

Human pro α 1(I) collagen: cDNA sequence for the C-propeptide domain

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We have previously constructed a cDNA clone pHCAL1, covering most of the C-terminal propeptide domain of human pro α 1(I) collagen mRNA, by inserting a 678 bp EcoRI-XhoI fragment of cDNA into pBR322 (1). Since the XhoI/SalI ligation prevented removal of the insert, we used the same strategy to obtain a similar clone in pUC8. RNA was isolated from fetal calvarial bones. The cDNA was digested with EcoRI and XhoI and fractionated on a 1% agarose gel. Fragments of 650-700 bp were cloned in pUC8 at the polylinker site, which now permits easy removal of the insert (2). The new clone was named pHCAL1U since the RNA was isolated from another individual.

Sequencing of pHCAL1U by the Sanger dideoxy method filled the two gaps (nucleotides 67 and 487) which remained in the previously published sequence (3). Three base differences were observed (shown above pHCAL1U sequence): two of these bordered the missing nucleotide (positions 66 and 68). The third difference (a transversion at nucleotide 616) changes the codon from TCC (ser, ref. 3) to ACC (thr), and may represent individual variation. The corresponding chick sequence also contains ACC (4). The approach outlined here is useful for studies on individual variation which is important to recognize when searching for disease-related mutations in type I collagen.

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38c  10   20   30   40   50   60   CNC70   80   90   100  110  120
CTGAGGTTGGACACCAACCCCTCAAGAGCCTGAGCCAGAGATCGAGAACATCCGGAGCCAGAGGGAAAGCCGGCAAGAACCCTGGCCGCTGACCTCAAGATGTGCCACTCTGAC
L E V D T T L K S L S Q Q I E N I R S P E G S R K N P A R T C R D L K M C H S D

78c  130  140  150  160  170  180  190  200  210  220  230  240
TGGAAAGATGGAGAGTACTGGATTGCCCAACCAAGGCTGCAACCTGGATGCCATAAAGTCTTCTGCACCATGAGAGCTGTGAGACTCGCTGTATACCCCACTCAGCCAGTGTGGCC
W K S G E Y W I D P N Q G C N L D A I K V F C N H E T G E T C V Y P T Q P S V A

118c 250  260  270  280  290  300  310  320  330  340  350  360
CAGAAGAACTGGTACATCAGCAAGAACCACCAAGGACAGAGGATGTCTGGTTCGGCGAGAGCATGCCGATGGATTCCAGTTCGAGTATGGGGGAGGGGCTCGACACCTCCCGATGTG
Q K N W Y I S K N P K D K R H V W F G E S H T D G F Q F E Y G G Q G S D P A D V

158c 370  380  390  400  410  420  430  440  450  460  470  480
GCCATCAGCTGACCTTCTCCGGCTGATGTCCACCGAGGCGCTCCCAAGATCACCTACCACTGCAAGAACAGCGGTGGCTTACATGGACCAGGAGACTGGCAACCTCAAGAAGGCCCTG
A I E L T F L R L H S T E A S Q N I T Y H C K N S V A Y M D Q Q T G N L K K A L

198c N490  500  510  520  530  540  550  560  570  580  590  600
CTCCTCAAGGGCTCCAAGAGATCGAGATCGCGCGGAGGGCAACAGCGCCTTCACTACAGGGTCACTGTGGATGGCTCACGAGTCAACCGGAGCCTGGGGCAAGACAGTATTGAA
L L K G S N E I E I R A E G N S R F T Y S V T V D G C T S H T G A W G K T V I E

238c 610  T 620  630  640  650  660  670
TAGAAAACCAAGAGCTCCCGCTCCCATCATGATGTGGCGCCCTTGGACGTTGGTCCCGAGACCAGGAATT
Y K T T K T S R L P I I D V A P L D V G A P D Q E F
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