# A rare paternity case with PP 99,99% and exclusion at three loci derived from the 33.15 sequence

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## INTRODUCTION

In general in cases of proven paterbnity (p > 99.99%) by analysis of conventional blood groups and the HLA system paternity exclusion by analysis of VNTR or STR-Loci is very rare. We present a case report with PP > 99.99% after analysis of 21 conventional systems, HLA, 5 SLP and 5 STR but 3 exclusions with SLP G3, MS1 and MS205 all derived from MLP 33.15.

**CASE:** Paternity quartet: Mother , child I, child II and putative father

## METHODS AND MATERIALS

Blood group antigens (ABO-, MNSs-, Rh-, Kell-, Duffy- and Kidd-system), plasma protein polymorphisms (PLG-, Gm-, Km-, C3-, Tf-, Pi-, Hp- and Gc-system) and erythrocyte enzyme polymorphisms (PGM-, GPT-, ACP-, AK-, ADA-, EsD- and PGD-system) were determined for each case following standard procedures. HLA-A, HLA-B and HLA-C antigens were typed by the microlymphocytotoxicity test (LCT).
VNTR (SLP/MLP) polymorphism analysis: Genomic DNA was extracted from 8ml of peripheral blood following the salting-out method. Electrophoresis conditions, hybridisation, southern blot transfer to nylon membranes (Quiabrane from Diagen Inc, Hilden, Germany) and detection of probes were performed as recommended by the manufacturers ( NICE™ ICI/CELLMARK, GenePrint Light™ Promega, (GTG)5 Fresenius, 33.15 ICI/CELLMARK). The results were analysed by an semiautomatic computerised system (DNA-Auswertungssystem Version 2.40 from M.Muche Immucor Medizinische Diagnostik GmbH, Rödermark, Germany) and the biostatistical calculation of paternity was performed with the Essen Möller probability (W).
STR polymorphism analysis: Fluorescence based PCR based analysis of STR loci was conducted on an 373A automated DNA sequencer (Perkin-Elmer) as described (Seidl 1995).The single/multi locus probes and STR polymorphism used in this study are summarized in table1. Chromosome staining: Staining of metaphase chromosomes in situ was performed according to standard protocols.

**Table.1**: SLP/MLP and STR polymorphism

| SLP | SLP | MLP | STR |
|---|---|---|---|
| yNH24 (D2S44) | MS1 (D1S7) | 33.15 | TH01 (11p15.5-p15) |
| MS31 (D7S21) | MS205 (D16S309) | (GTG)5 | D8S639 (8p21-p11) |
| MS43A (D12S11) | G3 (D7S22) | | CYP19 (15q21.1) |
| LH1 (D5S110) | | | ACPP (3q21) |
| PH30 (D4S139) | | | SE33 (ACTPB2) |

## RESULTS AND DISCUSSION

DNA analysis using three VNTR loci, G3, MS205 and YNH24 did give conclusive results for child I but revealed paternal fragments for child II regarding the loci G3 and MS205 that could not be observed in the putative father (Fig. 1). Extended VNTR analysis using 5 additional single locus probe (SLP's) (Table 1) exhibited a third mutation with probe MS1 affecting the paternal fragment of child II (Fig.1). Table 2 summarizes the results of the VNTR analysis. All potential

mutations were affecting paternal fragments of child II resulting in size differences of 110 (MS205), 110 (MS1) and 460 (G3) bp's between the observed and expected fragments and were reproducible with new DNA samples. Child II exhibited in all cases larger fragments sizes as we expected from the fragment sizes observed in the father.

**Table 2: Results obtained by the VNTR analysis of child I and II. Shifted fragments are printed in bold**

| SLP | Mother | Child I | Child II | Put.Father |
|---|---|---|---|---|
| MS205 | 2,38 / 3,10 | 1,75 / 3,08 | **1,86** / 3,09 | 1,75 / 2,92 |
| yNH24 | 2,83 / 3,24 | 2,89 / 3,22 | 2,82 / 4,04 | 2,89 / 4,05 |
| LH1 | 3,67 / 4,58 | 2,37 / 4,59 | 3,87 / 4,57 | 2,37 / 3,88 |
| pH30 | 2,90 / 4,59 | 2,90 / 17,09 | 4,58 / 16,86 | 3,60 / 16,98 |
| MS1 | 2,20 / 2,68 | 2,67 / 3,35 | 2,67 / **5,12** | 3,33 / 5,01 |
| MS43A | 9,81 / 10,76 | 10,47 / 10,77 | 9,14 / 10,66 | 9,14 / 10,58 |
| G3 | 1,67 / 9,43 | 1,67 / 6,16 | 9,45 / **10,39** | 6,15 / 9,93 |
| MS31 | 6,50 / 9,26 | 6,43 / 9,24 | 6,44 / 9,21 | 6,42 / 6,42 |

In contrast to the analysis of VNTR loci, the analysis of five STR-PCR loci (HUMTH01, D8S639, CYP19, ACPP and ACTBP2), 22 conventional serum protein and erythrocyte membrane systems including HLA-Class I (ABC) analysis did not reveal any exclusion patterns. Biostatistical calculation using the results obtained by the conventional, HLA and STR analysis resulted in an PP value of 99,99%. In addition, calculation of the results obtained from all analysed polymorphisms including the analysis of VNTR loci resulted in an PP value of 99,97% giving clear evidence for paternity.

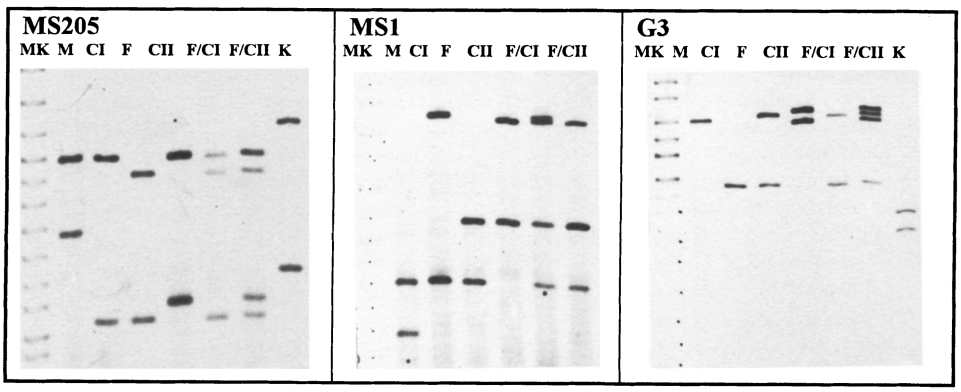


**Figure 1. Mutations observed with SLP's MS205, MS1 and G3. Marker (MK) Gibco-BRL bp ladder, mother (M), child I (CI), child II (CII), father (F), mixed samples father and child (F/CI F/CII), control cell line K562 (K)**

In order to verify the observed mutations in the VNTR systems, we performed DNA analysis using the multilocus probes 33.15 and $(GTG)_5$. The three SLP's MS1, MS205 and G3 have been originally cloned out from probe 33.15. In this respect, we were interested to study if the observed discrepancies in paternal fragment size of child II could be explained by a general shift of all bands generated with 33.15 or if there are single shifted bands that indicate a mutation event. Interestingly, with probe 33.15 we observed a single band difference between child II and the putative father that corresponds to the G3 mutation (Fig. 2). Unfortunately, the MLP 33.15 showed a very homogeneous pattern in the short fragment region, therefore we could not identify similar band shifts corresponding to SLP MS1 and MS205. In contrast, the results obtained from $(GTG)_5$ were in accordance with the expected paternity of the putative father for both children.

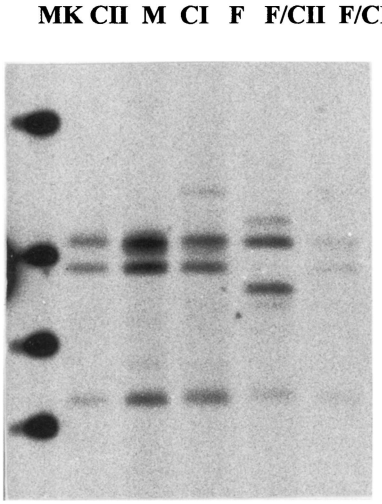


**Figure 2. Multilocus probe 33.15 reveals band shift corresponding to the G3 mutation in child II.**

Complex chromosome translocations may cause such phenomenon. Metaphase chromosome in situ staining results, however, did show regular caryotypes with no structural variations. In this respect, we interpretate the observed band shifts of child II as the result of at least three individual events leading to an increased fragment size of the paternal alleles. The mutation rate for SLP MS1 has been reported to be 4,16%, while the mutation rates of SLP's MS205 and G3 are 0,4% and 0,69%, respectively (Henke 1993, Jeffreys 1988, Royle 1992). Thus the possibility that these paternal mutations occur in one case is $1{,}15 \times 10^{-6}$ or 0,0001%. If 6 VNTR loci (including MS31, MS43A, LH1) are analysed the possibility of such an event is $0{,}96 \times 10^{-5}$ or 0,001%. Since the individual band shifts are relatively small (between 110 - 460 bp) the possibility that the enlarged fragments are due to the loss of a restriction enzyme site in the flanking region of the VNTR loci may not be very likely. Restriction enzyme sites in the human genome have in genral a distance between several thousand to 50 kb (Linn 1968, Smith 1970). Instead, we would propose two alternative explanations: a multiple unequal crossing over affecting the chromosomes 1 (MS1), 7 (G3) and 12 (MS205) occurred during paternal meiosis. An unequal crossing can result in a changed number of repeats in the core-sequence itself and thus explains the increased fragment sizes that we observed in child II. Alternatively a systematic slippage effect occurred during the last premeiotic paternal S phase. Both possibilities may represent rare event but emphasize the importance to combine the analysis of several VNTR loci and/or other genetic marker, i.e. STR or conventional marker, in the evaluation of paternity or human identification cases.

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