Short paper

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A V_H gene is located within 95 Kb of the human immunoglobulin heavy chain constant region genes

Using cosmids covering about 117 Kb upstream of the human immunoglobulin chain C_μ gene, we have identified a potentially functional V_H gene, belonging to the V_HVI subgroup. This V_HVI gene is only about 95 Kb from the C_μ gene and is probably the first functional V_H segment of the Igh locus. These results illustrate the proximity of the human V_H, D_H and J_H segments involved in creation of the complete heavy chain genes.

1 Introduction

Genetic recombination studies of the murine heavy chain locus (Igh) have indicated the possibility that V_H and C_H loci may be widely separated at a single autosomal band [1]. However, recent studies of human Igh have suggested that V_H may be closer, since a pseudo V_H segment has been identified about 100 Kb upstream of C_μ [2] and V_H V and VI genes have been reported within 240 Kb of C_μ [3]. Recently, it was also shown that the major D_H cluster maps to within 35 Kb of C_μ [2, 4] and that a second D_H cluster exists near the distal end of V_H [2]. The existence V_H segments in a set of cosmids covering the region upstream of human C_μ has been investigated and we now show a V_HVI subgroup gene within 95 Kb of C_μ. This provides the first physical linkage of immunoglobulin V_H and C_H in man.

2 Materials and methods

2.1 Hybridization procedures

Cosmid DNA was analyzed by filter hybridization [5] using an oligonucleotide probe specific for the framework III region of V_HVI [6], which was labeled using T4-polynucleotide kinase (New England Biolabs, Massachusetts, MA) and [γ^{32}P]ATP (Amersham Int., Amersham, GB) according to methods described in Carter et al. [7]. Filter blots were hybridized in 6 × SSC at 40 °C for 18 h and then washed in 5 × SSC at 65 °C to remove nonspecifically bound probe. Filters were exposed to X-ray film for 3 h at −70 °C.

Genomic DNA digests were transferred to Hybond N filters (Amersham) and analyzed by hybridization to the pUCRI 0.5/2 insert (V_HVI probe) labeled using random oligonucleotide labeling [8] and conditions described previously [9].

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Abbreviations: CDR: Complementary determining region **D:** Diversity **J:** Joining **C:** Constant **V:** Variable **H:** Heavy

2.2 Sequence of germ-line V_HVI gene

Subcloning was carried out into M13 and pUC vectors [10] and nucleotide sequence obtained by the dideoxy chain termination procedure [11] in M13 using the strategy shown in Fig. 1. DNA sequence was analyzed by the computer methods of Staden [12].

3 Results and discussion

3.1 Localization of V_HVI close to the major D_H locus

We have recently reported the isolation of six cosmids containing human D_H sequences that comprise two D_H loci termed the D_H major cluster and D_H minor locus [2]. Four cosmids (cosmids 21, 23, 24 and 25) define a region of about 117 Kb of the Igh locus and contain, in addition to the major D_H cluster, the C_μ constant gene, the J_H cluster and a pseudo V_H sequence about 100 Kb from C_μ (see Fig. 1). This pseudo V_H sequence was the only V_H segment detected in these cosmids using a V_HIII subgroup probe. It has, however, been shown recently [3, 15] that members of the V_HVI subgroup are located close to the J_H locus. The presence of such genes in the 117 Kb stretch of DNA covered by the cosmids [2] was investigated by hybridization with an oligonucleotide synthesized to part of the framework III region of the V_HVI gene sequence [6]. The result of this experiment showed that the probe detects a V_HVI gene in cosmid 23 (localized in a 0.8 Kb Eco RI restriction fragment). Restriction mapping data (not shown) places this fragment close to the 3′ of the pseudo V_H gene described previously. A plasmid subclone (pUCRI 0.5/2) containing this fragment was constructed from cosmid 23 DNA and the complete double-stranded nucleotide sequence of the V_H gene determined as shown in Fig. 1. The relevant part of this sequence is shown in Fig. 2, and confirms that a complete V_H gene is present in cosmid 23, and is located 95 Kb upstream of the C_μ gene. The genomic V_HVI sequence and position is the same as that for V_H sequence 6-1G1 [15] and a previously reported cDNA sequence [6], strongly suggesting that it is a functional V_H gene.

3.2 Relationship of V_HVI to other V_H genes

The genomic organization of the V_HVI described here shows that it contains an intron of 82 bp, which is typical of the intron

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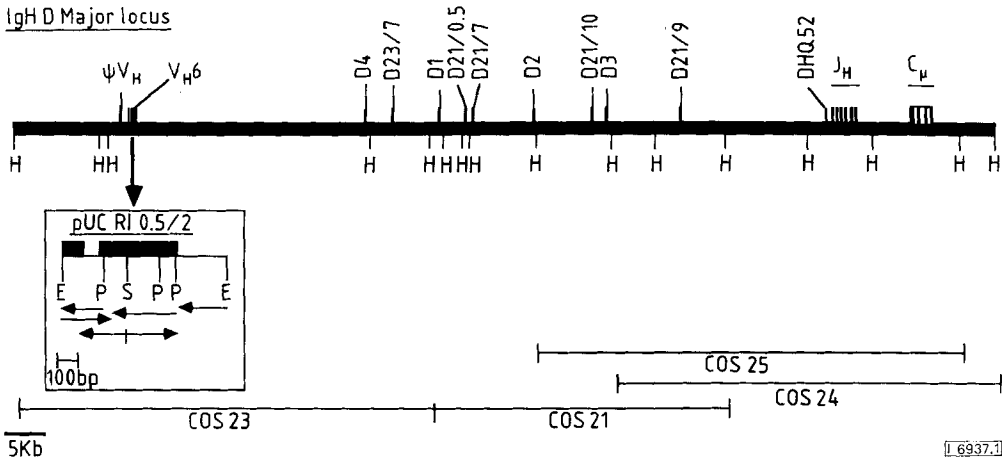

Figure 1. Organization of the region around the human major D_H locus. The map shows the major D_H locus and the posititions of the C_μ and J_H genes [13], members of the distinct D_H families [2, 14], the most proximal V_H genes and the position of Hind III restriction sites (H). The juxtaposition of cosmids 23 and 21 is known from the previously defined organization of D_H genes, D_1, D_2, D_3, D_4 [14], and has been recently confirmed by additional cosmids that overlap these two cosmids (LB and THR, unpublished data). The V_HVI gene was localized in cosmid 23 to a 0.8 Kb Eco RI restriction fragment (contained in plasmid subclone pUCRI 0.5/2) using an oligonucleotide probe specific for the framework III region of the V_HVI cDNA sequence of Schroeder et al. [6] (residues 295 to 340). The restriction map of the germ-line V_HVI gene and the sequencing strategy used (indicated by arrows) is shown in the insert panel and highlights the position of the first and second gene exons (filled in boxes) and gene intron. The characterization of the pseudo V_H has been described previously [2]. Restriction enzyme sites are: E = Eco RI; P = Pst I; S = Stu I.

found in V_H families I, II [17, 18] and IV [19] but not of V_HIII [20]. To determine the relationship of V_HVI to other V_H families, we have compared it to sequences of families V_HI, II, III, IV and V. This comparison shows that it is most closely related to the V_HIV subgroup. Comparison of the V_HVI sequence and a V_HIV sequence [19] is shown in Fig. 3. This comparison shows that the two genes are about 65% homologous with particularly strong homology featured in the framework I region (positions 184–273), a region already shown to be less well conserved when compared with V_HI, II and III sequences [6]. To try to assess the V_HVI repertoire, we have used the pUCRI 0.5/2 clone to probe genomic digests of unrearranged DNA (Colo 320 HSR and HeLa) using low stringency hybridization conditions. The results (Fig. 4) show that the V_HVI probe hybridizes to a number of fragments. Comparison of the Hind III digests (tracks 1 and 4) with the known Hind III fragment sizes of V_HIV genes [19] show that some of these are likely to be due to V_HIV cross-hybridization. Thus, the V_HVI gene family is small in comparison to the V_HIII family for example [20, 21], but apparently consists of more than the single gene identified within Cos23.

The result of analyses of regions just upstream of human J_H now shows that the major D_H cluster and J_H are within 20 Kb of each other [2, 4]. We have shown here that a V_H gene, probably the first V_H gene of the human Igh locus, is located within 95 Kb of C_μ. This is the first physical linkage of functional V_H and C_H segments, and shows that distances between these rearranging loci are not as great as previously imagined.

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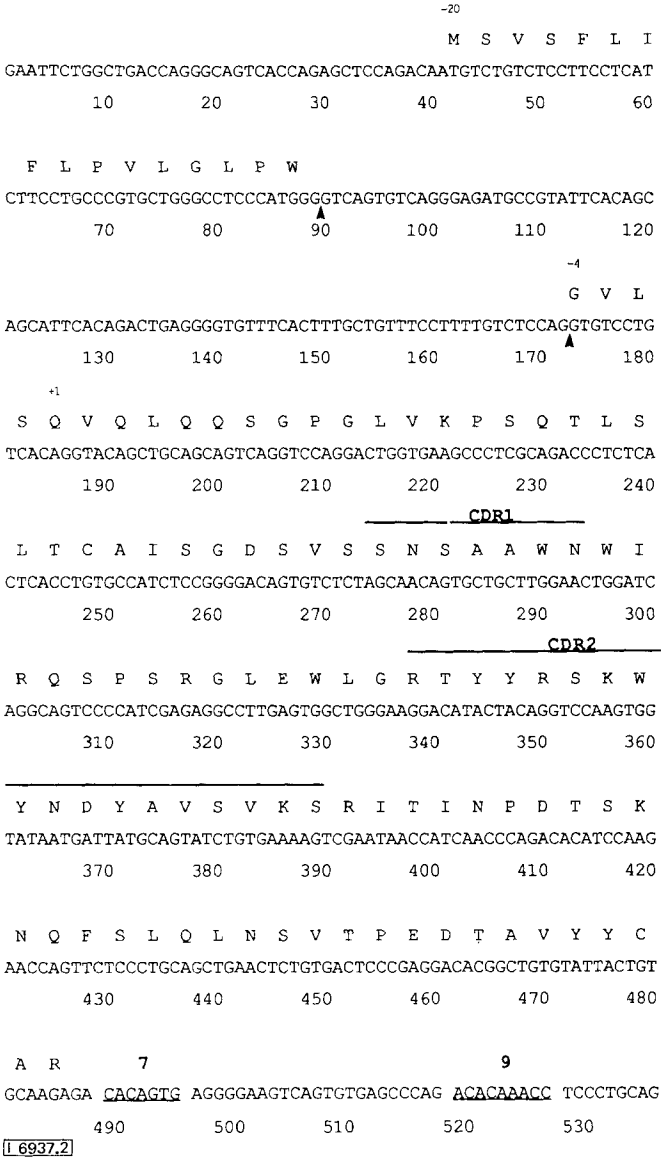

Figure 2. Nucleotide and derived protein sequences (in single letter code) of the germ-line V_HVI gene. Amino acids are numbered according to Kabat et al. [16] and the CDR1 and CDR2 regions are as designated by Schroeder et al. [6]. The positions of exon and intron junctions are shown by arrows and heptamer and nonamer recombination signals underlined and labeled accordingly.

```
VH IV  CATCCCTTTTCACTGCTCCGTACAAACGCACCACCCCCATGCAAATCCTCACTTAGGCGC
                10        20        30        40        50

VH VI                                                   GAATTCTGG
                                                        ::: ::
VH IV  CCACAGGAAGCCACCACACATTTCCTTAAATTCAGGTCCAACTCATAAGGGAAATACTTT
               70        80        90        100       110

       10        20        30        40        50        60
VH VI  CTGACCAGGGCAGTCACCAGAGCTCCAGACAATGTCTGTCTCCTTCCTCATCTTCCTGCC
       ::::      :   :   :  : :::  :::     :   :   :: :: ::::::
VH IV  CTGAGAGCTCATGGATCTCATGTGCAAGAACATGAAGCACCTGTGGTTCTTCCTCCTGCT
               130       140       150       160       170

       70        80        90        100       110       120
VH VI  CGTGCTGGGCCTCCCATGGGGTCAGTGTCAGGGAGATGCCGTATTCACAGCAGCATTCAC
        :::  ::  : :  ::: :     :   :::  :  : : :    : :   :   :  :
VH IV  GGTGGCGGCTCCCAGATGTGAATGTTTCTAGGATGCAGACATGGAGATATGGGAGGTGCC
               190       200       210       220       230

       130       140       150       160       170       180
VH VI  AGACTGAGGGGTGTTTCACTTTGCTGTTTCCTTTTGTCTCCAGGTGTCCTGTCACAGGTA
               : : :     : : : ::::    :::   :::: :::::::: ::: :
VH IV  TCTGATCCCAGGGCTCACTGTGGGTTTTTC----TGTTCACAGGGGTCCTGTCCCAGCTG
               250       260       270             280       290

      190       200       210       220       230       240
VH VI  CAGCTGCAGCAGTCAGGTCCAGGACTGGTGAAGCCCTCGCAGACCCTCTCACTCACCTGT
       ::::::::: :::: :: :::::::::::::::::: ::: :::::::: :: ::::::::
VH IV  CAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCACCTGC
       300       310       320       330       340       350

       250       260       270       280       290       300
VH VI  GCCATCTCCGGGGACAGTGTCTCTAGCAACAGTGCTGCTTGGAACTGGATCAGGCAGTCC
        :  :::: :: : :    ::   :: :  :::      :::  ::::::: : ::: ::
VH IV  ACTGTCTCTGGTGGCTCCATCAGCAGTAGTAGTTACTACTGGGGCTGGATCCGCCAGCCC
          360       370       380       390       400       410

       310       320       330       340       350       360
VH VI  CCATCGAGAGGCCTTGAGTGGCTGGGAAGGACATACTACAGGTCCAAGTGGTATAATGAT
       :::  ::  :: :: :::::: : :: :: :  :: :: ::    :     ::  :  :
VH IV  CCAGGGAAGGGGCTGGAGTGGATTGGGAGTATCTATTATAGTGGGAGCACCTACTACAAC
          420       430       440       450       460       470

       370       380       390       400       410       420
VH VI  TATGCAGTATCTGTGAAAAGTCGAATAACCATCAACCCAGACACATCCAAGAACCAGTTC
          :  :           :::::: : :::::   :  :::::: :::::::::: :::
VH IV  CCGTCCCTCAAG------AGTCGAGTCACCATATCCGTAGACACGTCCAAGAACCACTTC
          480             490       500       510       520

       430       440       450       460       470       480
VH VI  TCCCTGCAGCTGAACTCTGTGACTCCCGAGGACACGGCTGTGTATTACTGTGCAAGAGAC
       :::::: :::::: :::::::::  :::  ::::::::::::::::::::::: ::::::
VH IV  TCCCTGAAGCTGAGCTCTGTGACCGCCGCAGACACGGCTGTGTATTACTGTGCGAGAGAC
               540       550       560       570       580

       490       500       510       520       530
VH VI  ACAGTGAGGGGAAGTCAGTGTGAGCCCAGACACAAACCTCCCTGCAG
       :::::::::::: :: ::::::::::::::::::::::
VH IV  ACAGTGAGGGGAGGTGAGTGTGAGCCCAGACACAAACC
               600       610       620
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Figure 3. Comparison of nucleotide sequences of V_HVI and a V_HIV sequence [19]. This shows strong homology between the two V_H genes in the framework I region (positions 184–273 of V_HVI sequence) and at their 3′ ends including the region containing heptamer and nonamer recombination signals and the 23-bp spacer (positions 490–527 of the V_HVI sequence).

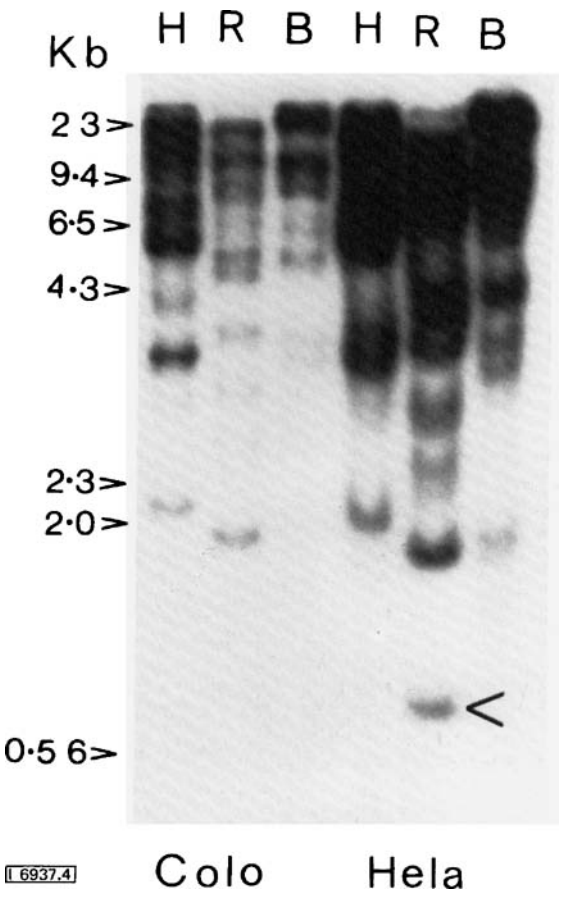

Figure 4. Hybridization of the pUCRI 0.5/2 insert (V_HVI probe) to germ-line genomic digests. Colo 320 HSR DNA (Colo) (5 μg) or HeLa DNA (HeLa) (5 μg) were digested with Hind III (H), Eco RI (R) or Bam HI (B) and transferred to Hybond N. The sizes of fragments were assessed by co-electrophoresis of λ DNA digested with Hind III and hybridization to the germ-line 0.6-Kb Eco RI fragment arrowed.

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