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SHORT COMMUNICATION

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DNA typing in cases of blood chimerism

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Abstract A chimera is an organism whose cells derive from two or more distinct zygote lineages. and therefore two different blood cell populations circulate in one individual. To point out the potential pitfalls in forensic analysis, a set of triplets (a girl and two boys) who revealed blood chimerism was investigated with four STR systems using PCR. The results indicated that a DNA profile based on DNA extracted from blood can lead to a false determination because the band pattern of each triplet contained a mixture of the original genotype and the genotype of the siblings. Additional investigations on biological materials other than blood must be made in order to find out the real genetic characteristics of each child.

Key words Blood chimerism · DNA typing · Forensic analysis · STR systems

Introduction

The presence of blood chimerism in human twins is a rare event and was first described by Dunsford et al. (1953) who discovered a mixture of two blood types in a healthy blood donor. Since then approximately 80 cases have been published.

According to Gill (1977) a spontaneous and an artificial chimerism can be distinguished. An artificial chimerism arises after allogenic bone marrow or blood stem cell transplantation. DNA fingerprinting and typing can be used to document donor cell engraftment in marrow transplant recipients (Ling Min et al. 1988). A spontaneous chimerism is due to blood vessel anastomoses between dissimilar twins in uterine life and a subsequent transfusion of red blood stem cells and lymphoid stem cells passing through foetal anastomoses. Some of the invading cells become established in the marrow of the host who throughout life continues to make cells with both genetic patterns (Tippett 1983). During this foetal stage a natural suppression of the immune response occurs and enables the organism to recognise cells as self which might otherwise be antigenic. Thus the genetically foreign cells are prevented from a immune response by the individuals own potential antigens (Tippett 1983). In humans this is the most common type of blood chimerism and is permanently established in an individual in contrast to the artificial chimerism.

A chimera differs from a mosaic which is formed of cells of a single zygote lineage and may arise at any stage of development as the result of an irregularity during the cell cycle, e.g. by somatic mutation or errors at mitotic phases (Gill 1977). However it is difficult to determine if an individual with two populations of cells is a mosaic or a chimera.

Investigations on chimeric twins using serological markers and blood group characteristics were described by Dunsford and Stacey (1957), Hansen et al. (1978, 1984), Hosoi et al. (1977) and Pausch et al. (1979). The results with DNA probes and the consequences for interpretation in forensic analysis were pointed out by Farber et al. (1989) and Hansen and Sondervang (1993). Dunsford and Stacey (1957) and Hansen et al. (1984) stated that the proportion of foreign cells in each individual can be variable and declined in the course of time. Thus different intensities and a higher number of signals can be obtained. Tippett (1983) pointed out that in some cases the proportion of foreign cell populations even exceeds the original genotype. According to Hosoi et al. (1977) the proportion of foreign cells depends on the period of the anastomoses. If it was only brief each twin should have more own genetic cells.

The occurrence of blood chimerism can lead to a false interpretation in the analysis of stain material in crime cases and in paternity testing because a mixed pattern of

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different genotypes can be obtained in one individual. In order to determine which DNA profile represents the true genetic line and to avoid a false determination, DNA typing of blood and other biological materials from a set of chimeric triplets has been carried out with STR systems and PCR and compared.

Materials and methods

For DNA analysis saliva and blood samples from each member of the chimeric family (parents, a girl and two boys) were collected. DNA was extracted according to the chelex protocol (Walsh et al. 1991) and the DNA was quantified by slot-blot analysis using the DNA quantitation system (Aces 2.0 Human DNA Quantitation System, Gibco BRL). PCR primers and reaction conditions for the STR systems applied have been described previously: amplification was performed with the HumAmelX/Y gender identification system (Mannucci et al. 1994) and the STR systems HumFES (Polymeropoulos et al. 1991), HumVWA (Kimpton et al. 1992) and HumTH01 (Edwards et al. 1992). Of the PCR product 3.5 µl was separated on horizontal polyacrylamide gels (9.0%T/0.9%C for HumAmelX/Y, 6.8%T/0.9%C for HumFES, 7.8%T/0.9%C for HumVWA and 7.5%T/0.9%C for HumTH01, 81 mM tris-formate buffer and 28 mM cyclohexamineethane sulfonic acid) according to Allen et al. (1989) using the Multiphor detection electophoresis chamber (Pharmacia). Maximum settings were 1000 V and 40 mA. PCR products were detected by silver staining.

Results and discussion

The investigation of blood with the three loci (HumAmelX/Y, HumVWA and HumFES) revealed a mixed band pattern for each child composed of his own genetic line and of the siblings whereas the saliva samples gave the original genotype of the individuals (Tables 1 and 2).

Table 1 Results of the DNA analysis from blood samples taken from the family

	HumTHO1	HumFES	HumVWA	HumAmel.X/Y
M	7	10a/11a	15/19	X
C_1	6/7	10a/11a/11	15/16/19	XY
C_2	6/7	10a/11a/11	15/16/19	XY
C_3	6/7	10a/11a/11	15/16/19	XY
F	6/8	11/11	16/19	XY

Table 2 Results of the DNA analysis from saliva samples taken from the family

	HumTHO1	HumFES	HumVWA	HumAmel.X/Y
M	7	10a/11a	15/19	X
C_1	6/7	10a/11	16/19	X
C_2	6/7	11a/11	15/16	XY
C_3	6/7	11a/11	15/16	XY
F	6/8	11/11	16/19	XY

HumAmelX/Y

DNA typing of the saliva with the gender identification system gave the expected results of a single band in the female members and a doublet in the male members of the chimeric family (Fig. 1 a). The blood samples also revealed the expected pattern in the parents and the male children, but the female child presented X and Y bands, typical for male individuals due to y cells circulating in the peripheral blood (Fig. 1 b). This result is in agreement with Farber et al. (1989) and Tippett (1983) who observed XY-lymphocytes in a karyotype analysis of female chimeric twins.

HumVWA

Analysis of DNA extracted from the saliva samples indicated a normal constellation for the whole family. The al-

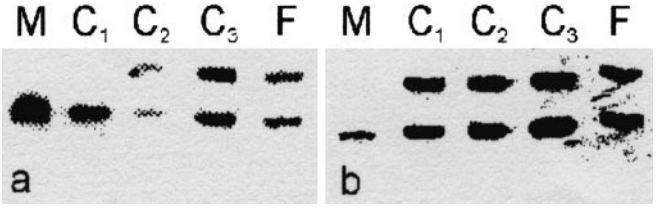

Fig. 1 a, b HumAmelX/Y: DNA profile of the saliva samples (**a**) from the chimeric triplets in comparison with the blood samples (**b**). Blood analysis revealed X and Y bands for the female child and the expected pattern from the saliva sample. M = mother of the triplets, C_1 = female triplet, C_2 and C_3 = male triplets, F = father of the triplets

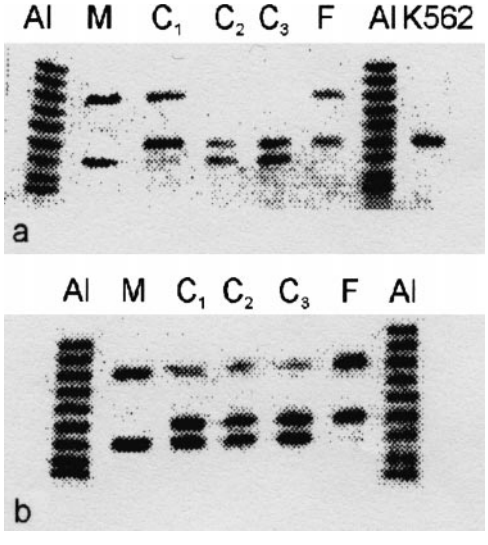

Fig. 2 a, b HumVWA: DNA profile of the saliva samples (**a**) from the chimeric triplets in comparison with the blood samples (**b**). Analysis of the blood samples of the children gave a mixture of the original and the foreign genotypes. The own genetic line of each was obtained by the saliva investigation. AI= allelic ladder, M = mother of the triplets, C_1 = female triplet, C_2 and C_3 = male triplets, F = father of the triplets, K562 = positive control

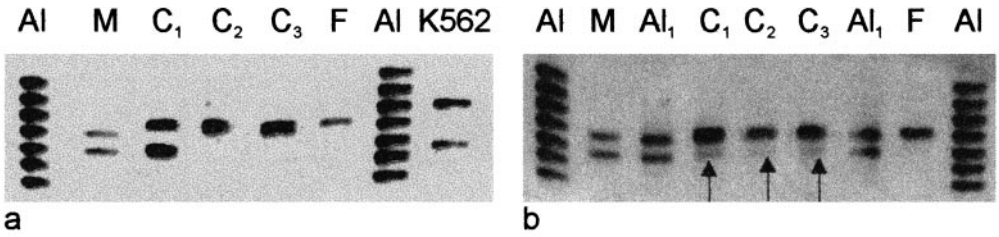

Fig. 3 a, b HumFES: DNA profile of the saliva samples (**a**) from the chimeric triplets in comparison with the blood samples (**b**). Investigation of the blood samples of each child presents a pattern of different genotypes composed of the individual and of the siblings. The saliva samples gave the expected band pattern. AI = allelic ladder, M = mother of the triplets, Al_1 = allelic constellation 10a and 11a, C_1 = female triplet, C_2 and C_3 = male triplets, F = father of the triplets, K562 = positive control. Arrows indicate weakly expressed bands of the triplets

leles 16/19 for the girl and 15/16 for the boys were observed (Fig. 2 a) whereas the blood analysis of all triplets showed besides the allele 16 a strong additional band (allele 15) and a weakly expressed band (allele 19) (Fig. 2 b).

HumFES

The allelic constellations of the saliva samples for the female child was 10a/11 and 11a/11 for the boys (Fig. 3 a). The results of the blood analysis (Fig. 3 b) revealed the same band pattern for each child, a weak 10a band and a strongly expressed 11a/11 combination.

HumTH01

The investigation of the saliva samples in comparison with the blood exhibited a normal constellation of DNA fragments between parents and children for both materials; no additional DNA bands could be found in the chimeric triplets.

Blood analysis with the STR systems HumVWA and HumFES gave the same results for all children both in intensity and allele constellations. The results indicated that the female sibling received a higher amount of haemopoietic stem cells from the male siblings (HumVWA:15 and HumFES: 11a, which were strongly expressed) but the males received a smaller number of stem cells from the female sibling (HumVWA:19 and HumFES:10a which were weakly expressed). This phenomenon was also observed by Hansen and Sondervang (1993) who demonstated a mixed pattern of DNA fragments of different intensities in each child using single locus probes.

Farber et al. (1989) compared the results of DNA fingerprinting from leukocytes of chimeric sibs and obtained an identical pattern for both twins. The original genotype of each which differs clearly could not be determined until the skin of both was analysed.

In Conclusion, when a mixed band pattern and different band intensities are obtained from a sample this observation should be taken into consideration for the interpretation, in order to avoid a false determination. Although the occurrence of blood chimerism is a rare event in humans it should be kept in mind in forensic DNA analysis.

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