Legal Medicine 60 (2023) 102158

Contents lists available at ScienceDirect

Legal Medicine

journal homepage: www.elsevier.com/locate/legalmed

Effects of DNA degradation and genotype imputation on high-density SNP microarray in pairwise kinship analysis

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ARTICLE INFO

Keywords:
Pairwise kinship analysis
high-density SNPs
DNA microarray
Imputation

ABSTRACT

High-density single nucleotide polymorphisms (SNPs) can detect distant relatives even in the context of pairwise kinship analysis. Although DNA microarrays conveniently generate genome-wide SNP data, they require large quantities of high-quality DNA. Genotyping data obtained from low-quantity and low-quality samples are likely unreliable owing to the incidence of no-called or mistyped SNPs. In this study, we examined the effects of insufficient sample densities and sample degradation on the efficacy of kinship analysis. While low DNA amounts had a minor effect, DNA degradation led to a significant increase in no-call rates and error rates. Posterior probabilities of kinship determination, calculated using the index of chromosomal sharing, were markedly lower in proportion to the no-call rates and error rates. We also investigated the effect of genotype imputation to complement the no-called genome data utilizing SNPs reference panels. We found that the posterior probability of the relative-assumed person increased with genotype complementation in case of mild degradation, even with mistyped genotypes. Therefore, DNA microarray with imputation is a promising method for analyzing forensic DNA samples taken from situations where DNA quantity and quality may be compromised, such as disaster victim identification using pairwise kinship analysis.

1. Introduction

Nowadays, DNA analysis is widely used in forensic casework, especially in kinship analysis. Kinship analysis is, for example, utilized in paternity testing, missing person identification, or disaster victim identification (DVI). In DVI, post-mortem samples and ante-mortem samples are collected from unidentified remains (e.g., bones, teeth, or nails) and family references, respectively [1,2]. Relationships between the samples are determined by calculating likelihood ratios (LR) or posterior probability. Although genotypes of short tandem repeat (STR) loci are the general materials for pairwise kinship analysis, most STR typing systems are only available for kinship determination of first-degree relations (i.e., parent–offspring and siblings); further relationships are thus difficult to conclude [3–5]. However, if all family members are missing because of the disaster, post-mortem samples should be compared with samples from distant relatives, such as nephews, nieces, or first cousins. Further, analysis of high-density single nucleotide polymorphisms (SNPs) has recently been found effective for distant kinship determination [6,7]. Therefore, in our previous study, we developed a new method for pairwise kinship analysis using the "index of chromosome sharing" (*ICS*), calculated from the detection of high-density autosomal SNPs [8].

Using the *ICS* method, we examined the genotypes of autosomal 174,254 SNPs and calculated the identity by state (IBS) of each SNP common between two individuals as well as the genetic length (centi-Morgan, cM) of IBS segments from each of the two individuals. To avoid coincidental sharing between the two, we defined the *ICS* value as the sum of the genetic length of segments longer than the threshold (i.e., 4 cM). We determined the threshold by varying the value (from 0 to 63 cM) and adopting the value showing the highest discrimination between the first-, second-, third-, fourth-, and fifth-degree relatives as well as the unrelated pair [8]. The *ICS* value indicates the expected shared genome portion of the pair. Using the log-normal distribution of the *ICS* value of each kinship pair estimated by simulation, we obtained the LR and posterior probability of the target pair. We could discriminate fifth-degree relatives from unrelated pairs using LR. We could also determine accurate kinship relationship up to third-degree relatives and distinguish between collateral and lineal relatives of the same degree

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https://doi.org/10.1016/j.legalmed.2022.102158
Received 2 September 2022; Received in revised form 5 October 2022; Accepted 11 October 2022
Available online 17 October 2022

[8,9].

DNA microarray is one of the most common methods for obtaining high-density SNP genotypic data; therefore, we used this method in our previous study [8]. Relatively large amounts of non-denatured, high-quality DNA samples are required for successful DNA microarray. For example, the Infinium HumanCore-24 BeadChip (Illumina, San Diego, USA) kit needs 200 ng of DNA per the manufacturer's protocol. In forensic work, obtaining sufficient high-quality DNA can be challenging, especially in the case of DVI, as the available DNA is often insufficient and/or degraded. Therefore, using DNA microarrays in such cases poses the risk that SNP genotypes could be more frequently unidentified and lead to false conclusions [10–12].

However, genotype imputation is widely used for improving genome coverage at untyped markers in genome-wide association studies (GWAS) [13]. Imputation methods include determining the linkage disequilibrium (LD) structure in a given genetic region to predict the possible alleles of untyped SNPs. With respect to imputation, we suppose that in many reference panels, such as the 1000 Genome Project [14], the study samples and controls are likely sampled from the same population and thus exhibit overlap in LD structure and haplotype distribution [15]. Therefore, the structure of the LD can be used to impute the alleles of an untyped SNP by finding the haplotype segments shared across reference panels and study samples [13]. The use of imputation methods to predict no-called SNP loci is expected to improve the efficacy of kinship analysis in cases where DNA samples are degraded. In this study, we first examined the effects of insufficient sample densities and sample degradation on the efficacy of the *ICS* method in kinship analysis. Furthermore, we examined the effect of SNP genotype complementation using imputation on no-called SNP loci.

2. Materials and methods

2.1. Estimation of no-call rate and error rate of insufficient and degraded DNA samples

2.1.1. DNA extraction and fragmentation

Blood samples were collected from three Japanese individuals. All participants gave written informed consent, and this study was approved by the ethics committee of the Graduate School of Medicine of Kyoto University. DNA was extracted using the GenElute™ Blood Genomic DNA Kit (Sigma-Aldrich, Burlington, MA, USA) and quantified using a NanoDrop Spectrophotometer (ND1000, Thermo Scientific, Bonn, Germany). The concentration of all extracted DNA samples was adjusted to 50 ng/μL.

DNA samples were then fragmented three times, with peak target sizes of 150 bp, 300 bp, and 450 bp, using an S220 Focused-ultrasonicator (Covaris, Woburn, MA, USA). The three target sizes were assumed as partial profile, almost full profile, and completely full profile with regard to general STR typing systems, respectively. The success of fragmentation was confirmed via gel electrophoresis, using the D5000 ScreenTape on 4200 TapeStation System (Agilent Technologies, Santa Clara, CA, USA). Thereafter, the three groups of fragmented DNA samples and unprocessed DNA samples were diluted to four concentrations: 50 (recommended DNA concentration for microarray), 12.5, 3.125, and 0.78125 ng/μL. In total, 16 groups of DNA samples were prepared from each individual.

2.1.2. Microarray genotyping

All DNA samples were genotyped using the Infinium HumanCore-24 BeadChip (Illumina, San Diego, USA). According to the manufacturer's protocol, 4 μL DNA samples were used in each run, and 200 ng of unprocessed DNA was used as the positive control (PC). Genotypes were called using the GenomeStudio Software (Illumina), and 174,254 autosomal SNPs were selected for further analysis, based on the amounts used in our previous study [8].

2.1.3. Model building for no-call rate and error rate

We calculated the no-call rate and error rate of each sample. The no-call rate (NR) was calculated as the ratio of no-called SNP loci to the total 174,254 SNP loci. The error rate (ER) was determined as the ratio of the number of SNP loci that were different from those in the PC (174,254 SNP loci). Finally, linear regression was used to determine the correlation between the NR and ER for each sample.

2.2. Generation of SNP genotypes assuming DVI cases

We used 67 actual relative samples from our previous study [8] to verify whether *ICS* was effective for degraded DNA samples. The 67 relative samples included collateral relatives up to the fifth degree: 20 sibling pairs (C1), 17 uncle-nephew pairs (*C*2), 14 first cousin pairs (C3), 10 first cousin once removed pairs (C4), and 7 s cousin pairs (C5). We also included 15 unrelated pairs (UN). For each related and unrelated pair, one of the genotypes was modified to represent an unidentified person in the case of DVI (i.e., degraded sample). No SNPs of the other sample were changed. We computationally generated the degraded DNA samples, thus masking the genotypes of randomly selected SNP loci according to the NR calculated in Section 2.1.3. Moreover, we randomly selected SNP loci according to the ER calculated in Section 2.1.3. and changed the genotype to give an error. In other words, we generated "a mistyped result" by changing the typed genotype from a homozygote (original genotype) to heterozygote or from a heterozygote (original genotype) to homozygote. For each pair, we simulated 15 different stages of NR and ER. All simulations were carried out using the open-source statistical software R, version 4.0.2 [16].

2.3. Kinship evaluation of degraded DNA samples

We determined kinship between each pair by calculating the *ICS* value according to our previous study [8], and the threshold of the length of IBS region was set to 4 cM. To determine the degree of kinship from an *ICS* value, we calculated the posterior probability from Bayes' theorem, assuming all degrees of relationship (i.e., the first-, second-, third-, fourth-, fifth-degree relative and unrelated pair) and using the log-normal distribution of the *ICS* value of each kinship pair estimated by simulation. The flat values of the prior probabilities were used because we assumed that all hypotheses were equally likely before obtaining the genotype data. We then evaluated the relationship by referring to the classical Hummel's predicates of paternity [17], which define a true relationship as having a posterior probability larger than 0.9.

2.4. SNP imputation

We computationally generated DNA genotypes modified to resemble degraded samples as in Section 2.2, to evaluate the effect of imputation on kinship analysis. As many bioinformatics tools require SNPs to be on the reference strand, we used the open-source whole-genome association analysis toolset, PLINK version 1.9 [18] to flip the SNPs that were not on the reference strand.

Genotypes were recommended to be converted into haplotypes in advance, which is known as "pre-phasing" [13], because imputation is used to estimate the unknown allele of a given SNP locus referring to the nearby known allele and the haplotypes of the reference panels on the same chromosome. In this study, we used SHAPEIT4 version 4.2.2 [19] for pre-phasing and used data from the 1000 Genome Project [14] as a reference.

Furthermore, we used imputation to predict the simulated no-called SNP loci using Minimac4 [13], also using the 1000 Genome Project [14] as the reference panel. After imputation, duplicated variants, non-SNP variants, and SNPs for which the minor allele frequency was less than 0.01, were removed. We finally selected 174,254 SNP loci, the same as in our previous study [8], from the remaining SNPs using PLINK version

2.0 [18]. Finally, we computed the *ICS* value and posterior probability between the actual pair as described in Section 2.3.

3. Results

3.1. No-call rates and error rates of ultrasonically fragmented and degraded DNA samples

We performed DNA fragmentation using an ultrasonicator and confirmed fragment lengths by gel electrophoresis. DNA fragmentation into 150 bp and 300 bp was conducted as per the manufacturer's instructions; however, as there were no instructions for DNA fragmentation into 450 bp, we adjusted the parameters to optimize the fragmented results that best fit 450 bp. The confirmed results are shown in Fig. S1.

We found that the NR and ER increased in accordance with the degradation level of the DNA samples (Fig. 1). For the smallest samples in quality and quantity (i.e., 150 bp fragmented, 3.125 ng input), the mean NR was 58.8% and the mean ER was 11.9%. We found that poor DNA quality (i.e., DNA fragmentation) rather than small DNA quantity, had a greater effect on the rate of mistyped genotypes. We constructed a linear regression model to describe the correlation, finding a positive correlation between NR and ER as depicted in Fig. S2.

3.2. Kinship analysis using the ICS method in artificially-degraded samples

The *ICS* values of relative pairs in each degree of kinship are shown in Fig. 2. Owing to the incidence of no-called and mistyped SNP loci in the degraded DNA samples, the genetic length of continuously shared segments between the pair was generally shorter, causing a decrease in *ICS* value. As the NR and ER values increased, the value of *ICS* dropped significantly. For sibling pairs (C1), the average *ICS* value of non-degraded pairs was 2733.6, whereas the average *ICS* value, calculated under simulated values of 60% NR and 12% ER, was 371.8. We observed the same pattern in all the relative groups, indicating that the decrease of *ICS* value might be a consequence of DNA degradation. Moreover, no apparent difference between *ICS* values of various degree of kinship could be observed with an increase in degradation.

As the result of the decreasing *ICS* value, the probability density obtained from the estimated log-normal distribution of the hypothesized relationship also decreased, as did the posterior probability (Fig. 3). When NR exceeded 5%, the posterior probability of all relative pairs except the C5 pairs decreased to less than 0.9, indicating that the relationship is unlikely to be proven according to Hummel's predicates [17].

3.3. SNP imputation

In our previous study [20], we examined the effect of imputation on no-call SNPs using artificially-degraded DNA samples. We found that imputation resulted in accurate complementation of the genotypes in degraded samples. In particular, for the most degraded sample, the concordance rate between degraded sample and the reference sample improved up to 37% through imputation. In this study, we further assessed the impact of imputed genotypes on the efficacy of *ICS* methodology applied in forensic cases where DNA samples may be sparse or of low quality (degraded).

The simulated, degraded genotypes of one of each of the related pairs and unrelated pairs were imputed, and the *ICS* values of the original (non-degraded) genotypes, the simulated degraded genotypes, and the imputed genotypes of each pair were calculated. Fig. 4 shows the variation among the three *ICS* values of the five collateral degrees of relative pairs and unrelated pairs. For the genotypes simulated in comparatively mild degradation (12.9% of NR and 1% of ER), the apparent improvement in *ICS* value could be seen after imputation, which shows recovery to as much as the same extent as that of the original genotypes (Fig. 4 (A)). However, in the case of severe degradation (25.9% of NR and 3.8% of ER), *ICS* values were not entirely recovered, even after imputation (Fig. 4(B)).

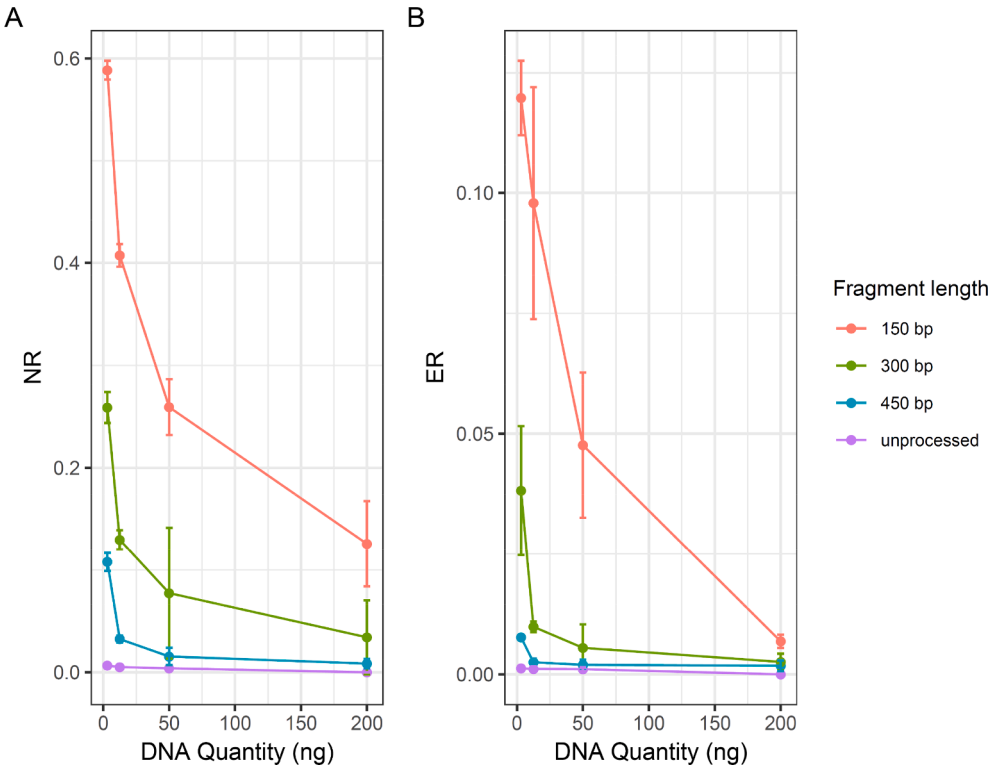

Fig. 1. Variation in (A) no-call rate (NR) and (B) error rate (ER) values with different DNA quantities within each DNA fragmentation stage. Plots and error bars show the mean values and standard deviations, respectively, across the three individuals.

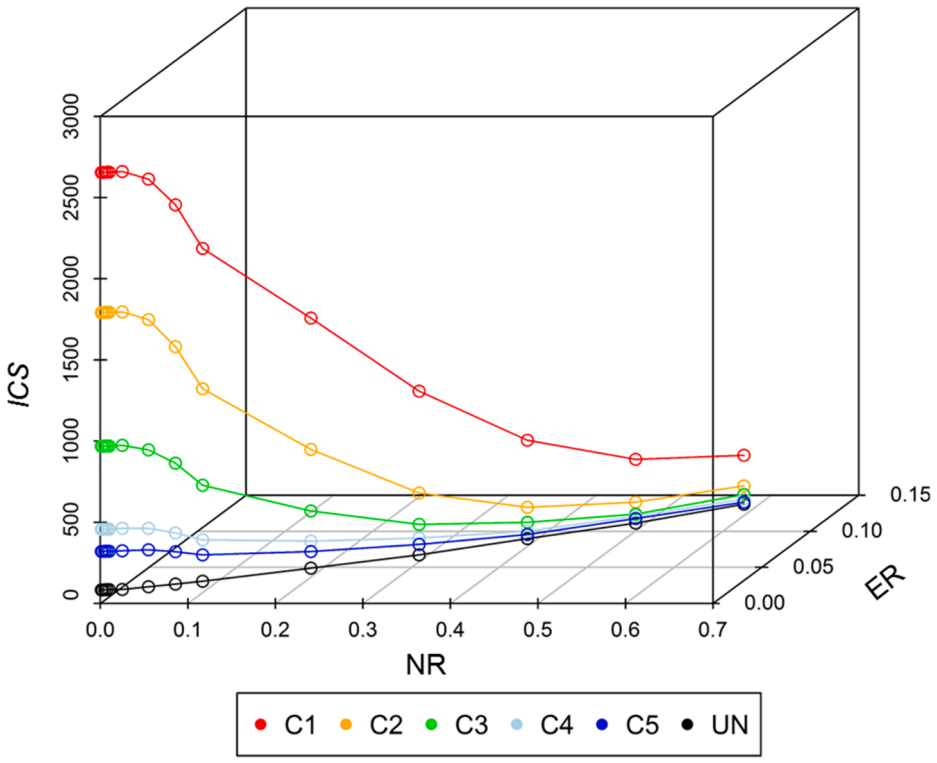

Fig. 2. Variation in index of chromosome sharing (*ICS)* values of the collateral relative pairs at 15 different stages of DNA degradation. Plots show the mean values of each degree of relationship.

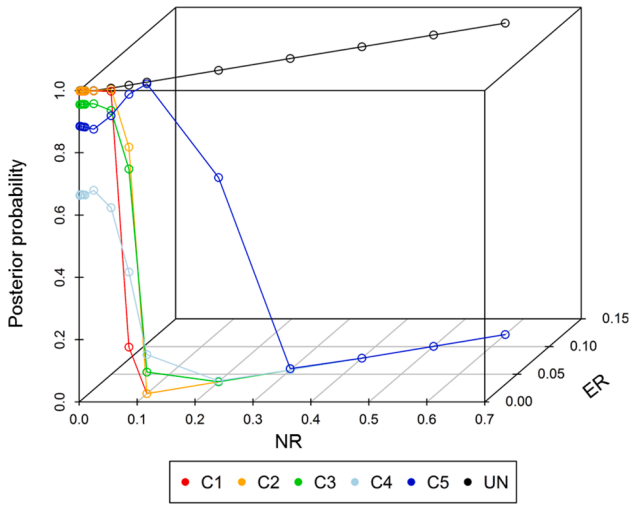

Fig. 3. Posterior probabilities of collateral relative pairs of different no-call rate (NR) and error rate (ER) values. Plots show the mean values of each degree of relationship.

Posterior probabilities were calculated using the above-mentioned *ICS* values. As shown in Table 1(a), posterior probabilities extensively increased in almost all relative pairs as a result of imputation. For the second cousin pairs (C5), although *ICS* values calculated using simulated degraded genotypes decreased, higher probability density values were obtained from the estimated log-normal distribution in C5, causing an increase in posterior probabilities. However, recovery to almost the same extent of original *ICS* values was observed after imputation, resulting in decreased posterior probabilities compared to those of the simulated degraded genotypes. The *ICS* value distribution of C5 is close to that of UN, and the width of the distribution is smaller than that of other kinship pairs (C1-C4), proving that the C5 relationship is more vulnerable to changes in *ICS* values. Conversely, although the *ICS* values of imputed genotypes were slightly recovered in the severely degraded simulation, no apparent elevation of posterior probabilities were found in all relative pairs except for the C5 pairs (Table 1(b)), indicating difficulties in kinship determination.

4. Discussion

Kinship analysis using DNA has proven to be a more accurate assessment method for relationships compared with the conventional method using blood type. However, in forensic genetics, obtaining sufficient high-quality DNA samples for genotyping can be challenging, especially in cases of DVI; thus, we must be able to analyze trace or degraded DNA samples to determine kinship. In this study, we investigated how varying levels of trace and simulated degraded DNA samples affect the accuracy of kinship analysis using the *ICS* method.

We found up to 58.8% no-called loci and 11.9% mistyped loci, depending on the degree of DNA sample degradation, when using DNA microarray to identify SNP genotypes. We further found that the higher the NR value, the higher the ER value. Consequently, an extensive decrease in *ICS* values could be seen in all relative pairs, regardless of the kinship degree. Moreover, reduction of posterior probabilities between the relative pairs was also recognized. When the NR exceeded 5% and the ER exceeded 0.5%, the posterior probabilities between relative pairs tended to drop below 0.9, indicating difficulty in determining the kinship between the two pairs according to Hummel's predicates of paternity [17].

We also investigated the complement of no-called genotypes by genotype imputation and its influence on *ICS* values under simulated degradation. Imputation can thus predict the no-called genotypes of degraded DNA samples correctly, compared to the results of non-degraded DNA samples [20]. Here, we found that if the NR of the DNA sample was up to 12.9%, the *ICS* value and posterior probability with the relative-assumed individual were improved by genotype

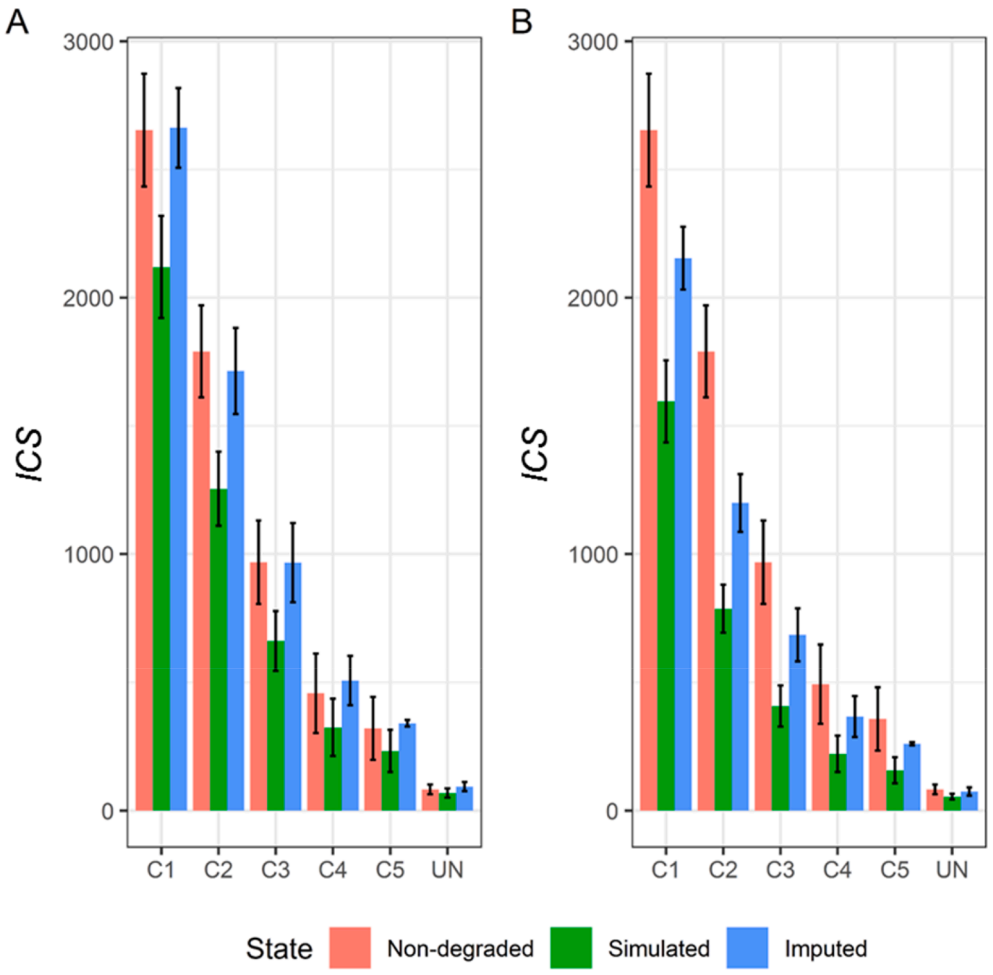

Fig. 4. Index of chromosome sharing () values calculated using three types of genotypes in different collateral relative pairs. Degraded genotypes were simulated separately with (A) no-call rate (NR) 12.9%, error rate (ER) 1% and (B) NR 25.9%, ER 3.8%. Bar plots and error bars show the mean values and standard deviations, respectively, for each degree of relationship.

Table 1
Posterior probability calculated for three types of genotypes in each relative pair.

a.

Kinship	*n*	posterior probability		
		non-degraded	simulated	imputed
C1	20	0.9979	1.34E-05	0.9982
C2	17	0.9993	0.0001	0.9967
C3	14	0.9722	0.034	0.9715
C4	10	0.8203	0.084	0.8525
C5	7	0.802	0.998	0.8687
UN	15	0.999993	0.999994	0.9999

b.

Kinship	*n*	posterior probability		
		non-degraded	simulated	imputed
C1	20	0.9979	4.28E-38	6.41E-05
C2	17	0.9993	6.01E-34	7.79E-06
C3	14	0.9722	2.44E-13	0.0723
C4	10	0.8203	8.54E-05	0.2433
C5	7	0.802	0.0354	0.9921
UN	15	0.999993	0.999993	0.9999

Note. Simulated genotypes denote DNA degradation genotypes simulated in (a) NR 12.9%, ER 1% and (b) NR 25.9%, ER 3.8%. Probabilities are the mean value of each degree of relationship.

complementation from imputation. In actual forensic casework, the degree of DNA degradation differs among samples. Although we cannot calculate the ER because the true genotype is unknown, we can estimate SNP genotyping performance from NR. Therefore, when the NR of the actual sample is below 10%, it is expected that pairwise kinship analysis using SNP microarray can be performed with high precision using imputation.

We further found that it is challenging to correct mistyped genotypes using imputation, as they can occur in actual forensic samples because imputation is essentially a genotype assumption carried out by comparing the typing results directly with a reference panel. However, owing to the incidence of mistyped loci in imputed genotypes, the length of each shared segment tended to decrease, leading to a reduction in respective *ICS* values. Although previous studies have shown that mistyped loci from severe DNA degradation or microbial contamination could be reduced in different kinds of samples using whole-genome sequencing or hybridization capture instead of DNA microarray [21–23], the current price for obtaining a whole-genome sequence is still too expensive for application in day-to-day forensic practice. Therefore, further research is necessary to identify methods of more accurate SNP genotyping for forensic application in future.

In conclusion, we examined the effects of insufficient sample densities and sample degradation on the efficacy of kinship analysis and provided findings for the practical application of this methodology to increase the accuracy of genotyping in cases where DNA samples may be sparse or degraded, such as in DVI cases. In future, we plan to evaluate

other approaches to continue improving the efficacy of imputation using the *ICS* method.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank the members of the Center for Genomic Medicine at the Kyoto University Graduate School of Medicine for their help with SNP imputation. The authors are grateful to the members of our laboratory for providing helpful feedback and support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.legalmed.2022.102158.

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