PCR Typing Including High Resolution Gel Electrophoresis Reveals new Alleles in the COL2A1 VNTR

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INTRODUCTION

The AT-rich variable number of tandem repeat (VNTR) near the collagen type II gene (COL2A1) is a candidate to increase the discriminative power in PCR based DNA typing. The aim of the present study was to investigate the COL2A1 VNTR in Norwegian family- and population materials, and to evaluate the system for forensic purposes.

POLYMERASE CHAIN REACTION (PCR)

PCR typing of the COL2A1 VNTR have been described by Priestly et al. (1990) and Wu et al. (1990). We did not, however, achieve high sensitivity and robustness with the published PCR. The primers were therefore redesigned by aid of computer programs (Oligo™ and GCG software). The new PCR primers had the sequences:

LCOL: 5'-ATCCCTGCCCCTGCTTCCTC-3'
HCOL: 5'-CACCTGCTCTCCTCCGACCC-3'

The PCR was performed as Perkin-Elmer Cetus recommend for their AmpliTaq™ with 1.5 mM $MgCl_2$ and a thermo profile including an annealing temperature of 58°C.

MATERIALS

The COL2A1 VNTR was studied in a Norwegian population material of 200 unrelated individuals and in 50 small Norwegian family groups.

NONDENATURATING POLYACRYLAMIDE GEL ELECTROPHORESIS

The electrophoresis of the PCR products was performed in a nondenaturating polyacrylamide gel (PAGel) in apparatus (V15-17) from Bethesda Research Laboratories.
Figure 1 is a picture of an ethidium bromide stained non-denaturating PAGel with COL2A1 VNTR PCR products from 13 unrelated individuals. Four of the allelic variants described by Priestly et al. (1990) are present in figure 1. We found a heterozygote frequency of 58% in our population material for this VNTR.

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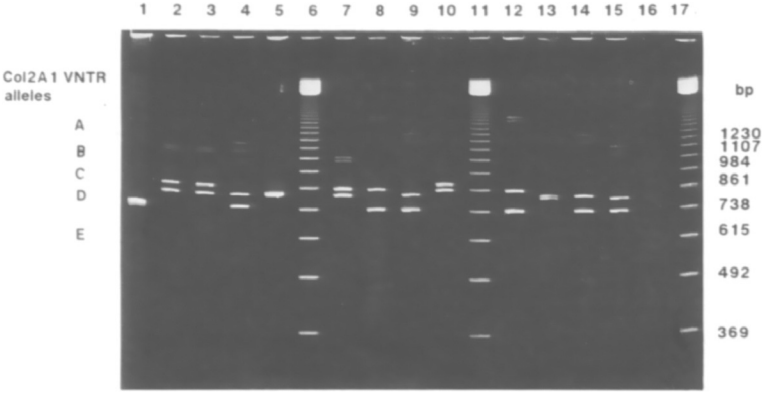

Fig. 1. Nondenaturating PAGel with COL2A1 VNTR PCR product of 13 unrelated individuals. Lane 6, 11 and 17 are molecular weight standard. Lane 16 is the negative control

DENATURATING POLYACRYLAMIDE GEL ELECTROPHORESIS

In the nondenaturating gel system there appeared several alleles which were difficult to classify because they migrated slightly different from standard alleles. The higher resolution power of the denaturating PAGel (sequencing gel) was necessary to resolve this problem. Prior to the electrophoresis the PCR product was labelled as described by Ludwig et al. (1989) by addition of an endlabelled internal primer before the last five cycles of the PCR. The internal primer had the sequence:

ICol: 5'-TCATGAACTAGCTCTGGTGG-3'

Figure 2 is a picture of the autoradiogram of one such gel. As compared to the nondenaturating gels, further subdivision of some alleles may seen. The denaturating gel system has so far revealed 16 allelic variants of this VNTR in the same Norwegian population material and a heterozygosity of 76.4%.

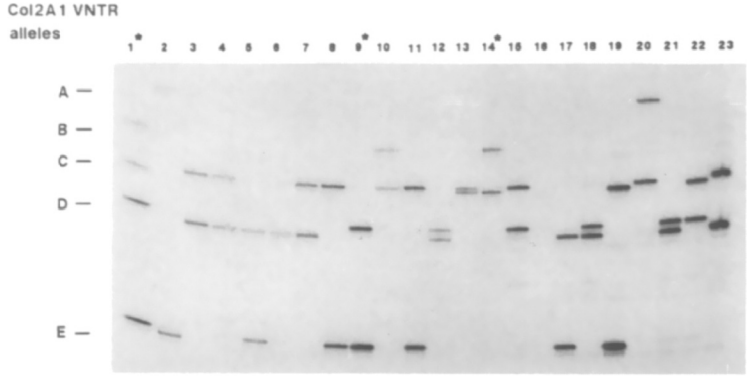

Fig. 2. Denaturating PAGel, which also includes all individuals in figure 1. The allelic ladder in lane 1 is a mixture of the PCR product from the individuals in lanes 9 and 14

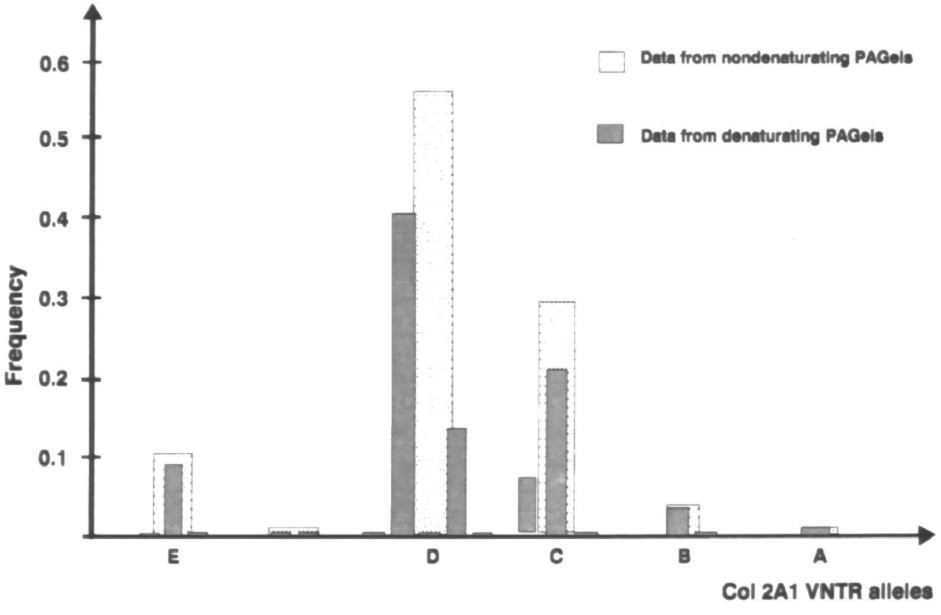

Fig. 3. This is a schematic drawing of the different allelic variants detected by the two resolution systems. In light colour bars are the alleles detected by nondenaturating PAGels. Norwegian gene frequencies are similar to those published by Priestly et al. (1990). The further subdivision of the allelic variants obtained in denaturating PAGel is represented by dark colour bars

Stoker et al. (1985) have shown that the COL2A1 VNTR is similar in construction to the Apo B VNTR. Our sequencing data support their findings. The small differences in size between the variable tandem repeat units, 31 and 34 bp, are visible only in the high resolution gels.
Sequencing of more alleles is necessary for further characterization of the Col2A1 VNTR, and should form basis for a more appropriate nomenclature.

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