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Genomic Organization and Amplification of the Human Keratin 15 and Keratin 19 Genes

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Keratin intermediate filaments are the major components of the cytoskeleton in epithelial cells. Mutations in keratin genes have been documented in many disorders of the skin, nails, hair, and mucous membranes. Although no mutations have been described in either keratin 15 or keratin 19, they are good candidates for other as yet uncharacterized genetic disorders of keratinization, particularly as the skin, nails, hair, and conjunctiva are sites of keratin 15 and 19 expression. To facilitate future mutation detection analyses, we have therefore characterized the intron–exon organization of the human keratin 15 and keratin 19 genes. The keratin 15 gene comprises 8 exons spanning approximately 5.1 kb on 17q21, and the keratin 19 gene consists of 6 exons covering approximately 4.7 kb on 17q21. We have also developed a PCR-based mutation detection strategy using primers placed on flanking introns followed by direct sequencing of the PCR products.

Key Words: **intermediate filaments; skin; nails; hair.**

The human keratins are a family of structurally related proteins that form intermediate filaments in epithelial cells (1) and are expressed in pairs of type I (acidic) and II (basic) polypeptides in a tissue and differentiation specific manner (2). The type I and type II keratin pairs form heterodimers through association of their α-helical rod domains, and polymerize further to form the characteristic 10 nm filaments (3).

There are approximately 30 different keratin genes and mutations in several of these genes have been shown to underlie a wide range of inherited diseases affecting skin, hair, and mucosal epithelium. These disorders include epidermolysis bullosa simplex (K5/14) (MIM 131760/131900/131800), bullous congenital ichthyosiform erythroderma (K1/10) (MIM 113800), epidermolytic palmoplantar keratoderma (K9) (MIM 144200), ichthyosis bullosa of Siemens (K2e) (MIM 146800), pachyonychia congenita type 1 (K6a/16) (MIM 167200), pachyonychia congenita type 2 (K6b/17) (MIM 167210), monilethrix (hK1/6) (MIM 158000), oral white sponge naevus, (K4/13) (MIM 193900) and Meesmann's corneal dystrophy (K3/12) (MIM 122100) (for clinicopathological review see (4)). Study of these keratin diseases has demonstrated that epithelial cells are fragile and lyse, clump or disaggregate under mild stress (5) when their cytoskeleton is perturbed.

Although no disease has yet been linked to either keratin 15 or keratin 19, both genes remain plausible candidates for other inherited disorders of mucocutaneous keratinization. Therefore, to aid future mutation analysis of the genes for keratins 15 and 19, we have elucidated the complete genomic organization of these genes and present conditions for the amplification of their individual exons.

MATERIALS AND METHODS

Characterization of human keratin 15 and 19 genomic structure. Primers based on the cDNA sequence of keratin 15 (GenBank No. X07696) and keratin 19 (GenBank No. Y00503) were used to amplify keratin 15 and 19 introns from control human genomic DNA (Roche Molecular Diagnostics, Lewes, England). Approximately 200 ng of genomic DNA was added to a premix containing PCR buffer (67 mM Tris-HCl pH 8.8, 16.6 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 0.17 mg/ml bovine serum albumin (Sigma, Poole, England), and 10 mM 2-mercaptoethanol), 10 nmol of each dNTP, 20 pmol of each primer in a total volume of 50 μl. After an initial denaturation at 95°C for 2 min, 2.5 units of Taq polymerase (Promega, Madison, WI) was added followed by 35 cycles of 95°C for 10 s, annealing temperature for 10 s, 72°C for 1 min, with a final incubation of 72°C for 5 min. The PCR products were examined by 3% agarose gel electrophoresis, purified using spin columns (Qiagen, Crawley, England) and directly sequenced using Big Dye terminators on an ABI 310 genetic analyzer (Perkin Elmer, Foster City, CA) (Table 1).

PCR amplification of genomic DNA. For direct amplification of the eight keratin 15 exons and the six keratin 19 exons from genomic DNA, primer pairs were positioned within the introns flanking the exonic sequences (Table 2). For PCR, 200 ng of genomic DNA was added to a premix containing PCR buffer (as above), 10 nmol of each dNTP, 20 pmol of each primer in a total volume of 50 μl. After an initial denaturation at 95°C for 2 min, 2.5 units of Taq polymerase

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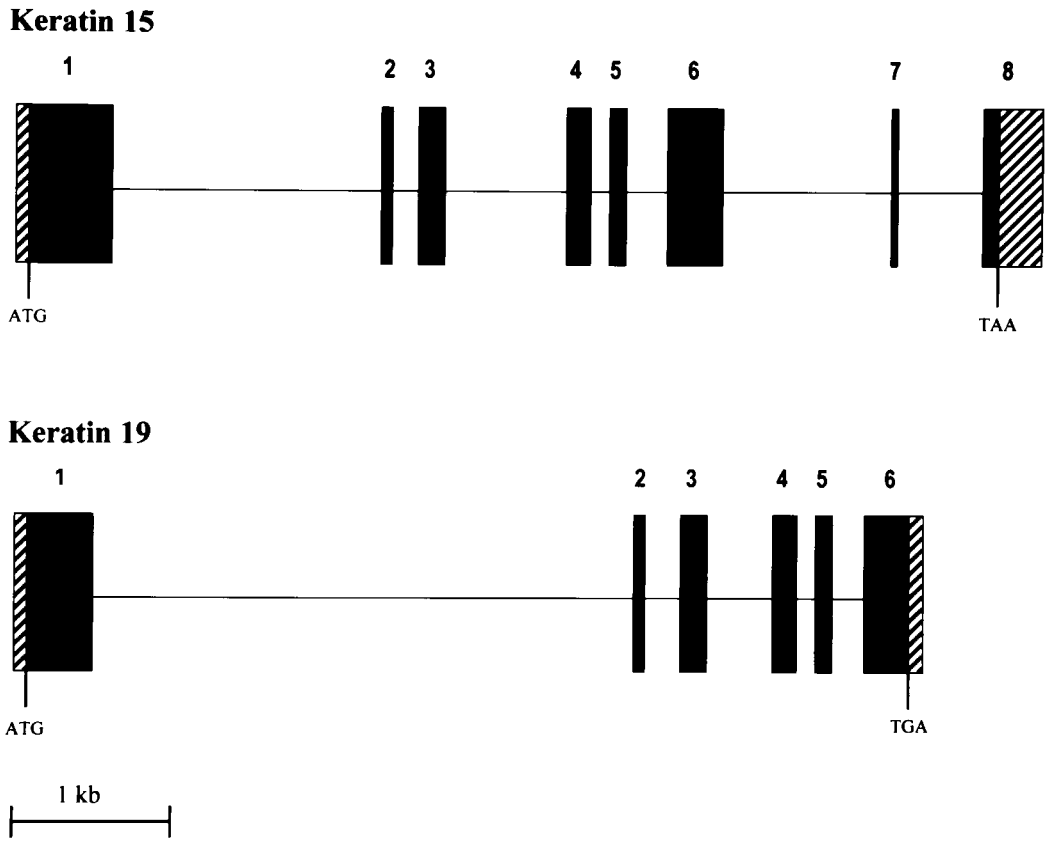

FIG. 1. Intron–exon organization of the human keratin 15 and 19 genes. Exons are represented by vertical boxes, introns by horizontal lines. The keratin 15 gene consists of 8 exons, 26–559 bp in size, spanning 5.1 kb of genomic DNA on 17q21, and the keratin 19 gene consists of 6 exons, 83–452 bp in size, spanning 4.7 kb of genomic DNA on 17q21 (Table 1). The positions of the translation-initiation and termination codons are indicated. The keratin 15 and keratin 19 gene sequences have been deposited in the GenBank database under Accession Numbers AF202320 and AF202321, respectively.

(Promega) was added followed by 35 cycles of 95°C for 10 s, annealing temperature for 10 s, 72°C for 30 s, with a final incubation of 72°C for 5 min. The annealing temperatures for each primer pair are displayed in Table 2. The PCR products were examined by 3% agarose gel electrophoresis, purified and directly sequenced as above.

RESULTS

Genomic Cloning of Human Keratins 15 and 19

To initiate genomic cloning of keratins 15 and 19, primers positioned within the respective cDNAs were used to amplify introns which were then sequenced. Analysis of sequence data revealed that keratin 15 consisted of 8 exons spanning approximately 5.1 kb of genomic DNA (Fig. 1). The exons varied in size from 26 bp (exon 7) to 559 bp (exon 1), and the introns ranged from 93 bp (intron 4), to 1165 bp (intron 1) (Table 1). Keratin 19 consists of 6 exons spanning approximately 4.7 kb of genomic DNA (Fig. 1). The exons varied in size from 83 bp (exon 2) to 452 bp (exon 1), and the introns ranged in size from 110 bp (intron 4), to 2550 bp (intron 1) (Table 1).

Development of a Mutation-Detection Strategy

To facilitate identification of pathogenic mutations in patients with potential candidate genodermatoses, we designed a strategy for detection of sequence variants in keratins 15 and 19. The approach consisted of PCR amplification of exons directly using genomic DNA as template (Fig. 2), with primers based on intron-specific sequences followed by direct sequencing. To allow PCR amplification of exons directly from genomic DNA, balanced primer pairs were designed on the basis of sequences close to the keratin intron-exon regions, although all primers were placed >85 bp away from intron-exon borders. The primer sequence information, the optimized amplification conditions, and the expected sizes of the PCR products are indicated in Table 2.

GenBank Accession Numbers

Human keratin 15 and keratin 19 genes have been deposited in the GenBank sequence database under

TABLE 1

Exon–Intron Boundaries of the Human Keratin 15 and 19 Genes

Exon	3′ splice acceptor sequence[a]	Exon size (bp)	5′ splice donor sequence[a]	Intron size (bp)
		Keratin 15		
1	Not determined	559	GACAAG**gt**gagtcctc	1165
2	tctcctgc**ag**ATCATG	83	GCTCAA**gt**gcgctccc	118
3	ctctcctc**ag**GTATGA	157	GAAGAG**gt**gagagctg	542
4	catcctgc**ag**GAGATG	162	AGCAAG**gt**gggcctta	93
5	tgacctac**ag**ACTGAG	126	AGCATG**gt**acggccct	191
6	actccccc**ag**AAAGCT	221	TGCCAA**gt**aggcgcct	797
7	tttctttc**ag**GATGGC	26	GGGAAG**gt**aggaaggc	528
8	ctctctct**ag**CCTCTT	375	Poly(A)	
		Keratin 19		
1	Not determined	452	GACAAG**gt**gggcggag	2550
2	tgccttcc**ag**ATTCTT	83	AACCAA**gt**gagtgtct	191
3	cctttgaa**ag**GTTTGA	157	GAGGAG**gt**gggtttga	302
4	atcttttc**ag**GAAATC	162	AGCCGG**gt**aggtagag	110
5	ctctctgc**ag**ACTGAA	126	AGCATG**gt**atgtgtcc	145
6	ctatcctc**ag**AAAGCT	380	Poly(A)	

[a] The consensus ag/gt splice sequences are shown in bold.

Accession Numbers AF202320 and AF202321, respectively.

DISCUSSION

Both keratin 15 (K15) and keratin 19 (K19) are type I intermediate filament proteins that do not have defined type II partners. Keratin 15 expression seems to signal an early stage in the pathway of keratinocyte differentiation that precedes the decision of a cell to commit to an epidermal or hair-like lineage (6). Keratin 15 is expressed primarily in the basal keratinocytes of stratified tissues (6) including fetal epidermis, fetal nail and the outer root sheath (7) and has been shown to be upregulated in K14 ablated hair follicles and downregulated in psoriasis and hypertrophic scars (7). By contrast, for keratin 19, its tissue distribution is not restricted to just stratified or simple-type epithelia. Keratin 19 has been detected in epidermal basal cells, in the bulge region of the hair follicle, in nipple epidermis (8), in the superficial layers of normal conjunctiva (9) and in human corneal epithelial cells (10). Keratin 19 is also different from the other keratins because it is naturally "tail-less" having a very short C-terminus (8). Because of the important role of keratins in creating stable cytoplasmic structures it is plausible that mutations in either keratin 15 or keratin 19 will lead to cell specific genetic disorders. Currently mutation screening of keratin 19 using cDNA as the template is severely compromised due to the presence of an intron-less pseudogene with very high homology to keratin 19 mRNA (11). Hence, characterization of the genomic organization of human keratin 15 and keratin 19 is necessary to allow identification of sequence variants in genomic DNA of patients with candidate diseases. These studies will lead to a better understanding of the importance of the entire intermediate filament cytoskeleton and associated connector molecules in maintaining the

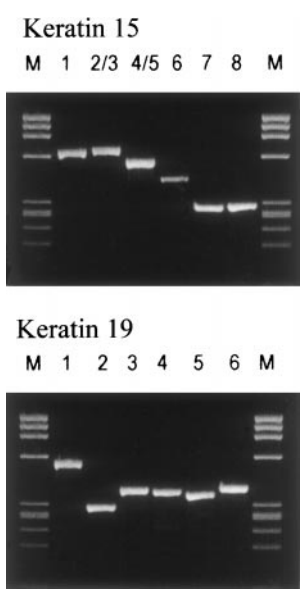

FIG. 2. PCR amplification of exons 1–8 for keratin 15 and exons 1–6 for keratin 19, using genomic DNA as template with the primer pairs shown in Table 2. The PCR products were examined by 3% agarose gel electrophoresis. The lanes M contain φX174 *Hin*dIII molecular weight markers.

TABLE 2

Primers for the Genomic Amplification of Human Keratins 15 and 19

Exon	Forward (5′ → 3′)	Reverse (5′ → 3′)	Product (bp)	Annealing temp. (°C)
		Keratin 15		
1	ctgagaactcacgggctcca (−31)[a]	taccagacagtcattgcctgg (+121)	650	58
2 + 3	aagtgaggctggactttgcc (−173)	cccactgacttaatctcgtga (+136)	667	58
4 + 5	ggcagagccaggattggcatg (−88)	ggcttgagaactccaaggctt (+86)	555	60
6	ctcaagaggtggttccctgaac (−103)	gtccctcaaggctgcttagg (+105)	429	60
7	cctagaggagtcaggtgtgtgg (−133)	ggaagaggcatctaatgaatg (+140)	299	56
8	gatctctggcagctcttgagg (−143)	ccagtcctccacttggcctg (1436)[a]	306	60
		Keratin 19		
1	ttgctccgtccgtgctccg (−26)[b]	ccacgtcctaacgggctcctg (+112)	558	64
2	gctggctagggctttggtctc (−128)	gcttcaggccatctaggctag (+96)	307	60
3	ctagcctagatggcctgaagc (−116)	ccatggtgagggtgcaccaag (+111)	384	60
4	gctagttactaggcctgcacctg (−122)	gggaataagcattgagtcagacc (+90)	374	60
5	gtaggtagagggaagcaggcac (−110)	gtgactcagcagagcctggttc (+123)	359	60
6	cacacttataccttgcagcctag (−106)	aaggacagcagaagccccag (1234)[b]	392	60

[a] In the keratin 15 cDNA (GenBank No. X07696) relative to the transcription start site.
[b] In the keratin 19 cDNA (GenBank No. Y00503) relative to the transcription start site.

structural integrity of the epidermis and other high stress epithelial tissues.

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