Human type III collagen 'variant' is a cDNA cloning artefact

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We have previously reported the isolation of two overlapping cDNA clones, pIII-33 and pIII-21, for human type III collagen (1). Although there is a single type III collagen gene and the cDNAs were derived from the same cell line (HFL-1, ATCC CCL153), we have shown that they differ by a single base in a region of overlap (2). As a result, the clones encode serine and proline respectively at amino acid 1011 of the helical domain and so an attempt was made to determine whether the variant was genuine or a cloning artefact.

To determine the corresponding DNA sequence in the cell line, a segment of the human type III collagen gene was amplified by the polymerase chain reaction and directly sequenced (3). Both alleles of the type III collagen gene should be amplified equally and if the variant is genuine, then bands would be detected in more than one lane at a position on the sequencing gel corresponding to the variant. Two primers (left: TCCCATTGGACCACCAGGGC and right: ATCCCAGCAATGGCAGCGGC), derived from the cDNA sequence 75 base pairs either side of the variant, were used in 30 rounds of amplification of 1 µg of genomic DNA. The amplification products were gel purified, reamplified through a further 15 rounds, re-purified and sequenced using one or other of the amplification primers as the sequencing primer.

The figure shows the sequence obtained using the left primer. A single band is detected at each position in the sequence including the point at which the variant was expected (indicated by the asterisk). A proline is encoded in the cell line at residue 1011. We conclude that the variant is not detectable in the cell line from which the cDNAs were derived and so is likely to be a cloning artefact.

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