Human Elastase 1: Evidence for Expression in the Skin and the Identification of a Frequent Frameshift Polymorphism

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Human pancreatic elastase 1 is a serine protease which maps to the chromosomal region 12q13 close to a locus for an autosomal dominant skin disease, diffuse nonepidermolytic palmoplantar keratoderma, and was investigated as a possible candidate gene for this disorder. Expression of two elastase inhibitors, elafin and SLPI, has been related to several hyperproliferative skin conditions. elastase 1 is functionally silent in the human pancreas but elastase 1 expression at the mRNA level was detected in human cultured primary keratinocytes. Antibody staining localized the protein to the basal cell layer of the human epidermis at a number of sites including the palmoplanta. Sequencing of genomic DNA from individuals with/without the keratoderma revealed a sequence variant, which would result in a premature truncation of the protein. This sequence variant, however, did not segregate with the skin disease and, indeed, was found to occur at a relatively high frequency in the population. Individuals homozygous for the variant do not have any obvious skin abnormalities. Based on the analysis of the secondary structure of the translated putative protein, the truncation is unlikely to result in knock-out of the elastase, but may cause destabilization of the enzyme–inhibitor complex. ***Key words: elafin/keratinocyte/protein truncation/serine protease. J Invest Dermatol 114:165–170, 2000***

The human elastase I gene (ELA1) maps to chromosome 12q13 (Davies *et al*, 1995) and could be regarded as a positional candidate for the autosomal dominant skin disease, diffuse nonepidermolytic palmoplantar keratoderma (NEPPK) (Kelsell *et al*, 1995, 1999). Elastase (EC 3.4.21.36.) is a member of a group of enzymes termed "serine proteases" which are characterized by the reactivity of a serine residue in the active site of the enzyme. Elastase breaks down elastin, the specific protein of elastic fibers, and digests other proteins such as fibrin, hemoglobin, and albumin. Three structurally related types of elastase, named elastases I, II, and III (or protease E), have been identified, with several isoforms being secreted by the mammalian exocrine pancreas (MacDonald *et al*, 1982; Kawashima *et al*, 1987; Shirasu *et al*, 1988; Tani *et al*, 1988). Despite the apparent structural integrity of the human elastase I gene (ELA1), it is reported to be transcriptionally silent (Tani *et al*, 1987; Kawashima *et al*, 1992). The evolutionary silencing of the human elastase I gene in pancreatic acinar cells appears to be due to mutations that inactivate enhancer and promoter elements (Rose and MacDonald, 1997) which are crucial for pancreas specific transcription (Roux *et al*, 1989; Swift *et al*, 1989, 1994; Rose *et al*, 1994; Kruse *et al*, 1995). Dot blotting of human tissue mRNAs from kidney, heart, liver, aortic intima, and peripheral blood lymphocytes failed to detect hybridization to ^{32}P-labeled human elastase I DNA probe, neither could elastase I mRNA be detected in the human kidney, liver, or heart by the more sensitive reverse transcription–polymerase chain reaction (PCR) technique (Kawashima *et al*, 1992). It should be noted that when in the literature human elastase 1 activity in the pancreas is described, it is in fact elastase IIA.

An elastase inhibitor termed elafin (Wiedow *et al*, 1990), also referred to as skin-derived anti-leukoproteinase (SKALP: Schalkwijk *et al*, 1990, 1991), has been isolated from the skin of patients with scaling skin disorders (Chang *et al*, 1990). Elafin has been shown to be a potent and specific inhibitor of porcine pancreatic elastase and human leukocyte elastase (Wiedow *et al*, 1990) as well as proteinase 3, an elastin degrading enzyme of neutrophils (Wiedow *et al*, 1991; Molhuizen *et al*, 1993). Another elastase inhibitor, secretory leukocyte protease inhibitor (SLPI) is also expressed in epidermal keratinocytes and upregulated in epidermal hyperproliferative skin disease (Wiedow *et al*, 1998; Wingens *et al*, 1998). Although elastase I is not expressed in human pancreas and other tissues, the striking conservation of the protein coding region and absence of any changes that could prevent correct RNA splicing or synthesis of a functional enzyme, suggest that this gene may in fact be functional, and that a candidate site for its expression is human skin where it may interact with elafin or SLPI. The latter hypothesis is supported by the fact that the crystal structure and the inhibitory mechanism of elafin was determined from its complex with porcine pancreatic elastase (Tsunemi *et al*, 1996)—the porcine orthologue of human elastase I.

This study reports the cloning and sequencing of a full-length transcript of pancreatic elastase I from cultured human skin keratinocytes. We also describe a nucleotide polymorphism in the coding region of the ELA1 gene, which would lead to truncation of the protein and may destabilize the enzyme–inhibitor complex.

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Abbreviations: ELA1, human pancreatic elastase 1; SLPI-secretory leukocyte protease inhibitor

MATERIALS AND METHODS

Immunohistochemistry Immunostaining was performed on 4–5 μm frozen skin sections on Superfrost Plus (''BDH'' Laboratory Supplies, Merck, Leicestershire, U.K.) microscope slides. Fresh skin was snap frozen in liquid nitrogen and stored at –70°C. The material was cryosectioned, the sections air dried and stained by standard immunohistochemistry protocols. The sections were stained using DAKO EnVision + System HRP (DAB) (DAKO, Carpinteria, CA) following the manufacturers instructions. The tissue sections were blocked with normal swine serum (diluted 1:5 in phosphate buffered saline) before incubation with the primary antibody. Anti-porcine pancreatic elastase rabbit monoclonal antibody (Chemicon International, Temecula, CA) was diluted 1:5000 in phosphate buffered saline with 0.1% BSA. Control sections were incubated with phosphate buffered saline, 0.1% bovine serum albumin instead of the primary antibody. Anti-human leukocyte elastase mouse monoclonal antibody from DAKO was used in dilutions 1:50 and 1:100.

RNA isolation Human skin primary keratinocytes were cultured with irradiated mouse 3T3 feeders as described in Navsaria *et al* (1994). Total RNA from confluent keratinocyte cultures and snap frozen tissue samples was isolated with RNAzol B (Tel-Test, Friendswood, TX) in a single-step procedure according to manufacturers instructions. The quality and integrity of the extracted total RNA were checked by visual inspection of the 18S and 28S rRNA in ethidium-bromide-stained agarose gels (not shown).

Reverse transcription–PCR and cDNA cloning For reverse transcription–PCR GeneAmp RNA PCR Kit (Perkin-Elmer) was used. Total RNA samples were heated to 65°C for 2 min to disrupt secondary structures which could mask the poly(A) segment of the mRNA and 1 μg of RNA pipetted directly into the master mix containing 2.5 μM oligo $d(T)^{16}$, 50 mM KCl, 5 mM $MgCl_2$, 10 mM Tris–HCl pH 8.3, dNTPs 1 mM each, RNase inhibitor 1 U per μl and 2.5 U per μl MuLV Reverse transcriptase, final volume 20 μl. The reactions were incubated at room temperature for 10 min for primer annealing and reverse transcription was performed for 30 min at 42°C in a hot top PCR machine, followed by 5 min at 99°C denaturation and 5 min at 5°C cooling step. For the PCR step, gene-specific primers and 1 unit of Taq polymerase were added and the reactions diluted to 2.2 mM $MgCl_2$ and 0.2 mM dNTP, in final volume of 100 μl. Amplification was carried out for 40 cycles, with denaturation at 95°C for 30 s, annealing 61°C for 1 min and extension at 72°C for 2 min in a Perkin-Elmer DNA thermal cycler.

Two overlapping PCR fragments covering the full length human pancreatic elastase I transcript were amplified using the primers pairs: forward primer 5′CAAGAAGGCAGTGGTCTACT 3′ and reverse primer 5′CTGACATCCAGAGCGAACTC3′ yielding a 639 bp N-terminal fragment and the forward primer 5′CAATGGGCAGCTGGCCCAGA3′ with the reverse primer 5′TCGCAAGTCCTATTGCAGATC3′ for the 370 bp C-terminal fragment. The 125 bp overlapping region of these two fragments contains a unique *Pst*I site. The PCR products were resolved in a 1.5% agarose gel. The fragments were gel purified using GeneClean (Bio101, Vista, CA) and subcloned into pGEM-T vector (Promega, Madison, WI). The clones were sequenced in both directions using PCR as well as additional internal primers. Further fragments from different primary keratinocyte cultures of different skin types were purified and sequenced directly; however, 45 PCR cycles were required to create enough 639 bp template for direct sequencing.

The reverse transcription–PCR primers for detection of human elastase II or elastase III were designed to recognize all known isoforms of the corresponding enzymes. The known mRNA sequences were aligned and primers chosen based on the mRNA full identity regions.

Reverse transcription–PCR primer sequences for elastase II (ELS2), based on mRNA sequences submitted to GenBank (accession numbers: D00236, E01220, M16631, M16652, M16653) were:
1 421 bp 5′ fragment: forward primer 5′ GCTGGAGCCCTCAG-TTGTGGG 3′; reverse primer 5′ TGTTGGGTAGAATGGTGCCGG 3′;
2 520 bp 3′ fragment: forward primer 5′ AGTCCGGCTCGCTG-GCAG 3′; reverse primer 5′ GCAATCACCGAATTGATCCAGTCG 3′.
Elastase III (EL3) (GenBank Accessions: M18692 J03516, E01257):
1 430 bp 5′ fragment: forward primer 5′ GCTCAGTTCCCTCCT-CCTTGTGG 3′; reverse primer 5′ ACCAGCCGGAGGGAGTGAGG 3′;
2 615 bp 3′ fragment: forward primer 5′ GCTCCCGGACCTACCA-GGTGG 3′; reverse primer 5′ TCGATCAGCACTGCCAGCTGG 3′.

Sequencing of genomic DNA DNA was isolated from human blood samples using Nucleon BACC3 DNA extraction kit (Nucleon Biosciences, Strathclyde, U.K.) according to the manufacturer's instructions. PCR reactions were typically performed with AB Thermostable DNA polymerase and reaction buffer (''Advanced Biotechnologies'', Epsom, Surrey, U.K.) at 60–62°C primer annealing.

Primers for the PCR amplification of the human elastase I from the genomic DNA primers were designed based on the sequence in the GenBank (Submission by Kawashima *et al*, 1992; accession numbers X62252–X62259). For the exons where only a few base pairs of flanking sequence were available in the public domain, primers were placed in the exon to amplify the introns. Long range Advantage Genomic Polymerase mix (Clontech, Palo Alto, CA) and 68°C annealing temperature was used for PCR of the long fragments. For intron 6 a Genome Walking approach (as described in Siebert *et al*, 1995) was required. The adaptor-ligated genomic DNA fragment libraries and adaptor specific primers were a generous gift from Dr Ian Gray. The primary PCR reaction was performed using the outer adaptor primer and an outer gene specific primer in the middle of the neighboring exon. Thereafter the primary reactions were diluted 50-fold and a ''nested'' PCR reaction was performed using corresponding inner primers. The nested PCR yielded clear single bands, which were column purified and sequenced directly.

Primer sequences designed based on the obtained intron sequences were used to screen the coding region and splice sites of the human elastase I for mutations/polymorphism. The primer pairs which amplify the eight exons of the ELA1 gene are shown in **Table I**.

The PCR products were purified using QIAquick PCR purification columns (QIAgen) and sequenced on both strands using the ABI PRISM Rhodamine or BigDye terminator sequencing ready reaction mix (Perkin Elmer) on an ABI 377 automatic sequencer.

Table I. Primer pairs which amplify the eight exons of the ELA1 gene

Exon no.	Primer	
1	Forward	5′ ATGGATGACAAGGGGTGCTCCC 3′
	Reverse	5′ GGGCCTGAATAGCCAGTGGCC 3′
2	Forward	5′ CTCAGAGAACTCACAGCTGGGCC 3′
	Reverse	5′ ACCACCTAAGCCTGATCCCATCC 3′
3	Forward	5′ CAGCTCNTAGGCTCAACTGATCCTC 3′
	Reverse	5′ GAGAGGGCAAAATTATATCCTCTTTCAG 3′
4	Forward	5 ‘CCATTCTCCTATCTCTAAAGTGGGC 3’
	Reverse	5′ CTCCTGGACGAATGAGCCAGC 3′
5	Forward	5′ GCTGCAATACCAATGTCCCACC 3′
	Reverse	5′ CCTGGTCTCTGGCCATAAGCAC 3′
6	Forward	5′ CCTGAGCTCAGCTTTCACGAGAG 3′
	Reverse	5′ AAGTGAGGGCATCGAGCAAGATC 3′
7	Forward	5′ TTTAGGATTCTGTTTCTCCTCCCTGC 3′
	Reverse	5′ AAGGAAGATGACGGCTTGCCC 3′
8	PCR forward	5′ GCTTGAGAGTTAGGTGAGGCTCTG 3′
	Reverse	5′ AGGGACCCCTGCTCTGGAGG 3′
	Sequenced forward with	5′ GCTCACGGCCATTTCAAGGTC 3′

RESULTS

A pancreatic elastase-like protein is present in normal human skin In order to test for the presence of elastase 1 in human skin, we used an antibody raised against the porcine pancreatic elastase, which has 89% amino acid identity with its human homolog (see alignment in **Fig 5*B***), to screen sections of normal human skin for the presence and localization of the ELA1 protein. The antibody detected expression of an elastase-like protein in the basal layer of normal human epidermis from breast, palm, sole, and foreskin. An example of the immunohistochemical staining of human sole skin with the anti-porcine pancreatic elastase antibody is shown in **Fig 1**. This protein was not recognized by the antibody directed against human leukocyte elastase (not shown). The fact that we used the anti-elastase antibody at such a low dilution (dilution 1:5000) makes it unlikely that the anti-porcine pancreatic elastase antibody cross-reacted with keratins. We had previously tested other rabbit antibodies and did not see similar staining even at lower dilutions of the antibody.

The presence of a pancreatic elastase like protein in human skin raises the question of whether the changes in the promoter enhancer region of the human elastase 1 gene (Rose and MacDonald, 1997) do not affect its expression in skin or have redirected expression from the pancreas to the skin. In order to shed some light on this question we have stained cryosections of pig and mouse skin with the same anti-porcine pancreatic elastase antibody (not shown). The antibody clearly stained the basal layers of these skin sections, but also the hair follicle and the sebaceous gland epithelia. These observations suggest, that a similar protein is present in the skin of other mammals which are known to express the enzyme in the pancreas.

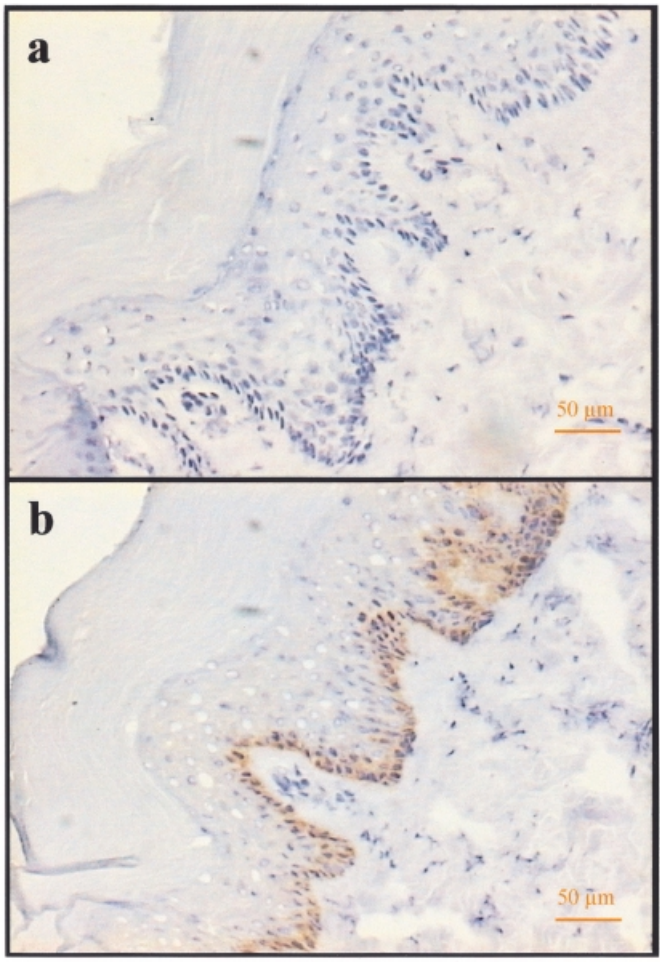

Figure 1. Immunostaining of normal human sole skin for pancreatic elastase like proteins. (*a*) Nonstained control section (secondary AB only). (*b*) Stained with anti-porcine pancreatic elastase rabbit monoclonal antibody. At antibody dilution 1:5000 a clear continuous staining of the basal cell layer of epidermis is observed.

Normal human keratinocytes express elastase I (ELA1) Owing to the high level of amino acid conservation between pancreatic elastases, cross-reactivity of the antibody with the other two known pancreatic elastases could not be excluded. Very weak staining of acinary cells in cryosections from human pancreas could be observed at higher concentrations of the antibody, although pancreatic elastase I has been shown to be absent in the human pancreas. This suggests that the antibody can also detect other pancreatic elastases. Therefore, we have made mRNA specific primers for all three known human pancreatic elastase genes (ELA1, ELS2, and EL3) to investigate mRNA expression in human cultured keratinocytes and fresh pancreatic tissue by reverse transcription–PCR. The two ELA1 specific fragments could be found only in the keratinocyte total RNA preparation (**Fig 2**, *lanes 1* and *2*). Reverse transcription–PCR amplified four fragments of the expected size (421 bp and 520 bp fragment of elastase II – **Fig 2**, *lanes 7* and *8* and 430 bp and 615 bp fragment of elastase III – **Fig 2**, *lanes 11* and *12*) from the total RNA preparation from human pancreas, but failed to detect any of these products in cultured human primary keratinocytes (**Fig 2**, *lanes 5*, *6*, *9*, and *10*). These primers were designed to recognize all known isoforms of human elastase II or elastase III, respectively (see *Materials and Methods*). Our data implies that the protein detected by the anti-porcine pancreatic elastase antibody in the epidermis is likely to be human elastase I.

cDNA sequence analysis The nucleotide sequence of the mature mRNA for human elastase I has not yet been reported (see *Introduction*) and only a synthetic putative mRNA sequence (accession number E01447), predicted from genomic sequence and its comparison with the nucleotide sequence of the rat elastase I

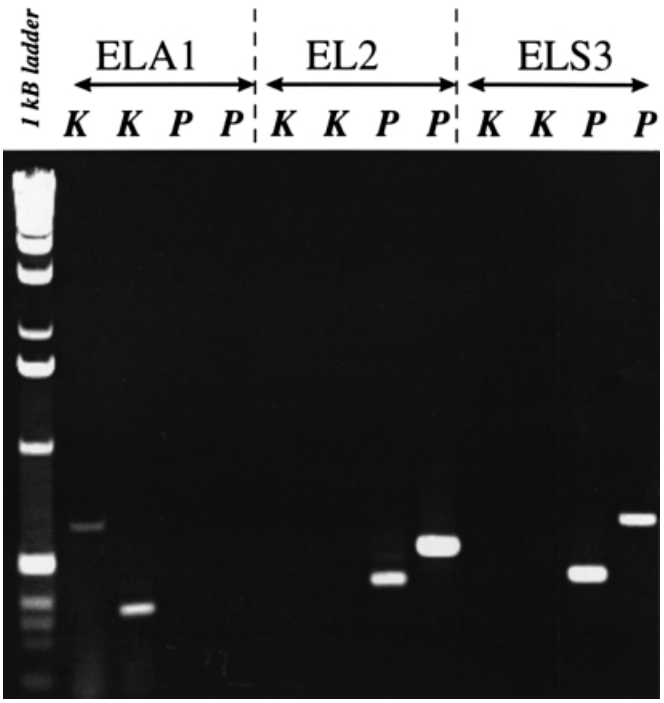

Figure 2. Reverse transcription–PCR of the known human pancreatic elastases I, II, and III from total RNA preparations. Primers were designed to amplify two different overlapping fragments of each elastase. K, keratinocytes; P, pancreas. PCR conditions: 45 cycles of 95°C 30 s, 61°C 60 s, 72°C 120 s. *Lanes 1* and *3*, 639 bp N-terminal PCR of ELA1; *lanes 2* and *4*, 370 bp C-terminal PCR of ELA1; *lanes 5* and *7*, 421 bp PCR of ELS2; *lanes 6* and *8*, 520 bp PCR of ELS2; *lanes 9* and *11*, 430 bp PCR of EL3; *lanes 10* and *11*, 615 bp PCR of EL3.

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                                                caagaaggcagtggtctactccatcggcaac

atgctggtcctttatggacacagcacccaggaccttccggaaaccaatgcccgcgtagtc
 M  L  V  L  Y  G  H  S  T  Q  D  L  P  E  T  N  A  R  V  V
ggagggactgaggccgggaggaattcctggccctctcagatttccctccagtaccggtct
 G  G  T  E  A  G  R  N  S  W  P  S  Q  I  S  L  Q  Y  R  S
ggaggttccggtatcacacctgtggagggacccttatcagacagaactgggtgatgaca
 G  G  S  R  Y  H  T  C  G  G  T  L  I  R  Q  N  W  V  M  T
gctgctcactgcgtggattaccagaagactttccgcgtggtggctggagaccataacctg
 A  A  H  C  V  D  Y  Q  K  T  F  R  V  V  A  G  D  H  N  L
agccagaatgatggcactgagcagtacgtgagtgtgcagaagatcgtggtgcatccatac
 S  Q  N  D  G  T  E  Q  Y  V  S  V  Q  K  I  V  V  H  P  Y
tggaacagcgataacgtggctgccggctatgacatcgccctgctgcgcctggcccagagc
 W  N  S  D  N  V  A  A  G  Y  D  I  A  L  L  R  L  A  Q  S
gttaccctcaatagctatgtccagctgggtgttctgccccaggagggagccatcctggct
 V  T  L  N  S  Y  V  Q  L  G  V  L  P  Q  E  G  A  I  L  A
aacaacagtccctgctacatcacaggctggggcaagaccaagaccaatgggcagctggcc
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cagaccctgcagcaggcttacctgccctctgtggactatgccatctgctccagctcctcc
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 H  G  V  T  S  F  V  S  S  R  G  C  N  V  S  R  K  P  T  V
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 F  T  Q  V  S  A  Y  I  S  W  I  N  N  V  I  A  S  N
ttttcctgagtccaacgaccttcccaaaatggttcttagatctgcaataggacttgcga
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Figure 3. Human elastase I cDNA as sequenced from primary keratinocytes and amino acid translation of the coding region. Underlined nucleotides denote differences between our sequence and the one proposed by Kawashima *et al* (1992; see text). The amino acids of the catalytic triad (His-63, Asp-111, and Ser-206) are printed in bold with a double underline. Bold script with a single underline highlights Val-227 and Thr-239 residues present at the mouth of the substrate binding pocket, which contribute to the substrate specificity of elastase I. The amino acids printed in bold italic are involved in the primary contact of the enzyme/inhibitor complex with elafin.

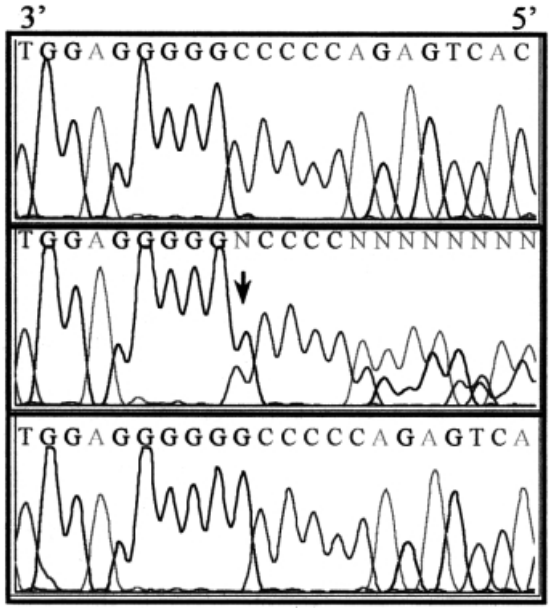

Figure 4. Sequencing traces of the polymorphic locus in exon 7 of ELA1. Wild-type (*top*), heterozygous (*middle*), homozygous individuals (*bottom*) for the variant. Insertion of an extra C is revealed as an extra G on the reverse strand (3′→5′), as shown with an *arrow*.

gene and rat cDNA is available (Kawashima *et al*, 1992) (and partial cDNA 142 bp fragment accession number D00159).

The usage of two alternative first exons has been suggested in the ELA1 gene (Kawashima *et al*, 1992; accession numbers X62258 and X62259, see **Fig 5*A***). We designed a gene-specific primer in the region upstream of the translation start codon, which is identical in both sequence variants. As mentioned above our two primer pairs (for sequences see *Materials and Methods*) amplified fragments of expected size: 640 and 370 bp, respectively, from the human keratinocyte total RNA (**Fig 2**, *lanes 1* and *2*). These fragments cover the full length of the gene coding sequence and were subcloned into pGEM-T. The 125 bp overlapping region of these two fragments contains a unique *Pst*I site. Sequencing of these fragments revealed mature human ELA1 cDNA 99% identical to the one predicted. The full length cDNA sequence of human elastase I (our submission to GenBank, accession number: AF120493) and the amino acid translation of the coding region is shown in **Fig 3**. Our cDNA sequence contains two nucleotide substitutions (t⇒c in exon 3 and g⇒a in exon 7) as compared with the published human elastase I genomic sequence(see **Fig 5*A***). These substitutions lead to the exchange of the coded amino acids: Trp-44 replaces the Arg residue and Gln substitutes for Arg-243. The same nucleotide differences were also found in all of our genomic DNA samples (see text below). These changes, however, may reflect an allelic polymorphism between Caucasian and Mongolian populations.

Although Kawashima *et al* (1992) have proposed two alternative splice variants of the exon1 of the gene (accession number X62258) differing by 40 bp in length, we could identify only the short form (see **Fig 5*A***), implying that the first splice site is preferentially used for mRNA splicing in keratinocytes.

Total RNA preparations of primary keratinocytes cultured from normal human skin of different body sites (breast, abdomen, palm), as well as from individuals of different ethnic and racial origin were positive for the two human elastase I reverse transcription–PCR fragments. A weak band of the reverse transcription–PCR product could also be obtained from normal skin total RNA samples.

An interesting polymorphism immediately downstream of the active site of the enzyme To investigate if mutations in ELA1 could underlie diffuse NEPPK, we have screened the coding region of the ELA1 gene (including splice sites) in genomic DNA from affected and unaffected family members.

The sequencing revealed an interesting polymorphism in exon 7. The genomic DNA sequence of a heterozygote and homozygotes for both alleles is shown in **Fig 4**. One allele is the result of an insertion of a C (shown as a G on the reverse strand in **Fig 4**, see also **Fig 5*A***) in a potential mutation ''hot spot'' comprised of a stretch of five guanine and followed by five cytosine nucleotides. This ''hot spot'' lies directly downstream from the sequence encoding the active site pocket of the enzyme (see **Fig 3**) and the insertion leads to a frameshift causing an early stop codon and shortening the putative 258 amino acid protein by 26 amino acids.

The protein truncating allele did not cosegregate with the disease, suggesting it may be a polymorphism. Screening of lymphocyte DNA from 80 additional unrelated normal individuals for this variant revealed the presence of this allele at a relatively high frequency with 43 homozygous for the ''wild-type'' allele, 31 heterozygotes and six homozygotes for the truncated variant. Individuals heterozygous and homozygous for this mutation appear normal with no obvious skin complaints. Unfortunately, no skin biopsies were available from the individuals who are homozygous for this polymorphism to investigate any subtle epidermal changes.

Owing to the unexpectedly high frequency of the protein truncating variant among apparently normal individuals, we have tried to evaluate the possible effect of the frameshift on the folding of the translated protein. The active site of ELA1 includes the amino acids of the catalytic triad (His-63, Asp-111, and Ser-206). These residues, as well as most of the cysteine residues (all but the last one), which stabilize the secondary structure of the protein, are coded upstream of the frameshift and remain unaffected (see alignments in **Fig 5*B***). We have analyzed and compared the secondary structures of the two putative gene products using the SOPM protein analysis program (Geourjon and Deléage, 1994). On a secondary structure level the polymorphism manifests itself in an excision of a short sheet–coil–turn–coil–turn–coil–sheet loop that precedes the terminal helix. This loop carries the key amino acid residues Val-227 and Thr-239 present at the mouth of the substrate binding pocket, which contribute to the substrate

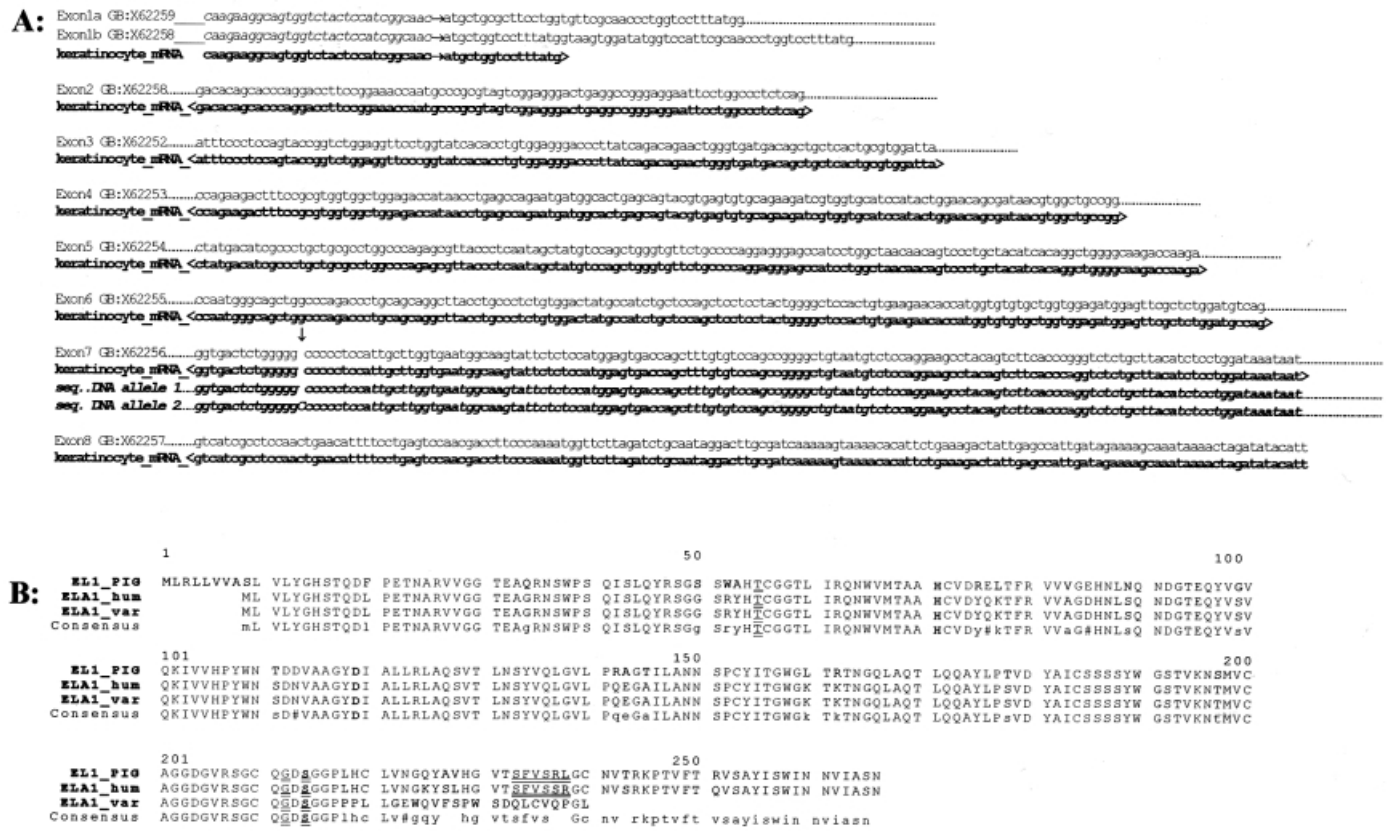

Figure 5. Sequence comparisons of Elastase 1. (*A*) Comparison of the earlier GenBank submissions of human elastase 1 exon sequence with our keratinocyte mRNA and blood DNA sequencing data (black bold print). → denotes the translational start point, >< show mRNA splice sites. The polymorphic site in exon 7 with an extra C insertion in the allele 2 is highlighted by an arrow (↓). Translation of the allele2 leads to the truncated ELA1 protein shown as ELA1_var in (*B*). Allele 1 corresponds to ELA1_hum in the (*B*) of the figure. (*B*) Multiple alignment of the porcine pancreatic elastase (EL1_PIG) and human elastase 1 (ELA1_hum) proteins with its truncated variant (ELA1_var). The amino acids of the catalytic triad (His-63, Asp-111, and Ser-206) are shown in bold. The amino acids printed in bold italic with double underline are involved in the primary contact of the enzyme/inhibitor complex with elafin.

specificity of elastase I (highlighted in **Fig 3**), as well as five of the eight amino acids involved in the primary contact of the elafin/elastase complex formation (see **Fig 5** highlighted also in **Fig 3**) (Tsunemi *et al*, 1996). These observations imply that the sequence variant might modify the substrate specificity of the enzyme and abolish the inhibitor binding capability.

DISCUSSION

We have demonstrated using immunohistochemistry that an elastase is localized in the basal cell layer of the mammalian skin. In human keratinocytes, we found the proposed shorter splice form of ELA1 gene (Kawashima *et al*, 1992), leaving the signal peptide of the translated product (only eight amino acids) too short for a secretory enzyme. Though not likely to be secreted, elastase 1 could be involved in the detachment of cells from the basement membrane upon their movement to the upper layers.

The secondary structure analysis of the truncated variant showed that it would not necessarily lead to the disruption of its vital function and could explain the absence of a clear phenotype associated with this variant. Inhibitor binding capability, however is likely to be abolished and abnormalities could be expected under conditions where blocking the enzyme activity becomes essential.

Elafin, the skin-derived elastase inhibitor, has been shown to be a potent and specific inhibitor of both the porcine homolog of ELA1 and human leukocyte elastase *in vitro* (Wiedow *et al*, 1990). Elafin is expressed by epidermal keratinocytes under hyperproliferative conditions such as psoriasis (Chang *et al*, 1990; Schalkwijk *et al*, 1990, 1991; Wiedow *et al*, 1993; Nonomura *et al*, 1994) and wound healing (van Bergen *et al*, 1996). It has also been reported to be present in many other adult epithelia that are exposed to environmental stimuli: tongue, plate, lingual tonsils, gingiva, pharynx, epiglottis, vocal fold, esophagus, uterine cervix, vagina, and hair follicles (Pfundt *et al*, 1996). In all these tissues, the presence of inflammatory cells is physiologic and elafin expression is believed to protect against leukocyte proteases, thereby helping to maintain epithelial integrity.

It is interesting to note that the antibody staining of normal skin in our experiments was mainly restricted to the basal, less-differentiated layer of the epidermis. Elafin on the contrary has never been found in the basal layer in any type of epithelial tissue and appears to be associated with the differentiation program (Alkemade *et al*, 1993, 1994a; Pfundt *et al*, 1996). Indeed elafin is virtually absent in normal human epidermis. The other known elastase inhibitor, SLP1, however, has been reported to be expressed in the basal keratinocytes (Wiedow *et al*, 1998; Wingens *et al*, 1998) suggesting that this may be the major elastase inhibitor in normal epidermis. Though there were no obvious pathogenic epidermal abnormalities associated with the truncated ELA1 variant, it is possible that carriers of this allele may be at greater risk of developing the common skin diseases such as psoriasis and eczema. Genetic and histologic studies will be required to investigate the role of ELA1 in these common epidermal disorders. Possible roles for elastase 1 in wound healing and cancer could also be explored (Alkemade *et al*, 1994b; Yamamoto *et al*, 1997).

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