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Paternity tests in Mexico: Results obtained in 3005 cases

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ABSTRACT

National and international reports regarding the paternity testing activity scarcely include information from Mexico and other Latin American countries. Therefore, we report different results from the analysis of 3005 paternity cases analyzed during a period of five years in a Mexican paternity testing laboratory. Motherless tests were the most frequent (77.27%), followed by trio cases (20.70%); the remaining 2.04% included different cases of kinship reconstruction. The paternity exclusion rate was 29.58%, higher but into the range reported by the American Association of Blood Banks (average 24.12%). We detected 65 mutations, most of them involving one-step (93.8% and the remaining were two-step mutations (6.2%) thus, we were able to estimate the paternal mutation rate for 17 different STR loci: 0.0018 (95% CI 0.0005–0.0047). Five triallelic patterns and 12 suspected null alleles were detected during this period; however, re-amplification of these samples with a different Human Identification (HID) kit confirmed the homozygous genotypes, which suggests that most of these exclusions actually are one-step mutations. HID kits with ≥20 STRs detected more exclusions, diminishing the rate of inconclusive results with isolated exclusions (< 3 loci), and leading to higher paternity indexes (PI). However, the Powerplex 21 kit (20 STRs) and Powerplex Fusion kit (22 STRs) offered similar PI ($p = 0.379$) and average number of exclusions (PE) ($p = 0.339$) when a daughter was involved in motherless tests. In brief, besides to report forensic parameters from paternity tests in Mexico, results describe improvements to solve motherless paternity tests using HID kits with ≥20 STRs instead of one including 15 STRs.

1. Introduction

DNA analysis probably has become the most powerful tool for solving forensic cases related to criminal activity and for biological kinship establishment. Most of the paternity tests involve comparison of genetic profiles between the alleged father and child (including or not the mother), followed by exclusion of paternity or confirmation that require the paternity index (PI) estimation.[1] Subsequently, probability of paternity (W) is computed applying the Bayes Theorem. Although the generally accepted minimum standard PI for inclusion of paternity is ≥ 100,[2] presently most laboratories require a PI ≥ 10000, which corresponds to W ≥ 99.99% assuming *a priori* probability of paternity of 0.5.[3] Biological kinship relationships can also be established by inclusion of additional relatives (reconstruction cases) and analysis of genetic markers with peculiar inheritance patterns. For instance, paternal kinship can be established between males carrying the same non-recombinant region of the Y-chromosome, and complex cases where at least one female is involved can be solved by means of X-linked STRs (X-STRs).[4]

Although PCR-based STR typing has been the method of choice for paternity testing,[5,6] STRs sporadically are affected by gametic mutations that influence the test interpretation. Therefore, different authors and organizations have estimated mutation rates (μ) for loci commonly used for Human Identification (HID) purposes.[7] Problems related to parentage testing gave rise to different national and international organizations, such as the International Society of Forensic Genetics (ISFG), Scientific Working Group on DNA Analysis Methods (SWGDAM), and the American Association of Blood Banks (AABB), among others. These groups have published recommendations and coordinated exercises to compare DNA typing results between participating laboratories.[1,3,6,8–12] However, these reports commonly omit information of some populations, mainly from developing countries. For instance, only two old reports exist regarding the non-paternity frequency in Mexico.[13,14] Consequently, the aim of this study is to analyze paternity testing data of a Mexican laboratory during a period of five years, such as type of paternity cases, non-paternity prevalence, number of exclusions and *a posteriori* information given by some of the HID kits employed in the lab, null alleles, triallelic patterns, and

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mutation rates.

2. Materials and methods

2.1. Information dataset

Information dataset was obtained from paternity cases performed over a period of five years by a Mexican laboratory of genetics (www.dnaprofile.com.mx). During the database creation, personal information of individuals involved in paternity cases was not shared according to legal regulations (Ley federal de protección de datos personales, México). The laboratory participates in two quality control proficiency tests annually organized by the Spanish and Portuguese-Speaking Working Group of the International Society for Forensic Genetics (https://ghep-isfg.org), and by the Sociedad Latinoamericana de Genética Forense (http://slagf.org.ar/a2/).

2.2. Genotyping

DNA extraction was carried out with the DNA IQ™ System (Promega Corp.) from saliva swabs or from dried blood spotted on FTA paper. For PCR amplification, the following commercial HID kits were employed according to the supplier instructions: AmpF*l*STR® Identifiler®, AmpF*l*STR® Yfiler® (Applied Biosystems), PowerPlex® 16,PowerPlex® 21, PowerPlex® Fusion, PowerPlex Y-23 (Promega Corp.), and Investigator Argus X-12 (Qiagen). These kits were gradually incorporated into the lab for paternity testing resolution. Amplified products were analyzed by capillary electrophoresis either with the ABI Prism™ 310 or 3130 Genetic Analyzers (Applied Biosystems). The Genemapper 3.2 software and the corresponding allelic ladders included in the kits were employed for allele calling.

2.3. Criteria for paternity testing conclusions

Exclusion of paternity was concluded when more than two mismatches were detected.[1] However for the cases with 1 or 2 isolated exclusions we applied the following criteria: 1) we discarded genotyping mistakes; 2) for PI computation in presence of isolated mutations, we used the corresponding mutation rate (μ) and power of exclusion (PE) as recommended by the AABB (PI = μ/PE). More than two mutation steps were considered out of the stepwise mutation model (SMM), which is commonly accepted for STR loci.[15] 3) residual PI was computed to assess the probable paternity relationship considering mutations of multiple steps applying the Brenner's formula: (1/2) μ (1/2) $(1/10)^{s-1}$ [16]; an PI < 0.001 was considered sufficient to rule out paternity; [1] 4) we evaluated the null allele presence in alleged father and child (false homozygous) estimating the PI by means of the appropriated formulas.[17,18] In addition, in motherless cases based on 15 STRs displaying low residual PI with isolated exclusions by mutations or null alleles, the paternity test report suggested the inclusion of the mother to reach definitive conclusions. Conversely, when we failed to exclude paternity, the PI and probability of paternity (W) was estimated with the Excel worksheet PATPCR version 2.02 Juan A. Luque.[19] For this purpose, different STR allele frequencies from Mexican Mestizo populations were used according to the geographic origin of the case, and the time the STR databases were available.[20] For mutation rate estimation by locus, we used the whole dataset of cases analyzed during the period 2010–2015.

When DNA samples were available, we tried to confirm the null allele presence by re-amplification with another HID kit to obtain a different amplicon size for the involved STR locus. In complex parentage cases or when exclusions could have been explained both by null allele and mutation, PI was estimated with the program Familias.[21]

2.4. Statistical analyses

Descriptive statistics was applied to the following information captured in a Microsoft Excel spreadsheet: type of cases (trio and motherless, mainly), genetic system (autosomal STRs, Y-STRs, and X-STRs) and commercial HID kits employed, final conclusion (paternity/exclusion), number of loci detecting the exclusion, estimated PI and W values for confirmed paternity cases, prevalence and type of mutations, inference of null alleles, and observed triallelic patterns. In order to evaluate the performance of different HID kits, we compared average number of exclusions and PI values between Identifiler (15 STRs) PowerPlex 21 (20 STRs) and PowerPlex Fusion (22 STRs). PowerPlex 16 data were not considered because of the limited number of cases analyzed with this kit. For comparison purposes, only motherless cases were considered because of their large number that allow getting stronger conclusions. For comparison purposes of the Powerplex Fusion kit, which includes the Y-STR DYS391 that provides extra male-specific information, the cases were divided by the child gender. Similarly, we only included cases with complete DNA profiles, without mutations and/or null alleles. For the PI comparison between kits, U Mann-Whitney test was performed using the SPSS 19.0 for Windows, and it was graphically represented in Box-and-Whisker plots. Finally, the mutation rate was calculated as the number of mutations between the number of meiosis. The 95% confidence intervals (CI) were calculated based on the binomial distribution and obtained via the website: http://statpages.org/confint.html.

3. Results and discussion

3.1. Type of DNA parentage tests

We collected 3005 cases from different states of Mexico during a period of five years (2010–2015) (Supplementary Table S1). Motherless were the most common tests (77.27%), followed by trios including the mother (20.7%) (Table 1). The remaining 2.04% included different reconstruction cases where the alleged parent is missing and additional relatives were used to evaluate the biological kinship. Most of these cases were solved with lineage markers according to the analyzed biological relationship. For instance, Y-STRs were used in cases involving male relatives who shared the same Y chromosome (sibling, grandfather-grandchild, and uncle-nephew). For this purpose, we employed the Y-filer kit and PowerPlex Y-23 system. Recently, X-STRs were incorporated for solving complex kinship cases involving a least one woman where they can be more informative than autosomal STRs,[22] such as paternal half-sisters, paternal grandmother-granddaughter, and uncle-niece (Table 1). Moreover, before the implementation of X-STRs into the lab, some complex kinships such as paternal half-sisters, grandparents-grandchildren, uncle-niece, and aunt-nephew were solved with autosomal STRs using the Familias software to estimate the LR.[19]

This is the first report from Mexico that describes the types of cases analyzed in a paternity testing laboratory. Motherless were the most frequent cases in this Mexican lab; unfortunately, for comparison purposes, we did not find any report specifying these frequencies from paternity testing labs either from Mexico or Latin America. Despite the lower statistical weight offered by motherless tests,[5,23] this option was preferred probably by the following reasons: i) it is cheaper that when mother is included (trio); ii) the father does not want that the mother realize about it; iii) mother's participation is not obligatory because the large majority of the tests are for personal (no legal) purposes. By the way, we are uncertain of the paternity test purpose (legal or personal) because most of the biological samples analyzed in this Mexican lab were collected by associated labs; thus, this topic is not further discussed.