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CASE REPORT

Didier Hatsch,[1,2] Ph.D.; Christine Keyser,[1] Ph.D.; Rémi Hienne,[2] Ph.D.; and Ludes Bertrand,[1] M.D., Ph.D.

Resolving Paternity Relationships Using X-Chromosome STRs and Bayesian Networks

ABSTRACT: X-chromosomal short tandem repeats (X-STRs) are very useful in complex paternity cases because they are inherited by male and female offspring in different ways. They complement autosomal STRs (as-STRs) allowing higher paternity probabilities to be attained. These probabilities are expressed in a likelihood ratio (LR). The formulae needed to calculate LR depend on the genotype combinations of suspected pedigrees. LR can also be obtained by the use of Bayesian networks (BNs). These are graphical representations of real situations that can be used to easily calculate complex probabilities. In the present work, two BNs are presented, which are designed to derive LRs for half-sisters/half-sisters and mother/daughter/paternal grandmother relationships. These networks were validated against known formulae and show themselves to be useful in other suspect pedigree situations than those for which they were developed. The BNs were applied in two paternity cases. The application of the mother/daughter/paternal grandmother BN highlighted the complementary value of X-STRs to as-STRs. The same case evaluated without the mother underlined that missing information tends to be conservative if the alleged father is the biological father and otherwise nonconservative. The half-sisters case shows a limitation of statistical interpretations in regard to high allelic frequencies.

KEYWORDS: forensic science, Bayesian network, X-STR, paternity test, likelihood ratio

DNA parentage testing and forensic identification rely mainly on autosomal short tandem repeat (STR) genotyping. In some complicated paternity relationships, autosomal STRs (as-STRs) do not produce high paternity probabilities, but no evidence of exclusion is found. X-chromosomal STRs (X-STRs) can be used to advantage in this situation. As X-STRs are inherited differently by male and female offspring, they may be more informative about a suspected relationship than as-STRs. X-STRs have already been used in some paternity cases allowing likelihood ratios (LRs) to be increased (1,2). However, the use of such markers is dependent on the use of mathematical formulae for calculating probabilities under two alternative hypotheses. As a rule of thumb, the more complex the alleged paternity relationship is, the more challenging the calculation will be. Scientists might benefit from graphical representation to help them estimate complex probabilities more easily. A Bayesian network (BN) is a graphical tool that represents independent and probabilistic variables (3). A BN is a graphic system of nodes and directional arrows that is constructed by the analyst so as to model a set of random variable and contains the possible states of that variable as well as a conditional probability table. The conditional probabilities of a node depend on the states of its "parent" nodes (the ones from which directional arrows arise). When applied to kinship studies using STRs, a specific BN can be modeled upon a specific pedigree. The BN describes alleged blood relationships, specific rules of inheritance of alleles (genes). Rules can include various inheritance schemes such as autosomal, patrilineal (Y-chromosomal), X chromosomal and matrilineal (mitochondrial). Exceptions to the rules (mutations, null alleles, dropout, etc.) can be included, as well. Allelic types/locus are entered as the BN's data for the case. The BN considers alternative hypotheses (e.g., paternity by the alleged father vs. paternity by a random man) and a LR is computed for all the data. The system provides simplicity, speed and commercial availability. BNs have been used successfully in several paternity cases and case specific BN models have proven to be modular and flexible (4).

In this paper, two BNs have been adapted to a daughter–grandmother relationship and a half-sister/half-sister relationship. The use of these two models in two actual cases highlights their utility but also points out some limits in statistical interpretation.

Materials and Methods

DNA Extraction and STR-typing

DNA was extracted from blood (QIAamp® Blood kit, Qiagen, Courtabeuf, France) or buccal swabs (Cleanmix® kit, Talent, Triest, Italy) according to the manufacturers' recommendations.

Autosomal STRs were studied using the Powerplex®16 mutliplex kit (Promega, Charbonnieres, France) or the AmpF/STR® SGM plus mutliplex kit (Applied Biosystems, Foster City, CA). X-STRs were investigated by means of the Mentype® Argus X-UL mutliplex kit (DXS8378, DXS7132, HPRTB, and DXS7423). Polymerase chain reactions were performed following the manufacturer's protocol.

PCR products were analyzed on an ABI Prism 3100 (Applied Biosystems) automated DNA sequencer. Fragment sizes were determined automatically using GeneMapper software, and by comparison with allelic ladders provided in each kit.

Bayesian Network

The BNs were constructed following guidelines (5). They were implemented in the computer program Hugin Lite™ v5.7 (http://

[1]Institut de Médecine Légale, EA 3428, 11 rue Humann, 67085 Strasbourg Cedex, France.
[2]Laboratoire CODGENE, 11 rue Humann, 67085 Strasbourg Cedex, France.

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www.hugin.dk). Frequency tables from the Mentype® Argus X-UL protocol were used in the BNs.

Results and Discussion

Bayesian Network Construction

Construction of BNs in paternity testing follows some simple rules. The basic building block is composed of three nodes that model a person: the genotype (gt) node is dependent on a maternal gene (mg) node and a paternal gene (pg) node (Fig. 1). The term "gene" refers to an STR allele. Establishing a paternity relationship between a child and his father depends on the association between the child's gene nodes and the four gene nodes of his parents. If one parent is not known, the gene node of that person will contain a table of allele frequencies. Constructing BNs for X-STR interpretation differs from the classical construction scheme. As a male offspring's genotype nodes do not include pg, the gt node consists only of a mg which is itself dependent on the mg and pg of his mother. Thus, to simplify the construction, the male gt is directly dependent on the mg and pg of his mother. Under this model, two BNs were built. The first one, termed BN-A, is designed for the interpretation of paternity cases when the father is not available for typing but the paternal grandmother is (Fig. 2). The second one models a two half-sisters relationship linked by a common father (BN-B, Fig. 3). In either BN, the node hypothesis, "the alleged father is the biologic father," could have two states: Yes / No, each state has a 50% chance *a priori*.

BN-A was developed for paternity determination conditional on the following trio: the daughter, the mother, and the paternal grandmother. This network, however, is applicable to more than just the case for which it was initially developed. Indeed, grandmother–daughter kinship probability can also be computed when only daughter and grandmother genotypes are introduced into the network. Moreover, it can be used in classical trio/duo cases, by setting the grandmother's genotype to a homozygote, thus simulating an alleged father genotype.

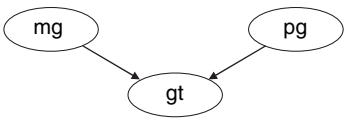

FIG. 1—*Basic building block for a Bayesian Network. gt:genotype, mg: maternal gene and pg: paternal gene.*

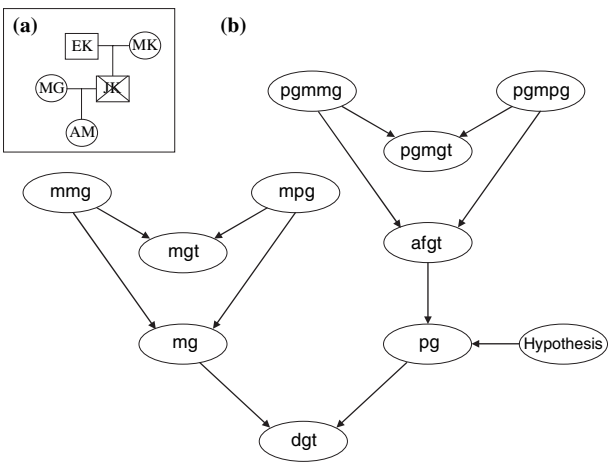

FIG. 2—*(a) Pedigree diagram corresponding to case 1. (b) X-Chromosomal Bayesian network derived from the pedigree diagram (BN-A). Node name convention: the names are formed by a combination between a prefix and a suffix. The prefix corresponds to individuals (d: daughter; m: mother; af: alleged father; pgm: putative grandmother) and the suffix corresponds to either an allele (mg: maternal gene; pg: paternal gene) or a genotype (gt: genotype).*

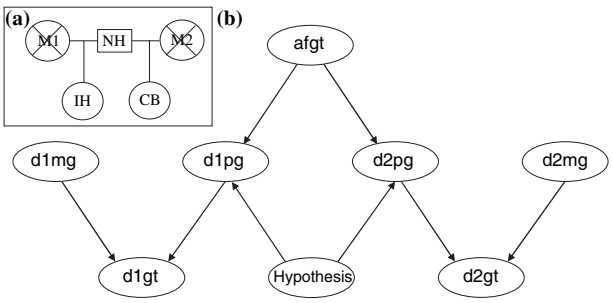

FIG. 3—*(a) Pedigree diagram corresponding to case 2. (b) X-Chromosomal Bayesian network derived from the pedigree diagram (BN-B). Node name convention: the names are formed by a combination between a prefix and a suffix. The prefix corresponds to individuals (d: daughter; m: mother; af: alleged father; pgm: putative grandmother) and the suffix corresponds to either an allele (mg: maternal gene; pg: paternal gene) or a genotype (gt: genotype).*

The two networks were validated by comparing the resulting LR for the different genotype combinations with published formulae (1,6).

Case 1

AM claims to be the daughter of MG and the deceased man JK. However, the parents of JK, EK and MK, contest the alleged paternity (Fig. 2*a*). Blood samples were collected from the four different persons and as-STRs were typed. No exclusions were found and a LR (PI) of 99 was calculated over the 10 loci of the AmpF*l*STR® SGM plus kit. Such a LR results in a paternity probability of 99.00%, which is too low to draw a conclusion for legal purpose in France. Therefore, X-STRs were genotyped for the mother, the daughter, and the alleged paternal grandmother. Once again, no genetic inconsistencies with paternity were detected. The combined LR of four X-STRs loci were estimated to be 45 using the BN (Fig. 2*b*). As a result, the cumulative LR over all available loci rose to 4455, corresponding to a paternity probability of 99.98%. This probability is sufficiently high to support the legal claim of JK's paternity. This case highlights the complementary role that X-STRs can play in some complex paternity situations.

In order to test the same BN for a motherless case, X-STRs LR calculations were repeated without giving any indication of the mother's (MG's) genotype to the network. Three loci showed lowered LRs, but the LR of HPRTB increased from 1.81 to 4.20. This result is explained by the presence of the low-frequency allele: 15 (frequency of 0.076). With knowledge of the mother's genotype, only allele 12 could be transmitted by the paternal grandmother (Table 1). However, in the motherless case, either allele 12 or 15 could be inherited from the paternal grandmother. The increased LR is due to the low frequency of allele 15. As there are misleading situations where either exclusion can be missed or higher LR can be found in motherless cases, caution is warranted in their interpretation (7,8).

Case 2

Two alleged half-sisters (IH, CB) suspected common paternity by NH. After gathering informed consent, samples were taken

from the trio using buccal swabs (Fig. 3*a*). Autosomal STRs were determined to verify paternity. Paternity probabilities of 99.98% and 99.99% were respectively found for IH and CB. X-STRs were typed for this trio and no exclusions were found (Table 2). BN-B was used to investigate the half-sister elationship in a fatherless situation. A LR of 2 was found combining the four X-STRs. This low LR is explained by the high frequencies of the alleles transmitted by the father (Table 2). Indeed, except for DXS7423, the alleles found in the three other loci are the most frequent ones.

Although X-STRs added little information in resolving this relationship, it is evident that, when common alleles are transmitted by an alleged father, additional locus markers may be required for case resolution.

Another bias could be operating in this case: the allelic frequencies given by the manufacturer may be in error because no data are available for the French population. Use of population data from Germany could conceivably affect the LR calculation.

Conclusions

Bayesian networks for two complex paternity cases were constructed using X-STRs. They were validated against known formulae. Such graphical/statistical tools are both useful and flexible. Indeed, one BN, which was designed for trio daughter/mother/paternal grandmother, also could be used for trio daughter/mother/alleged father. In addition, the network is applicable to motherless cases.

If X-STRs proved themselves to be useful complements to as-STRs, however, there are cases where high or low allele frequencies reduce their value. For example, in motherless cases, exclusions may be missed or falsely higher LR could appear due to rare alleles.

cTo overcome these limitations, more X-STRs could be studied. X chromosome haplotyping also might be of value (1). Thus, new BNs could be developed to integrate linked and unlinked X-STRs.

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Additional information and reprint requests:
Didier Hatsch, Ph.D.
Institut de Médecine Légale
EA 3428, Université Louis Pasteur
11 rue Humann
67085 Strasbourg Cedex
France
E-mail: didier@hatsch.net