HETERODUPLEX ANALYSIS IS A RAPID METHOD FOR THE DETECTION OF SUBALLELES CAUSED BY MIXED LENGTH AND SEQUENCE VARIABILITY IN SHORT TANDEM REPEAT SYSTEMS

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INTRODUCTION

STR polymorphisms differ in the number of the tandem repeats. However, in addition, a microheterogenity, as far as the sequence variation is concerned, has been detected in some systems (HumVWA and others) (Möller 1994). The aim of our paper is to demonstrate the usefulness of the heteroduplex (HD) analysis (HDA) for the detection of suballeles in DNA systems such as HumVWA (Kimpton 1992), HumCD4 (Edwards 1991) and Hum Dys19 (Roewer 1992). The HDA is a well-established technique (Wilkin 1993) but, to our knowledge, HDA is not usual in forensic application. During PCR amplification, the DNA products are submitted to a melting and a reassociation process. In case of a homozygous genotype, the reassociation produces only homoduplexes. However, if there are DNA fragments with different numbers of repeats and / or significant differences concerning the sequence, the reciprocal association produces two or more types of HDs in addition to homoduplexes. On dependency on the extension of the missmatch area, the HDs migrate noticeably slower than homoduplexes in the nativePAGE. Thus, HD can provide several pieces of information which remain hidden, if only measurements of the length are performed.
Y-chomosomal markers occur in hemizygous state, that means that natural HDs are not existent. We show that the construction of artficial heterozygous genotypes makes the STR Dys 19 accessible to HDA.

MATERIAL and METHODS

During routine work using VWA and CD4, our attention was focused on the allele length assessment and the HD variabillity as well. Conspicous HD variants were checked by an electrophoretic site-to-site comparison with well studied samples (Fig 1). If a special genotype produces an atypical HD, sequencing can clarify which of the 2 alleles involved carries a sequence variation. For this purpose, the stained bands were dissected, reamplified and readed using the A373 sequencer in combination with the Taq cycle sequencing technique (APPLIED BIOSYSTEMS). For the construction of artificial genotypes, we mixed the reamplified single allele products (Fig 2) in new combinations. HD formation occurs if the mixture is submitted to 5 PCR cycles omitting the enzyme.

RESULTS and DISCUSSION

HDA in VWA: The gel depicted in Fig. 1 shows natural VWA genotyps containing the allel 17 in combination with the other alleles. Obviously, homozygous genotypes such as 17 / 17 do not produce considerable HDs. In addition, the alleles 14, when combined with other ones, tend to make no or only weak HDs. HDs which are mutated with regard to the distances between the bands (but less with regard to the intensity) reflect the sequence deviations in one of the alleles.
The Fig.3 shows a gels with artificial arrangements of interesting allele products. Several fragments chosen from genotypes with common and conspicous HDs as well were combined with a common and a variant allel 17 fragment. Some pieces of information can been drawn from this gel:
1.: In spite of the fact, that the common allel 17 was mixed with its variant counterpart, a formation of HDs in the mixed 17 common and 17 variant is only very weak. This indicates that the sequence heterogenity is not compellingly conspicuous by HD formation.
2.: Despite the fact that the combination of the different allel 16 with the common allel 17 reveals a gross HD difference, the combination with the variant allele 17 variant shows only a discrete band distance deviation.
3.: The combination of 17 common and 15 variant alleles produces HDs which are similar to those of the reciprocal situation 17 variant and 15 common.
4.: Leaving aside the situation in homozygous genotypes with regard to the length there is a good chance of detecting the sequence variants investigated here, since all combinations of the allele 17 variant differ

from the combinations with the allele 17 common with regard to the HD band distances. In principle results in experiments using other alleles confirm these statements (not shown).
In the VWA System, we have found a considerable variability in the alleles 15, 16, and 17, and low or no variability in the alleles 14 , 18, 19 and 20 up to now.
HDA in CD4: Fig.4 shows an arrangement of natural genotypes. Only within the genotypes 108/ 88 bp, there are some intensive variations but no distance mutations. Sequence analysis and the construction of artificial genotypes as well (not shown) revealed that variability in HD intensity does not definitively reflect the existence of sequence variations. Thus, our study into 400 alleles from European population and 50 Asian alleles could not detect any suballeles in CD4.
HDA in Dys 19: Artificial combinations of the allele D with all further frequent alleles can reveal that there is no variability in the HDs formation.(Fig 5). This investigation has been conducted on more than 100 alleles. Therefore, we are strongly convinced that in DYS 19 the occurrence of suballeles is a rare event.
Paternity testing: In the pedigree of Fig.6. VWA allele lenght assessment includes the putative father for both children as the possible true father. By HDA the putative father is excluded to be the true father for one of them due to the fathers' common HD type and the rare HD in the 2nd child (lane 4). This has been confirmed by sequencing data and by exclusions in further systems.

Symbols used in the figures: = > homoduplexes; * HDs; C= common HD type; R= rare HD type

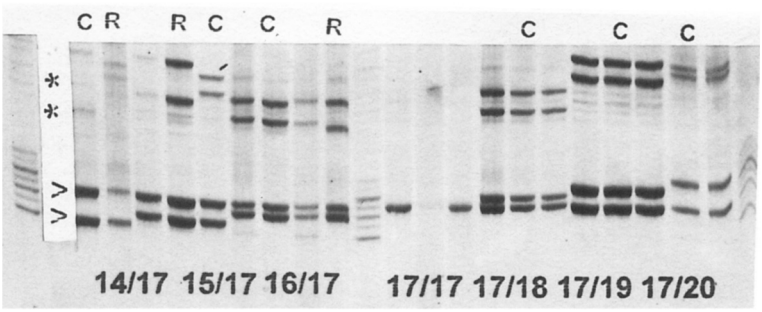

Fig.1: VWA genotypes involving allel 17 with common (C) and rare (R) HD types.

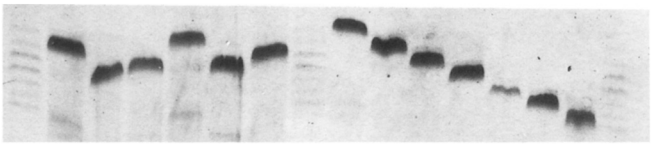

Fig.2: Sinlge VWA allele fragments produced by reamplification of chosen bands from probands with common and rare HD type.

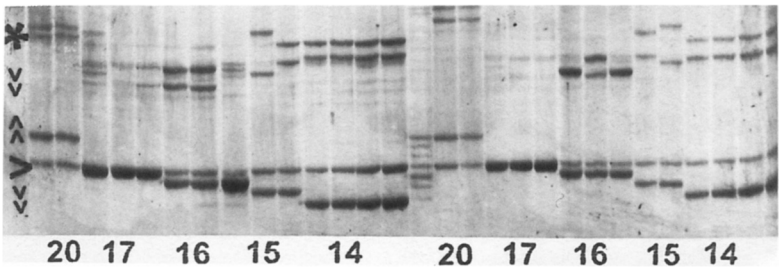

Fig. 3: Artificial genotypes in VWA established by mixing a common (left) and a rare (right) variant of the allele 17 with an ensemble of common and rare alleles.14-20.
Allele 17: TCTA $(TCTG)_4$ $TCTA_{11}$; Allele 17 var: TCTA $(TCTG)_3$TCTA $_{12}$

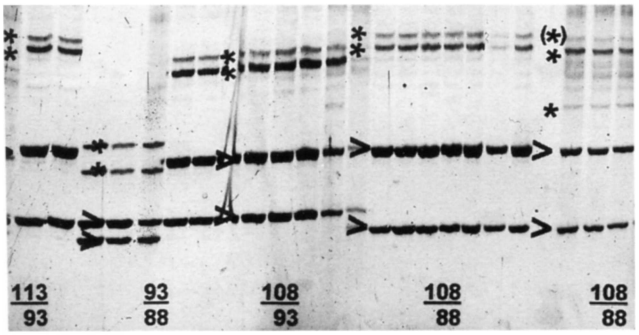

Fig. 4: Natural genotypes of the STR CD4 chosen from routine work

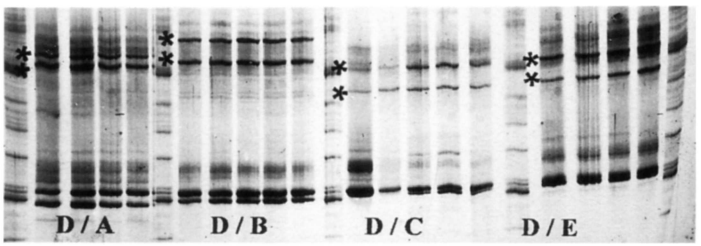

Fig. 5: Artifcial genotypes in Dys 19 including the alleles A-D are lacking HD variabillitiy

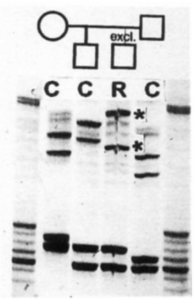

Fig. 6:
HDA in VWA paternity testing: Paternity exclusion cannot be drawn in length assessment but in HDA and by sequencing
Allele 16 (C): TCTA $(TCTG)_4$ TCTA $_{11}$
Allele 16 (R): TCTA $(TCTG)_3$ TCTA $_{12}$

Conclusions:

1.: HDA helps to detect the most of the existing suballeles within alleles of equal length.
2.: As work and material do not cause high costs, HDA is highly recommended if extended population genetic studies of variability have to be made within the main alleles.
3.: HDA can provide increased information in routine work without additional expenditure of work and material. However, the inclusion of HD information into the expertise requires a detailed investigation into the suballele.

References

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