relatively acidic (pI = 4.5–5.5), whereas type II keratins (K1–K9) are larger (53–68 kD) and more basic (pI = 5.5–7.5). Usually keratins are expressed as specific pairs consisting of one type I and one type II polypeptide, both of which are essential for filament formation (Hatzfeld and Franke 1985; Eichner et al. 1986) and, as such, characterize biochemically the type of epithelial differentiation. For example, K5 and K14 are synthesized in the basal cell layer of all stratified squamous epithelia, while, in the course of stratification, terminally differentiating epidermal cells express K1 and K10, and suprabasal—i.e., maturing cells of nonkeratinizing squamous epithelia—express K4 and K13 (Quinlan et al. 1985; O'Guin et al. 1987).

On the basis of the analysis of many individual specimens of human upper-digestive-tract epithelia (Wild and Mischke 1986) and epidermis (Mischke and Wild 1987), we have recently reported on interindividual variations within the respective keratin patterns as caused by electrophoretic—i.e., polymorphic—variants of keratins K1, K4, K5, and K10. In the present paper we include observations on more than 1,000 individual samples of squamous nonkeratinizing epithelium to allow for the calculation of allele frequencies of K4 and K5. In addition, both the concurrence of the observed frequencies with the frequencies predicted by the Hardy-Weinberg equation and the transmission of keratin alleles K4a/K4b and K5a/K5b as Mendelian traits lend strong support to the hypothesis that these keratin genes are polymorphic and that the variants are products of codominant alleles.

Received September 6, 1989; revision received November 2, 1989.

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0002-9297/90/4603-0018$02.00

Material and Methods

Tissues and Preparation of Keratin-enriched Protein Fractions

Palatine tonsils were obtained from 1,299 patients

(49.3% female and 50.7% male, aged 3–62 years with a predominance in the first and second decade) who underwent elective tonsillectomy. Specimens were heated at 60°C for 5 min to allow for easy dissection of the squamous nonkeratinizing epithelium lining the surface of the human upper digestive-tract (Milstone and McGuire 1981; Wild and Mischke 1986). Epithelium for family studies was obtained by scraping the mucosa of the buccal pouch with spatulas. Epithelial tissue samples were homogenized and extracted in the presence of protease inhibitors according to a method described elsewhere (Wild and Mischke 1986). The resulting Triton/high-salt-resistant pellet, highly enriched for keratins, was solubilized in sample buffer for gel electrophoresis.

Gel Electrophoresis, Immunoblot Analysis, and Densitometry

One-dimensional SDS-PAGE according to Laemmli (1970) was performed according to a method described elsewhere (Wild and Mischke 1986; Mischke and Wild 1987). Proteins from unstained gels were transferred electrophoretically onto nitrocellulose filters (Towbin et al. 1979) and incubated with a polyclonal rabbit anti-keratin antiserum exclusively immunoreactive to type II keratins (Mischke and Wild 1987). Densitometry was done with the LKB Ultroscan-XL laser densitometer and the GSXL software (version 1.64).

Results

Variation and Frequency of Human Keratins K4 and K5

Analysis of keratin-enriched cytoskeletal residues obtained from human upper-digestive-tract squamous nonkeratinizing epithelia by high-resolution SDS gel electrophoresis revealed pronounced differences among different individuals. Within the characteristic polypeptide pattern consisting of the type II keratins K4, K5, and K6 and type I keratins K13 and K14, an interindividual variation was seen for K4 and K5. This was brought about by protein bands showing a slight difference in apparent molecular mass and presenting either as a doublet (fig. 1, lanes 1–3 for K4; fig. 1, lanes 1, 4, and 7 for K5) or as a single band with the lower (fig. 1, lanes 4–6 for K4; fig. 1, lanes 2, 5, and 8 for K5) or the higher (fig. 1, lanes 7–9 for K4; fig. 1, lanes 3, 6, and 9 for K5) electrophoretic mobility.

Laser densitometric scans indicated a 1:1 ratio of the doublet polypeptides which, combined, were present in grossly the same amount as the respective single bands (fig. 2). In all, these data suggested that both

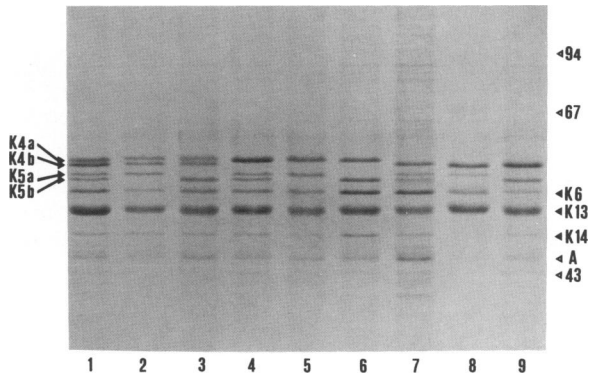

Figure 1 One-dimensional SDS-PAGE analysis of cytoskeletal proteins of human squamous nonkeratinizing epithelium, showing the interindividual variation of keratin patterns due to polymorphic keratins K4 and K5 (Coomassie blue–stained gel). All samples were obtained from the surface epithelium lining the palatine tonsils, except for the sample shown in lane 8, which is from buccal mucosa. Keratins (K) are designated according to the catalogue of human cytokeratins (Moll et al. 1982), with the suffixes a and b added to indicate the two allelic variants of K4 and K5. A denotes residual human actin, and the numbers denote the positions of the molecular-weight standards used (94 = phosphorylase b, 94 kD; 67 = BSA, 67 kD; 43 = ovalbumin, 43 kD).

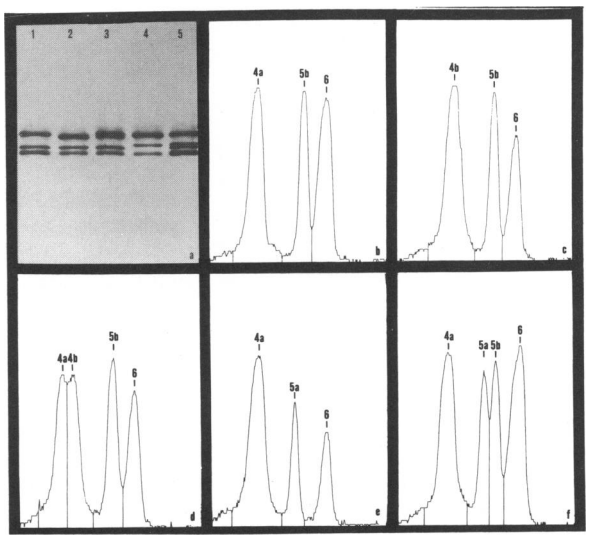

Figure 2 Densitometry of type II keratins. Cytoskeletal proteins obtained from tonsillar epithelium of five different individuals were separated by SDS-PAGE, transferred electrophoretically onto nitrocellulose filters, and reacted with a polyclonal rabbit anti-keratin antiserum (*a*). The densitometric scans in *b–f* correspond to lanes 1–5 of *a*, respectively. The mean integrals for K4a and K4b in *d* were 26.5% and 25.7%, respectively, and those for K5a and K5b in *f* were 17.4% and 17.6%, respectively. The values for K4a were 41.3% (*b*), 58.7% (*e*), and 34.9% (*f*), and that for K4b was 49.5% (*c*). K5b gave values of 24.8% (*b*), 26.7% (*c*), and 25.8% (*d*), and K5a gave a value of 20.0% (*e*). The values for K6 were 34.8% (*b*), 23.8% (*c*), 22.1% (*d*), 21.4% (*e*), and 30.1% (*f*).

keratin genes are polymorphic and express codominant alleles. Individuals displaying keratin doublets may therefore be interpreted as being heterozygous—and those showing just one keratin species may therefore be interpreted as being homozygous—for the respective variant allele a or b.

Table 1 summarizes the results obtained in a survey of 1,299 different individuals. Since the population analyzed was not strictly Caucasian, allele frequencies were calculated for German individuals, others (17.8%; mostly from Turkey), and the total, as presented in table 2. The resulting allele frequencies indicated slightly different distribution equilibria among these samplings.

The observed frequencies of particular keratin patterns agreed with the frequencies predicted by the Hardy-Weinberg equation, as shown in table 3, thus supporting our hypothesis that the variant polypeptides are products of polymorphic genes.

Pedigree Analysis of Keratins K4 and K5

To test the genetic hypothesis and to establish the mode of inheritance, we have analyzed the distribution of the polymorphic keratins within three German families. Two of these pedigrees are shown in figure 3. The data demonstrated that the phenotype distribution within the families could be explained, without exception, by genetic transmission of the variants as an autosome-linked Mendelian trait. This conclusion was particularly supported by the following observations: (1) when both parents expressed the same homozygous phenotype, only offspring with the same phenotype was observed; (2) if the parents expressed different homozygous phenotypes, the heterozygous phenotype was observed in the child (fig. 4, K4); and (3) the parental combination of a homozygous and a heterozygous phenotype resulted in children with the same homozygous (fig. 4, K5) or the heterozygous phenotype.

Table 1

Variation and Frequency of Keratins Expressed in Human Squamous Nonkeratinizing Epithelia

Observed Keratin Phenotype (*N*)	Frequency (%)	Assumed Keratin Genotype
4a (924)	71.1	4a 4a
4b (41)	3.2	4b 4b
4a + 4b (334)	25.7	4a 4b
5a (10)	.8	5a 5a
5b (1,095)	84.3	5b 5b
5a + 5b (194)	14.9	5a 5b
6 (1,299)	100	6 6
13 (1,299)	100	13 13
14 (1,299)	100	14 14

Table 2

Allele Frequencies of Polymorphic Keratins

Sampling	Allele Frequency			
	4a	4b	5a	5b
German (1,068)	.856	.144	.087	.913
Other (231)	.766	.234	.063	.937
Total (1,299)	.840	.160	.082	.918

Discussion

In the present study we have extended our observations on the two polymorphic human keratins K4 and K5, both of which displayed two alleles (a and b). Accordingly, we have observed three phenotypes for each gene, two representing the homozygous (a or b) and one representing the heterozygous (a + b) situation. Of the possible nine classes of keratin patterns in human squamous nonkeratinizing epithelium resulting from combinations of three phenotypes for the two genes K4 and K5, eight were observed in our sampling of 1,299 unrelated individuals. The ninth, being expected for 2/10,000 individuals, was found among the participating family members (fig. 1, lane 8, and fig. 4, lane 1).

The genetic basis of these protein variations was demonstrated in family studies, which showed the genetic transmission of the variants as autosomal Mendelian traits. The observed 1:1 ratio of the variants in

Table 3

Comparison of Observed and Expected Frequencies for Particular Keratin Phenotypes

Keratin Phenotype	No. Observed	Frequency Observed (%)	Frequency Expected (%)
4a + 4b 5a + 5b	39	3.00	4.05
4a + 4b 5a	3	.23	.18
4a + 4b 5b	292	22.48	22.65
4a 5a + 5b	146	11.24	10.62
4a 5a	7	.54	.47
4a 5b	771	59.35	59.46
4b 5a + 5b	9	.69	.38
4b 5a	...	...	.02
4b 5b	32	2.46	2.16

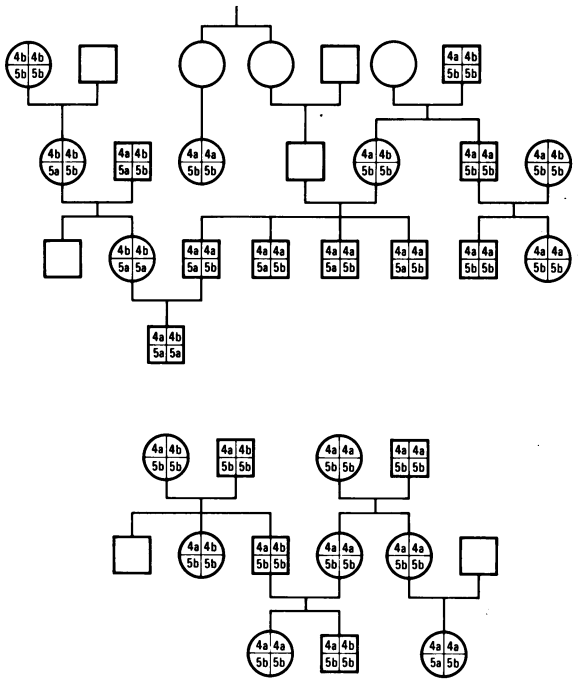

Figure 3 Segregation of alleles for K4 and K5 in two families. Cytoskeletal proteins of tissue samples obtained from buccal mucosa were analyzed by SDS-PAGE, and the particular keratin pattern was determined.

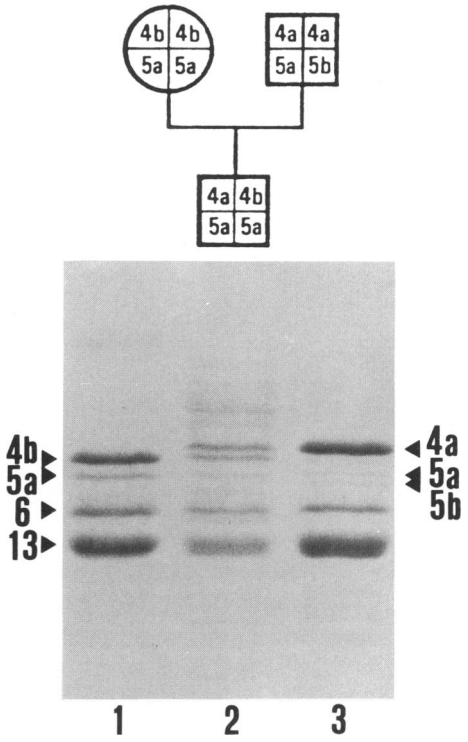

Figure 4 Detail of pedigree analyses, illustrating the segregation of alleles. SDS-PAGE-separated and Coomassie blue–stained keratins of buccal epithelium from a mother homozygous for K4b and K5a and from a father homozygous for K4a and heterozygous for K5a + K5b are shown in lanes 1 and 3, respectively. Their son (lane 2) was homozygous for K5a and, as expected, heterozygous for K4a + K4b. The amount of K5 may fluctuate among samples taken by scraping of buccal mucosa, because of the variable inclusion of cells from the deeper, K5 and K14 synthesizing, layers.

the heterozygous phenotype, reflecting the codominant expression of these keratin alleles, also supported the genetic hypothesis.

Genetic polymorphism—i.e., the presence, at a given locus, of two or more alleles in a population—is well documented. While the level of protein polymorphisms is high for enzymes and serum proteins, it is lower for structural proteins. However, reports on polymorphisms among intermediate filament proteins have been published. Willard (1976) has shown one of the three neurofilament polypeptides in rabbits to be polymorphic, and Comings (1982) has reported on a polymorphic variant of human glial fibrillary acidic protein.

In the context of the understanding that intermediate filament proteins—and, even more, the members of the keratin family—are closely related among each other and have evolved through common ancestors (Steinert and Roop 1988), genetic polymorphisms may not be surprising but may rather reflect ongoing evolutionary processes leading to diversification and specialization. In particular, tandem repetition of sequence motifs that is followed by mutational changes has been suggested to contribute to such a diversity within the variable N- and C-terminal domains of keratin proteins (Sun et al. 1984; Blumenberg 1988) and may well also be responsible for the observed alteration in apparent electrophoretic mobility of the polymorphic variants. Yet, the differences between the alleles of keratins K4 and K5 may not be caused by deletions or insertions but rather by amino acid substitutions, as their migration behavior is strongly dependent on gel composition (Wild and Mischke 1986; Mischke and Wild 1987).

The excellent fit between the expected and the observed frequencies of keratin phenotypes (table 3) suggests that the K4 and K5 genes are not closely linked and are independently transmitted. This lack of linkage disequilibrium is particularly noteworthy because it has been speculated, on the basis of recent keratin mapping data, that all keratin genes of the same sequence type are clustered—i.e., that type I genes are localized on human chromosome 17 and that type II genes are localized on chromosome 12 (Lessin et al.

1988; Romano et al. 1988; Rosenberg et al. 1988; Popescu et al. 1989). Specifically, as the K4 gene has already been mapped to chromosome 12 (Romano et al. 1988), our data would then indicate that the K5 keratin gene resides on another chromosome. Alternatively, linkage equilibrium could also be achieved for two linked genes, if the mutations that led to their alleles occurred a very long time ago.

In addition to its significance in population genetics, the keratin polymorphisms may also be useful in forensic analyses and in disputed-paternity cases. With the advent of an increasing number of cloned and sequenced human keratin genes (for a review, see Steinert and Roop 1988) it should be possible to identify the respective alleles and to elucidate the particular significance of polymorphic keratins—e.g., for functional diversification of keratins.

Acknowledgments

We are grateful to all members of our families for their kind cooperation in this study. We particularly appreciate one reviewer's comment pointing to the lack of linkage disequilibrium between K4 and K5 and thank Dr. H. Neitzel (Human Genetics Department, Free University) for consolidating our conception.

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