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Case Report

Limitation of Regular Autosomal STR Testing for Paternity within an Isolated Population

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In order to determine paternity by genetic testing, the Paternity Index (PI) and probability of paternity are calculated using likelihood ratio method. However, when it is necessary, additional testing can be performed to validate the genetic relationship. This research demonstrates autosomal short tandem repeat (STR) results of Jeju Island population in order to determine genetic relationship. Two notable cases showed that despite the acceptable PI value obtained from STR testing, average of 12 mismatches were found in total of 169 autosomal single nucleotide polymorphism typing. Such cases imply that cautious statistical approach is necessary when determining genetic relationship, especially within an isolated population group. Moreover, this would suggest that a further research and investigation are needed in order to understand the population structure of Korea.

Key Words : Single nucleotide polymorphism, Paternity test, Oligonucleotide array sequence analysis

Introduction

The genetic testing for paternity is based on autosomal short tandem repeat (STR) markers, and the genetic relatedness is statistically presented. However, when the typical testing does not guarantee the paternal relationship, additional genetic analysis can be introduced to improve the result. Generally, additional STR typing with increased number of markers is performed to increase the power of discrimination,[1)] or paternal and maternal lineage testing is utilized to prove the relationship indirectly. However, these methods may not be suitable when it is difficult to test with additional STR markers, such as the samples from old body remains or when the test for maternal and paternal lineage does not provide significant information. Recent studies show that single nucleotide polymorphism (SNP) markers are also utilized as a complementary tool for paternity

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test.

When presenting a degree of genetic relatedness through STR typing, probability is calculated based on the allele frequency at each locus of two individuals within a population group sharing common alleles. When calculating, it is assumed that the population satisfies the condition as ideal population, such as random mating, an infinitely large population and that all alleles are independent to one another. However, because not all the populations are ideal, the coancestry coefficient, estimated at population-level, is considered for calculating relatedness among the alleles within a group. Nevertheless, the group that is not completely independent to or difficult to associate to mainland Koreans may possess distinctive genetic characteristics compared to main population. In addition, limitations may apply for general statistical evaluation for such population and moreover, there is no sufficient statistical data to explicate such findings.[2]

Jeju Island is a small isolated group with limited interaction with other regional groups for a long period of time, thus fails to satisfy the condition as ideal population. Therefore, within this population group, acquired partial STR profiles can be unreliable and inadequate to conclude the paternal filiation even with acceptable Paternity Index (PI) or likelihood ratio (LR) values. Furthermore, for cases that have failed to obtain full STR profile from the bone sample, it was obligatory to perform additional genetic test, especially for Jeju population with distinctive environmental factors. In this study, those deferred cases were selected to perform additional SNP typing. As a result, additional SNP testing rejected two paternity cases regardless of acceptable PI value obtained via STR testing. A further assessment of relatedness is discussed in this study.

Case Report

Previously, STR testing was performed to identify the victims of Jeju 4.3 incident. Samples from victims' bone remains and DNA from their family members' are collected for analysis, but few cases showed inconclusive results. For two of those cases, additional Y-STR and mtDNA analysis could not be taken into account due to the fact that bone sample of putative father needed to be compared with a female child. Those undetermined cases were then applied to newly developed DNA typing method using SNP markers for further analysis.

DNA samples were extracted from bone and blood samples using phenol-chloroform extraction method[3] and Maxwell 16 Blood DNA Purification Kit (Promega, Madison, WI, USA), respectively. For reproducibility, DNA samples were extracted in replicates for both samples, and named bone (1st), (2nd) and putative daughter (1st), (2nd), respectively in two independent cases.

Fifteen STR loci were amplified using AmpF/STR Identifiler polymerase chain reaction (PCR) amplification kit (Applied Biosystems, Foster City, CA, USA). For PCR reaction ABI GeneAmp PCR System 9700 (Applied Biosystems) was used, and the amplified products mixed with Hi-Di formamide were separated by capillary electrophoresis using 3130 genetic analyzer (Applied Biosystems). Then the result was analyzed using GeneMapper ID software version 3.2.1 (Applied Biosystems).

PI values were calculated using the allele frequency database for the Korean population.[4] The probability of paternity was calculated from cumulative PI which was the product of the individual PIs.

SNP marker testing was performed using AccuID chip (DNA Link Inc., Seoul, Korea),[5] which consists of probes for 169 autosomal SNP markers on array. The probes were designed in triplicate for each SNP markers on array. The experimental protocols were provided by manufacturers.[5] The definitive call was determined only when two or more calls were identical among read calls.[6]

The STR typing provided full profiles of 15 STR loci from the tested samples except for one bone sample with only a partial profile (Table 1). The cumulative PI and probability of paternity were calculated based on

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the results, which were 87,763 and 99.998% in case 1 and 3,333 and 99.970% in case 2, respectively. These values were statistically significant to verify paternity.[7]

In SNP typing of case 1, out of 169 SNPs, 121 and 107 in bone samples, and 149 and 151 in blood samples were typed from each replicates. For test of case 2, 103 and 110 SNPs in bone samples and 137 and 156 SNPs in blood samples were typed (Table 2). All of the typed SNP calls showed a 100% matching call rate. The comparisons of typed markers between bone and blood samples were conducted in total of four possible combinations, paired as bone (1st)-putative daughter (1st), bone (1st)-putative daughter (2nd), bone (2nd)-putative daughter (1st), bone (2nd)-putative daughter (2nd). As a result, 73–86 markers in case 1 and 64–80 markers in case 2 out of 169 SNPs were commonly typed, whereas 10 and 13 in case 1 and 11–16 markers in case 2 showed discrepancies. The distribution of these mismatch SNP loci did not depend on a specific chromosome, but rs7031829 locus was commonly found mismatched region in both cases (Table 3).

Table 1. The Paternity Index and Probability of Two Cases in STR Typing

Parameter	Case 1	Case 2
D8S1179	5.556	3.125
D21S11	0.668	1.664
D7S820	1.645	2.101
CSF1PO	2.066	–*
D3S1358	1.468	1.425
TH01	2.045	–*
D13S317	2.377	2.242
D16S539	1.462	1.462
D2S1338	4.152	–*
D19S433	0.765	1.866
vWA	1.214	–*
TPOX	17.190	1.520
D18S51	2.874	2.385
D5S818	0.988	2.174
FGA	3.546	4.444
Cumulative Paternity Index	87,763.027	3,333.725
Probability of Paternity (%)	99.999	99.970

STR, short tandem repeat.
*Inapplicable alleles to paternity test due to lack of results from bone sample.

Discussion

Typical paternity testing is performed using autosomal STR markers, and statistical probability of paternity is calculated based on LR or PI. The accepted value of LR or PI for paternity is usually greater than 100 or 1,000 when declaring the paternal relationship.[7] In this research, LR value obtained from autosomal STR testing using 15 markers confirmed the paternity with a PI value greater than 1,000. However, when repeated with autosomal SNP testing, the result displayed more than 10 SNP markers that cannot be shared between parent and child, thus failed to validate paternity.

The paternity testing based on only LR value may lead to a wrong conclusion, like some cases that have

Table 2. The Comparison of Potential Paternity Relation in Two Cases Using Commonly Typed Markers from Bone-Putative Daughter Pairs

Case No.	Comparison trial* (bone-putative daughter)	Typed SNP		Compared marker†	Mismatch marker‡
		Bone	Putative daughter		
1	1st-1st	121	149	86	13
	1st-2nd	121	151	85	13
	2nd-1st	107	149	73	10
	2nd-2nd	107	151	75	10
2	1st-1st	103	137	64	15
	1st-2nd	103	156	78	16
	2nd-1st	110	137	69	11
	2nd-2nd	110	156	80	12

*Crossover comparison of bone and putative daughter samples among replicates in two independent cases.
†The number of common single nucleotide polymorphism (SNP) markers typed in both blood sample from a putative daughter and bone sample.
‡The number of non-identical SNP markers that are difficult to prove paternity relationships among the compared markers.

Table 3. The Result of Unmatched SNP Markers Between Bone and Putative Daughter Sample in Two Cases

Locus	Bone (1st)	Bone (2nd)	Putative daughter (1st)	Putative daughter (2nd)
Case 1				
rs10495682	CC	CC	TT	TT
rs17111166	GG	GG	AA	AA
rs1998033	TT	TT	CC	CC
rs2022958	GG	GG	TT	TT
rs2241225	CC	CC	TT	TT
rs2817438	CC	–	TT	TT
rs297013	AA	–	GG	GG
rs409492	AA	–	GG	GG
rs4907923	CC	CC	TT	TT
rs547041	GG	GG	AA	AA
rs7027501	CC	CC	TT	TT
rs7031829*	AA	AA	GG	GG
rs9373570	GG	GG	AA	AA
Case 2				
rs10448261	AA	AA	GG	GG
rs1150461	GG	GG	AA	AA
rs11689668	AA	AA	GG	GG
rs1211780	GG	GG	TT	TT
rs12534955	TT	–	CC	CC
rs12607426	GG	GG	AA	AA
rs12672158	GG	GG	AA	AA
rs12965342	CC	CC	TT	TT
rs1340562	AA	AA	CC	CC
rs1539525	AA	AA	GG	GG
rs1546833	TT	–	CC	CC
rs2425427	CC	CC	TT	TT
rs2613019	TT	TT	CC	CC
rs2685441	CC	CC	–	TT
rs7031829*	GG	–	AA	AA
rs914532	CC	–	TT	TT

SNP, single nucleotide polymorphism.
*Common locus found in both cases.

been reported. According to Gonzalez-Andrade et al.,[8] a case had PI of 13,811 for alleged father 1 and 35,322 for alleged father 2, thus failed to exclude neither of two fathers. However, the additional test using Y-STR markers indicated that all of the tested Y chromosomal alleles were shared between only alleged father 2 and the child. The personal interview and pedigree study later confirmed that father 1 and father 2 were in fact half-brothers.[8] Another case stated it is possible that putative father candidate and his biologically related brother may show almost no difference in PI and a probability high enough to declare paternal relationship.[9] Moreover, a recent simulation study on Korean kinship using STR markers showed that LR value of full-siblings was similar to or even greater than the value between a parent and a child.[10]

A remarkable finding is that despite the fact that subjects of this research claimed to have no possible kinship with one another, the result showed high enough PI value to declare genetic relatedness; nonetheless, there is no reference made regarding such outcomes. The existence of sub-population in Korea, including in Jeju Island, has not been reported yet. A particular small population group like single-clan village may have characteristics of a sub-population, but statistical factors like coancestry coefficient in such population have never been investigated. This research denotes a possible existence of a sub-group in Korea, expressing greater

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genetic relatedness with each other than those in the ideal population. When determining paternity within an isolated group, cautious approach is, therefore, required due to the fact that the typical threshold of LR value may lead to misinterpretation.

When it is difficult to confirm genetic relatedness between father and child only using autosomal STR typing, Y-STR marker testing or additional STR testing with biological mother is performed. However, additional analysis can be challenging when child is a female or when DNA sample from a biological mother cannot be collected. The additional Y-STR typing and mtDNA testing could not be applied to relate between putative father and a female child. These limitations can be statistically compensated by increasing the number of STR, but mutation rate also increases along with the increased number of markers. Therefore, SNP typing presented in this study can only serve a supplementary tool, providing additional information for paternity analysis.

Furthermore, the result of SNP typing in bone samples provided in this study showed improvements in comparison to the previously reported results[6] due to a development of technique and research. According to previous study, only 75–94 SNPs out of 169 markers were typed, whereas 103–121 SNPs were successfully typed in this study with no discrepancies between experimental replicates caused by phenomenon called allele drop out. A further research with continuous technical development is anticipated to increase the efficacy of DNA chip based on SNP markers.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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