Evaluating the effect of additional forensic loci on likelihood ratio values for complex kinship analysis

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Abstract

A study was conducted to evaluate the effect of adding autosomal STRs to the 13 U.S. core loci for complex kinship analysis. Additional genetic information may increase likelihood ratio values for true relationships in a pedigree, while reducing the chance of identifying false relationships. The clear discrimination of true versus false relationships is important for complex kinship cases, such as familial searches, paternity testing, missing persons work, and immigration testing. Allele frequency data was available for 46 forensic autosomal STR loci from U.S. Caucasian, Hispanic, and African American population samples. These loci were from commercial amplification kits (Identifiler®, PowerPlex® 16 System, and PowerPlex® ESI/ESX 17 Systems) and an in-house assay (NIST 26plex). With the large number of loci, various sets of loci were selected to simulate genotype pairs for related and unrelated individuals. The relationships evaluated were parent-offspring, full siblings, and half siblings. The expected likelihood ratio distributions were compared across sets of 13, 20, and 40 STR loci to evaluate the discrimination power gained by adding markers to the core U.S. and European forensic loci. Increasing the number of STR loci resulted in increased discrimination of true and unrelated pairs, although the results were more dramatic for parent-offspring and full siblings than for half siblings. With a likelihood ratio threshold of one, the use of 40 STR loci produced robust discrimination of parent-offspring (no false inclusions or exclusions) and full sibling pairs (less than 1% false inclusions and exclusions). However, half siblings demonstrated overlapping likelihood ratio distributions with 40 loci, resulting in false inclusion and exclusion rates of 5-7%. Suggestions are provided to further improve the discrimination power of autosomal STR loci for kinship determination.

Introduction

Since their selection in November 1997, genetic information from a core set of 13 short tandem repeat (STR) loci have been required for upload of DNA profiles to the national DNA database (Butler 2006). The 13 U.S. core loci used by the National DNA Index System are: CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, and D21S11. These loci are commonly referred to as the 13 Combined DNA Index System (CODIS) loci. Forensic laboratories analyze the CODIS loci to identify the perpetrator of a

crime by directly matching an evidence profile and a suspect profile or by searching an evidence profile against an offender database. If a match is found between two DNA profiles, then the rarity of the genotype is determined by calculating the random match probability (RMP) (Butler 2009). To determine how much more likely it is that the evidence sample originated from the suspect than from a random individual, a likelihood ratio (LR) is used. In the forensic case, a LR is a comparison of the probabilities of the evidence under two alternative hypotheses. The numerator (prosecution's) hypothesis is that the DNA from the crime scene came from the suspect, while the denominator (defense's) hypothesis is that the DNA originated from an unrelated, random individual in the population. Thus, the LR is calculated as 1/RMP (Butler 2009).

In addition to individual identification for forensic purposes, the U.S. core loci are routinely used to evaluate the relatedness between individuals. Instead of identifying a perfect match between two DNA profiles, kinship analysis assesses any shared genetic information between putative relatives under alternative hypotheses or by estimating an unknown relationship (Weir et al. 2006). Applications for kinship analysis include civil paternity disputes, criminal paternity cases, immigration applications, missing persons cases, disaster victim identifications, and familial searches of offender databases.

In cases of alleged paternity, a putative father-child relationship can be tested using information obtained from DNA typing and statistical analysis. Typically, DNA samples from the mother, child, and alleged father(s) are tested with 15-20 forensic STR markers. If the alleged father is indeed the biological father, then at each tested marker the child will share one allele with the mother and one allele with the father (barring mutations). These shared alleles are identical by descent (IBD) since the allele originated from a common ancestor in the father-child and mother-child pairs. For pairs of relatives, the probabilities of sharing alleles IBD are known (Weir et al. 2006, Table 1). Using the genetic data, population allele frequencies, hypothesized relationships, and associated IBD probabilities, an LR calculation will evaluate how strongly the genetic data support the purported relationship (Evett and Weir 1998, Buckleton et al. 2005, Weir et al. 2006, Gjertson et al. 2007). In the case of a paternity test, a LR is commonly referred to as a paternity index (AABB 2009).

Figure 1 illustrates how the forensic core competency is being expanded from direct matching with 13 STR loci to applications of complex kinship analysis and familial searching for which indirect matches are made and uncertainty increases. As opposed to forensic identifications, no standard set of loci exists for kinship testing applications. However, for many kinship analyses, commercial forensic PCR kits are used to type 15 STR loci—13 CODIS loci plus D2S1338/D19S433 or Penta D/Penta E, depending on the manufacturer. In most paternity trio cases, 15 forensic loci are sufficient to provide positive proof of paternity (Poetsch et al. 2006). However, in cases where a relationship cannot be confirmed or refuted with statistical certainty with 13-15 STR loci (e.g., deficient paternity, pairs of relatives, or possible mutation events), additional STR loci or alternative DNA markers may be required (Wenk et al. 2003, Poetsch et al. 2006, Betz et al. 2007, AABB 2009). With familial searches and disaster victim identifications, a one-to-many search in a database of 13-15 STR profiles will produce a large number of fortuitous matches for close biological relatives, such as parent-offspring and full siblings (Brenner and Weir 2003, Bieber et al. 2006, Reid et al. 2008, Curran and Buckleton 2008). Furthermore, the number of false positive matches increases when the alleged genetic relationship becomes more distant due to the reduced amount of IBD allele sharing. However, tests of more distant relationships, such as for half siblings or uncle-nephew, may be of interest for inheritance disputes and immigration testing, for example. Additionally, the use of additional loci may decrease the number of false positive relationships and provide more support for true relationships than the 13-15 forensic STR loci currently used for familial searches in the U.S.

In April 2005, the European Network of Forensic Science Institutes selected five STR loci (D12S391, D1S1656, D2S441, D10S1248, and D22S1045) to add to their existing European Standard Set of seven STRs (TH01, vWA, FGA, D8S1179, D18S51, D21S11, and D3S1358) (Gill et al. 2006a, 2006b). Germany and several other European countries test the highly polymorphic locus SE33 (Hill et al. in press). To provide maximum overlap of core markers among the European community, STR kit manufacturers have produced multiplex PCR kits that include the 12 ESS loci, D16S539, D2S1338, D19S433, and SE33. In addition to the expanded set of European loci, 26 STR loci have been characterized by NIST for use in forensic typing, and an NIST in-house multiplex PCR assay has been developed with 25 of these loci (Hill et al. 2008, Hill et al. 2009). Combining the loci from the expanded ESS, the commercial U.S. and European kits, and the NIST-developed assay, 46 unique STR loci are available to the forensic and kinship testing communities. For the major U.S. populations, allele frequency data have been collected for these 46 loci (Butler et al. 2003, Hill et al. 2008, Hill et al in press, unpublished data). For challenging kinship

cases, the large set of available genetic data can be used to determine whether additional loci, beyond the 13-15 STR loci currently tested, can improve the discrimination of true relatives from unrelated individuals by reducing false inclusions and exclusions.

Ideally for use in forensic and kinship analyses, genetic markers on the same chromosome should be more than 50 centimorgans (cM) apart in genetic distance (approximately 50 Mb in physical distance). This distance ensures full recombination (recombination frequency = 0.50) and thus independent inheritance of alleles at multiple markers. The new ESS locus D12S391 occurs on the short arm of chromosome 12 and is only 6.3 megabases (Mb) from the established vWA STR locus that is widely used (Phillips et al. in press). Recent studies have detected significant linkage disequilibrium (O'Connor et al. in press) and linkage (recombination frequency estimate of 0.108, Budowle et al. in press) between the D12S391 and vWA loci. Consequently, the single-locus genotype probabilities for D12S391 and vWA should not be multiplied to determine the match probability of an autosomal STR profile when unrelated or related individuals are involved. More research is needed to determine the effect of linkage disequilibrium and linkage on match probability calculations. Until then it is convenient to simply exclude D12S391 from consideration as an additional marker to improve complex kinship analysis since vWA has been a core U.S. and European locus for years.

Materials and Methods

Allele frequencies for 46 STR loci were available from approximately 600 NIST U.S. population samples including Caucasians, Hispanics, and African Americans (Butler et al. 2003, Hill et al. 2008, Hill et al. in press, unpublished data). The NIST allele frequency data are available at http://www.cstl.nist.gov/biotech/strbase/NISTpop.htm. For the current study, the following marker systems were used:

- AmpFlSTR® Identifiler® (Applied Biosystems, Foster City, California): CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, D2S1338, and D19S433 (Butler et al. 2003)
- PowerPlex® 16 System (Promega Corporation, Madison, Wisconsin): CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, Penta D, and Penta E (unpublished data)
- PowerPlex® ESI 17 System (Promega): D1S1656, D2S441, D2S1338, D3S1358, D8S1179, D10S1248, D12S391, D16S539, D18S51, D19S433, D21S11, D22S1045, FGA, TH01, vWA, SE33 (Hill et al. in press)
- NIST 26 miniSTR loci: D1GATA113, D1S1627, D1S1677, D2S441, D2S1776, D3S3053, D3S4529, D4S2364, D4S2408, D5S2500, D6S474, D6S1017, D8S1115, D9S1122, D9S2157, D10S1248, D10S1435, D11S4463, D12ATA63, D14S1434, D17S974, D17S1301, D18S853, D20S482, D20S1082, D22S1045 (Hill et al. 2008)

Allele frequencies of the three major U.S. population groups were kindly imported into DNA-VIEW™ v. 29.23 (Charles Brenner, Oakland, California) by Dr. Charles Brenner. In the Kinship Simulation module of DNA-VIEW™, pairs of genotypes were simulated using population-specific allele frequencies for sets of 13, 20, and 40 STR loci (Table 2). Genotype pairs were simulated as true parent-offspring, true full siblings, true half siblings, and unrelated individuals. For each simulation, a LR was calculated that compared the hypotheses of a specific relationship versus no relationship. The true relative genotype pairs were evaluated with a numerator hypothesis corresponding to their actual relationship (e.g., for a simulated parent-offspring pair, the LR evaluated the probabilities of the genotypes given a parent-offspring relationship versus no relationship). The unrelated genotype pairs were evaluated in separate simulation modules using the hypotheses of parent-offspring, full siblings, or half siblings versus no relationship. Although no mutations were created during genotype simulations, LR calculations accounted for possible mutation events as per AABB recommendations (AABB 2009). For each of the three relationship scenarios, 1000 independent genotype simulations and LR calculations were performed. However, due to the large range of LR values for full sibling comparisons and the resulting ragged LR distributions, 5000 simulations were necessary to increase the density of data points and to produce smoother curves, which allowed for a more direct visual comparison between test schemes (data not shown). For each set of simulations, log LR values were graphed to produce expected LR distributions in Excel®.

Results

To assess the discrimination power gained by increasing the number of STR loci for kinship analysis, pairs of genotypes with defined relationships (parent-offspring, full siblings, half siblings, and unrelated persons) were simulated using NIST U.S. Caucasian, Hispanic, and African American allele frequencies for 13, 20, and 40 STR loci (Table 2). For each simulation, LR values were calculated to compare the probabilities of the genotype pair under the hypotheses of a specific relationship versus no relationship. The distributions of LR values for each type of true relative pair and marker set were graphed separately for Caucasians, Hispanics, and African Americans (Figures 2-4, respectively). As the number of loci increased, the LR distributions shifted to the right for true relatives, indicating larger LR values that give more strength to the hypothesized relationship. The increase in LR values was greater for true parent-offspring and full sibling relationships than for half sibling relationships. All LR values were greater than one for true parent-offspring relatives for 13, 20, and 40 STR loci, while the LR distributions shifted below one for true full and half siblings, indicating a region of LR values that supported the hypothesis of no relationship. Additionally, the breadth of the true relative distributions increased with the number of loci typed, demonstrating a larger range of possible LR values when additional loci were typed.

By the definition of a LR, any value greater than one supports the numerator hypothesis of a specific relationship. Conversely, any value less than one supports the denominator hypothesis of no relationship. By defining a specific LR threshold, the proportion of expected false inclusions (unrelated persons that appear related) and expected false exclusions (true relative pairs that appear unrelated) can be estimated from the simulation data. Additionally, the distributions of LR values can be graphed for unrelated persons and true relatives (Figure 5). Graphing the distributions allows for a convenient evaluation of whether increasing the number of STR loci, beyond the core 13 STRs used for forensic typing in the U.S., can produce robust discrimination of true relative pairs and unrelated persons. If the unrelated and related distributions overlap, then a range of LR values exists where false positive or false negatives can occur for a given set of loci, allele frequencies, and relationship scenario. For kinship analysis, one hopes to achieve complete separation of the distributions to reduce the chance of falsely identifying individuals as related or unrelated.

Discrimination among U.S. populations

To compare the power of various numbers of loci to discriminate true relatives from unrelated persons, distributions of LR values were graphed for related and unrelated genotype pairs under each relatedness scenario and allele frequency dataset. Figures 6-8 provide the simulation results using 13 STR loci for Caucasians, Hispanics, and African Americans, respectively. Similarly, LR distributions for 20 loci are shown in Figures 9-11, and results for 40 loci are shown in Figures 12-14. For each relationship scenario evaluated with 13, 20, or 40 loci, there was little difference between LR distributions with allele frequencies of the three population groups. In particular, the median LR values and breadth of the distributions were similar for specific relationship scenarios among the three populations. However, median LR values were slightly larger for African American and Hispanic genotypes than for Caucasian genotypes for a specific relationship scenario. For example, for true parent-offspring genotypes with 13 STRs, the median LR values were 10,000 for Caucasians; 15,000 for Hispanics; and 19,000 for African Americans (Figures 6-8, respectively). In the case of evaluating true full siblings with 20 STRs, the median LR values were 680,000 for Caucasians; 800,000 for Hispanics; and 1,750,000 for African Americans (Figures 9-11, respectively). For true half sibling genotypes with 40 STRs, the median LR values were 300 for Caucasians, 310 for Hispanics, and 610 for African Americans (Figures 12-14, respectively). Although differences were observed for median LR values between population groups, the proportion of false inclusions and exclusions remained similar between the groups (Table 3).

Since the LR distributions varied more with the number of loci and type of relationship than between population groups, data analysis efforts were focused on evaluating the differences between expected LRs when 13, 20, and 40 STR loci were used to assess specific relationships. The Caucasian dataset will be used as an example to illustrate the power of increasing the number of STR loci to discriminate true relatives from unrelated individuals. Results from analyses using Hispanic and African American genotypes are provided in the accompanying figures and Table 3.

Discrimination potential of 13 STR loci using Caucasian data

For a given set of loci, LR distributions exhibited increasing overlap between true relative and unrelated pairs when the hypothesized relationship became more distant. Using Caucasian 13-locus genotypes, LR values did not overlap between unrelated and related parent-offspring comparisons (Figure 6). However, the distributions were nearly overlapping, indicating a chance for false inclusions to occur. Note that false exclusions would only occur for a parent-offspring pair if a meiotic mutation was present and not accounted for in the likelihood ratio algorithm. No false positive and false negative parent-offspring relationships were indicated with 13 loci since LR values for unrelated pairs were less than one while values for true relatives were greater than one (Figure 6, Table 3). However, unrelated and related full and half sibling comparisons produced LR values that overlapped in the region defined by 0.01 to 1,000 (Figure 6). For full sibling comparisons, this area of uncertainty produced false positives and false negatives with a frequency of 0.027 and 0.033, respectively (Table 3). More extreme than the full sibling scenario, LR distributions overlapped for unrelated and related half sibling comparisons, producing false positives and false negatives with a frequency of 0.155 and 0.173, respectively (Figure 6, Table 3). The use of 13 locus profiles produced false inclusions and exclusions for pairs of full and half sibling relatives.

Discrimination potential of 20 STR loci using Caucasian data

The performance of 20 STR loci was investigated to determine if additional loci could improve the discrimination of true relatives from unrelated individuals. As with 13 loci, no overlap was observed between LR distributions of unrelated and related parent-offspring comparisons (Figure 9). Consequently, no false positive and false negative parent-offspring relationships were indicated with 20 loci (Table 3). As compared to results for full and half sibling hypotheses with 13-locus genotypes, the areas of overlap diminished with 20 loci, although the ranges of LRs defining the overlapping regions remained the same for full and half sibling comparisons (Figure 9). For full sibling comparisons, the reduced area of uncertainty produced false exclusions and false inclusions with a frequency of 0.006 and 0.008, respectively (Table 3). For half sibling comparisons, the reduced area of uncertainty produced false positive and false negative relatives with a frequency of 0.075 and 0.104, respectively (Table 3). The discrimination of parent-offspring, full siblings, and half siblings was improved with 20 STR loci due to increasing LR values of true relative pairs and decreasing LR values of unrelated individuals.

Discrimination potential of 40 STR loci using Caucasian data

An expanded set of 40 STR loci was used to evaluate the power to identify true relatives from unrelated individuals. As with the smaller numbers of loci, no overlap was observed between LR distributions of unrelated and related parent-offspring pairs (Figure 12). All unrelated comparisons produced LRs equal to zero when evaluated as parent-offspring with 40 loci. No false positive and false negative parent-offspring relationships were indicated with 40 loci (Table 3). When a full sibling relationship was evaluated with 40 loci, the area of overlap between unrelated and related distributions was reduced when compared with results for 13 or 20 loci. Moreover, the region of overlap decreased to LR values between 0.1 and 10 (Figure 12). For the full sibling hypothesis, the reduced area of uncertainty produced false positive and false negative relatives with a frequency of 0.001 and 0.002, respectively (Table 3). For half sibling comparisons, the reduced area of uncertainty was defined by the same LR range as with 13 and 20 loci (Figure 12) and produced false positives and false negatives with a frequency of 0.051 and 0.066, respectively (Table 3). The use of 40 STR loci provided robust discrimination of parent-offspring and full sibling relatives from unrelated individuals. Although 40 loci reduced the frequency of false inclusions and exclusions for half sibling comparisons to ~5-7%, discrimination was not markedly improved with a LR threshold of one when compared to 20 loci.

Discussion

Challenges of kinship analysis with limited numbers of STR loci

This study confirmed previous work that the 13 forensic STR loci are not sufficient to definitively discriminate between pairs of close relatives and unrelated individuals and may lead to false inclusions and exclusions (Wenk et al. 2003, Poetsch et al. 2006, Allen et al. 2007, Pu and Linacre 2008, González-Andrade et al. 2009). Although all true parent-offspring comparisons produced LRs that supported relatedness, a few false positives were observed

when unrelated pairs had LR values that favored a parent-offspring relationship. The chance for false inclusions (albeit small) illustrates the difficulty of determining paternity when deficient (motherless and reverse paternity) cases are attempted with a small set of STR loci (Wenk et al. 2003, Poetsch et al. 2006, González-Andrade et al. 2009). For full and half sibling cases, the chances of false inclusions and exclusions are even greater than for parent-offspring. True siblings and more distant relatives may not share an allele at every locus, potentially resulting in LR values that favor no relationship. Conversely, unrelated individuals can share alleles by chance to generate positive LR values that support relatedness.

In addition to the challenge of identifying close relatives through a pairwise (one-to-one) comparison with 13 loci, the task is more difficult when relatives are compared using a database (one-to-many) search (Figure 1). Databases are used to identify relatives using familial searches (Bieber et al. 2006, Reid et al. 2008, Curran and Buckleton 2008), missing persons cases (Gornik et al. 2002), and mass disaster identifications (Brenner and Weir 2003). When comparing one 13-15 locus profile against many profiles, the chance increases that two unrelated individuals will appear related due to fortuitous allele sharing. This will be especially problematic when testing for relationships in which allele sharing is not required at every locus (e.g., full siblings and more distant relationships).

Increased discrimination power with additional STR loci

To increase the discrimination power of identifying close relatives with 13 forensic loci, additional loci may be tested. This study shows that increasing the number of typed autosomal STR loci to 40 resulted in complete discrimination of true parent-offspring relatives from unrelated individuals. In fact, no unrelated pairs had a LR value greater than zero. Thus, when evaluating 40 markers for a parent-offspring relationship, there were no instances in which pairs of unrelated individuals shared an allele at every locus by chance. In full sibling comparisons, 40 STR loci produced robust discrimination of true relatives from unrelated individuals with false inclusion and exclusion rates of ~0.1%. Compared to typing with 13 or 20 STR loci, the use of 40 loci improved the discrimination of true half siblings and unrelated individuals. However, the false inclusion and exclusion rate of 5-7% reflected the lower power of this set of loci to identify half siblings due to the reduced amount of IBD allele sharing in half siblings. Additional STR loci would be required for further discrimination of half siblings and more distant relatives (Blouin 2003, Wilkening et al. 2006). In cases with one-to-many comparisons, the use of 40 STR loci could reduce fortuitous allele sharing between pairs of unrelated individuals, particularly for parent-offspring and full siblings where IBD allele sharing is higher than for more distant relationships (Weir et al. 2006).

When using additional loci for relatedness testing, one caveat to consider is that there are more opportunities for meiotic mutations to occur in true relatives. The recommendation currently employed—that at least two STR mutations are required to exclude a parent-offspring relationship (AABB 2009)—would likely need to be adjusted to allow for more potential mutations between true relatives. In this study, genotypes were not simulated with mutation events but possible mutations were accounted for in the LR algorithms. Further research is needed to evaluate the occurrence and impact of multiple mutations when genotyping large numbers of STR loci for kinship determination. Alternatively, SNP loci could be added to increase the discrimination power of forensic STR loci (Børsting and Morling 2010) due to the 5-fold lower mutation rate of SNPs compared to STR loci (Butler 2009).

Increased discrimination power with lineage markers

Unlike diploid autosomal markers, which are independently assorted during meiosis, loci on the Y chromosome and on the mitochondrial genome (mtDNA) are transmitted unchanged (barring mutation) from generation to generation. Y-chromosome haplotypes and mitochondrial sequences of hypervariable regions I and II (HVI/II) have proven highly informative for identifying related individuals, even distant relatives (Ginther et al. 1992, Junge et al. 2006, Butler et al. 2007). Y-haplotypes identify all relationships between males who are linked only by males. If a paternal relative exists in a database, a Y-STR match is always made (barring mutation). MtDNA sequences identify all relationships between any persons, male or female, who are linked only by females, including mother-son, mother-daughter, full siblings, maternal half siblings, maternal uncle-nephew, male or female cousins related entirely through mothers, and so on. If a mitochondrial relative exists in a database, an mtDNA sequence match is always made (barring mutation). The power to detect paternal or mitochondrial relatives depends on the population frequency of the shared Y-STR haplotype or mtDNA sequence. Population studies have indicated that 96% of Y-haplotypes defined by 17 STR loci are unique and all Y-haplotypes are individually rare (frequency of less than 1%) (Butler et al. 2007). Similarly, population studies have shown that approximately 70% of individuals have private

mtDNA haplotypes in samples from the three major U.S. populations (Budowle et al. 1999). In sampled Caucasians, one mtDNA HVI/II haplotype has a frequency of 7%; all other haplotypes are much more rare (Parsons and Coble 2001, Allard et al. 2002). All mtDNA haplotypes are individually rare in African and Hispanic samples (Allard et al. 2005, Allard et al. 2006). MtDNA sequences are particularly useful for identifying maternal half siblings or other relationships for which persons do not share a Y chromosome.

Increased discrimination power with additional relatives and non-genetic information

Before attempting a kinship analysis, it is necessary to determine the discrimination power of a particular set of loci. As was performed in this study, simulation experiments are valuable tools to evaluate the expected range of LR values for a defined set of loci, relationships, populations, and statistical algorithms (Blouin 2003). Depending on the simulation results, one could determine if additional loci, family members, or non-genetic data are needed to meet a predefined level of certainty. In some cases it is not always possible to type additional loci to increase the discrimination power due to limited resources or the type of relationship in question (e.g., distant relatives such as second cousins). The use of additional known or putative relatives can increase the power of correctly inferring relatedness (Nothnagel et al. 2010). Examining multiple relatives, such as several full siblings, can identify Mendelian discrepancies that may be missed in a pairwise analysis (Browning and Thompson 1999). Alternatively, non-genetic information may be available to improve confidence in a test result. Metadata, such as age, sex, ethnicity, and legal documentation, can be helpful in familial searching cases and immigration testing (Sheehan and Egeland 2007). When non-genetic information is used, Bayesian statistics are required to incorporate prior probabilities for the non-genetic with likelihood ratios for the genetic data (Shoemaker et al. 1999).

Defining thresholds for discrimination

Based on the definition of a LR, a LR threshold was selected in this study to define a positive or negative relationship as being greater than or less than one, respectively. However, in practice, different LR thresholds may be required to achieve particular specificity and sensitivity thresholds (Gaytmenn et al. 2002). If higher LRs are used, there will be a trade-off between decreasing false inclusions and increasing false exclusions. A balance must be sought and may vary across particular applications (e.g., paternity testing, immigration testing, familial searching, etc.). As opposed to a discrete LR threshold, a grey zone approach can be helpful for defining a likelihood ratio range that does not eliminate uncertainty about the relationship status (Coste and Pouchot 2003, Giroti et al. 2007).

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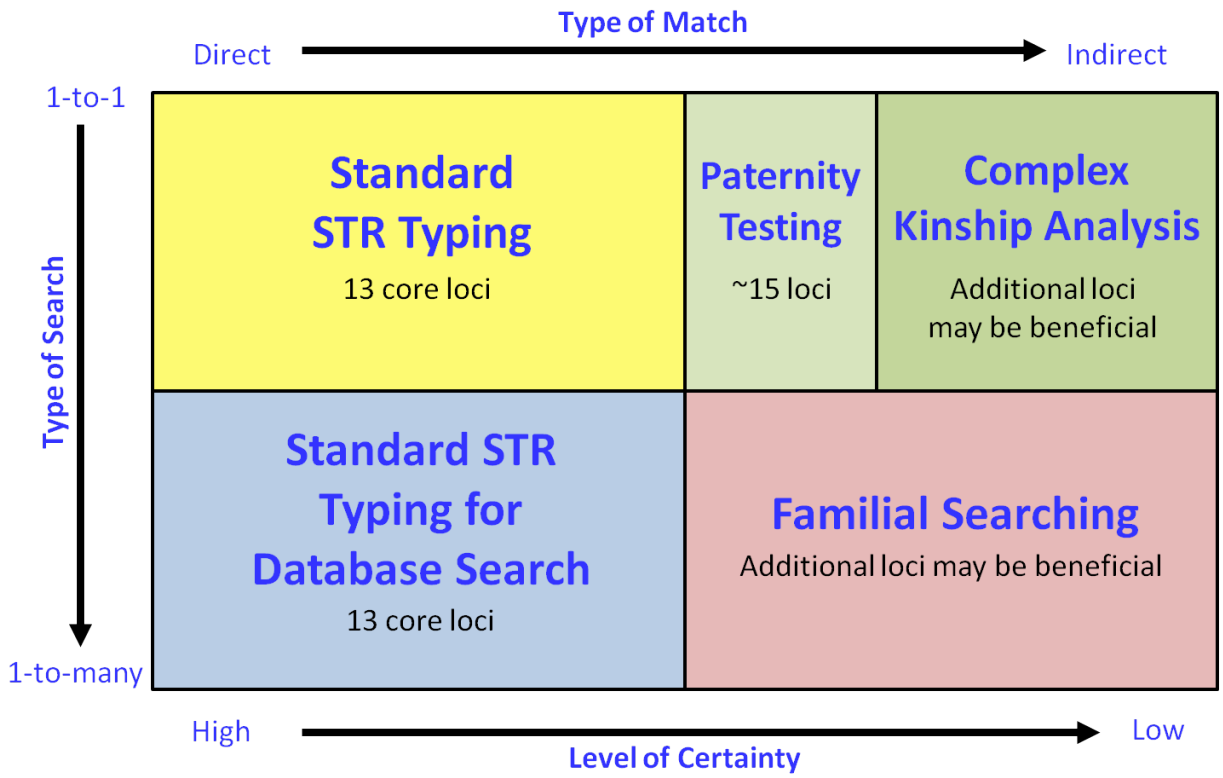

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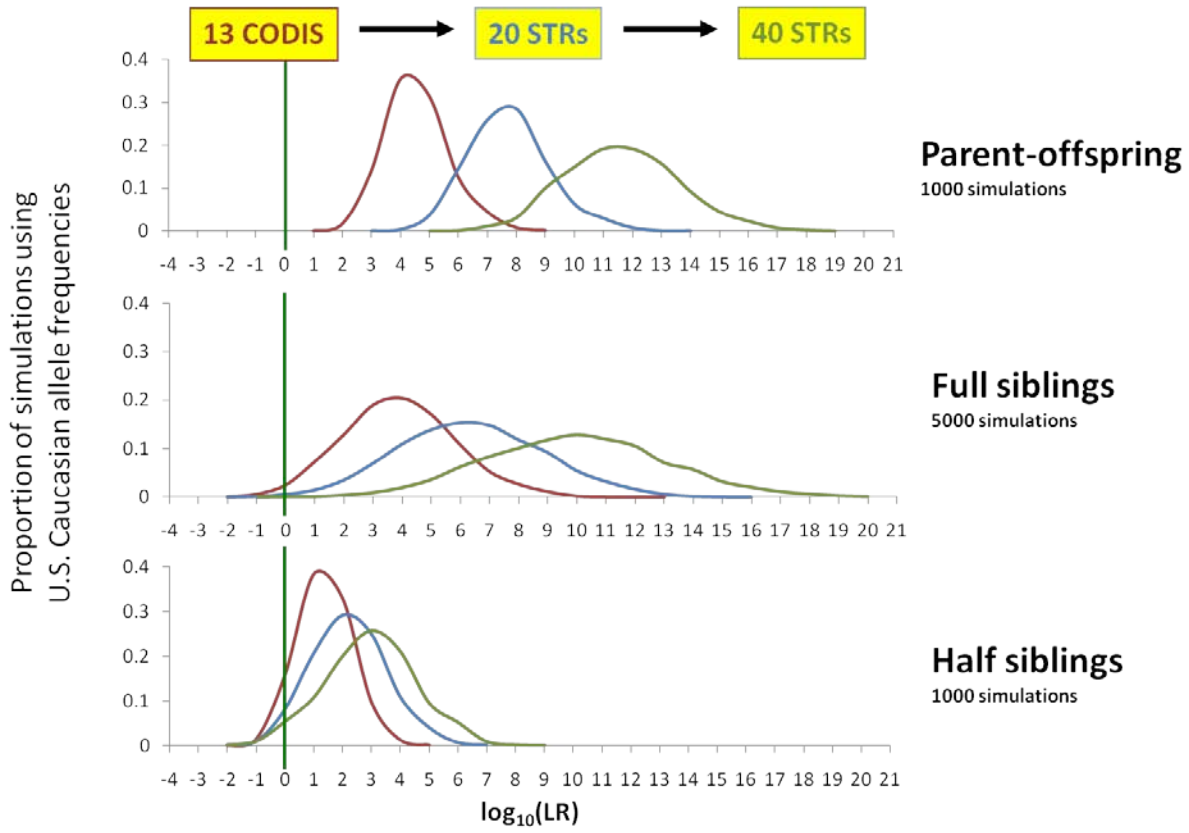

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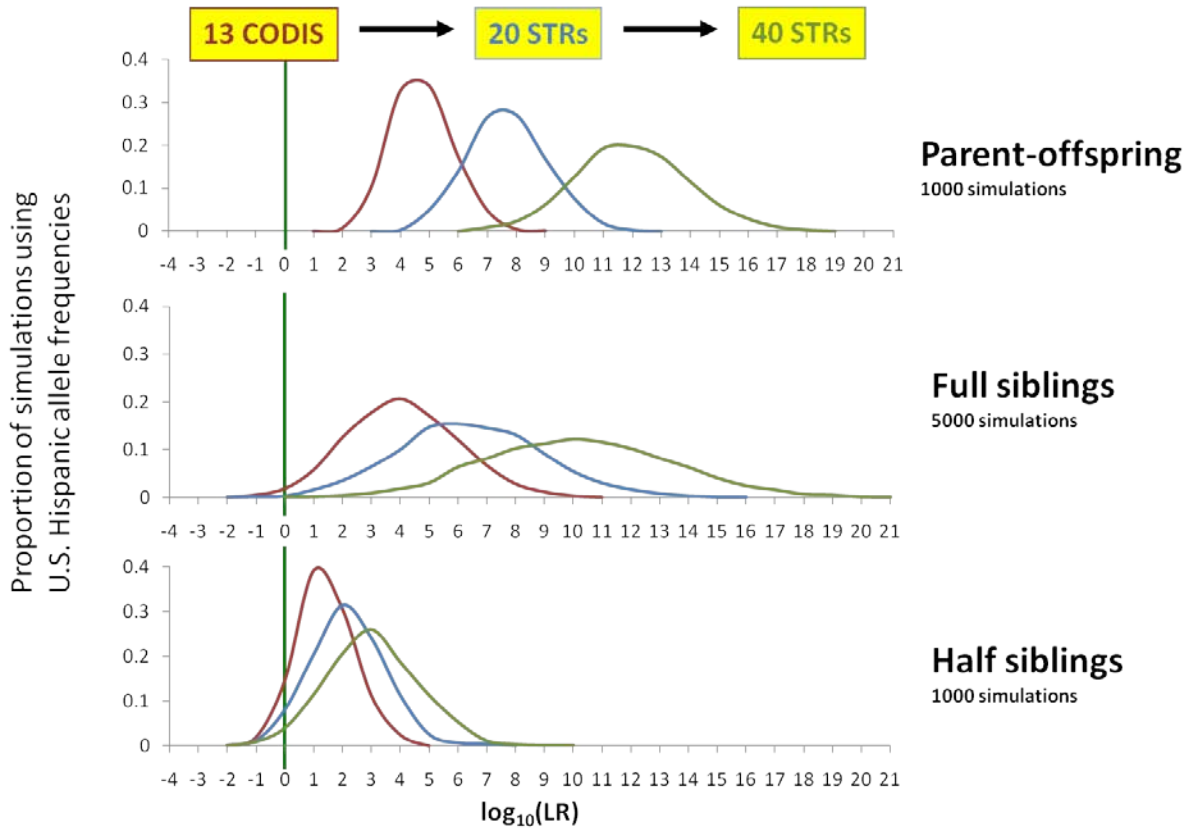

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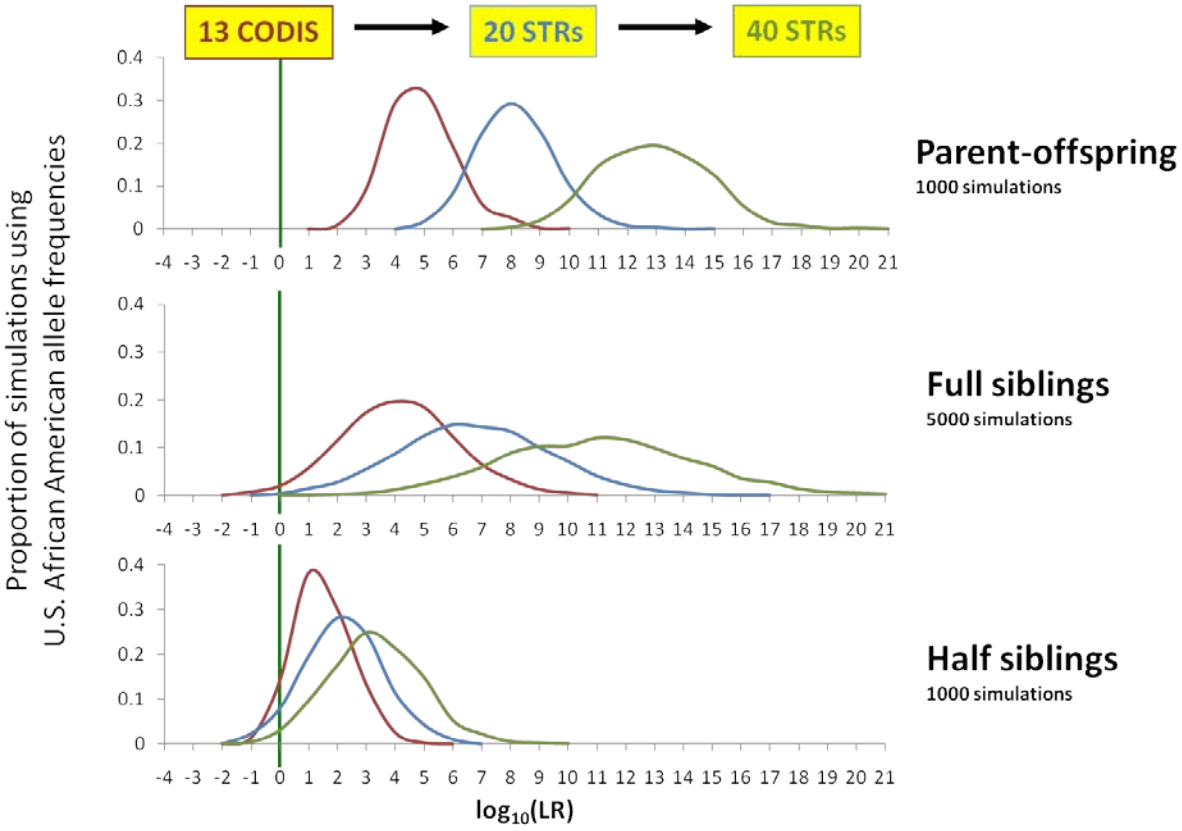

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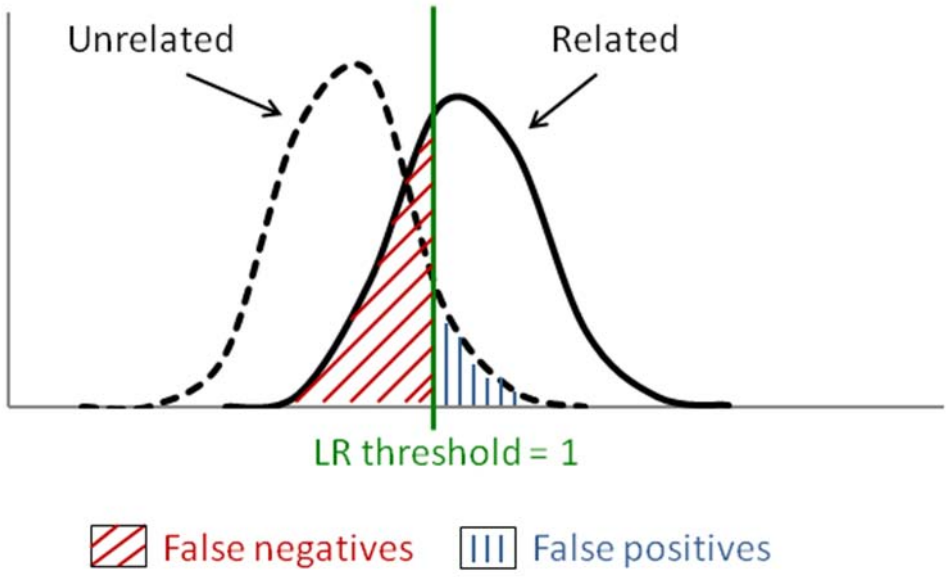

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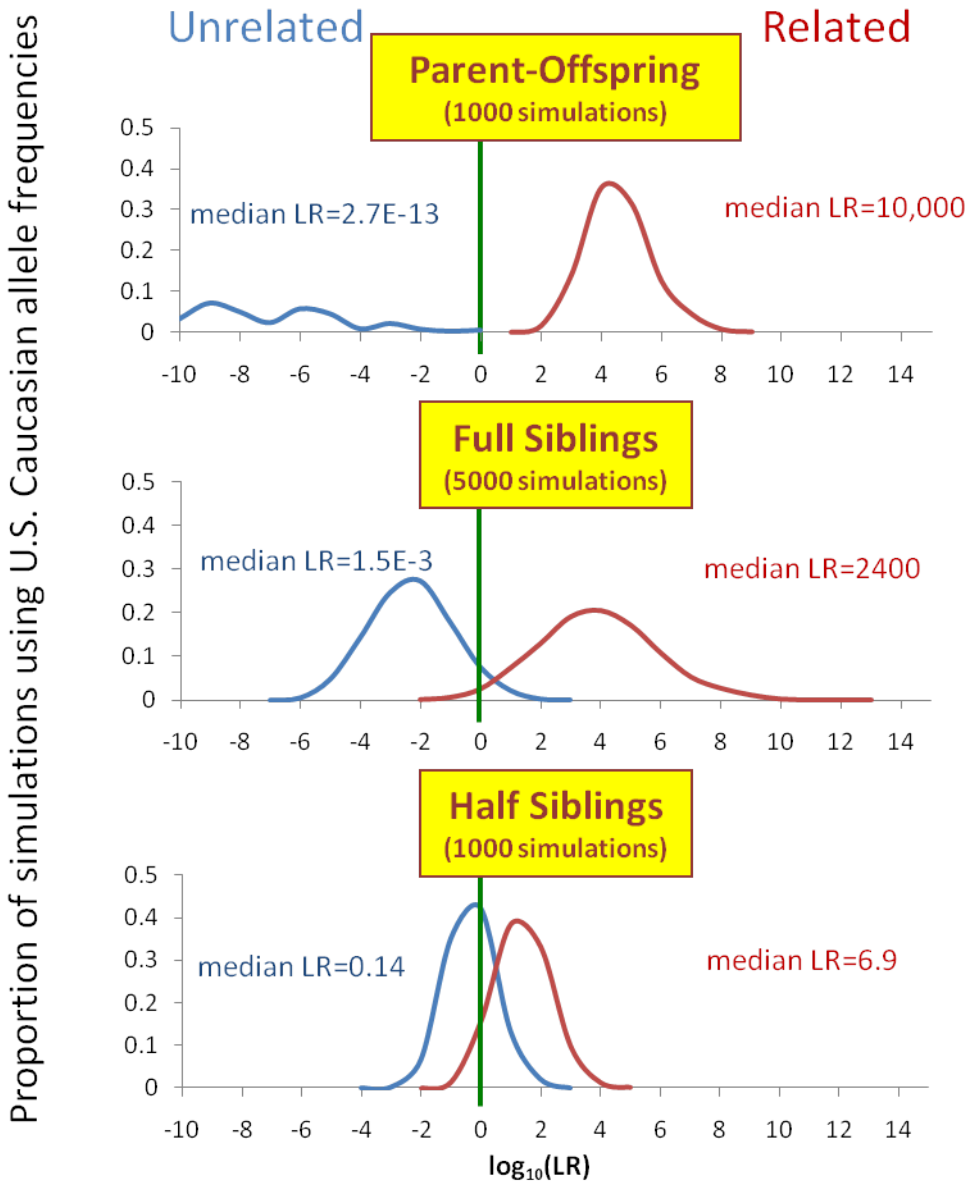

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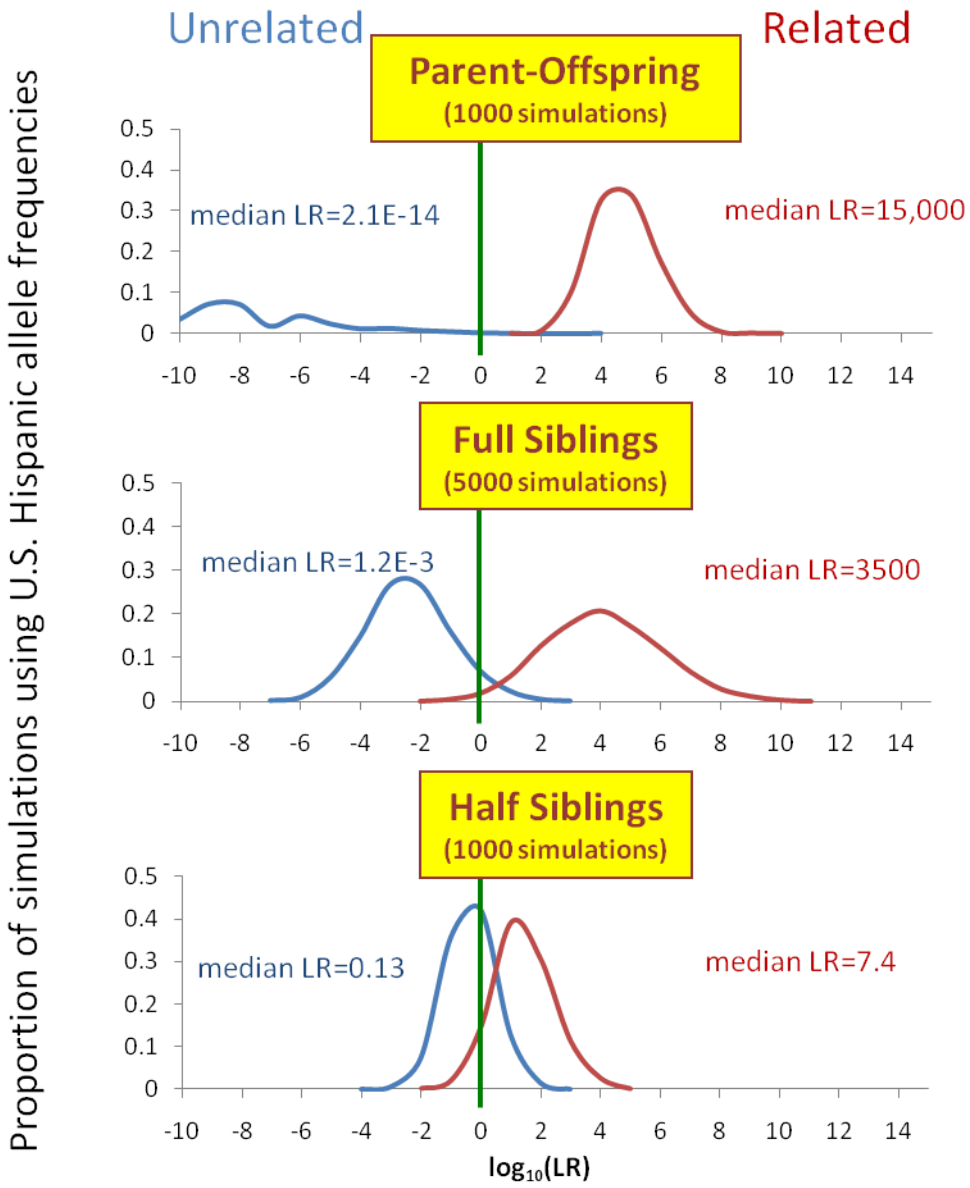

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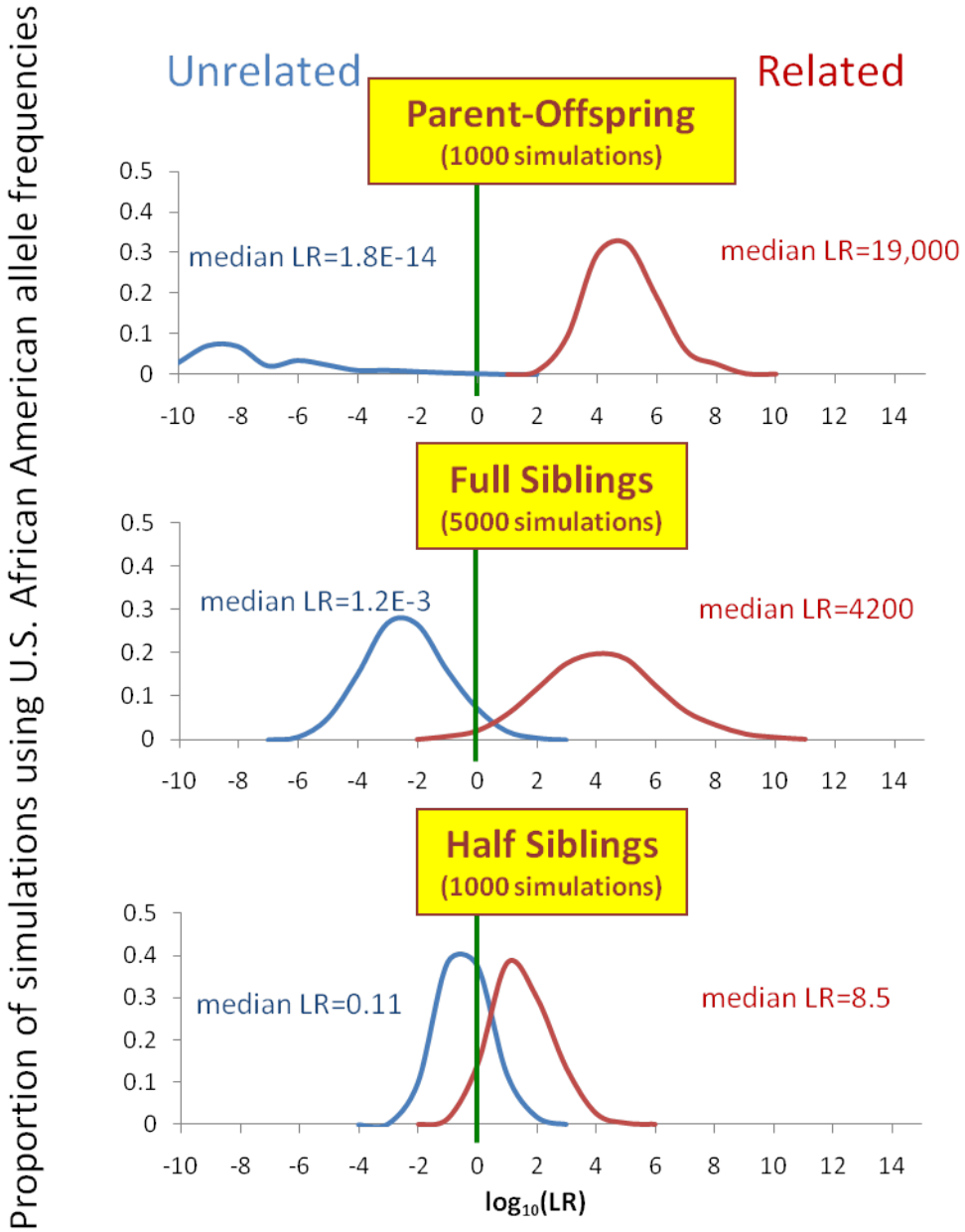

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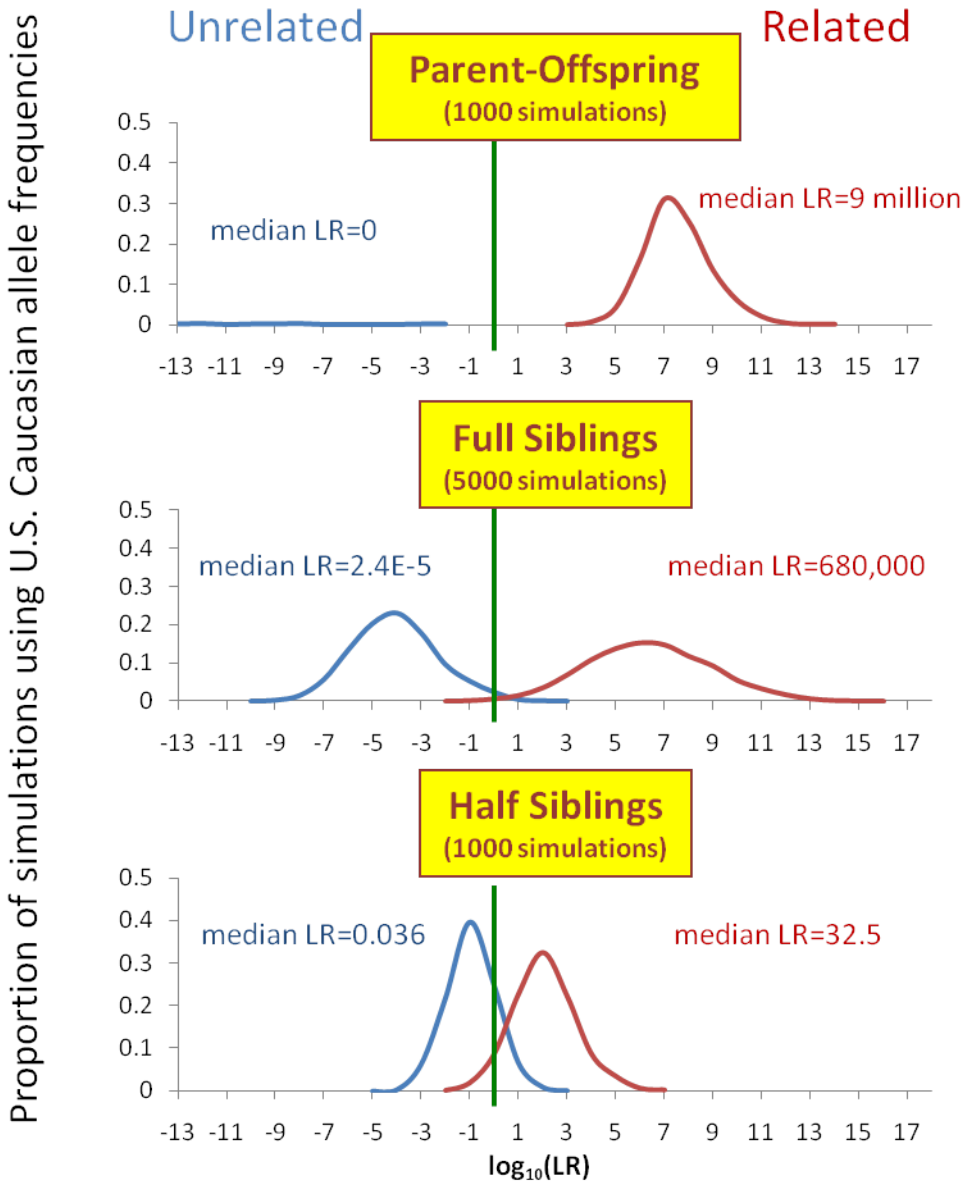

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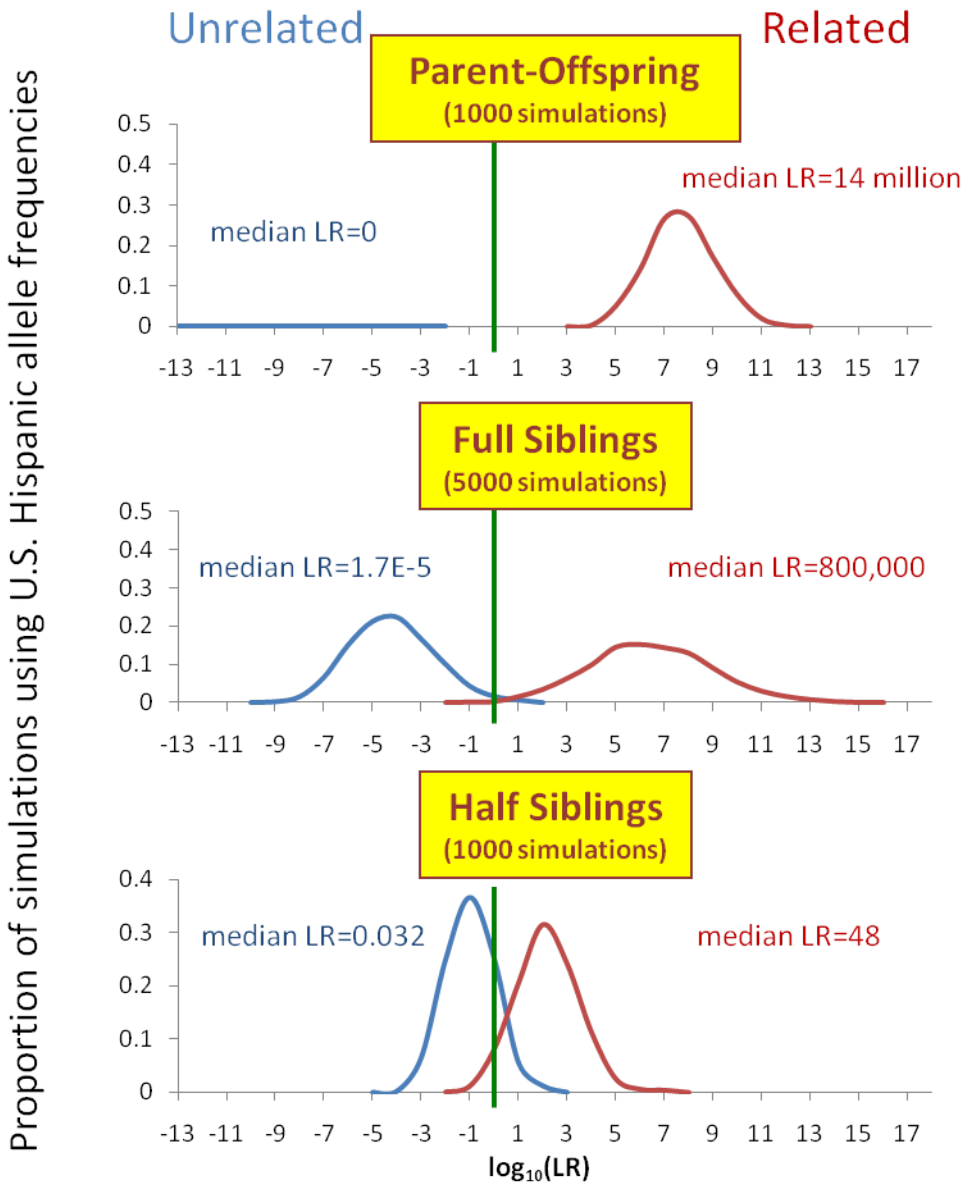

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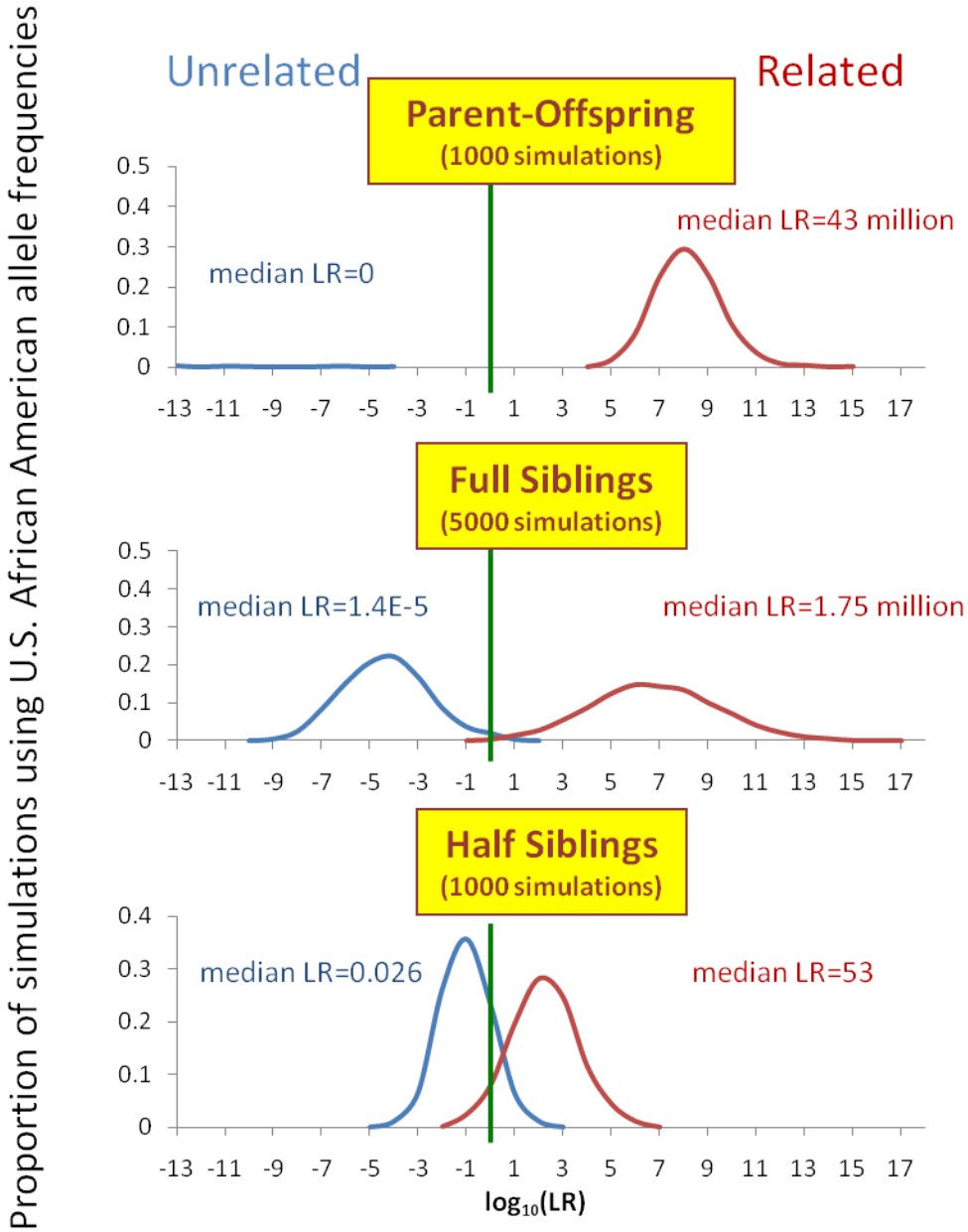

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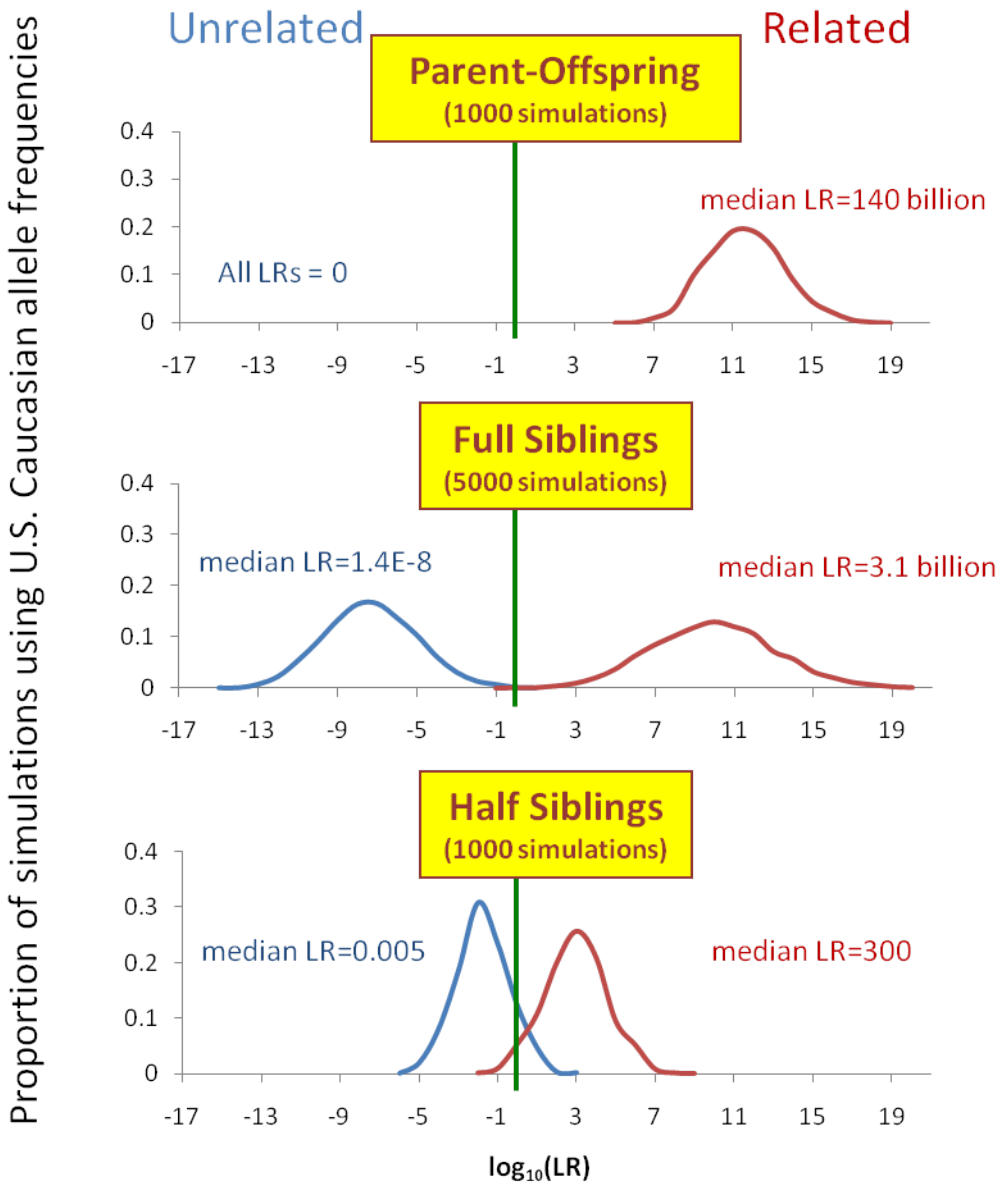

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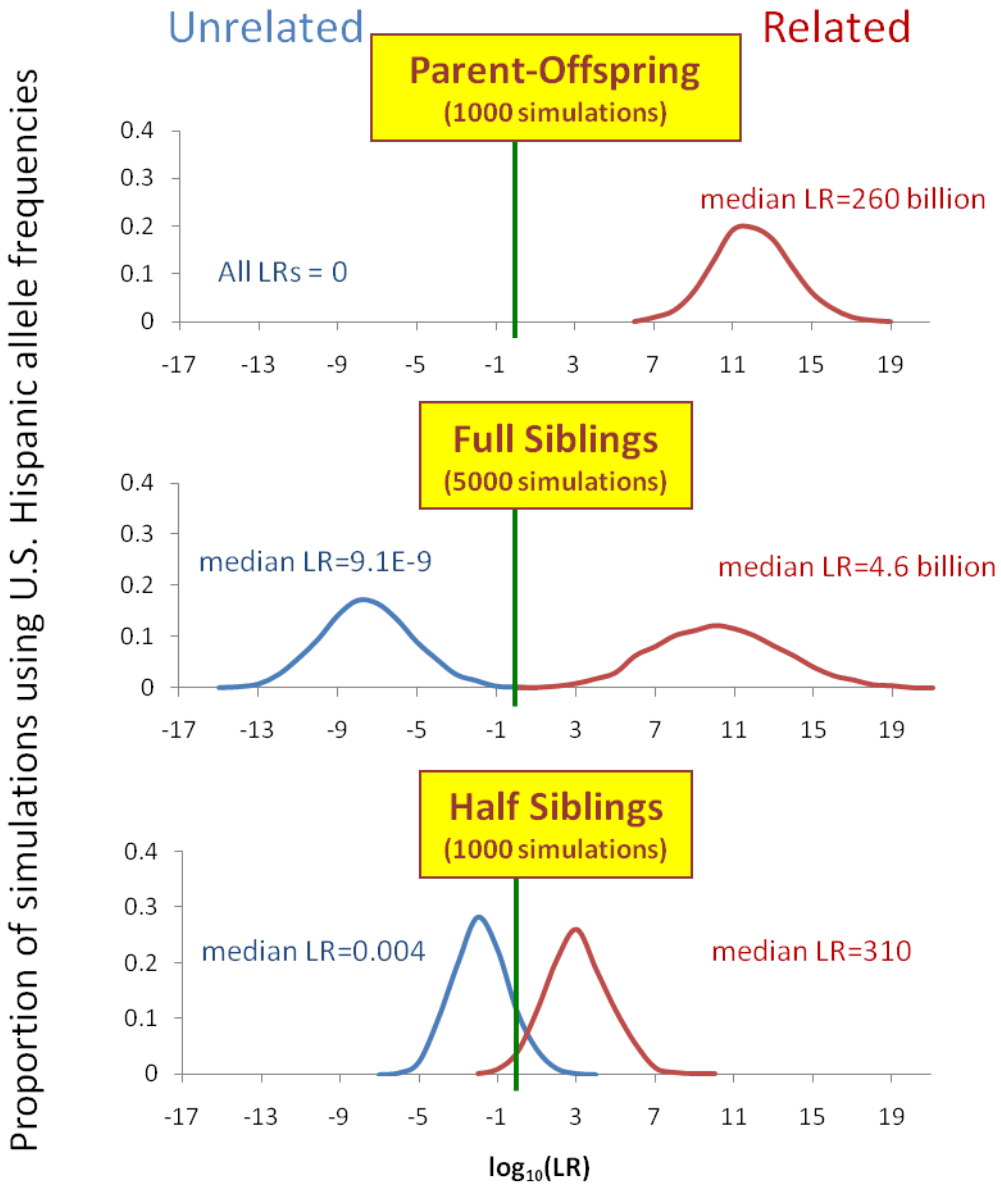

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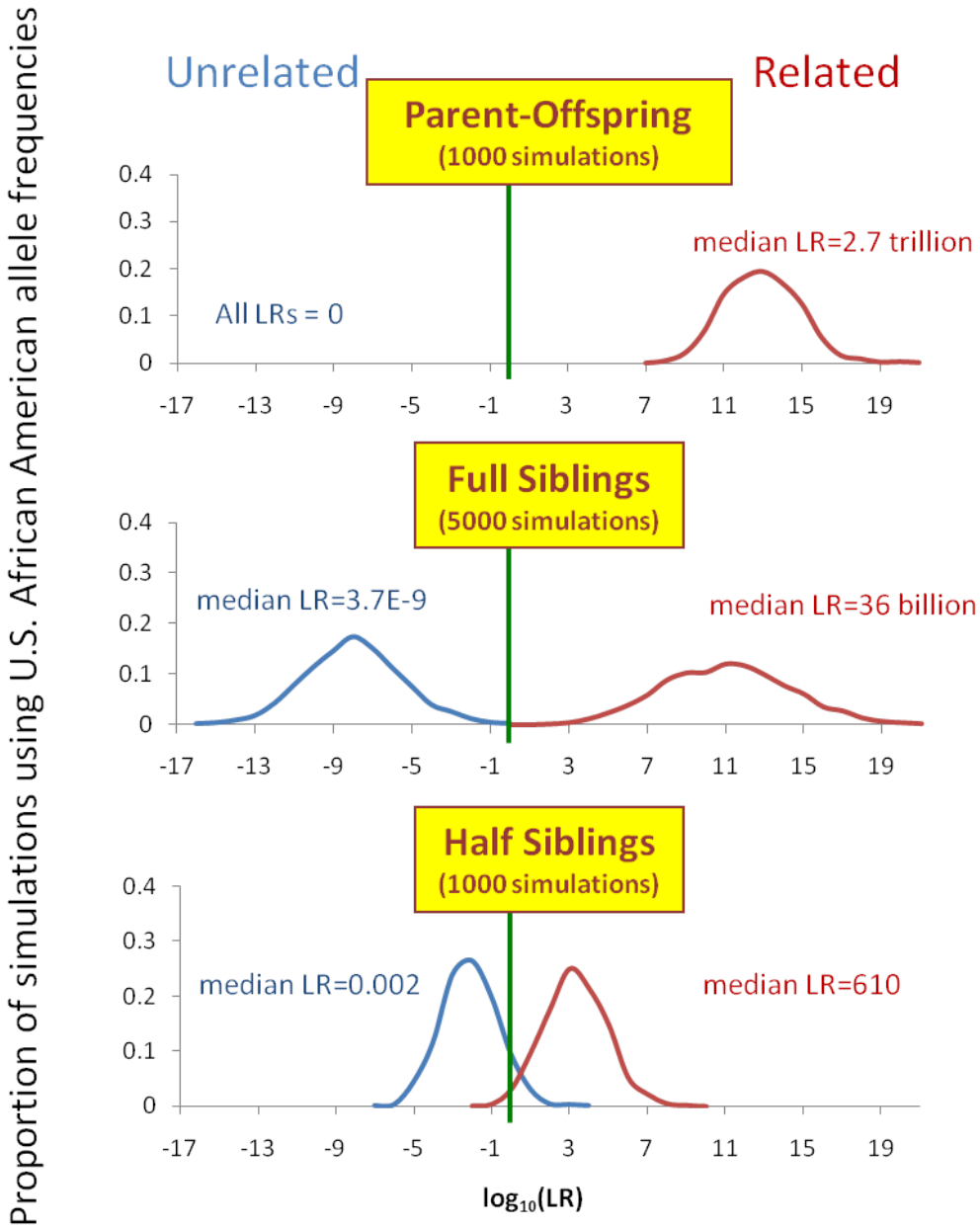

Table 1. Identity by descent (IBD) probabilities for pairs of non-inbred relatives (Weir et al. 2006). The values k_0, k_1, and k_2 correspond with the probabilities of sharing zero, one, and two alleles IBD, respectively.

Relationship	**k_0**	**k_1**	**k_2**
Identical twins	0	0	1
Parent-offspring	0	1	0
Full siblings	1/4	1/2	1/4
Half siblings	1/2	1/2	0
Avuncular	1/2	1/2	0
Grandparent-grandchild	1/2	1/2	0
First cousins	3/4	1/4	0
Double first cousins	9/16	3/8	1/16
Unrelated	1	0	0

Table 2. List of 13 U.S. core STR loci and expanded sets of 20 and 40 STR loci used in this study. The 12 European Standard Set loci are composed of the 11 loci in bold plus D12S391.

13 Loci	20 Loci	40 Loci
TPOX	TPOX	TPOX
CSF1PO	CSF1PO	CSF1PO
D5S818	D5S818	D5S818
D7S820	D7S820	D7S820
D13S317	D13S317	D13S317
FGA	**FGA**	**FGA**
vWA	**vWA**	**vWA**
D3S1358	**D3S1358**	**D3S1358**
D8S1179	**D8S1179**	**D8S1179**
D18S51	**D18S51**	**D18S51**
D21S11	**D21S11**	**D21S11**
TH01	**TH01**	**TH01**
D16S539	D16S539	D16S539
	D2S1338	D2S1338
	D19S433	D19S433
	D2S441	**D2S441**
	D10S1248	**D10S1248**
	D22S1045	**D22S1045**
	D1S1656	D1GATA113
	SE33	D1S1627
		D1S1677
		D2S1776
		D3S3053
		D3S4529
		D4S2364
		D4S2408
		D5S2500
		D6S474
		D6S1017
		D9S1122
		D9S2157
		D10S1435
		D11S4463
		D12ATA63
		D14S1434
		D17S974
		D17S1301
		D18S853
		D20S482
		D20S1082

Table 3. Frequency of false positive and false negative relatives defined with a likelihood ratio threshold equal to one. Genotypes of true relatives or unrelated persons were simulated from NIST U.S. Caucasian, Hispanic, and African American allele frequency data for 13 U.S. core STR loci, 20 STR loci, and 40 STR loci. The pairwise relationships evaluated were parent-offspring (PO), full siblings (FS), and half siblings (HS).

	13 Loci			**20 Loci**			**40 Loci**		
	PO	FS	HS	PO	FS	HS	PO	FS	HS
False positives									
Caucasian	0	0.027	0.155	0	0.006	0.075	0	0.001	0.051
Hispanic	0.002	0.026	0.143	0	0.007	0.067	0	0.001	0.058
African American	0.002	0.025	0.140	0	0.004	0.074	0	0.001	0.039
False negatives									
Caucasian	0	0.033	0.173	0	0.008	0.104	0	0.002	0.066
Hispanic	0	0.026	0.168	0	0.006	0.094	0	0.001	0.050
African American	0	0.029	0.156	0	0.005	0.105	0	0.001	0.036