

Combined STR VNTR and MVR Typing and mtDNA Sequencing, led to the Identification of Human Remains Emergent from the AMIA Explosion in Buenos Aires

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Introduction

On July 18th, 1994, the building of a Jewish Association (AMIA) was blown by a bomb in Buenos Aires. The attack resulted in more than 90 fatal and over 300 injured victims and the complete destruction of the building. A high number of fragmentary human remains were found. National authorities required us to characterize over 80 human remains and to identify victims by comparison with family groups searching for missing relatives.

Combined molecular typing approaches, including seven STRs, four VNTR and PCR-MVR, were used, supplemented with mtDNA sequencing. The strategy employed sped up the identification process. The high efficiency of sample handling and molecular typing systems employed is due in part to a previous experience, the bombing attack against the Israeli Embassy occurred in 1992, after which our laboratory also contributed to the identification of remains (Corach et al., 1994).

Materials and Methods

Strategy.

1-DNA was extracted from samples immediately after receiving them from the morgue.

2-A set of seven microsatellite systems were typed, including two sex chromosome specific-STRs that additionally allowed rapid gender determination.

3- All samples displaying identical STR genotypes were further analyzed by means of typing with four minisatellites.

4- Samples that could not be typed with minisatellites due to degradation (absence of signal) or severe mismatch were then typed by means of PCR-MVR.

5- Some remains, discovered several months after the disaster, required to be analyzed by mtDNA control region sequencing.

DNA Extraction: CTAB extraction protocol (Corach et al., 1995) was used.

STR Analyses. The following STRs were employed: HUMTHO-1, HUMFABP, HUMRENA4 and HUMHPRTB (Edwards et al., 1992) HUMVWA (Kimpton et al., 1992), HUMFES/FPS (Polymeropoulos et al., 1991) and the Y specific STR Y27H39 (Roewer et al., 1992). PCR reactions were carried out in a Perkin Elmer Thermal Cycler. A triplex comprising variable sex chromosome-specific sequences HUMHPRTB / Y27H39 and a monomorphic Y specific sequence located at Yq12qter (Kogan et al., 1992) allowed typing and gender determination. All reaction mixtures included a 0.02 mCi/sample α -P32-dATP. Reaction volume was 30 μ l.

Amplicon Evaluation by PAGE. Amplicons were separated in denaturing 5% Acrylamide:Bisacrylamide (38:2) gel in an S2 sequencing electrophoretic apparatus (BRL Life Technologies, USA). Gels were run at 1,500 Volts (constant voltage). After electrophoresis, gels were exposed overnight to radiographic film at -70°C.

VNTR Analyses. Remains displaying identical STR genotypes were Hae III-digested, analyzed by means of Southern Blot and probed sequentially with four minisatellites :YNH-24 (D2S44), PH-30 (D4S139), MS-1 (D1S7) and LH-1 (D5S110). The first was radiolabeled

with α -dATP P³² by random priming, and the other three were chemiluminiscent Alkaline Phosphatase-linked oligo probes. Detection of signals was by autoradiography and by ACES (LifeTechnologies, BRL) respectively.

MVR Analyses. Samples showing identical STR genotypes and similar VNTR patterns denoting single band or complete profile shifts, or that could not be VNTR typed due to severe degradation, were selected for MVR evaluation. An MVR Kit was kindly provided by Dr. Alec Jeffreys and CellMark Diagnostics. Samples were PCR-amplified, Southern blotted, probed and detected according to the protocol supplied. Interpretation of results was based on Jeffreys et al.,(1991).

mtDNA Sequencing. Samples recovered several months after the explosion, were initially typed with all STRs and compared with the samples typed previously. Samples displaying identical STR genotypes were chosen for amplification of mtDNA control regions 1 and 2(Orrigo and King,1991). Sequencing was performed by using DS DNA Cycle Sequencing System (BRL, LifeTechnologies,U.S.A)

Results and Discussion

STR typing was attained, at least partially, in 100% of the samples analyzed. However, a 77% were completely typed; the remainder 23% failed to be fully evaluable. Under identical PCR conditions, some STRs were more efficient than others as regards amplification.

Combined sex chromosome microsatellites allowed to getting a two-fold purpose in order to contribute, on the one hand, with to the typing of the remains and, on the other, with to gender determination. This procedure showed that 25% of the samples belonged to females, 69% to males and 6% remained undetermined on account of partial typing. Genotype comparison allowed grouping the remains according to the number of samples displaying identity. Genotypes were loaded on a Fox Pro-based program in order to establish identity and detect kinship with potential relatives. These and victims that shared STR alleles were then investigated by VNTR analysis. In some cases, inclusions were further confirmed by mtDNA sequencing of putative mothers and the remains of their offsprings. However, in some others, inclusion based on STR typing were shown to be excluded by VNTR typing. In addition, genotype identity allowed us to correlate remains previously identified by conventional means with unidentified ones. This permitted confirm the identity of 14 remains. In order to evaluate the STR applicability for identification in complex forensic case work, confirmation of the above results was performed by means of minisatellite VNTR evaluation of those remains displaying identical STR genotypes. Most samples tested with four different probes agreed with STR information. Nevertheless, absence of bands as well as a band or profile shift was observed. Such results may be expected when dealing with DNA obtained from decomposed cadaveric material. PCR-MVR typing and mtDNA sequencing evaluation were applied to these samples so as to verify their genetic identity. In all instances in which VNTR typing failed in confirming STR data, those approaches permitted confirmation of the results. Multiplex reactions, which sped up the typing process, and the combination of sex chromosome specific sequences, also contributed to the molecular determination of sex and to the identification of fragmentary human remains (Corach et al.,1995). The identification potential of STR systems will be correlated with the number of systems to be employed. Since the aim of this paper is the evaluation of STR systems in a complex forensic case work, conventional VNTR analyses, mtDNA sequencing and the novel MVR-PCR systems were used as verification approaches. A remarkable result arose as a by-product of the STR confirmation: the high efficiency of MVR-PCR typing. Two simultaneous amplifications, a southern blot and probing can generate as much information as that provided by many STR systems put together. As the analytical range of this system is 300 bp to 2 kb and a high

number of hypervariable alleles can be scored, its applicability to forensic sample analysis should be considered.

Acknowledgements: This investigation was supported in part by a grant from UBACyT FA131.

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