

CASE REPORT**CRIMINALISTICS**

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Detection of Genotype Recycling Fraud in U.S. Immigrants*

ABSTRACT: Relationship testing laboratories provide genetic evidence to support or refute claims of kinship between U.S. citizen petitioners and potential immigrant beneficiaries. One female beneficiary presented a male amelogenin type and alleles at 15 autosomal loci that were identical to an alleged brother's. Laboratory records showed that her alleged father had petitioned to have 15 children emigrate from Ghana. The petitioner's 15 paternity indices exceeded 10^5 , but the children shared only four short tandem repeat (STR) profiles, suggesting fraudulent reuse of genotypes in this alleged pedigree (AP). To determine the extent of this "genotype recycling," I examined the laboratory's 555 APs from Ghana and 532 control APs from Nigeria. Seventeen Ghanaian APs (3.1%) but no Nigerian APs showed genotype recycling. Of 90 tested people in the 17 APs, 56 shared identical STR profiles with others in their AP. Of these 56 people, 10 were petitioners with unexpectedly high parentage indices. Seven of 56 had amelogenin types that disagreed with their declared genders. Database searches for identical multilocus genotypes in allegedly different people would best detect this fraud.

KEYWORDS: forensic science, DNA typing, relationship testing, genotype recycling, immigration fraud, short tandem repeats, F13A01, F13B, FESFPS, LPL, Penta B, Penta C, D10S1248, D2S441, D22S1045, TPOX, TH01, vWA, D16S539, D7S820, D13S317, D5S818, CSFIPO, PentaE, D18S51, D21S11, D3S1358, FGA, D8S1179, PentaD, Amelogenin

Alleged spouses of permanent resident aliens or naturalized U.S. citizens (petitioners) are given high priority among immigrant applicants (beneficiaries) who use family-based immigration visas. Thus, many legal challenges in immigrant families involve marriage fraud. High immigration priority also is given to the parents or children of petitioners, but siblings and other relatives are assigned lower priorities (1). Some people who claim blood relationships may be unrelated to the petitioner but are strongly motivated to impersonate first-degree relatives. For example, among immigrants from nations where polygamy is permitted, younger wives of a male petitioner may claim they are his daughters. Other people who are more distant blood relatives of a petitioner may claim closer relationships. For instance, older and younger siblings who successfully impersonate a parent and child would improve their chances of immigration or accelerate the process (2).

When documentation of an alleged blood relationship is considered insufficient or possibly bogus, accredited relationship testing laboratories in the United States are asked to provide genetic and statistical evidence to support or refute the claim. It is important to note that laboratory findings of parentage exclusion or low probability of relationship can have explanations other than fraud—some nonrelationships are mistaken beliefs. A man who raised a child may not be aware of his nonpaternity until genetic testing is carried

out as part of immigration procedures. Similarly, two unrelated children who were raised from infancy in the same household may falsely believe that they are siblings.

One kind of immigration fraud, identity theft, occurs when one person pretends to be another by using a name other than his own. A second kind of deception involves substitution of the biologic sample of a close blood relative in place of one from a more distant relative or an unrelated person. The substitution causes genetic analysis to produce a falsely increased probability of a claimed relationship and an increased chance of immigration. A genetic relationship testing laboratory cannot observe the sample substitution, but it may detect evidence of it when two or more people demonstrate identical multilocus DNA profiles: "genotype recycling." The purposes of this paper are to report an occurrence of systematic genotype recycling, to describe its attributes, and to offer ways to detect and prevent it.

Index Case

The test battery chosen for relationship tests is the PowerPlex[®] 16 System (Promega Corp., Madison, WI) that uses 15 autosomal loci to calculate a combined relationship index. Amelogenin testing for determining gender is part of this multiplex of short tandem repeats (STRs), and it is used for forensic identifications. Amelogenin test results may not be examined carefully, however, when the multiplex is used for relationship studies. Nevertheless, in one immigration case, the DNA of an adult alleged daughter was observed to have a male amelogenin type (X, Y). Furthermore, follow-up examination of her 15 autosomal STR loci revealed that all the woman's alleles were identical to those of her alleged brother. Both alleged sibling beneficiaries were hoping to emigrate

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from Ghana, and their petitioning alleged father was a naturalized U.S. citizen.

All laboratory records were retrieved that bore the petitioner's name to help determine whether there had been a laboratory error. Surprisingly, seven different records of paternity tests were found that dated between 1999 and 2009. In total, the petitioner had claimed paternity of 15 alleged children. While it is common for petitioners to request immigration of several beneficiaries on different dates, it was most unusual that the petitioner had paid extra fees to have his DNA re-extracted and his genetic STR tests repeated seven times.

The petitioner's single-parent paternity index (PI, likelihood ratio) of each child exceeded 10^5 , but closer examination of the genotypes of the alleged children revealed that they exhibited only four different DNA profiles. In this alleged pedigree (AP), four groups of 2–5 people shared a genotype (Table 1, AP #16).

Methods

To determine the extent of "genotype recycling," a sample of 555 APs from Ghana, the country of the index case, was examined for shared genotypes among petitioners and/or beneficiaries. As controls, 532 APs from nearby Nigeria were examined for genotype recycling too. All APs in the study had been examined by the author's laboratory.

The laboratory's sample processing is designed to prevent sample mislabeling and misidentification, but STR tests of any AP members who shared genotypes were repeated to assure that misidentification or mistyping had not occurred. Stored DNA samples were retested when allegedly different people had identical profiles, but all pairs of initial and repeated results were identical.

Once genotype recycling was ascertained, the implicated APs were examined to determine whether there were simple ways to screen for identical DNA profiles when testing different members

of an AP: first, the parentage indices (PIs) of 10 petitioner–beneficiary (parent–child) pairs from Ghana that used recycled genotypes were compared with the PIs of 10 petitioner–beneficiary pairs that were truly parent and child. Extraordinarily large PIs are expected in genotype recycling because the identical "alleged parent" will possess both of the alleged child's obligate parental alleles at every genotyped locus, whereas there is usually one obligate allele per locus in a biologic parent. Second, the frequency of discrepancies between amelogenin type and claimed gender was determined in all people who used recycled genotypes.

Alleged pedigrees in which there was genotype recycling were called to the attention of the Diplomatic Security Service and the Fraud Prevention Service of the U.S. State Department (3). The United States, in turn, notified Ghanaian law enforcement authorities to carry out an investigation. Initial follow-up included the collection of new specimens under direct observation in one recent AP. This time, buccal swabs were collected instead of blood specimens. The second specimen collection, labeling, packaging, and shipping were directly witnessed by an American officer with security clearance to assure that the chain of custody of samples remained intact. All collection, processing, and testing procedures met AABB standards (4).

Only one AP with genotype recycling was needed to enable law enforcement to apprehend the phlebotomist(s) engaged in actively substituting the blood samples, and labor, processing, and test expenses were too great for the re-evaluation of more than one AP. Therefore, a case was chosen that involved four people and two highly suspected recycled genotypes (Table 1, AP #2). In this AP, one genotype was shared by the petitioner mother and a beneficiary alleged son with an apparent female amelogenin type. The other recycled genotype was shared by two beneficiaries, an alleged son, and an alleged daughter with an apparent male amelogenin type.

The buccal samples were tested, and their STR typing results were compared with the STR results of the original blood samples. Nine additional loci were added to the original 15 when retesting the petitioner and the two alleged children who had shown recycled genotypes. These STR loci were F13A01, F13B, FESFPS, LPL (GenePrint[®]), Penta B, Penta C (PentaBEC[®]); Promega with allelic ladders manufactured in-house from samples provided with consent), D10S1248, D2S441, and D22S1045 (home brew MiniSTR multiplex, allelic ladders manufactured in-house, custom primers by Applied Biosystems Inc. [ABI], Foster City, CA) (5). Commercial internal lane standards were used in all tests (Promega's Internal Lane Standard 600 for PowerPlex 16, PentaBEC, and GenePrint systems and ABI GeneScan-500 LIZ for the MiniSTR system). PCR products were detected by capillary electrophoresis using an ABI Prism 3130-Avant[®] and data collection software versions v3.0 or 3100-Avant[®] using data collection v1.0. Analyses were performed using ABI GeneMapper[®] ID v3.2 software. All laboratory procedures have been validated, met with accrediting body requirements (ASCLD/LAB and AABB), and complied with proficiency test requirements.

Results

Among 555 APs from Ghana, there were 17 in which two or more putative relatives presented identical 15-locus DNA types (3.1%). In these 17 APs, there were 56 people who shared genotypes with one or more other people. The genotype recycling in the 17 APs is summarized in Table 1. There were no APs from Nigeria that showed genotype recycling.

Two patterns of recycled genotypes were evident. In one, a beneficiary's blood sample was substituted and retested as the blood of

TABLE 1—Seventeen alleged pedigrees in which genotype recycling was evident.

Alleged Pedigree (AP)	No. of People in the AP	No. of People/AP Sharing 1 Genotype	Alleged Relatives Who Shared 1 Genotype	Petitioner & Beneficiary
1	3	2	Father–Daughter	Yes
2	4	2	Mother–Son	Yes
			Son–Daughter	No
3	3	2	Father–Son	Yes
4	5	2	Daughter–Daughter	No
5	3	2	Son–Daughter	No
6	3	2	Mother–Daughter	Yes
7	3	2	Mother–Daughter	Yes
8	3	2	Father–Son	Yes
9	5	2	Father–Son	Yes
10	5	2	Son–Son	No
11	8	2	Father–Daughter	Yes
		2	Father–Son	Yes
12	6	2	Son–Son	No
13	4	2	Daughter–Daughter	No
14	4	2	Son–Daughter	No
15	8	3	Son–Daughter–Son	No
		4	Daughter–Son–Daughter–Son	No
16	17	4	Son–Son–Son–Daughter	No
		4	Son–Son–Son–Son	No
		5	Daughter–Daughter–Son–Son–Son	No
		2	Father–Son	Yes
17	5	2	Father–Son	Yes

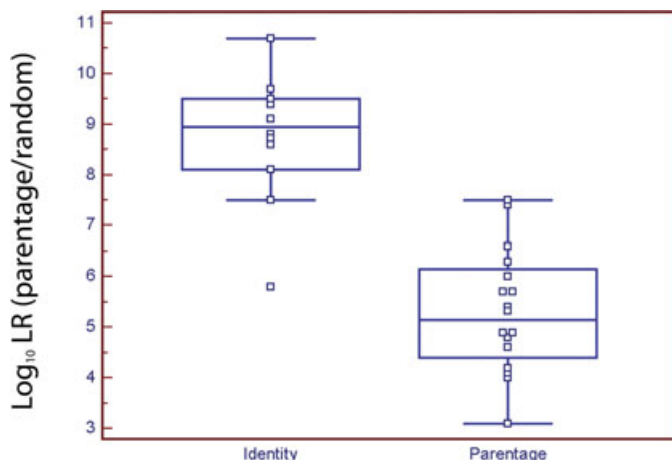


FIG. 1—Combined 15-locus, one-parent parentage indices of 10 cases in which the same genotype was used for both petitioner and beneficiary (identity) and 10 unexcluded parent-child cases (parentage). Indices are generally higher with genotype recycling (Identity), but there is overlap.

one or more other beneficiaries. This pattern was seen 12 times in nine APs. Two to five beneficiaries in an AP shared a single genotype. Usually, the identical samples were tested on different dates so that direct comparison of the indistinguishable genotypes was not possible by an observer of laboratory results. A subset of this pattern included seven examples of a beneficiary of one gender whose sample was used by one or more beneficiaries of the opposite sex. In the second pattern, a petitioner’s sample was tested as a beneficiary’s (i.e., the petitioner or his/her blood had to be transported from the United States to Ghana to be substituted for a beneficiary’s sample). Identical profiles appeared side by side on these original laboratory reports. Additionally, the parentage indices were unusually high (Fig. 1) in cases where an alleged parent petitioner and alleged child beneficiary shared one genotype. In retrospect, the second pattern had occurred 11 times in 10 APs. (Despite the clues, laboratory workers had initially missed the evidence of genotype recycling.)

The initial STR results (from blood) and repeated results (from buccal swabs) of the petitioner and three beneficiaries of the AP designated by the United States for fraud investigation (AP #2 in Table 1) indicated that one male beneficiary’s sample appeared to have been his own, but two blood substitutions were evident because retests showed gender-appropriate amelogenin types that had been inappropriate after the initial tests. The new results proved that the alleged mother’s (petitioner’s) own sample had been substituted for the sample of an alleged son and another alleged son’s blood had been reused for the sample of the petitioner’s alleged daughter.

In addition to the reversal of amelogenin types, retests of the two beneficiaries suspected of using recycled genotypes had multilocus DNA profiles that differed from the original ones at 4/15

loci in the alleged daughter and at 11/15 loci in the alleged son. However, there was insufficient evidence to exclude the petitioner’s maternity of either alleged child because there were no genetic inconsistencies between the alleged mother and the alleged daughter and only two inconsistencies (possible mutations) between the petitioner and her alleged son.

Curiously, the recollected samples of both suspect children showed a greater proportion of the 15-locus genotypes that were identical to the petitioner’s than the proportion seen in true parents and children: the alleged daughter had alleles that were identical to her alleged mother at 7 of 15 autosomal loci (46.7%) and the alleged son had alleles identical to his alleged mother at 11 of 15 loci (73.3%). Even after examination of nine more STR loci, the alleged son shared both alleles per locus with the petitioner at 14 of 24 loci (58.3%) and the alleged daughter shared both alleles with her alleged brother at 11 of 24 loci (45.8%). These results suggested a diagnosis of full sibship between the petitioner and both beneficiaries because samples from Ghana of true parent-child pairs ($n = 34$) and true full sib pairs ($n = 30$) show distinctly different frequencies of loci with identical genotypes. The mean number of genotypically identical loci per 15 tested was 5.3 (35.3%) in full sibling pairs, and the range was 1–9 per 15, twice that in parent-child pairs (mean 2.6/15 or 17.3%, range 0–6 per 15). Under the hypothesis that the petitioner was a full sibling of each beneficiary, the sibling indices of the suspect alleged son and daughter were both $>10^9:1$.

Discussion

When a relationship analyst finds identical 15-locus genotypes in two purportedly different people, the best explanation is a duplication of results following inadvertent or intentional testing of two samples from the same biologic source. Except for monozygotic twins, two people will not present identical genotypes at 15 independent STR loci. Whereas close relatives are more likely to possess the same alleles at one locus than more distant relatives or unrelated people, the probability that they will have identical genotypes at 15 tested loci is the minuscule product of the probabilities of the individual loci. On the other hand, finding a high proportion of identical one-locus genotypes after repeated typing was evidence that the petitioner and her two alleged children were related as full siblings (6). In outbred families, full siblings are the only relatives who can inherit two alleles per locus that are identical by descent, and the probability that sibs will share an identical genotype at each locus is greater than 25%. (See Table 2 and reference 6.) A high proportion of loci with identical genotypes could occur in two people as a result of consanguineous mating or population substructure, but high proportions of homozygous loci would be expected, too (7).

In retrospect, laboratory searches for systematic genotype recycling might have been carried out sooner than they were. The U.S. Customs and Immigration Service (USCIS) had already suspected fraud involving blood relatives pretending to be parents and

TABLE 2—Probability that one person will possess the identical one-locus genotype of the other*.

	Parent and Child	Two Full Siblings	Two Half Siblings	Two Unrelated People
Genotype				
Heterozygous (P/Q) [†]	0.5(p + q)	0.25 + 0.25p + 0.25q + 0.5pq	0.25p + 0.25q + pq	2pq
Homozygous (P/P) [†]	p	0.25 + 0.5p + 0.25p ²	0.5p + 0.5p ²	p ²

*The probability of an identical 15-STR profile is the product of individual STR probabilities.

[†]The respective frequencies of codominant alleles P and Q are p and q, respectively. The electrophoretic phenotype “P” is assumed to be homozygous genotype P/P and not P/null.

children, and accordingly, the posterior probability (prior = 50%) required as evidence of parentage was increased by USCIS (Watson R. "Change in State Department standards for verifying parentage." March 15, 2001, personal communication) from 99% (PI = 100) to 99.5% (PI = 200). Recently, some investigators have suggested routinely testing alleged parent-child pairs for other blood relationships, including full sibs (8), but in the United States, these alternate hypotheses are only considered upon specific request of an embassy. Furthermore, laboratory workers might have observed the occasional discrepancies between amelogenin types and genders declared by people and documented on laboratory requisitions. Failure to notice them occurred because amelogenin types were not considered relevant in kinship analyses and the busy laboratory staff was not looking for evidence of a problem that had not been encountered previously.

The underlying cause of systematic genotype recycling is fraudulent specimen handling at overseas collection sites. Accredited laboratories control the specimen collections of petitioners at U.S. sites, but are unable to assure the identification of specimens collected abroad. An impartial third party can ascertain that samples are correctly collected, labeled, and mailed, however. After suspected fraud was reported by the author's laboratory to USCIS, that agency changed its operating procedures, not only in the suspected center, but in all overseas collection centers. Phlebotomists were changed too at the Ghana site where systematic genotype recycling had been detected. Presently, specimen collection quality is controlled by an officer of the embassy who directly observes and supervises buccal sampling, specimen labeling, and mailing so that the entire chain of custody is assured.

Despite the collection oversight, genotype recycling will probably recur as a sporadic rather than a systematic problem. Relationship testing laboratories should be able to institute several policies and procedures that might detect it: first, after specimens have been collected, file searches for recurring family names could detect APs that should be carefully reviewed for the presence of identical genotypes in alleged relatives. However, name searches are nonspecific and insensitive. Names may recur because of legitimate re-examinations as new beneficiaries are tested and both given names and surnames are common in particular ethnic populations. Use of aliases or variant spellings also may cause a failure to detect recurrent names, and visual examinations are slow and subject to failure. Second, a laboratory might look for (i) discrepancies of gender between amelogenin tests and the sex declared on laboratory requisitions, (ii) very high parentage indices, and (iii) reports containing visibly identical multilocus profiles. However, many recycled genotypes involve members of the same sex, parentage indices overlap with those found in true parentage (Fig. 1), and it is improbable that identical results in two people would be found when they appear on different reports on different dates. Third, genotype recycling could be detected by a laboratory information

system that compares the DNA profile of each individual tested with all the profiles stored in the laboratory's immigration database. Because a petitioner could intentionally use more than one laboratory or collection center, an interlaboratory information system would be optimal.

No laboratory system will be able to detect all fraud that involves tampering with sample identification—an impostor beneficiary might obtain a specimen from a petitioner's untested blood relative who has not emigrated and the impostor could still enter the United States successfully.

However, the combination of direct oversight of specimen collections overseas and laboratory vigilance stateside should be sufficient to prevent genotype recycling fraud.

Conflict of interest: The authors have no relevant conflicts of interest to declare.

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