Analysis of Sibling Pair Relationships of Balineses Indonesia,Using 12 STR Loci for Human Identification Process

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Abstract

Use of STR locus in studying the relations between siblings for the need of ethnic genetic database in Indonesia for identifying disaster victims, criminals and Probation Test (paternity test) is not well established compared to other countries. Targeted outcomes are STR typing and profiling of the human genome for individual identification and kin relations. The aim of this study was to analyse kin relationships among siblings by assessing and determining a better STR locus and the level of allelic distribution among full siblings in Balinese people of Indonesia. From a collection of 10 Balinese families, we took a sample of 4 people for each family consisting of the father, mother, and two children. Kin relationships were analysed by using 13 STR loci technique (CSF1PO, F13B, FES, TH01, TPOX, VWA, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, and D21S11). Through the use of 13 autosomal STR loci, this study provides evidence that the strength of sharing 2 alleles can be used as a reference in that siblings have high kinship relations. Based on the results of this study, STR loci D8S1179 and S21S11 are recommended when typing the Balinese.

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Introduction

Geographically, Indonesia is located at the encounter of three major active plates which are the Indo-Australian Plate, the Eurasian Plate and the Pacific Plate. The geographical location of Indonesia causes Indonesia to be more vulnerable to geological and hydro-climatological disasters [1]. Based on the 2018 data from the National Disaster Management Agency (BNPB), the incident of disasters recorded in Indonesia was 3,397 and the number of people recorded as either dead or missing was 3,874. Between 2009 and 2018, Indonesia experienced disasters with varied impact ranging from infrastructure damage to loss of lives [2].

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The process of personal identification is often met with challenges since there is no genetic information from parents[3]. To overcome this problem, siblings may be used to identify the victims during a disaster or criminal investigation. Kinship analysis using siblings has more problems than identification tests using parents as a comparison because there no obligation between siblings to have the same allele. Other than that, full siblings probably have two identical alleles with offspring from the same ancestors at the given locus because they have zero allele. Thus, the lack of alleles distributed at each particular locus does not exclude two people from being related [4].

At present, the STR multiplex locus (73Plex (full 73-loci multiplex) or as part of 20 loci (20Plex) is very polymorphic thereby making it possible to increase a clear resolution in mixed samples from two individuals. STR loci used in 73 Plex has substantial sequence variations that produce high heterozygosity for several loci have reduced the spread of the allele length so as to

facilitate a specific look at the repetition unit. In its use, this multiplex as a complementary tool, has the potential to increase resolution by reducing the number of alleles distributed between individuals compared to the core STR system currently in use [5].

The application of STR in analysing kin relations has not been executed in Indonesia. The use of STRs in Indonesia is hindered because of many ethnic groups that are distributed in different geographical and diverse cultural locations. As a result, a lot of time is needed to prepare supporting tools for personal identification, genetic mapping, ethnic profiling and kinship analysis. This study aims to explain the percentage distribution of alleles among siblings using 13 STR loci in ten families consisting of the father, mother, and two biological children in each family. The main objective of this study was to determine the percentage of allele distribution among siblings and see the STR locus/loci with high differentiating ability between siblings of the Balinese tribe.

Materials and methods

Study participants

The samples size for this study was 20 siblings (10 sibling pairs) taken from a total of 40 people (10 families) of native Balinese. To be included in this study, a family was supposed to belong to at least three generations of native Balinese with two biological children. Those families who were not meeting this inclusion criteria were excluded from this study. As a result, four individuals from each family were made into one project consisting of father, mother and two siblings. The father and the mother were used as controls/references for allele sharing between siblings. This study was approved by the Health Research Ethics Commission, Faculty of Dentistry, Universitas Airlangga with ethical clearance number 256 / HRECC.FODM / IV / 2018. All participants in this study agreed and allowed the publication of results. Participants' identities and confidentiality were respected.

Specimen collection

Blood samples were drawn from the peripheral using a syringe into EDTA containing tubes. The tubes were labelled with the letters F (father), M (mother) and S (sibling).

DNA extraction

DNA was extracted by using DNAzol as previously explained in[6–8]. After DNA isolation, 50 µL of distilled water was added to each DNA pellet.

DNA Amplification using STR-PCR

DNA was amplified by using STR-PCR (PowerPlex® 21 Systems, Promega, USA) targeting 13 STR autosomal loci (CSF1PO, F13B, FES, TH01, TPOX, vWA, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, and D21S11). The amplification process was done using a Bio Rad T100TM PCR machine for a total duration of 2 hours and 7 minutes. The temperature settings for PCR were set as follows: 96°C for 2 minutes, then 94°C for 1 minute, 64°C for 1 minute, 70°C for 1.5 minutes, for 10 cycles, then 90°C for 1 minute, 64°C for 1 minute, 70°C for 1.5 minutes, for 30 cycles. The amplicons were stored at 4°C according to the protocol[9–11]. Amplicons were visualised on 6% Silver Nitrate stained polyacrylamide gel electrophoresis (Bio-Rad Mini-PROTEAN®) [12].

Results

As can be seen from Table 1, a low percentage (10%) of the zero-alleles shared at the D16S359 and VWA loci while the other seven loci did not show any similarities. For sharing one allele, percentages greater than or equal to 50 were observed at all loci with percentages of 50% (D13S317 and D16S359), 60% (F13), 70% (TH01), and 90% (FES). To share the two alleles, high percentages were observed in D5S818, VWA, D18S51, D7S820 with 70% and D8S1179, D21S11 with 80% respectively (Table 1). for the mean, two alleles share a percentage of 56.15%, one allele shares 42.30%, and zero allele shares 1.53 (table 1). whereas (table 2) describes the percentage of each sibling pairs with very high results. Eight families have a percentage of 100% and only two families have a percentage of 92.30%.

num	Loci	2 Allele Sharing	1 Allele Sharing	0 Allele Sharing	1+ 2 Allele Sharing
1	TPOX	60	40	0	100
2	TH01	30	70	0	100
3	D13S317	50	50	0	100
4	D5S818	70	30	0	100
5	D8S1179	80	20	0	100
6	D16S539	40	50	10	90
7	VWA	70	20	10	90
8	FES	10	90	0	100
9	CSF1PO	60	40	0	100
10	F13	40	60	0	100
11	D18S51	70	30	0	100
12	D7S820	70	30	0	100
13	D21S11	80	20	0	100
Average percentage		56,15	42,30	1,53	98,46

Table 1. Two alleles, high percentages were observed in D5S818, VWA, D18S51, D7S820 with 70% and D8S1179, D21S11 with 80% respectively.

family	12 STR loci allele sharing (1 or 2 allele)	Allele percentage
1	13 loci	100
2	12 loci	92,30
3	12 loci	92,30
4	13 loci	100
5	13 loci	100
6	13 loci	100
7	13 loci	100
8	13 loci	100
9	13 loci	100
10	13 loci	100

Table 2. Percentage of each sibling pairs with very high results.

Discussion

These observations show the basic law used to assess the probabilities of common alleles in tested and reference samples that were inherited with the Identical By Decent (IBD) allele. There is a 25% chance that two siblings will inherit the two IBD alleles from ordinary parents, a 50% chance that two siblings will inherit one IBD allele from parents, and a 25% chance of two siblings inheriting no IBD allele from parents, hence will not share alleles at the diploid locus.

The possibility of siblings not sharing an allele (25% of no allele sharing) at one of the two loci poses significant problems in identifying dead individuals from mass disasters when there is only one sibling who is alive. While the probability that one sibling will inherit zero IBD allele from his/her parents is constant at 25% for each locus, the probability that two full siblings will not share alleles / loci partly depends on polymorphism information content (PIC) or heterozygosity [13]. When testing for relations using siblings, sensitivity variations depend threshold of certainty, that is the higher the certainty threshold, the lower the sensitivity. High sensitivity is when the number of false negatives is low and high specificity is when the number of false positives is low [3]. Although it is not an obligation for siblings to have the same allele, the existence of allele sharing in siblings can be used test if the siblings are related as shown in the conducted research [14].

Research which was carried out by Butler, states that regions which contain nucleotide repeats such as STR sequences are very useful for forensic experts because the variations are good markers for human identification.The parameters in the identification process using STRs are allele frequency, homozygosity and heterozygosity, effective numberof alleles (n), Polymorphism Information Content(PIC), power of discrimination (DP) and Power of Exclusion (PE)[15,16]. STR loci for the process of human identification was used to compare the population of Indonesia and Bangladesh by looking at allele frequency in siblings and also using the Power of Discrimination for the two tribes [17]. The use of STR-CODIS in Indonesian population has shown the possibility of similarities with other Asian countries especially on the CSF1P0 and D16S539 loci. When compare to other Asian countries, the locus D3S1358 was found to have high similarity with Vietnam, FGA with Korea and D8S1179 with Japan [18]. The percentage result of 10 Balinese families shows a very high number at 5 STR loci. This study is similar to the research conducted by Agung sosiawan who observed VWA and TH01 as the main locus of sibling sharing [19].

Research on Balinese natives was carried out using STR and compared with other residents outside the Bali region or who were not Balinese. This research is almost the same as the one conducted by Hameed, who observed D16S539, THO1, VWA, D5S818, D8S1179, D3S1357 and CSF1PO as main loci in shared amongst siblings [16]. Research on the indigenous Balinese in the Gilimanuk area was carried out using the STR locus TPOX and TH01 which showed genetic variation [20]. Other studies that have been carried out are able to identify and recommend loci that can be used for the process of identifying human Minangkabau residents [21].

Based on previous research, we tried to see the allele frequency among siblings of the Balinese population by observing 13 STR loci.

The main objective of this study was to determine the distribution of alleles in Balinese siblings. The Balinese are one of the largest ethnic groups in Indonesia who have a tendency to work outside the home. The use of STR is expected to take advantage of many existing loci to reduce false positives in the allele distribution of each sibling. Small variations in allele distributions amongst siblings was also shown to be influenced by endogamy marriages in which the researchers recommended the use of 25 loci for Lebanese population (21). Forensic cases such as human identification for fire victims use siblings to compare to the victims by utilising the Y chromosome for identifying whether the siblings are from one father [22]. Quite a number of research has been done to assess the relationship between siblings among others mainly based on heterozygosity and Combined Sibship Indices (CSIs) [23]. Research using STR to see genetic diversity in Indonesia has been carried out to see the extent of genetic mixing between Javanese and Arab tribes [24]. When assessing sibling relationships, one aspect to pay attention to is to minimize false positives and increase the number of loci to be examined. Based on the results of the study, for the Balinese ethnic group, we recommend the use of loci D8S1179 and S21S11 which were found to have a high percentage.

Conclusions

The findings of this study are useful for adding to the literature and also analyzing the relationship between ethnic siblings in Indonesia. We highly recommend using a larger sample size in the future as this will greatly assist the identification of victims in Indonesia, which is a disaster-prone country.

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Declaration of Interest

All the authors of this paper do not have any conflict of interest.

References

1. Paidi. Pengelolaan manajemen risiko bencana alam di indonesia. *Widya*. 2012;Nomor 321(83):37. http://e-journal.jurwidyakop3.com/index.php/majalah-ilmiah/article/download/63/59.
2. Pahleviannur MR. Edukasi Sadar Bencana Melalui Sosialisasi Kebencanaan sebagai Upaya Peningkatan Pengetahuan Siswa terhadap Mitigasi Bencana. *J Pendidik dan Ilmu Sos*. 2019;29(1):49-55.
3. Gaytmenn R, Hildebrand DP, Sweet D, Pretty IA. Determination of the sensitivity and specificity of sibship calculations using AmpF/STR profiler plus. *Int J Legal Med*. 2002;116(3):161-164. doi:10.1007/s00414-001-0273-8
4. Wenk RE, Traver M, Chiafari FA. Determination of sibship in any two persons. *Transfusion*. 1996;36(3):259-262.
5. Novroski NMM, Wendt FR, Woerner AE, Bus MM, Coble M, Budowle B. Genetics Expanding beyond the current core STR loci : An exploration of 73 STR markers with increased diversity for enhanced DNA mixture deconvolution. *Forensic Sci Int Genet*. 2019;38(September 2018):121-129. doi:10.1016/j.fsigen.2018.10.013
6. Chomczynski P, Mackey K, Drews R, Wilfinger W. DNAzol ® : A Reagent for the Rapid Isolation of Genomic DNA. *Biotechniques*. 1997;22:550-553.
7. Chen H, Rangasamy M, Tan SY, Wang H, Siegfried BD. Evaluation of five methods for total DNA extraction from western corn rootworm beetles. *PLoS One*. 2010;5(8):1-6. doi:10.1371/journal.pone.0011963
8. Song Y, Fahs A, Feldman C, et al. A reliable and effective method of DNA isolation from old human blood paper cards. *Springerplus*. 2013;616(2):1-7.
9. Aslam N, Rahman Z, Riaz-Ud-DIn S. Optimization of PCR Conditions to amplify Short Tandem Repeats (STR) of Human Genomic DNA. *Int J Agric Biol*. 2002;4(1).
10. Joshi M, J.D D. Polymerase Chain Reaction: Methods, Principles and Application. *Int J Biomed Res*. 2010;1(2):81-97.
11. Lorenz T. Polymerase Chain Reaction : Basic Protocol Plus Troubleshooting and Optimization Strategies. *J Vis Exp*. 2012;63:115. doi:10.3791/3998
12. Benbouza H, Jacquemin J, Baudoin J, Guy M. Optimization of a reliable , fast , cheap and sensitive silver staining method to detect SSR markers in polyacrylamide gels. *Biotechnol Agron Soc Environ*. 2006;10(2):77-81.
13. Lee JC, Lin Y, Tsai L, Lin C, Huang Y, Hsieh H. A novel strategy for sibship determination in trio sibling model. *Croat Med J*. 2012;53:336-342. doi:10.3325/cmj.2012.53.336
14. Tzeng CH, Lyou JY, Chen YR, Hu HY, Lin JS, Wang SY. Parentage Testing. *Transfusion*. 2000;40:840-845.
15. Butler JM, Hill CR. Biology and Genetics of New Autosomal STR Loci Useful for Forensic DNA Analysis. In: *Forensic Science Review*. Vol 4049. Central Police University Press; 2012:15-26. doi:1042-7201/24-01/Jan. 2012/15–26
16. Hameed IH, Ommer AJ, Murad AF, Mohammed GJ. Allele frequency data of 21 autosomal short tandem repeat loci in Mesan and Basra provinces in South Iraq. *Egypt J Forensic Sci*. 2015;5(4):150-156. doi:10.1016/j.ejfs.2014.10.003
17. Dobashi Y, Kido A, Fujitani N, Hara M, Susukida R, Oya M. STR data for the AmpFLSTR Identifiler loci in Bangladeshi and Indonesian populations. *Leg Med*. 2005;7:222-226. doi:10.1016/j.legalmed.2005.04.001
18. Untoro E, Surya D, Pu C, Wu F. Allele frequency of CODIS 13 in Indonesian population. *Leg Med*. 2009;11:S203-S205. doi:10.1016/j.legalmed.2009.01.007.

19. Sosiawan A, Yudianto A, Furqoni AH, Martin S, Nzilibili M. Full-sibling allelic frequency and sharing among Madurese : STR technique by 12 locus and the sex-typing amelogenin gene. *Egypt J Forensic Sci*. 2019;9(38):1-10.
20. Koesbardiati T, Yudianto A, Murti DB, Suriyanto RA. Loci STR CODIS (TH01,TPOX) Genetic Variation on Gilimanuk Man (Bali Islands). 2013;33(2):133-150.
21. Hidayat T, Susanti R. Analisis genetik lokus CSF1PO, TH01, dan TPOX short tandem repeats pada etnis minangkabau. *Maj Kedokt Andalas*. 2018;41(3):94-100. doi:10.25077/mka.v41.i3.p94-100.2018
22. Maeda K, Murakami C, Irie W, et al. The case of 2 siblings that identified not only by DNA profiling. *Forensic Sci Int*. 2015;5:555-556. doi:http://dx.doi.org/10.1016/j.fsigss.2015.09.219
23. Reid TM, Ph D, Wolf CA, Kraemer CM, Lee SC, Ph D. Specificity of Sibship Determination Using the ABI Identifiler Multiplex System. 2004;49(6):2-4.
24. Sari NK. Determining the Genetic Similarities and Variability of Javanese and Arab Ethnic Families with DNA Fingerprint in Malang East Java Indonesia. *J Ilm Sains*. 2017;17(1).