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SHORT COMMUNICATION

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Molecular analysis of the human orosomucoid gene ORM1*Q0$_{köln}$ responsible for incompatibility in a German paternity case

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Abstract In a German paternity test, an alleged father was excluded only by reverse homozygosity of ORM1 phenotypes (mother ORM1 S, child ORM1 S and alleged father ORM1 F1) out of the 28 classical and DNA markers investigated. Without the ORM1 system the biostatistical probability of paternity was calculated to exceed 99.9999%. The intensity of the immunoprinted bands of the ORM1 protein for the child and alleged father after isoelectric focusing appeared to be reduced to about half. To identify a possible null allele, gene-specific amplification followed by single-strand conformation polymorphism and sequencing analyses were carried out. Deletion of one of the two copies of a 4 bp direct repeat sequence (GTCT) in exon 4 of the consensus sequence of ORM1*F1 was observed in the child and alleged father. Thus, the sharing of a rare mutant gene, ORM1*Q0$_{köln}$, increased the probability of paternity.

Key words Isolated exclusion · Null allele Orosomucoid gene · Paternity case · Reverse homozygosity

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Introduction

When an isolated exclusion is observed in paternity tests, attention must be paid to the occurrence of a de novo mutation or a null allele, which bring about false incompatibility between an alleged father and child. Conversely, elucidation of a cause at the DNA level can increase the probability of paternity.

In a paternity test, we encountered an apparent incompatibility in the orosomucoid (ORM) system. Human plasma ORM, also known as α_1-acid glycoprotein (AGP), is a mixture of products of the two closely linked loci, ORM1 and ORM2, in a molar ratio of 3:1 (Yuasa et al. 1986). The two ORM proteins are encoded by the two tandem genes AGP1 and AGP2. Each gene consists of six exons and five introns, and encodes 183 amino acids (Dente et al. 1985, 1987). The two loci are very polymorphic and a number of variant alleles have been identified in various populations. The three common alleles at the ORM1 locus, ORM1*F1, ORM1*F2 and ORM1*S were shown to arise from two point mutations occurring at the codons for amino acid positions 20 in exon 1 and 156 in exon 5 of the AGP1 gene (Yuasa et al. 1997). In addition, non-expression of genes at both loci have also been observed in population and family studies and paternity testing by isoelectric focusing (IEF) and occur at a polymorphic frequency in some populations (Escallon et al. 1987; Umetsu et al. 1988, 1995; Yuasa et al. 1990a, 1990b). Null alleles, however, are rare in Europeans (Kasulke and Weidinger 1990). In this study we describe the molecular basis of an allele responsible for the aberrant inheritance of ORM1 phenotypes observed between an alleged father and a child in a German paternity case. This is the first time an ORM1 null allele has been investigated at the DNA level.

Materials and methods

Blood samples were obtained from a Caucasian trio in a German paternity case. ORM phenotyping was performed by three IEF

techniques: IEF of desialyzed samples in the presence and absence of detergent (Yuasa et al. 1993) and of alkylated samples in the presence of urea (Rocha et al. 1993; Dülmer et al. 1998). In some experiments a carrier ampholyte with a broad pH range of 3.5–9.5 (Pharmacia, Uppsala, Sweden) was also used. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out as described previously (Yuasa et al. 1995). Genomic DNA was extracted by a standard method. Gene-specific amplification and the nested polymerase chain reaction (PCR) of six exons were carried out as described previously (Yuasa et al. 1997) with a modification: a new reverse primer ORM1-E6R3 (5′-AAGTGAGGGAAAAAGCTGAG-3′) was used instead of ORM-E5R2 to obtain gene-specific fragments containing exons 4–6. Single-strand conformation polymorphism (SSCP) analysis and direct sequencing of the PCR products have also been described in detail (Yuasa et al. 1997). To amplify a small fragment containing a mutation site, a new forward primer ORM1-Köln (5′-GACCTACATGCTTGCTTTTG-3′), corresponding to the sequence of codons 108–115 was used in combination with the ORM-E4R primer (Yuasa et al. 1997). This primer set was also used to estimate the frequency of the mutated gene in 172 unrelated German and 235 unrelated Japanese DNA samples.

Results

Electrophoretic study of ORM proteins

The testing of the three individuals showed no exclusion from fatherhood in 15 classical systems (ABO, MNS, RH, HP, GC, TF, PI, AHSG, ORM2, F13B, C1R, ACP, PGM1, AK and ADA) and 12 DNA markers (D1S7, D7S21, D12S11, D16S309, D5S110, D1S80, D17S5, TH01, LDLR, HBBG, D7S8, and HLA-DQA). A paternity probability of more than 99.9999% was calculated. However, ORM1 phenotyping by the three different IEF techniques followed by immunodetection revealed an identical result: ORM1 S in the mother and child and ORM1 F1 in the alleged father (Fig. 1). An apparent incompatibility was observed in the ORM1 system between the child and alleged father but the intensities of the immunoprinted bands in both the child and alleged father appeared to be reduced to about half. The IEF in a broad pH range showed no additional band. In the SDS-PAGE no elongated or truncated proteins were observed. These findings suggested that they shared a null allele.

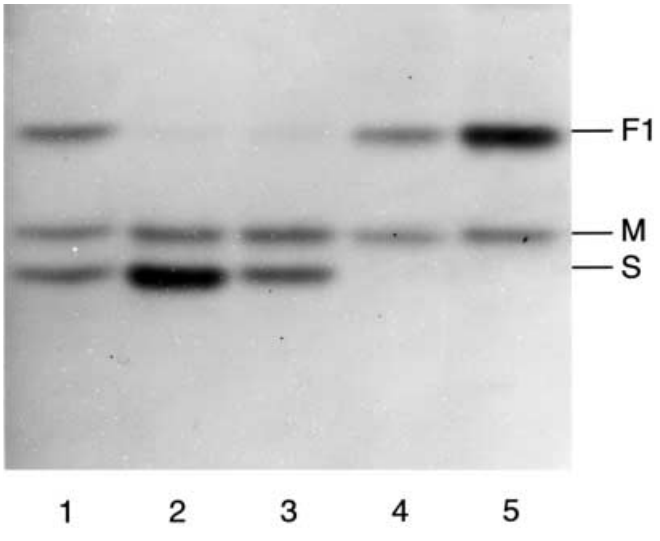

Fig. 1 Immunoprinted banding patterns of desialyzed ORM proteins after isoelectric focusing in the presence of detergent (Method A, Yuasa et al. 1993). *Anode at top. Lanes: 1* ORM1 F1-S/ORM2 M (control), *2* S/M (mother), *3* S/M (child), *4* F1/M (alleged father), *5* F1/M (control)

SSCP analysis

The PCR products containing each exon of the respective genes were analysed by non-radioactive SSCP under various conditions. Altered migration patterns were observed in the fragments for exons 1 and 4. The SSCP patterns of exon 1, where a nucleotide substitution distinguishing ORM1*F1 and ORM1*S resides, showed that the mother and alleged father had patterns identical to ORM1 S and ORM1 F1 homozygotes, respectively. However, the child showed the same SSCP pattern as the ORM1 F1-S heterozygote, being inconsistent with the IEF results (Fig. 2 a). The PCR fragments for exon 4 obtained from the child and alleged father showed the same altered migration patterns (Fig. 2 b). No mobility shifts were observed in the other exons of AGP1 and AGP2 genes. These SSCP results suggest that the child and alleged father share a gene with a small mutation in exon 4, originating from ORM1*F1.

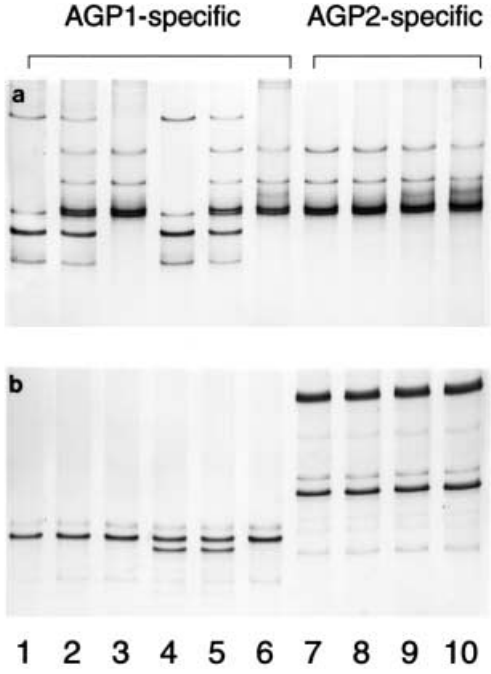

Fig. 2 Single-strand conformation polymorphism (SSCP) analysis of AGP1 gene-specific and AGP2 gene-specific products for exons 1 **a** and 4 **b** by electrophoresis on polyacrylamide gels (12%T, 3%C) without glycerol. ORM1*S has the same sequence in exon 1 as the AGP2 gene (ORM2*M). *Lanes 1 and 7* ORM1 F1/ORM2 M, *2* F1-S/M, *3* S/M, *4 and 8* alleged father, *5 and 9* child, *6 and 10* mother

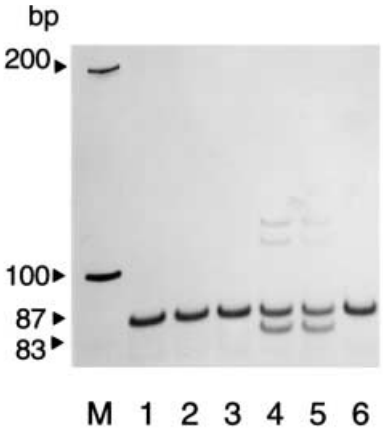

Fig. 3 Electrophoresis of fragments obtained using a primer set, ORM1-Köln and ORM-E4R. *Lanes: 1* ORM1 F1/ORM2 M, *2* F1-S/M, *3* S/M, *4* alleged father, *5* child, *6* mother, *M* 100-bp ladder marker

Sequencing

The AGP1 gene-specific products for exon 4 indicated by SSCP analysis were subjected to direct sequencing. The nucleotide sequences of the products from the child and alleged father were heterozygous from codon 126 when using a forward primer, and from codon 125 when using a reverse primer, suggesting that a frameshift mutation due to a small deletion had occurred in the mutant gene. To obtain fragments for the mutant gene, PCR was performed using a primer set made up of ORM1-Köln and ORM-E4R. Two bands different in length were obtained, and one short fragment was characteristic of the child and alleged father (Fig. 3). Each band was excised from gel and subjected to reamplification with the same primer set. Sequencing of both strands revealed that a deletion of one of the two copies of a 4 bp direct repeat sequence (GTCT) within codons 124–127 occurred in the short mutant fragment (Fig. 4). Since this deletion introduced a frameshift, an amino acid sequence was predicted using GENETIX-MAC, version 9 (Software Development Co., Tokyo, Japan). Consequently, the original stop codon in a normal cDNA sequence (Dente et al. 1986) was abolished and no new stop codon appeared at least up to the poly(A) addition site.

Estimation of the frequency of the mutated gene

The frequency of the mutated gene was estimated in a total of 407 DNA samples from German and Japanese individuals. As expected, no mutated gene was observed, suggesting that it is very rare.

Discussion

Although a number of DNA polymorphisms including variable number of tandem repeat (VNTR) and short tandem repeat (STR) polymorphisms are nowadays applied to the fields of forensics, the value of classical markers with a relatively high polymorphism has not decreased, especially in paternity tests, because of the low mutation rates. However, null and deficient alleles with a rare frequency have been observed in most classical markers. We encountered a reverse homozygosity between the child and alleged father, caused by a null allele in the ORM1 system. To solve the apparent incompatibility, SSCP analysis was performed revealing a cryptic allele exclud-

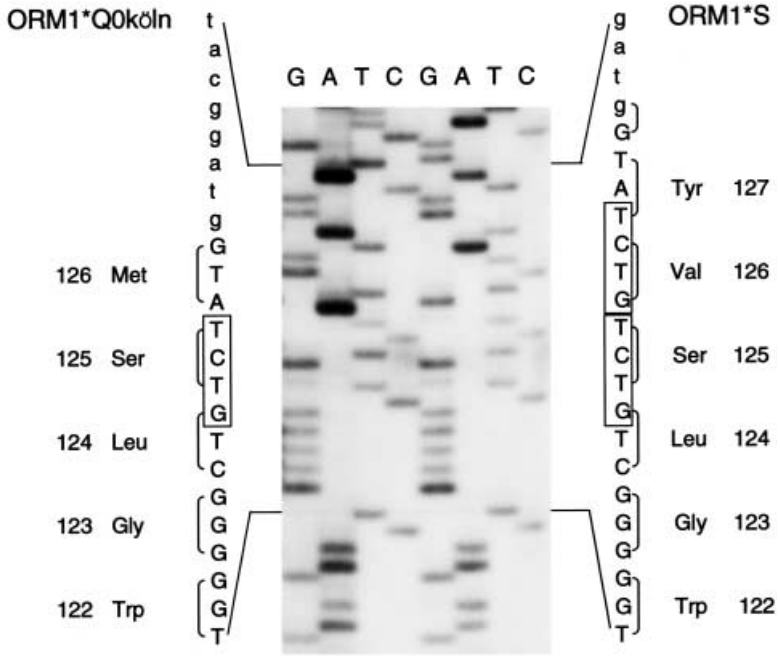

Fig. 4 Sequence of AGP1-gene specific products for exon 4 from the child (The codon numbers and the corresponding amino acids of ORM1*$Q0_{köln}$ and ORM1*S are listed on the left and right, respectively. A 4 bp (GTCT) deletion is observed in the mutant gene, ORM1*$Q0_{köln}$. This sequence was registered in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession number AB014887)

ing the incompatibility. Subsequent sequencing revealed that both individuals shared a deletion of one of the two copies of a 4 bp direct repeat sequence, explicable by a slipped strand mispairing mechanism (Efstratiadis et al. 1980; Krawczak and Cooper 1991). The frequency of the mutated gene is very rare in German and Japanese populations. Thus, the discovery of a rare sequence in both individuals evidently increased the probability of paternity. We designated the null allele ORM1*$Q0_{köln}$ after the birthplace of the alleged father.

The 4 bp deletion found in the present mutant gene is unique, because it abolished the stop signal, in contrast with a 2 or 5 bp deletion at the same mutation site, which produced a frameshift leading to an early termination. Generally, frameshift mutations do not only bring about a change in amino acid sequences downstream from the mutation sites, but also produce a new stop signal, leading to the synthesis of truncated proteins in most cases, and elongated proteins in the remainder. Only a few cases have been reported where the original stop codon was abolished through a point mutation event. The α2-globin gene in Hb Constant Spring and Hb Seal Rock, however, has another stop signal between the original stop codon and poly(A) signal. As a result, an elongated polypeptide is observed in these patients (Clegg et al. 1971; Kosasih et al. 1988; Merritt et al. 1997). A novel mutation found recently in the adenine phosphoribosyl-transferase (APRT) gene also abolished the original stop codon through a point mutation event. In this case there was no stop codon between the original stop codon and poly(A) addition site. The mRNA level for APRT was approximately 25% of those from the control subjects, but no APRT proteins were detected (Taniguchi et al. 1998). The present mutant gene also removed the stop codon upstream from the poly(A) addition site. Although it was not determined that this gene is completely unable to synthesize the ORM1 protein, a similar mechanism would affect the present mutant gene, i.e. the mutation probably leads to a complete loss of the ORM1 protein. This must be the reason why the reverse homozygosity was observed between the child and alleged father.

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