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Can we use forensic human identification techniques to detect homologous blood transfusion in doping control? A pilot study.

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Introduction

Homologous blood transfusion is a banned practice that athletes may use to improve the delivery of oxygen to tissues. At present, the method of detection is based on the identification, by cytofluorimetry, of mixed populations based on differences of expression of minor blood group antigens on erythrocytes (RBCs) surface [1]. The current screening test is performed testing 8 antigens belongings to four different human blood group (big C, small c and big E of the Rh group, Jka and Jkb of the Kidd group, Fya and Fyb of the duffy group and big S of the MNS group). An individual can either express or not blood group antigens on the surface of his/her red blood cells. In all cases in which donor and receiver have different antigen expression pattern, a double population of cells (expressing and non-expressing) can be detected by cytofluorimetry. The ability of this method to clearly identify double populations of RBCs depends on the number of blood group antigens analyzed: the broader the range of antigens tested, the higher the probability to detect “donor cells” into receiver's blood.

Based on the blood samples analyzed by our laboratory for blood transfusion in 2010, we built up a database and found that many blood samples, coming from different athletes, shared precisely the same eight antigens pattern. In order to improve the discriminating power of the method, we first decided to expand the number of antigens analysed and found that, as expected, the frequency of individuals sharing the same haplotypes decreased but, after all, a certain number of individual with the same exact haplotypes still persisted.

We therefore evaluated the possibility to explore a different approach to detect homologous blood transfusions, using DNA forensic human identification techniques.

Forensic human identification is based on the analysis of portions of DNA called microsatellites (Short tandem repeats, STRs) [2]. Each locus STR is constituted by a wide number of alleles on the basis of the number of the repeated sequences. Several STR loci are present on different human chromosomes (fig.1).

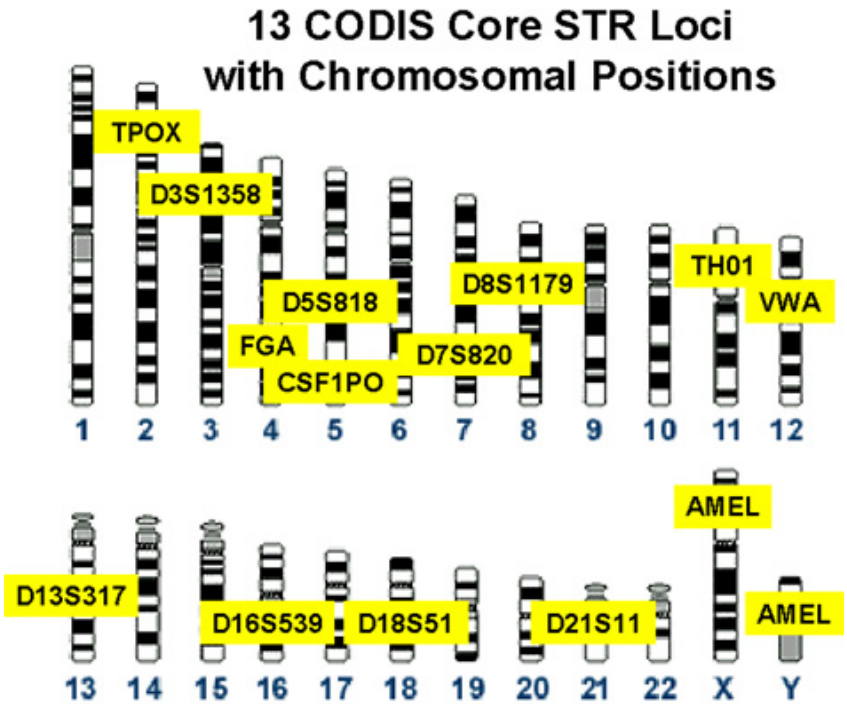

Fig.1: Position of 13 CODIS (Combined DNA Index System) STR loci on human chromosomes. STR by CODIS are widely used in human forensic DNA identification worldwide.

Since 16 loci are normally tested in human identification (13 CODIS loci plus 3 supplementar loci), the probability that two different individual share the same haplotype pattern is virtually null. DNA obtained from different sources (blood, urine, buccal swab etc.) is amplified by Polymerase Chain Reaction (PCR) into a master mix containing the primer set of all the 16 loci of the identification panel (D8, D21, D7, CSF, D3, TH01, D13, D16, D2S, D19, vWA, TPOX, D18, D5, FGA and Amelogenin used for sex determination). Each primer is specific for a certain locus. PCR is specific and generates a very high number of fragments containing the alleles of interest. Amplified samples are then analyzed in a capillary electrophoresis system to separate the fragments locus by locus. Alleles detection is made by a five-colour detector system. A size standard constituted by fragments of known molecular weight is added to the mixture to align the runs. An allelic ladder sample is also used to get the correct typization by comparison with the unknown sample.

The study of mixed samples in forensic human identification is helpful in detecting samples suspected for blood transfusion. An individual's DNA profile comprises a collection of genotypes, one for each marker. Each genotype consists of an unordered pair of alleles, one inherited from the father and one from the mother (though one cannot distinguish which is which). When both alleles are identical the individual is homozygous at that marker, and only a single allele value is observed; else the individual is heterozygous. In a diploid genome like

the human one, only these two conditions are possible. The presence of three or four alleles at certain locus can be due to contamination or can be the consequence of a transfusion practice.

Experimental

Flow Cytofluorimetry: blood samples were analysed for blood transfusion detection using FC500 Beckman Coulter cytofluorimeter. Classical 8 minor blood group antigen panel has been expanded analyzing 5 more antigens (small e, small s, big Kell, Lewis a and Lewis b) improving the sensitivity of the method. Samples sharing identical blood group haplotypes were mixed at 40%, 25%, 10% and 5% of the donor and re-analyzed for homologous blood transfusion detection.

Forensic human identification: DNA was extracted and purified from whole blood mixed samples at 40%, 25%, 10% and 5% using a Prepfiler Forensic DNA Extraction Kit (Applied Biosystems). DNA was subsequently amplified with Polymerase Chain Reaction (PCR) using AMPFlSTR Identifiler PCR amplification Kit (Applied Biosystems). PCR was performed with 1ng estimated DNA. A total of 28 cycles were used for amplification. Separation of amplified STR fragments was made with capillary electrophoresis system ABI Prism 310 (Applied Biosystems) [3]. Runs were made at 15kV, 60°C, injection time 5sec. Alleles identification at each locus has been made by confrontation to an allelic ladder using Gene Mapper ID v.3.2 software.

Results and discussion

We have considered 58 samples from different individuals. 22 individuals shared an identical haplotype with at least another individual of the database (37,9% of the total). Identical haplotypes belonged to eight different groups of haplotypes.

As expected, for each of the six samples mixed at 5% of the donor, no double population was found for each of the 13 antigens analyzed (fig.2, left). On the contrary, the analysis based on forensic identification showed that it is possible to detect a mixed sample in all cases considered (fig.2, on the right is one example). We identified triple and quadruple alleles at various loci to assess that a sample is the result of a mixture. We found an average of 10 triple and quadruple alleles (and minimum number of 6) for a mixed sample in which the donor's contribution is at 5%. We also calculated the percentage frequency of the appearance of triple and quadruple alleles in all loci analyzed (excluding Amelogenin locus that only serves for sex determination). By doing this, we found that loci such as D3S, TH01, D5S, FGA are more informative for our purpose as they always present a triple or a quadruple allele in all cases considered in our database. On the contrary, loci such as D8 and CSF seem to be less informative in particular when the contribution of the donor is at 5%.

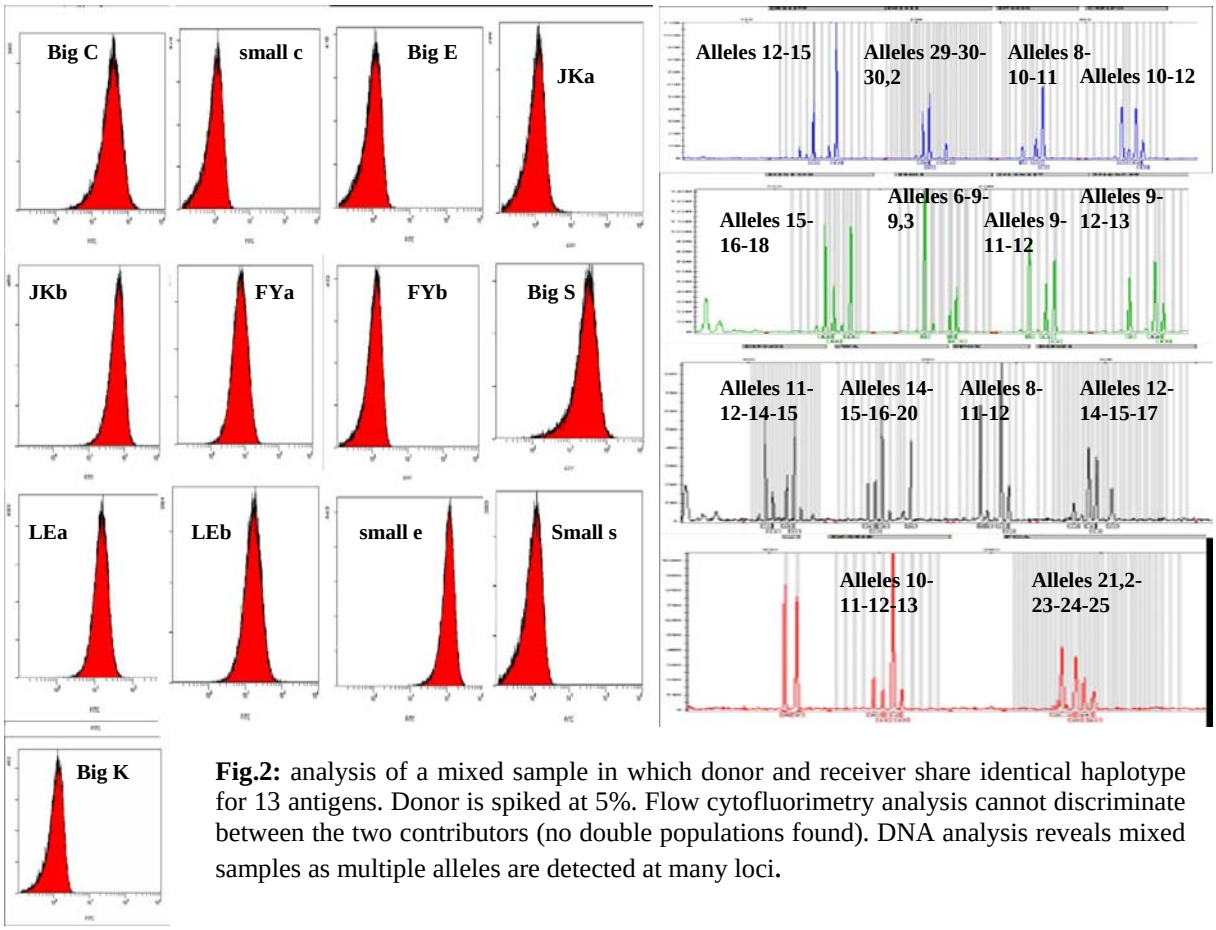

Fig.2: analysis of a mixed sample in which donor and receiver share identical haplotype for 13 antigens. Donor is spiked at 5%. Flow cytofluorimetry analysis cannot discriminate between the two contributors (no double populations found). DNA analysis reveals mixed samples as multiple alleles are detected at many loci.

In summary, although DNA forensic human identification is normally applied to the study of mixed samples as a consequence of contamination, this study demonstrates that this technique can be a powerful tool also to be applied in the detection of homologous whole blood transfusions with a very high power of discrimination among transfused samples. This is achieved thanks to the high number of loci analyzed by and most of all thanks to their high polymorphism. A possible limitation of this test can emerge when RBC concentrates are used for transfusion in place of whole blood. However, residual leucocytes are always present into concentrates eyrthrocytes' blood bags so allowing DNA from the donor to be detected anyway into recipient's blood. These encouraging results lead us to delve into the developing of a complete method based on these techniques.

References

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