Immunogenetics (1998) 48: 40–46

ORIGINAL PAPER

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A new apparently functional *IGVK* gene (Vk*La*) present in some individuals only

Received: 21 August 1997 / Revised: 23 December 1997

Abstract We describe a hitherto unknown functional *IGKV* gene, Vk*La*, belonging to the *IGKV1* subgroup with exon 2 having only 94% similarity to the closest known *IGKV* gene, *1–13/1D-13* (*L4/L18a*). Genomic DNA sequences spanning from 5' of the decanucleotide box to 3' of the heptamer (649 bp) were cloned and sequenced from four individuals. The new gene encodes the conserved amino acids in the exons and contains no apparent defects in known regulatory intron sequences such as pd-box, dc-box, TATA-box, CCCT-elements, splice-sequences, initiation codon, and heptamer sequence. Vk*La* is therefore potentially functional and, correspondingly, we found transcripts of properly rearranged Vk*La* with somatical hypermutations. Vk*La* was found in 12 of 57 (21%) healthy Caucasians by a nested polymerase chain reaction and subsequent sequencing of exon 2. This finding shows that there is more inter-individual variation in the available *IGKV* gene repertoire than was hitherto assumed. Finally, we describe a minor correction in the *IGKV1D-43* (*L23*) gene sequence.

Key words Immunoglobulin · Genes · Kappa light chain · Human · Antibody repertoire

The nucleotide sequence data reported in this paper have been submitted to the EMBL/GenBank nucleotide sequence databases and have been assigned the accession numbers HSY14861 (Vk*La*E03), HSY14862 (Vk*La*E07), HSY14863 (Vk*La*E25), HSY14864 (Vk*La*E34), HSY14865 (the germline gene Vk*La*), HSY14866 (Vk*La*V16), HSY14867 (Vk*La*V19), HSY14868 (Vk*La*V82), HSY14869 (Vk*La*V85), HSY14870 (Vk*La*V87), HSY14871 (Vk*La*V88), and HSY14872 (Vk*La*V94). In this report the *IGK* gene names used in the IMGT database [IMGT 1997 (http://im-gt.cnusc.fr:8104); Lefranc et al. (1998)] have been employed. The nomenclature of Zachau and co-workers (1996) is indicated in parentheses

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Introduction

Most of the kappa immunoglobulin (*IGK*) light chain genes are located on chromosome 2 at 2p11.2 in a main gene cluster comprising 76 known *IGKV* genes, five *IGKJ* genes, and one *IGKC* gene (Zachau 1996). Of the *IGKV* genes, 34–38 are considered functional, while 32–35 are considered to be pseudogenes. The remaining six or seven genes have open reading frames but do have minor defects that make use of the genes unlikely [IMGT 1997 (http://im-gt.cnusc.fr:8104); Feeney et al. 1997; Juul et al. 1997a]. A further 27 *IGKV* genes – referred to as orphons – have been found outside the *IGKV* gene cluster. Most of the orphons are pseudogenes, with three of them described as ORF: V3/OR2-268 and V1/OR2-108 (Huber 1990; Huber et al. 1994) and V1/OR2-0. The 800 kilobases (kb) of DNA separating the *IGKJ* proximal and the *IGKJ* distal part of the main *K* gene cluster and the regions immediately flanking it have not been found to contain further *IGKV* genes (Zachau 1996). On the basis of hybridization and sequencing of cosmid clones, some investigators have concluded that all potentially functional *IGKV* genes are likely to have been found (Huber et al. 1993). However, the existence of further *IGKV* genes in close proximity to the main gene cluster cannot be completely excluded (Zachau 1996).

In a recent study of the expressed kappa light chain repertoire, we found the expression of germline *IGKV* genes hitherto known in minor defect versions only (Juul et al. 1997a), among these *1–8* (*L9*). During analysis of the germline *IGKV* gene *1–8*, we found – and describe here – a new *IGKV* gene that appears to be functional. We have temporarily named the new gene Vk*La* until its location has been determined and a more systematic name can be assigned.

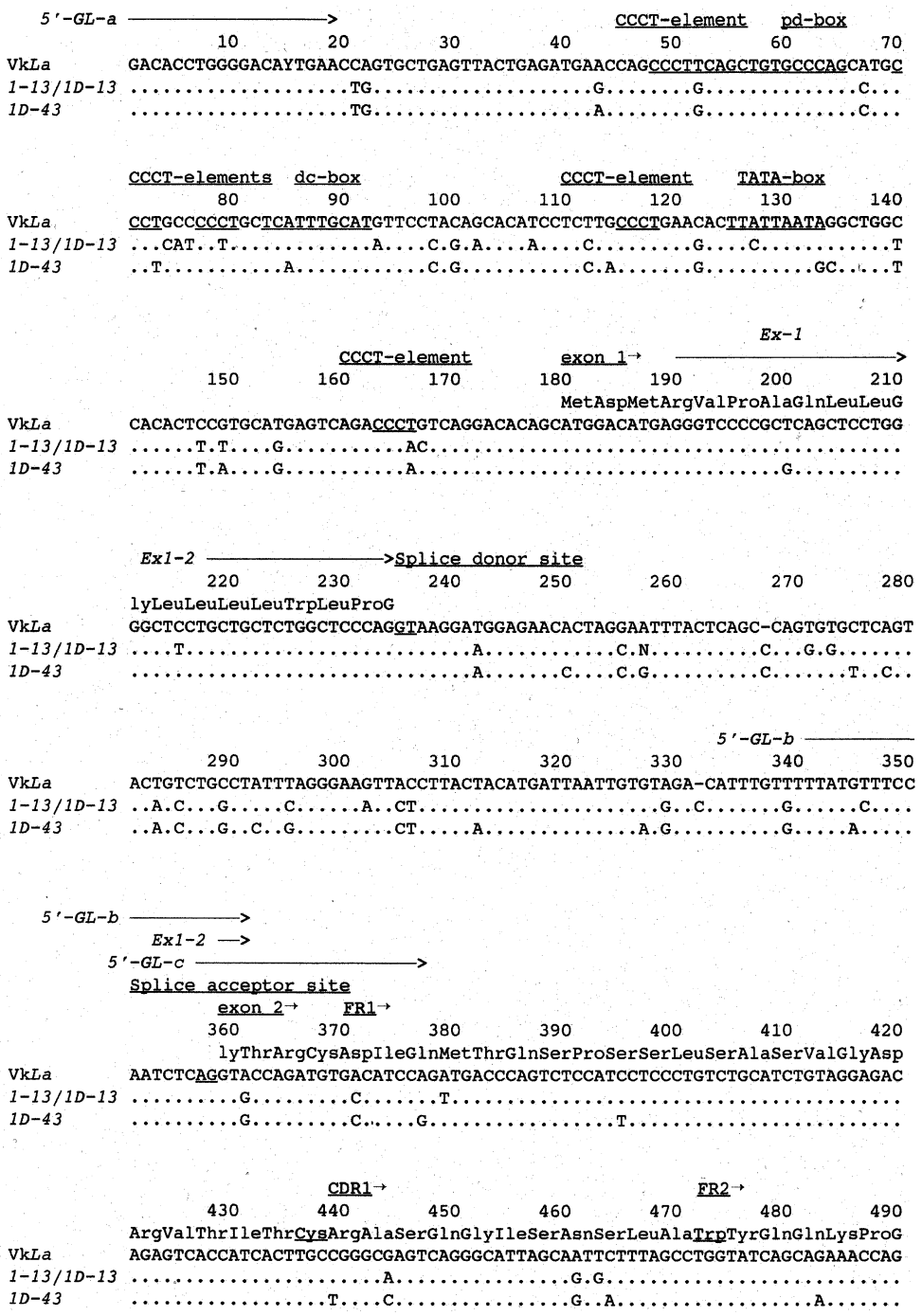

Fig. 1 (For continuation and legend see page 42)

Materials and methods

Source of DNA

Blood samples were obtained from healthy Caucasian donors, and genomic DNA was isolated from blood cells by a salting out method (Miller et al. 1988).

Polymerase chain reaction of genomic DNA

Two polymerase chain reaction (PCR) procedures were used. PCR1 yielded a 686 base pair (bp) product and was performed in a total volume of 50 µl with 0.5 µg genomic DNA as template using 1.25 units of *Taq* polymerase (Perkin Elmer, Norwalk, Conn.), PCR buffer (Perkin Elmer), and dNTP in a final concentration of 0.2 mM each. Ten picomoles of the following primers were used: Upstream degenerated primer: 5'GL1-a: 5'-ATGGATCCGACACCTGGGGACA(T/C)TGAA-3' (*Bam* HI site underlined) and 3'GL1-a: 3'-GGGAATTCGGGGTTTTTGTTTGGGTG-3' (*Eco* RI site underlined), see Fig. 1 (the primer sequences are not included in the sequence submitted to the EMBL database). PCR1 was performed under the following conditions: after an initial denaturation for 4 min at 94 °C, 35 PCR cycles were performed consisting of 1/2 min of denaturation at 94 °C, 1 min of annealing at 50 °C, and 2 min of elongation at 72 °C. Finally, the product was extended for 5 min at 72 °C.

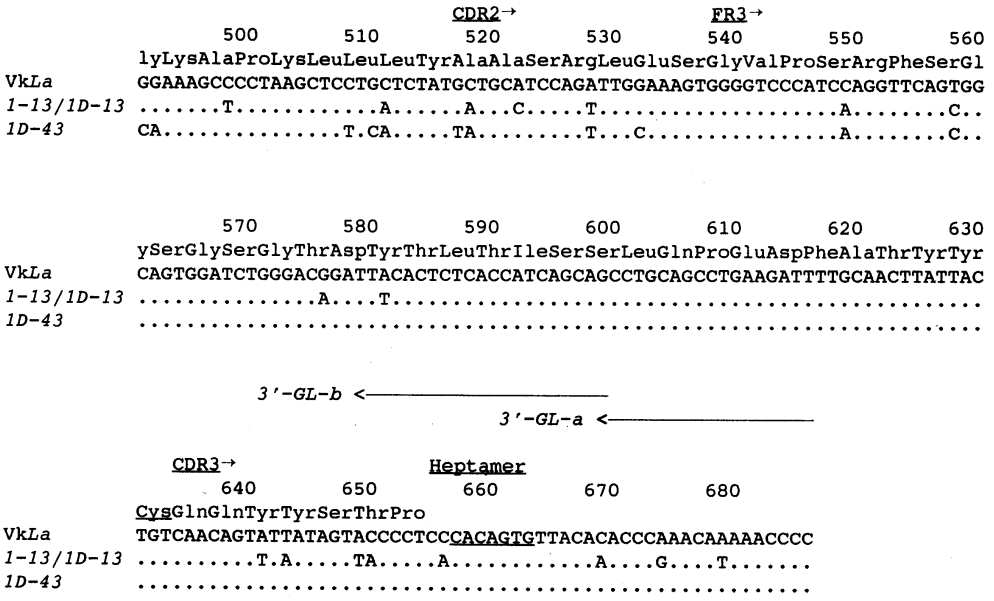

Fig. 1 The Vk*La* gene sequence. For comparison, sequences of the *IGKV* genes *1–13/1D-13* and *1D-43* are given. A *dot* indicates identity with the Vk*La* sequence. A *dash* indicates a missing nucleotide. An "*N*" indicates that the nucleotide has not been determined. The start positions of the exons and exon regions as well as regulatory sequences in non-coding parts of Vk*La* are indicated. An amino acid translation of the exons of Vk*La* is indicated and amino acid residues C23, W41, and C104 are *underlined*. Primers are shown as *arrows,* with their names given in *italics*

The PCR2 procedure was a semi-nested PCR amplifying the exon 2 sequence and was used to determine the frequency of Vk*La*. The first PCR was performed with 0.5 µg of genomic DNA as template in a volume of 15 µl and the same concentrations of the other ingredients as in PCR1. One microliter of product from the first PCR was carried over to the next PCR which was performed with the same concentrations of buffer, *Taq* polymerase, and dNTP in a volume of 15 µl. Five picomoles of each primer was used: in the first PCR the upstream primer was the 21-mer 5'-GL-b: 5'-TTATGTTTCCAATCTCAGGTA-3' and the downstream primer was the 21-mer 3'-GL-b: 5'-GTGTAA-CACTGTGGGAGGGGT-3'. The second PCR used the same downstream primer and the following 21-mer upstream primer 5'-GL-c: 5'-AGGTACCAGATGTGACATCCA-3'. The primers were chosen to yield as much specificity as possible by discriminating the *V*-genes in the 3' end. The primers were located in positions 341–361, 669–649, and 357–377 (see Fig. 1), respectively (these positions equal positions 322–342, 650–630, and 338–358 in EMBL/GenBank submission HSY14865, respectively)

The first PCR was performed for 20 cycles with an annealing temperature of 63 °C and the second PCR was performed for 15 cycles with an annealing temperature of 67 °C. The other cycling conditions were as in PCR1. The PCR products were checked by agarose gel electrophoresis.

The quality of the DNA samples used in PCR2 was ensured by an *IGKV 1–9* (*L8*) PCR performed as described elsewhere (Juul et al. 1997a). All DNA samples yielded a strong band in this PCR.

Cloning and sequencing of PCR products from germline genes

Two procedures were used for the cloning of PCR products. In the first, the PCR product was isolated by preparative agarose gel electrophoresis. The amount of relevant PCR product was estimated and then cut out and purified using the Qiaex kit (Qiagen, Chatsworth, Calif.). The Qiaex-purified DNA and the cloning vector Bluescript KS+ (Stratagene, La Jolla, Calif.) were digested with *Bam* HI and *Eco* RI enzymes in OnePhorAll buffer (Pharmacia, Brussels, Belgium) for 2 h at 37 °C. The cut PCR product was divided between three vials and the cut vector was added to each vial to an estimated molar PCR product: vector ratios of 3:1, 1:1, and 1:3, respectively. The DNA mixture of each vial was purified separately with the Qiaex kit and eluted in 20 µl of water. Subsequently, 2.5 units T4 DNA ligase, ligase buffer (both Amersham, Bucks, UK), hexamine cobalt chloride (final concentration: 1 µM), and rATP (final concentration: 0.5 mM) were added to a final volume of 30 µl. Ligation was performed overnight at 14 °C. After ligation, the three mixtures were pooled, yeast tRNA was added (Gallagher et al. 1987) to a final concentration of 0.5 µg/µl, and the nucleic acids were chloroform/phenol extracted. Afterwards, glycogen and ammonium acetate were added to final concentrations of 20 ng/µl (Walsh et al. 1992) and 2.5 M, respectively. Subsequently, the nucleic acids were precipitated by the addition of 2.5 volumes of 99% ethanol. After a final wash with ethanol, the pellet was dried and redissolved in 10 µl of sterile water. One microliter of this solution was used for electroporation into *Escherechia coli* JM 105 by means of an Electro-Porator as described by the manufacturer (Invitrogen, San Diego, Calif.).

The other procedure used to clone PCR products was a T-overhang cloning vector system (TA cloning kit, Invitrogen, De Schelp, The Netherlands, and pGEM-T Vector Systems, Promega, Madison, Wis.). Between 1 and 3 µl of unpurified PCR product was cloned without prior purification as instructed by the manufacturers.

Expression of VkLa

In order to evaluate Vk*La* expression, peripheral blood mononuclear cells (PBMC) were isolated using density gradient centrifugation on Lymphoprep (Nycomed, Oslo, Norway). RNA was isolated from PBMC by the guanidinium-thiocyanate method (Chomczynski and Sacchi 1987), and cDNA synthesis was performed with RNA from 5×10^5 cells by use of an RT-PCR kit (Perkin Elmer). For the cDNA synthesis, a kappa constant region-specific 18-mer primer was used (5'-ACAGAGGCAGTTCCAGAT-3', being complementary to positions 406–389 in EMBL/GenBank submission J00241). Nested PCR was used because of the expected relatively rare occurrence of sequences originating from Vk*La* (Juul et al. 1997a). The PCRs were performed employing the same conditions as described for the germline gene, with the following exceptions: the first PCR was carried out with an annealing temperature of 55 °C in a volume of 100 µl using the following primers. The upstreamprimer was the 21-mer Ex-1: 5'-GGGTCCCCGCTCAGCTCCTGG-3' identical to a signal peptide sequence (positions 190–210 in Fig. 1 equal to positions 171–191 in EMBL/GenBank submission HSY14865) and downstream primer *IGKC* (5'-CCAAGCTTCATCAGATGGCGGGAAGAT-3', being complementary to positions 378–360 in EMBL/GenBank submission J00241). Of the product from the first PCR, 2 µl was carried over to the second, which was performed with an annealing temperature of 60 °C for 30 cycles and employing the nested 20-mer upstreamprimer Ex1-2 5'-GCTGCTCTGGCTCCCAGGTA-3' (identical to positions 218–234 and 359–361 in Fig. 1 equal to positions 199–215 and 340–

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IGKV1cons                  CTGGTGCTGAGTTACTGAGATGAGCCAGCCCTGCAGCTGTGCCCAGCCT
Our 1D-43-like sequence    ......................A......................
Published 1D-43 sequence   G.G.A.GTCGACT.T..AGGAT.CC.............
pSVB plasmid polylinker    C.G.A.GTCGACT.T..AGGAT.CC
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Fig. 2 The 5' end of an *IGVK* gene *1D-43*-like sequence (the consensus sequence of 11 clones) compared with the published *1D-43* sequence (accession number HSIGKL23), the consensus sequence of functional *IGKV1* genes (*IGKV1*cons), and with a part of the polylinker region of the pSVB plasmids. A *dot* indicates identity with the *IGKV1*cons sequence. The pd-box is *underlined*

342 in EMBL/GenBank submission HSY14865) and the same downstream primer as the first PCR. The PCR products were cloned with a T-overhang cloning vector system as described above.

Cloning and sequencing

The electrotransformed bacteria were plated on 2×YT plates (Sambrook et al. 1989) containing 50 μg of ampicillin per ml and an indicator system for β-galactosidase. The next day, colorless colonies were picked and transferred to a liquid 2×YT medium and allowed to grow overnight. Plasmid DNA was isolated as described (Jones and Schofield 1990). Sequencing of the cloned inserts was performed by automatic sequencing using a 373 DNA Sequencer with dye primers or dye terminators (Applied Biosystems, Perkin Elmer). As sequencing primers, T7 (5'-AATACGACTCACTATAG-3'), T3 (5'-ATTAACCCTCACTAAAG-3') and an internal kappa primer (5'-CTGCTGATGGTGAGAGT-3' (complementary to positions 599–583 in Fig. 1 equal to positions 580–564 in EMBL/GenBank submission HSY14865) were used.

Results and discussion

The germline gene

Two clones from PCR1 products from each of ten healthy Caucasian individuals were sequenced. All clones from four individuals contained a hitherto unknown sequence which we named Vk*La.* The clones of the six remaining individuals yielded other known sequences only (see below). Further Vk*La* sequences were obtained from the four individuals possessing Vk*La* (Vk*La*+), contributing a total of nine, eight, four, and three Vk*La* sequences, respectively. The Vk*La* sequence spans 649 bp from 30 bases 5' of the pd-box to just 3' of the heptamer. The consensus sequence is shown in Fig. 1. In 11 of the 24 Vk*La* sequences we recorded a total of 13 deviations from the consensus sequence, interpreted as *Taq* errors yielding an error rate of 0.08% in agreement with our own and others' estimates (Juul et al. 1997a; Tindall and Kunkel 1988). The remaining 13 sequences were all identical to the consensus sequence. An EMBL database search for sequences matching the Vk*La* exons found maximum similarity to the *Vk* genes *1–13* (*L4*) and *1D-13* (*L18*) with 94% identity with these genes in the compared sequence (see Fig. 1). The Vk*La* gene fits with the structural characteristics of the *IGKV1* subgroup ([6.3.7]) as described in the IMGT database (IMGT 1997). Furthermore, Vk*La* contains the features of a functional *IGKV* gene with regulatory noncoding DNA sequences such as pd-box, promoter supporting CCCT- sequences, dc-box, TATA-box, splice donor site, and splice acceptor site (Högbom et al. 1991; Schäble and Zachau 1993; Schäble et al. 1994). As seen in Fig. 1, the sequences of these regulatory sequences are not fully conserved but Vk*La* does in all positions share sequence with at least one *IGKV* gene considered potentially functional. Correspondingly, the conserved amino acids of the exons (Kabat et al. 1991) were also encoded by Vk*La,* including residues C23, W41, and C104, which are important for the stability of the Ig domain structure (Kirkham and Schroeder 1994). We conclude that Vk*La* has all the structural requirements of a functional *IGKV* gene.

Whether Vk*La* is simply an allelic variation of a known *IGKV* gene or an entirely new one is not yet completely certain. However, the low degree of similarity of exons (94%) between Vk*La* and the closest other known *IGKV* gene strongly suggests that Vk*La* is not just an allelic variation. The similarity of exons between the known *IGKV* gene alleles is in all cases above 98% with a median of 99.6%.

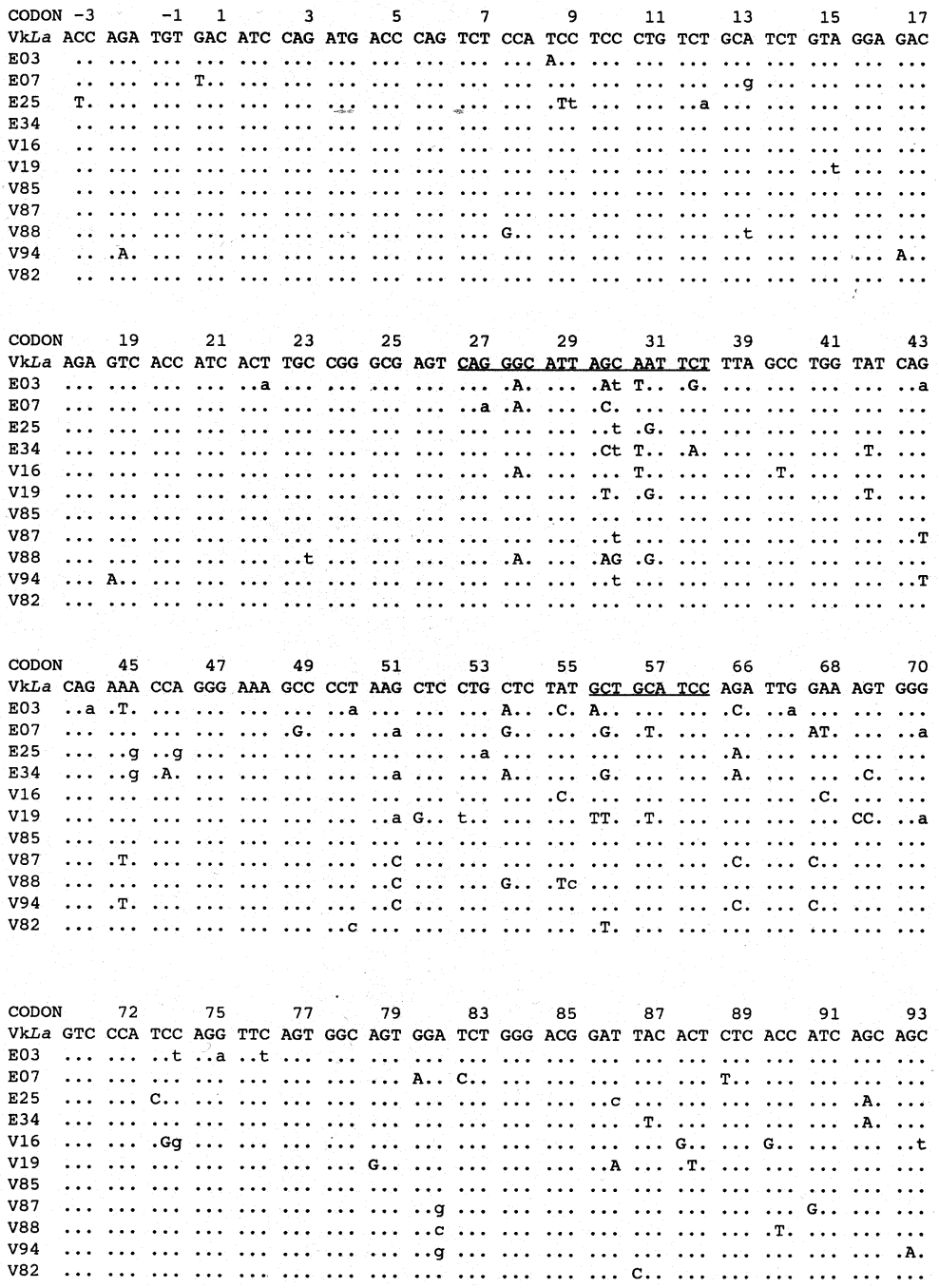
Of the 12 sequenced clones from the VkLa– individuals, one sequence was identical to the published *IGKV 1D-8* (*L24)* gene sequence, and the 11 other sequences all had high similarity to the *IGKV* gene *1D-43* (*L23*). The *1D-43* sequence is given in Fig. 1 for the purpose of comparison.

The consensus sequence of the *1D-43*-like clones was identical to the only published *1D-43* sequence from the 3' end of the pd-box through the heptamer. However, the sequence differed considerably from the published *1D-43* sequence in the 5' end (see Fig. 2). It is remarkable that our sequence is almost identical to the *IGKV* family I consensus sequence in that area. A similarity search in the NCBI database with the 25 5' end bases of the published *1D-43* sequence (Schäble and Zachau 1993) showed identity of the 24 3' end bases with the polylinker region of the pSVB plasmids (see Fig. 2) and identity of the 22 3' end bases with the multiple cloning site of several other cloning vectors: M13, pUC, pTUGAS, pIC-20R, and pTL-8.

Table 1 Distribution of apparently functional RNA-derived sequences assigned to differrent *IGKV* genes on the basis of highest degree of overall similarity. A total of 48 sequences were obtained from two Vk*La*+ individuals and one Vk*La*– individual

Individual	Vk*La* status	Vk*La*	1–27	1–16	1–5	1D-13	1–9	1–33/1D-33	1–39/1D-39
E	Vk*La*+	4	4	0	3	2	1	1	2
V	Vk*La*+	6	0	1	4	0	2	1	0
I	Vk*La*–	0	3	0	12	0	1	0	1

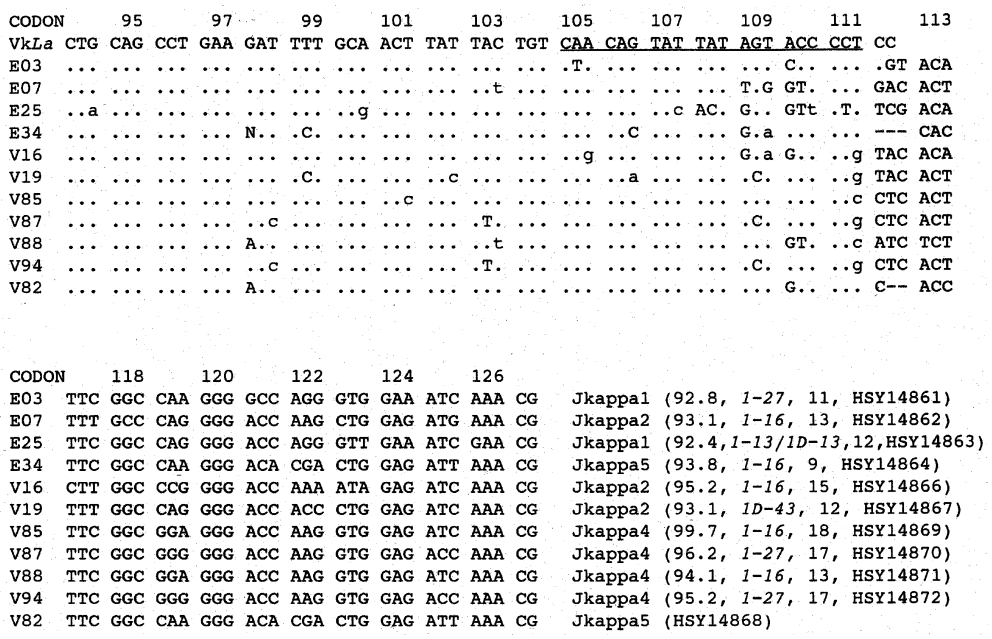
Fig. 3 (For continuation and legend see page 45)



Therefore, it seems likely that the 5'end of the published *IGKV* gene *1D-43* sequence is derived from a cloning vector, and that our sequence represents that of the actual *1D-43* (Fig. 1).

Expression of VkLa

Rearranged sequences on the RNA level spanning from codon –3 to codon 127 were sought from three individuals: two Vk*La*+ and one VkLa–. The parts of the sequences derived from *IGKV* genes (codons –3 through 110) were assigned to the most similar *IGKV* gene and rearranged versions of the Vk*La* were found in both investigated Vk*La*+ individuals, but not in the VkLa– individual, see Table 1. The sequences assigned to Vk*La* are shown in Fig. 3. Their consensus sequence was identical to Vk*La*. The average similarity to Vk*La* of the sequences assigned to this gene was 94.6%, which is in close agreement with the average similarity of 95.0% of *IGKV* transcripts to the assigned germline genes described earlier (Juul et al. 1997a). It appears very likely that these sequences did originate from Vk*La* because on average they differed further by 9–18 bp from the second most similar *IGKV*



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CODON      95       97       99      101      103      105      107      109      111      113
VkLa CTG CAG CCT GAA GAT TTT GCA ACT TAT TAC TGT CAA CAG TAT TAT AGT ACC CCT CC
E03  ... ... ... ... ... ... ... ... ... ... ... ... .T. ... ... ... ... C.. ... .GT ACA
E07  ... ... ... ... ... ... ... ... ... ..t ... ... ... ... ... T.G GT. ... GAC ACT
E25  ..a ... ... ... ... ... ..g ... ... ... ... ... ... ..c AC. G.. GTt .T. TCG ACA
E34  ... ... ... ... N.. .C. ... ... ... ... ... ... ..C ... ... G.a ... ... --- CAC
V16  ... ... ... ... ... ... ... ... ... ... ... ..g ... ... ... G.a G.. ..g TAC ACA
V19  ... ... ... ... ... .C. ... ... ..c ... ... ... ..a ... ... .C. ... ..g TAC ACT
V85  ... ... ... ... ... ... ... ..c ... ... ... ... ... ... ... ... ... ..c CTC ACT
V87  ... ... ... ... ..c ... ... ... ... .T. ... ... ... ... ... .C. ... ..g CTC ACT
V88  ... ... ... ... A.. ... ... ... ... ..t ... ... ... ... ... ... GT. ..c ATC TCT
V94  ... ... ... ... ..c ... ... ... ... .T. ... ... ... ... ... .C. ... ..g CTC ACT
V82  ... ... ... ... A.. ... ... ... ... ... ... ... ... ... ... ... G.. ... C-- ACC

CODON    118      120      122      124      126
E03  TTC GGC CAA GGG GCC AGG GTG GAA ATC AAA CG   Jkappa1 (92.8, 1-27, 11, HSY14861)
E07  TTT GCC CAG GGG ACC AAG CTG GAG ATG AAA CG   Jkappa2 (93.1, 1-16, 13, HSY14862)
E25  TTC GGC CAG GGG ACC AGG GTT GAA ATC GAA CG   Jkappa1 (92.4,1-13/1D-13,12,HSY14863)
E34  TTC GGC CAA GGG ACA CGA CTG GAG ATT AAA CG   Jkappa5 (93.8, 1-16, 9, HSY14864)
V16  CTT GGC CCG GGG ACC AAA ATA GAG ATC AAA CG   Jkappa2 (95.2, 1-16, 15, HSY14866)
V19  TTT GGC CAG GGG ACC ACC CTG GAG ATC AAA CG   Jkappa2 (93.1, 1D-43, 12, HSY14867)
V85  TTC GGC GGA GGG ACC AAG GTG GAG ATC AAA CG   Jkappa4 (99.7, 1-16, 18, HSY14869)
V87  TTC GGC GGG GGG ACC AAG GTG GAG ACC AAA CG   Jkappa4 (96.2, 1-27, 17, HSY14870)
V88  TTC GGC GGA GGG ACC AAG GTG GAG ATC AAA CG   Jkappa4 (94.1, 1-16, 13, HSY14871)
V94  TTC GGC GGG GGG ACC AAG GTG GAG ACC AAA CG   Jkappa4 (95.2, 1-27, 17, HSY14872)
V82  TTC GGC CAA GGG ACA CGA CTG GAG ATT AAA CG   Jkappa5 (HSY14868)
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Fig. 3 Eleven rearranged sequences assigned to Vk*La*. Two individuals (E and V) contributed with four and seven sequences, respectively. The sequence of the CDRs are *underlined*. A *dot* indicates identity with the Vk*La* sequence. A *dash* indicates missing nucleotide. An "*N*" indicates that the nucleotide has not been determined. *Uppercase* letters indicate mutations resulting in amino acid replacement and *lowercase* letters indicate silent mutations. Codons have been *numbered* according to instructions in the IMGT database. The assigned *IGKJ* gene is indicated at the *end* of each sequence. In *parentheses* are indicated similarity to Vk*La* in%, the second most similar *IGVK* gene, the number of bp by which the sequence deviates more from this *IGVK* gene than from Vk*La*, and the accession number of the sequence, respectively. Note that *V82* has a frameshift in the *V-J* joining region

gene. None of the sequences contained stop codons and all but one were properly rearranged to a *IGKJ* gene. Codons 113–127 of the transcripts were assumed to originate from *IGKJ*. Four *IGKJ* genes were represented among the transcripts, leaving out only *IGKJ* 3 which appears only rarely in the average normal expressed repertoire (Juul et al. 1997a). One sequence had a frameshift in the rearrangement position (V82), which was not unexpected, as it was reported in four of 103 rearrangements from the normal repertoire (Juul et al. 1997a). In accordance with expectations, the Vk*La*-derived sequences showed higher R/S ratios and lower overall similarity in the CDRs compared with the FRs (3.9 vs 1.6 and 88% vs 96%, respectively). The fact that no sequences from the VkLa– individual could be assigned to Vk*La* emphasized that the sequences from the Vk*La*+ individuals assigned to Vk*La* are not just sequences from other genes that by chance had mutated to resemble Vk*La* and therefore were selected by our PCR. If the VkLa– individual had had the same opportunity of producing Vk*La*-like transcripts, the possibility of not recording any such sequence would be $P = (1–0.24)^{17} = 0.009$ given that Vk*La*-like sequences were expressed with the frequency of individual E (4:17 = 0.24).

We therefore conclude that in Vk*La*+ individuals, this *V*-gene is undergoing proper rearrangement and somatic hypermutations and thus appears functional.

The consensus sequence of the RNA-derived sequences assigned to Vk*La* was identical to Vk*La*. This further substantiates the notion that the sequences originate from Vk*La*.

Frequency of VkLa

PCR2 was initially carried out with DNA from 12 individuals. Among these were three of the Vk*La*+ individuals and five of the VkLa– individuals studied with PCR1.

PCR2 was reproducible and yielded a visible PCR product on an agarose gel with DNA from Vk*La*+ but not from VkLa– individuals. DNA from these 12 individuals was used as controls when we performed PCR2 with DNA from a further 57 individuals. Of the 57 PCRs, 44 yielded no product, 11 yielded a strong band of the expected size, and two yielded a weak band of the expected size. The PCR2 product of all 13 samples yielding a band was cloned and sequenced. The 11 individuals yielding a strong band and one of the individuals yielding a weak band all turned out to possess Vk*La*. A total of 29 clones were sequenced and all sequences were Vk*La,* suggesting that the PCR2 is Vk*La*-specific. Despite several attempts, it was not possible to clone the PCR product from the last individual yielding a very weak band. The most likely explanation is that this individual does not possess Vk*La*. Hence, we estimate the frequency of the Vk*La*+ phenotype to be 12/57 = 21% (95% confidence limits 11–34%). It should be noted, however, that this is a minimum estimate, since it cannot be completely excluded that some of the individuals not yielding a PCR2 product do possess Vk*La.*

In conclusion, it has been suggested that all functional *IGKV* genes have been described (Zachau 1996). However, we present evidence here for a new functional *IGKV* gene,

Vk*La*, which is expressed on the RNA level and is present in about one-fifth of the investigated individuals. These findings emphasize that there may be significant differences in the available *IGKV* gene repertoire among individuals and that the *IGKV* gene cluster is not yet fully described. The fact that Vk*La* has not been found earlier is probably because earlier studies seem to have employed DNA from only one or very few individuals. Studies of the variable genes in the *Ig* heavy chain locus have shown considerable insertion/deletion polymorphism (reviewed by Cook et al. 1994). A deletion polymorphism is known in the *IGKV* gene cluster (haplotype 11) and this study suggests that further such polymorphism may exist here.

The location of Vk*La* in the genome remains to be determined, but the fact that Vk*La* is expressed on the RNA level suggests that it is located in the main *IGKV* gene cluster on chromosome 2.

Acknowledgments We thank Lene Pedersen and Ingrid Alsing for their excellent technical assistance. This study was supported by the Danish Hospital Foundation for Medical Research, Region of Copenhagen, The Faroe Islands and Greenland, The Foundation of Gerda and Aage Haensch, The Danish National Association against Lung Disease, The Foundation of Consul Ehrenfried Owesen and widow, The Novo Nordisk Foundation, and The Danish Medical Research Council grants nos. 9503237 and 9503060.

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