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Characterization of human 12-lipoxygenase genes

(platelet/pseudogene/promoter/chromosome localization)

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ABSTRACT **Two human 12-lipoxygenase enzyme (arachidonate:oxygen 12-oxidoreductase, EC 1.13.11.31)-related genes were characterized from 13 distinct clones isolated from three genomic bacteriophage and cosmid libraries. A complete gene (12-lipoxygenase gene 1) spanning ≈17 kilobases and consisting of 14 exons with sequence matching the cloned platelet/human erythroleukemia (HEL) cell cDNA sequence was identified. Several consensus sites for transcription factors and two potential transcription initiation sites within the 5′ flanking region, encompassing the putative promoter region, were identified. A segment of a second, probable pseudogene (12-lipoxygenase gene 2), which displays ≈85% identity to gene 1 within exon sequences, was also characterized. The presence of two 12-lipoxygenase genes was also substantiated by Southern blot analysis of total human genomic DNA. Exon–intron boundaries for the 12-lipoxygenase genes were located in the identical corresponding positions to the previously cloned human 5-lipoxygenase and rabbit 15-lipoxygenase genes, indicating a highly related gene family. Three lipoxygenase genes (12-lipoxygenase genes 1 and 2, 15-lipoxygenase) were localized to human chromosome 17, whereas the most unrelated lipoxygenase (5-lipoxygenase) was mapped to chromosome 10 by PCR analysis of a human–hamster somatic hybrid DNA panel. 12-Lipoxygenase gene 1 expression could be detected in human erythroleukemia cells, platelets, and human umbilical vein endothelial cells with certainty by reverse transcription-PCR analysis. There was no detectable 12-lipoxygenase gene 2 expression in several tissues and cell lines.**

The 12-lipoxygenase enzyme (arachidonate:oxygen 12-oxidoreductase, EC 1.13.11.31) catalyzes the transformation of arachidonic acid into 12(*S*)-hydroperoxyeicosatetraenoic acid and its 12(*S*)-hydroxy derivative, 12-hydroxyeicosatetraenoate (12-HETE) (1). Although these are the most abundant products of arachidonate metabolism formed in platelets (2), their biological function remains unclear. One possibility is that 12-lipoxygenase products modulate the transformation of the membrane glycoprotein IIb/IIIa complex that occurs with platelet activation, revealing cryptic binding sites for fibrinogen (3, 4). Synthetic 12-HETE has been reported to regulate the expression of this complex in a stereospecific manner in tumor cells (5), and the platelet enzyme has been reported to translocate (6), in a calcium-dependent manner, from the cytosol to the cell membrane. 12-Lipoxygenase products (synthetic 12-hydroperoxyeicosatetraenoic acid and its hepoxilin derivative) influence ion-channel conductivity in *Aplysia* (7) and hippocampal neurons (8).

A second form of the 12-lipoxygenase has been identified in porcine leukocytes. This form differs from the platelet form, sharing 65% amino acid identity (9–11). Bovine tracheal epithelial tissue exhibits a 12-lipoxygenase that is immunologically distinct from the human platelet form but closely related to that found in porcine leukocytes (12). We have failed previously to identify mRNA for the platelet form in human epithelial cells using RNA blot analysis (10).

These studies raise the possibility of the existence of more than a single 12-lipoxygenase gene within a given species. There is now good evidence for the existence of two related genes for prostaglandin G/H synthase (cyclooxygenase), the other major arachidonate-metabolizing enzyme in platelets (13, 14). We have undertaken the characterization of the 12-lipoxygenase gene(s)¶ to address the issue of potential genetic heterogeneity and to investigate their organizational and spatial relationships with other lipoxygenase genes.

MATERIALS AND METHODS

Isolation and Characterization of Genomic Clones. Three human genomic libraries (library 1, Stratagene, catalog 946203; library 2, Clontech, catalog HL1067j; library 3, male human leukocyte genomic library in cosmid vector pWE15, from John W. McLean, Genentech) were screened by standard procedures (15) with ^{32}P-labeled 12-lipoxygenase cDNA probes prepared by random priming as described (16). Various restriction fragments were subcloned into the plasmid vector pGEM3z (Promega) for mapping and into M13mp18 and M13mp19 vectors for sequencing by the Sanger dideoxynucleotide chain-termination method (17). Intron sizes were estimated by either restriction mapping, PCR analysis, or complete sequencing.

Southern Blot Analysis of Total Genomic DNA. DNA isolated from human leukocytes was digested with various restriction endonucleases, electrophoresed in a 0.7% agarose gel, and transferred to a nylon membrane. The membrane was hybridized (15) with a ^{32}P-labeled *Bgl* II/*Eco*RI 12-lipoxygenase cDNA [positions 411–852, including 9 base pairs (bp) from exon 3, exons 4–6, and 45 bp of exon 7] and subsequently, after probe removal, with a PCR-derived DNA that included exons 9–11 (positions 1178–1540; see ref. 10). The membrane was washed to 0.1× standard saline citrate/0.1% SDS at 65°C and exposed at −70°C for 1–3 days.

Reverse Transcriptase-PCR Analysis. Total RNA isolated from various human tissues and cells (18) was subjected to first-strand cDNA synthesis and PCR analysis as described (19). Primers used for 12-lipoxygenase gene 1 amplification were 5′-TGGACACTGAAGGCAGGGGCT-3′ and 5′-GGCTGGGAGGCTGAATCTGGA-3′ and were designed to be mRNA specific (10, 19). The primers for 12-lipoxygenase gene 2 amplification were 5′-CCAATGTGATCCAAAGAGG-3′ and 5′-AGTTGGAATCGGAGTTTCAG-3′. Amplification was for 40 cycles at 94°C for 50 s, 55°C for 1 min,

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Abbreviations: HEL, human erythroleukemia cell; 12-HETE, 12(*S*)-hydroxyeicosatetraenoate.

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¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. M87004).

and 72°C for 1 min. Five-microliter aliquots were electrophoresed in a 2% agarose gel (NuSieve, FMC). After transfer to nylon, hybridization was done with a full-length human 12-lipoxygenase cDNA.

Chromosomal Localization of Lipoxygenase Genes. A panel of 25 human–hamster somatic hybrid DNAs were analyzed by PCR to determine the localization of lipoxygenase genes. PCR was done in a volume of 100 μl with 10 pmol of lipoxygenase-specific probes from exon 14, for 40 cycles at 94°C for 45 s, 60°C for 45 s, and 72°C for 1 min. The primers and expected size of the amplified products are as follows: 5-lipoxygenase, CTGTTCCTGGGCATGTACCC and GACATCTATCAGTGGTCGTG, 465 bp; 12-lipoxygenase, GTGCCTCTGGGGCACCACA and AGAGCCTTAGCAGCAGAG, 281 bp; 15-lipoxygenase, GTGGCTGTGGGCCAGCATGAG and GTGAGACTCTGTCTTAACAAC, 563 bp. Amplified products were visualized by ethidium bromide staining of 10-μl aliquots electrophoresed in a 2% NuSieve/1% SeaKem agarose gel. Some samples were subjected to restriction enzyme digestion to verify authenticity of the amplified product. After transfer to nylon membranes, hybridization was done with 5-, 12-, and 15-lipoxygenase probes (9, 20, 21) under high-stringency conditions.

RESULTS

Isolation and Characterization of 12-Lipoxygenase Genomic Clones. Initially, two human genomic libraries in bacteriophage vectors were screened (1.5×10^6 plaque-forming units each) with a full-length 12-lipoxygenase cDNA and subsequently with several smaller probes. Seven clones (three unique) were plaque-purified from library 1, and nine clones (eight unique) were plaque-purified from library 2. Restriction fragment mapping combined with Southern blot analysis and DNA sequencing revealed two sets of overlapping clones; set 1 clones—CG24, CG27, CG28, and 15B—which contain exons 12–14 (see below and Fig. 1) and set 2 clones—CG35, CG37, CG93, CG95, Gp34, and 921—which contain 12-lipoxygenase-related sequence. One clone, CG23, did not overlap with either set and was found to possess exons 1–8, including ≈3.5 kilobases (kb) of 5′ upstream DNA, encompassing the putative promoter region of the 12-lipoxygenase gene. To isolate the missing gene section a human cosmid library was screened with a full-length cDNA. Two clones, CS1 and CS5, were isolated, mapped (Fig. 1), and partially sequenced. Clone CS1 overlapped with both CG23 (confirmed by sequencing a 250-bp segment within intron H of both clones; data not shown) and set 1 clones and included exons 9–14. The 5′ end of clone CS5 began 6 bp within exon 9, 3.5 kb downstream of clone CS1.

Partial Sequence and Organization of Human 12-Lipoxygenase Genes. Southern blot analysis of total genomic DNA (Fig. 2) with two different 12-lipoxygenase cDNA probes, combined with restriction mapping and limited sequencing of the isolated genomic clones, clearly indicated the presence of two 12-lipoxygenase genes. The intron/exon boundaries and all exons for gene 1 were sequenced (see Fig. 6). All introns conform closely to the GT-AG rule (22), and surrounding sequences are closely related to splice junction consensus sequences (23). The gene is ≈17 kb in length and is divided into 14 exons. All exon sequences are identical to the previously published human erythroleukemia (HEL)/platelet 12-lipoxygenase cDNA sequence (10) with the exception of three nucleotides (see Fig. 3 legend). Exons range in size from 82 bp (exon 3) to 493 bp (exon 14), and most introns are small (<1.1 kb).

The set 2 clones (gene 2) contained a region corresponding to exons 7–8 and 10–11 of gene 1 but with a distinctly different restriction map (Fig. 1). These clones likely represent part of a 12-lipoxygenase pseudogene (see *Discussion*).

The 5′ End of 12-Lipoxygenase Gene 1. The nucleotide sequence of a 578-bp region upstream from the ATG initiator codon was determined (Fig. 3). The sequence is (G+C)-rich, especially close to the start codon (≈80%). Three GC boxes (GGGCGG), potential sites of interaction with the Sp1 transcription factor, are present, and a "TATA-like box" is present (TTTAAA) at position −96. There are two near matches to the AP-2 consensus sequence CCCCCAGAC at position −531 and TCCCAGGC at position −511 (consensus CCCCAGGC). There are three CACCC repeats within a 23-nucleotide region (−286 to −263), a motif found in the 5′ upstream regions of globin genes and the 15-lipoxygenase gene (24). No major homologies appear with the 5′ upstream

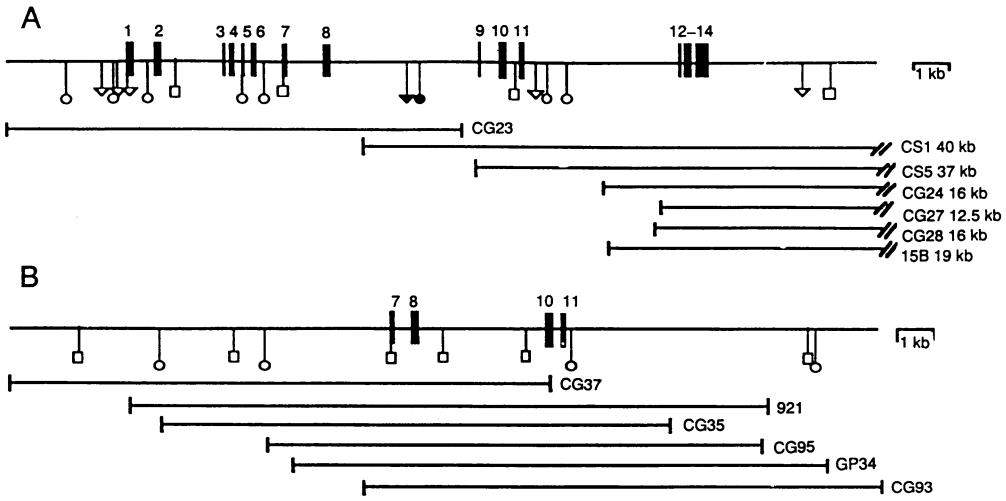

FIG. 1. Human 12-lipoxygenase gene loci. (*A*) (*Upper*) Relative exon positions within 12-lipoxygenase gene 1 are indicated by shaded bars. Positions of all restriction endonuclease cleavage sites for *Eco*RI (□), *Sac* I (○), and *Sfi* I (▽) within the designated region are shown; no *Sal* I sites were found. Two additional sites (*Pst* I, ▼; *Hin*dIII, ●) found in clones CG23 and CS1 are noted, and the region between them was sequenced to establish the correct overlap within intron H. (*Lower*) Relative position of clones within the gene locus obtained by screening library 1 (15B), library 2 (CG23, CG24, CG27, CG28), and library 3 (CS1, CS5). The 3′ end of all clones, except CG23, is omitted and their approximate sizes are indicated at right. (*B*) (*Upper*) Relative exon positions of 12-lipoxygenase gene 2 are displayed on the long line. All restriction sites for *Eco*RI (□) and *Sac* I (○) are shown. No *Sfi* I and *Sal* I sites were found. (*Lower*) Six overlapping clones were isolated and mapped (921, GP34, library 1; CG35, CG37, CG93, CG95, library 2).

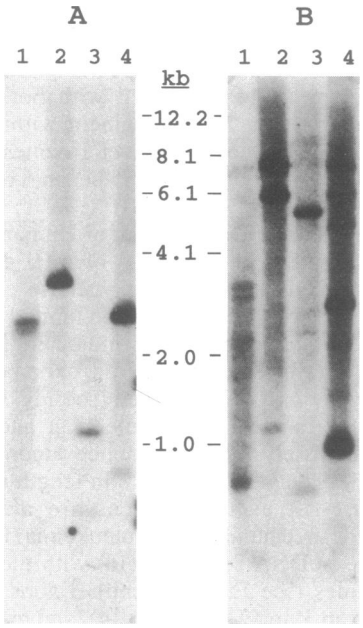

FIG. 2. Southern blot analysis of total genomic DNA. Restriction endonuclease-digested DNA (20 μg) was electrophoresed in a 0.7% agarose gel, transferred to nylon membrane, and probed with ^{32}P-labeled 12-lipoxygenase cDNA comprising primarily exons 4–7 (*A*), or an exon 9–11 12-lipoxygenase cDNA (*B*). Lanes: 1, *Msp* I; 2, *Eco*RI; 3, *Bgl* II; 4, *Eco*RI/*Bam*HI. Membrane in *A* was exposed for 20 hr; membrane in *B* was overexposed for detection of smaller bands in lanes 1 and 3 (3 days). The intensely hybridizing bands correlate with the restriction map of 12-lipoxygenase gene 1 clones. Weaker hybridizing bands in *B* can be correlated with the restriction map of 12-lipoxygenase gene 2 clones.

regions of human platelet factor 4 (25), human platelet glycoprotein IIb (26), and human platelet thrombomodulin (27), genes specific to the megakaryocyte/platelet lineage.

Primer-extension analysis was done to map the sites of transcription initiation in HEL cells. Two premature termination stops (positions −9 and −26; Fig. 3) occurred in (G+C)-rich regions during reverse transcription; however, there were two additional weak bands (positions −62 and −88; see Fig. 3) not present in the yeast tRNA sample (data not shown), which could represent the 5′ end of transcripts in HEL cells.

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AGGTACTGGAGGACACATGGGCCGCGCCGGGTCTAGCCGCGGGACATCCCCAGAC  -524
CCCAGCACGGCCTCCCAGGCCACCTTCCAGAAGCCGGCCCACTGCCTTCCCCGGG  -469
TCCCAGCCCAGAAAAGCGGAGACCTTCCCCTCCGTTTGGAAGATGGACTCATCCC  -414
CTACTTCCTCCTGGCTCCCCAGAAGACCTGGAGACCTCGGGAAGTGTTCTCATCT  -359
ATGTTCGCTCCAACCCGGGGGCTCAGGCCCACCTCGATTCCGCCTTCCCGGGAGG  -304
TCCAGGAAGGTCCCGCCGCACCCACCCCGTTGGCCTCACCCTGGCTCGGGCCCCT  -249
TTATCATCCTGCAGCTACGCTCCGACCAGGAGGCACAGCCAGTCCCCACAGCCGG  -194
CTCTACCGCTCTGCTCGCAGGTTTGGGCTAGTCTGGGCGGGGACTTGGGAGCGC  -139
CTAAAACTTGCGAGGAGGGCGGGGCCGCAGACCGGTCCTTTAAAGGTTGGAAGTG  -84
GCCCCGAGGCGCCGGCAGCCCTGGGCGGTCCCGGGCAATCGCACAGGACCCGGCT  -29
                      -1 1
CCCCTCGCCTAAGCTGCTGGGGGGCGCC ATGGGCCGCTACCGCATCCGCGTGGC   26
                             MetGlyArgTyrArgIleArgValAl
CACCGGGGCCTGGCTCTTCTCCGGGTCGTACAACCGCGTGCAGCTTTGGCTGGTC  81
aThrGlyAlaTrpLeuPheSerGlySerTyrAsnArgValGlnLeuTrpLeuVal
GGGACGCGCGGGGAGGCGGAGCTGGAGCTGCAGCTGCGGCCGGCGCGGGGCGAG  135
GlyThrArgGlyGluAlaGluLeuGluLeuGlnLeuArgProAlaArgGlyGlu
gtcagcgcgg
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FIG. 3. Nucleotide sequence of the 5′ flanking region and exon 1 of 12-lipoxygenase gene 1. Numbering starts at the ATG initiation codon. Three potential Sp1 sites and a TATA-like box are boxed. Two regions similar to the AP-2 consensus sequence are underlined. The three nucleotides GGA previously found at the extreme 5′ end of the cloned HEL cDNA (10) were not present in the genomic DNA and probably resulted from a cDNA cloning artifact. ●, Potential transcription initiation sites in HEL cells.

Chromosomal Localization of Lipoxygenase Genes. The chromosomal localizations of the lipoxygenase genes were determined by using a panel of somatic cell human–hamster hybrid DNA samples. PCR amplification of a 281-bp 12-lipoxygenase DNA specific to exon 14 was amplified in a normal human genomic DNA sample and hybrid clones 811 and 937 (Fig. 4*B*) and was undetectable in the remaining 22 hybrid and hamster DNA samples. The 281-bp band could be cleaved by *Pvu* II digestion to 129- and 152-bp fragments, the expected sizes for a 12-lipoxygenase DNA from gene 1. These results allowed us to assign the 12-lipoxygenase gene 1 unequivocally to human chromosome 17. Similarly, the human 15-lipoxygenase gene was assigned to chromosome 17 (Fig. 4*C*). We have also assigned the 12-lipoxygenase gene 2 to human chromosome 17 because a ≈800-bp DNA band, specific to gene 2, spanning part of exons 7 and 8 and the intervening sequence, was amplified in the normal genomic DNA sample and in hybrid clones 811 and 937 (data not shown). Interestingly, the 5-lipoxygenase gene, which differs the most from the other lipoxygenases, was assigned to human chromosome 10. A 465-bp exon 14-specific band was detected in the normal human genomic DNA sample and hybrid clones 860, 983, and 1079 (Fig. 4*A*).

Expression of 12-Lipoxygenase Genes in Various Tissues and Cells. Previously, we have detected expression of the human 12-lipoxygenase gene only in platelets and HEL cells by RNA blot analysis (10, 11). Hybridization to the 2.4-kb 12-lipoxygenase transcript was not evident in samples from several other tissues by this method. Additionally, 12-lipoxygenase mRNA was shown to be present in unstimulated HEL cells at ≈five copies per cell by quantitative PCR analysis (19). The 12-lipoxygenase gene 1 mRNA was detected in the present study in all tissues and cells examined by PCR (Fig. 5*A*). Although quantitative analysis was not done, the low levels detected in placenta, liver, lung, and leukocytes could have resulted from platelet contamination of the tissue or cell preparations. The 12-lipoxygenase mRNA, however, was definitely present in human umbilical vein endothelial cells (passage 4) because platelet contamination would not be a contributing factor. Genomic DNA is not a potential contaminating source of amplifiable DNA (Fig. 5*A*, lane 9) because the primers are mRNA/cDNA specific.

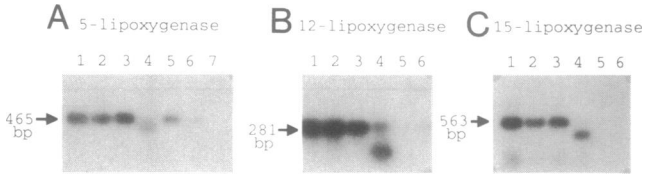

FIG. 4. Chromosomal localization of human lipoxygenase genes by PCR analysis of human–hamster hybrid DNAs. A panel of hybrid DNAs was analyzed by PCR with exon 14 gene-specific oligonucleotide primers. Products from the reaction were electrophoresed in a 2% NuSieve/1% SeaKem agarose gel. After transfer to nylon membrane, hybridization was done with 5-lipoxygenase cDNA (*A*); 12-lipoxygenase cDNA (*B*); or 15-lipoxygenase cDNA (*C*). Sizes of amplified products are shown by arrows. Lanes: (*A*) 1, human genomic DNA; 2, hybrid clone 860; 3, hybrid clone 983; 4, DNA from amplified sample (lane 2) cleaved with *Xho* I; 5, hybrid clone 1079; 6, hybrid clone 212 (one of 22 negative samples); 7, hamster DNA. (*B*) 1, human genomic DNA; 2, hybrid clone 811; 3, hybrid clone 937; 4, DNA from amplified sample (lane 2) cleaved with *Pvu* II; 5, hybrid clone 507 (negative); 6, hybrid clone 909 (negative). (*C*) 1, human genomic DNA; 2, hybrid clone 811; 3, hybrid clone 937; 4, DNA from amplified sample (lane 2) cleaved with *Bgl* II; 5, hybrid clone 324 (negative); 6, hybrid clone 423 (negative). Not all negative samples are shown. The 5-lipoxygenase gene was assigned to chromosome 10, and the 12- and 15-lipoxygenase genes were assigned to chromosome 17.

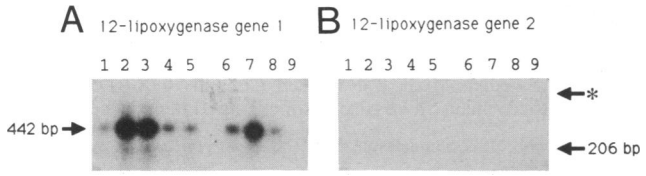

FIG. 5. Reverse transcription-PCR analysis to detect expression of the 12-lipoxygenase gene 1 (*A*) and gene 2 (*B*) expression in various human tissues and cells. PCR was done on identical cDNA samples synthesized from total RNA using 12-lipoxygenase gene 1- and gene 2-specific primers. The amplified products were electrophoresed in a 2% NuSieve agarose gel and probed with equal amounts of ^{32}P-labeled 12-lipoxygenase cDNA. Blots were exposed for 6 hr at −70°C. Lanes: 1, placenta; 2, umbilical vein endothelial cell; 3, phorbol 12-myristate 13-acetate-stimulated HEL cell; 4, unstimulated HEL cell; 5, liver; 6, lung; 7, platelet; 8, leukocyte; 9, genomic DNA. Position of the 12-lipoxygenase gene 1-amplified product is shown at left and is mRNA specific (i.e., no genomic DNA amplification). No hybridization to an expected 206-bp 12-lipoxygenase gene 2 band was seen, even with prolonged exposure (3 days). However, some amplification of an ≈800-bp (*) band from contaminating genomic DNA within the RNA samples was seen, which was clearly evident upon longer exposure.

Expression of the 12-lipoxygenase gene 2 was also investigated in the same tissue and cell RNA samples by using specific primers. There was no evidence for amplification of the expected 206-bp band (Fig. 5*B*). However, an ≈800-bp hybridization-positive band, indicative of genomic DNA contamination, was evident in most samples upon longer blot exposure.

DISCUSSION

A complete human 12-lipoxygenase gene, including the putative promoter region, has been isolated on several overlapping genomic clones (Fig. 6). The gene is ≈17 kb in length and is divided into 14 exons in a manner analogous to the human 5-lipoxygenase (16) and rabbit 15-lipoxygenase (24) genes. Exon sequencing indicated that this gene corresponded to the previously cloned platelet/HEL cell cDNA (10, 11). A portion of a second gene (12-lipoxygenase gene 2), highly related to the 12-lipoxygenase gene 1 within exon sequences, was also isolated (Fig. 7). This gene was clearly

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Exon/size(bp)          Intron/size(kb)

1(197) GGCGAG    gtcagcgcgg A(0.55)  gcctgcacag    GAGGAG
       GlyGlu                                      GluGlu
2(202) GGCACCG   gtgagcaggc B(1.6)   gtctccccag CCCGCCTG
       GlyThrA                                     laArgLeu
3(82)  ATCTACTG  gtgaccaccc C(0.12)  caactcctag   CTGGGCC
       IleTyrCy                                    sTrpAla
4(123) AAGGCAGG  gtgagaaaag D(0.20)  acctgcctag   GGCTCTG
       LysAlaGl                                    yAlaLeu
5(104) CTGGCTG   gtcagtggtt E(0.25)  cccaccaaag AGAAGGTT
       LeuAlaG                                     luLysVal
6(161) CTTCAG    gtacctctcc F(0.7)   ctgtccccag   AATGGT
       LeuGln                                      AsnGly
7(144) ATCCAG    gtaagggccc G(1.1)   tttctcccag   ATTCAG
       IleGln                                      IleGln
8(210) TTCAAG    gtacttatta H(4.0)   tcctctccag   TTCCTG
       PheLys                                      PheLeu
9(87)  GATAAG    gtgaggaggg I(0.57)  cctgtcccag   GCAGTG
       AspLys                                      AlaVal
10(170)ATTGCCAG  gtgagtaagg J(0.43)  ccctgggcag  GTATGTG
       IleAlaAr                                    gTyrVal
11(122)GACCGAG   gtaagatcca K(4.2)   tcctgcccag GTTTCCCT
       AspArgG                                     lyPhePro
12(101)GGCCAG    gtatggacag L(0.10)  cttcctccag   CTGGAC
       GlyGln                                      LeuAsp
13(171)GACATG    gtgagagggg M(0.12)  tctgtctcag   GTGCCT
       AspMet                                      ValPro
14(493)....TTCCAATAAAACTTTATGGACACTGAGATATGAATGTTACATC
                                    ↑
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FIG. 6. Intron/exon structure of human 12-lipoxygenase gene 1. Intron (lowercase letters) and exon (capital letters) junction boundaries of the 14 exons and 13 introns are displayed. Approximate intron sizes were determined by restriction mapping, PCR, or direct sequencing. The polyadenylylation signal is underlined, and the ↑ indicates the site of poly(A) addition.

much more similar to the 12-lipoxygenase than to either the 5- or 15-lipoxygenases by sequence analysis. This gene was not expressed in platelets, HEL cells, human umbilical vein endothelial cells, placenta, lung, liver, and leukocytes. Additionally, the gene was lacking an exon 9-like sequence and contained an uncommon splice junction at the 5′ end of exon 8; furthermore the translated sequence of exon 8 would contain two altered histidine residues (His-360 → Tyr and His-383 → Arg). There are five highly conserved histidines in all plant and animal lipoxygenases (16, 28, 29), contained within exons 8 and 9 of the three known genomic sequences; some of these histidines appear to be involved in binding the nonheme iron atom (30, 31). One of the putative altered histidines (His-360) corresponds to a residue critical to 5-lipoxygenase activity (30, 31). We believe that 12-lipoxygenase gene 2 represents a 12-lipoxygenase pseudogene that prob-

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                              EXON 7
12LX-1 GAATTCCAGCCAACGTGATCCGAGGAGAGAAGCAATACCTGGCTGCCCCCCTCGTTATGCTGAAGATGGAGCCCAATGGGAAGCTGCAGCCCATGGTCATCCAG
12LX-2 GAATTCCAGCCAATGTGATCCAAAGAGGGAAGCAGTACCAGGCTGCAGCCCTCGTCATGCTGAAGATGGAGCCTCCTGGGAAGCTGCTACCCATGGTCATCCAG

12LX-1 gtaagggccc---- 1.1 kb  ----tttctcccag
12LX-2 gtgagggccc---- 0.55 kb ----ctccttctgg

                              EXON 8
12LX-1 ATTCAGCCTCCCAGCCCCAGCTCTCCAACCCCAACACTGTTCCTGCCCTCAGACCCCCCACTTGCCTGGCTCCTGGCAAAGTCCTGGGTCCGAAATTCAGATTTC
12LX-2 CTTCAGCCTCCCAGCTTCAGCTCTCCCACCCCAATACTGTTCCTGCCCTCAAATCCTTTGCTTGCCTGGCTCCTGGCTAAATCCTGGATCCTGAAACTCCGATTC

12LX-1 CAACTGCACGAGATCCAGTATCACTTGCTGAACACTCACCTGGTGGCTGAGGTCATCGCTGTCGCCACCATGCGGTGCCTCCCAGGACTGCACCCATCTTCAAG
12LX-2 CAACTGCACGAAGCTCCGATACTATTTGCTGAACACTCATCTCGTAGCTGAAGTCATTGCTATTGTCACCATAATGTGCCTCCCACGTTTGCGCCCTGTCTTCAAG

                              EXON 10
12LX-1 cctgtcccag GCAGTGAGCACAGGTGGAGGGGGCCATGTACAGTTGCTCCGTCGGGCGGCAGCTCAGCTGACCTACTGCTCCCTCTGTCCTCCTGACGACCTGG
12LX-2 cttgtcccag GCAGTGAGCACAGGTGGAGGGGGCCATGTGCAGTTGCTCCATTGGGCAGCGGCTCAGCTGACCTGCTGCTCCCTCTGTTGTCCTGATGACCTGG

12LX-1 CTGACCGGGGCCTGCTGGGACTCCCAGGTGCTCTCTATGCCCATGATGCTTTACGGCTCTGGGAGATCATTGCCAG gtgagtaa-- 0.43 kb--ctgggcag
12LX-2 CTGCCTGTGGCCTGCTGGGACTCCCACGTGCTGTCCATACCCATGATGCTTTAGGGCCCTGGGAGATTATTACCCA gtaggatg-- 0.13 kb--ctgggcag

                              EXON 11
12LX-1 GTATGTGGAGGGGATCGTCCACCTCTTCTACCAAAGGGATGACATAGTGAAGGGGACCCTGAGCTGCAGGCCTGGTGTCGGGAGATCACGGAGGTGGGGCTGTG
12LX-2 GTGTGTGGAG---ATCGTCCACCTCCTCCACCAAAGGGATGACACGGTGAGGGTGGACCCTGAGCTGCAGGCCTGGGATCAGGAGATCATGGAGGTGGGGCTGTG

12LX-1 CCAGGCCCAGGACCGAG gtaagatcca
12LX-2 CCAGGCCCAGGACCGAG gtaagatctg
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FIG. 7. Comparison of nucleotide sequences of 12-lipoxygenase genes 1 and 2. Exon sequences between the *Eco*RI site of exon 7 to the end of exon 11 are shown. There was no evidence for exon 9 within gene 2. Intron boundaries are shown as lowercase letters, and approximate intron sizes are noted. Underlined nucleotides denote amino acid changes between the two genes, if gene 2 sequence were translated to peptide sequence in the same frame as for gene 1. Positions of four conserved histidines, within exon 8, found in all lipoxygenases but absent in two positions of gene 2, are boxed. Three nucleotide differences, one in exon 9 (C → T, position 1166) and two within exon 8 (T → G, position 1092; G → A, position 965) were found between our previously published cDNA sequence (ref. 10) and gene 1. Two of these polymorphisms were noted by Izumi *et al.* (11).

ably arose through a gene duplication event. These two lipoxygenase genes, as well as the 15-lipoxygenase gene, have all been mapped to human chromosome 17. Previously, we had mapped the human cyclooxygenase gene to chromosome 9 by the same methods (32). Interestingly, the gene for platelet glycoprotein IIb resides also on chromosome 17 (33). 12-HETE, a product of the platelet 12-lipoxygenase, apparently modulates expression of the glycoprotein IIb/IIIa complex by an unknown mechanism (5).

The 5′ flanking region of the 12-lipoxygenase gene 1 is somewhat related to the corresponding regions of the 5- and 15-lipoxygenase genes. Their 5′ noncoding sequences are short (<90 nucleotides) and very (G+C)-rich. All have GC boxes (3 for 12-lipoxygenase, 10 for 5-lipoxygenase, and 1 for 15-lipoxygenase), potential sites for binding the transcription factor Sp1. These genes lack a CCAAT motif in the canonical position, and the 12- and 15-lipoxygenase genes have a "TATA-like" sequence upstream from the transcription initiation sites. Phorbol ester treatment up-regulates 12-lipoxygenase expression in HEL cells (10, 11). This regulation could be mediated through interaction with one or both of the AP-2 consensus sequences in this putative promoter region. The significance of these motifs as well as the extent of platelet/megakaryocyte-specific nuclear factors controlling expression of this gene will await further functional characterization of the promoter.

Most studies to date that suggest the presence of 12-lipoxygenase isoforms have used bovine tissues (12, 34). Various biochemical and immunological criteria give evidence for three forms in this species. There has been little convincing evidence for the existence of 12-lipoxygenase in human tissues, except in platelets and HEL cells. Previously, we were unable to detect 12-lipoxygenase mRNA in human liver, lung, adrenal gland, aorta, and UCLA-P3 lung epithelial cells (10). These negative findings may have been due to the relative insensitivity of the RNA blot analysis or, alternatively, to a very low expression level. Indeed, we found the 12-lipoxygenase mRNA in HEL cells to be of low abundance by quantitative PCR analysis (19). In the present experiments we have definitively found evidence for 12-lipoxygenase mRNA in human umbilical vein endothelial cells. The formation of small amounts of 12-HETE in these cultured cells has been reported (35). Perhaps some low levels of 12-lipoxygenase are expressed in other human tissues because we consistently observed a 12-lipoxygenase-specific PCR-amplifiable band. However, we cannot exclude the possibility of platelet contamination in these tissue preparations. Previously, there has been evidence for 12-lipoxygenase enzyme activity in rat adrenal and pancreatic tissues, whereby 12(*S*)-hydroperoxyeicosatetraenoic acid may modulate angiotensin II-induced aldosterone (36) and insulin (37) secretions, respectively. 12-Lipoxygenase enzyme has also been immunolocalized in several porcine tissues (38), including parenchymal cells of the anterior pituitary gland (39). Metabolites of 12-lipoxygenase are released by rabbit corneal tissues in response to injury (40). Further studies involving *in situ* hybridization will be required to resolve the extraplatelet expression of 12-lipoxygenase in human tissues.

In conclusion, we have isolated and partially characterized two human 12-lipoxygenase genes, one functional and one an apparent nonprocessed pseudogene. The availability of 12-lipoxygenase genomic clones will enable experiments that study the factors regulating formation of 12-lipoxygenase products, hopefully leading us to understand the role of this enzyme in human biology.

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