CASE REPORT

X-chromosome Short Tandem Repeats Resolved Complex Kinship Cases in Malaysia: Case Reports

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ABSTRACT

The article reports the first use of X-chromosome short tandem repeats (X-STR) in two Malaysian cases *viz*. disputed siblings and deficiency maternity (maternal grandparents). X-STR profiling from blood on FTA cards using 12 loci successfully proved half-siblings relationships and established the maternal grandmother-child relationship, highlighting the potential of X-STR analysis in resolving kinship investigations for forensic casework in Malaysia.
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INTRODUCTION

Human identification using autosomal short tandem repeats (STR) has been the popular choice for convicting and/or exonerating suspects, disaster victim identification and kinship investigations. In certain kinship cases, autosomal STR markers maybe insufficient to provide conclusive results. This is because in many kinship cases, DNA specimens from both parents and siblings are not always available for comparison. The emergence of X-STR in resolving complex kinship investigations (e.g. cases involving half-siblings or paternal grandmother/granddaughter relationships) prove pertinent due to its unique mode of inheritance. Unlike Y-STR and mitochondria DNA which only provide paternal and maternal information, respectively, the X-STRs provide information on genetic material inherited from both parents in female samples and exclusively from the mother in male samples (1). When examining paternal half-sisters or paternal grandmother/granddaughter relationships, the existence of a single X chromosome in males that doesn't recombine during meiosis (passed on to all female descendants as a haplotype) has shown as significant to establish the relationship. In contrast, the pair of X chromosomes in females recombine with one another directly during meiosis and therefore, the mother's recombined X chromosome copy is randomly passed on to her sons and daughters (2).

A search in the Scopus databases using the two words combinations with the logical operator ("X-STR" AND "case report") produced only 29 publications involving X-STR analyses, 11 of which reported real caseworks. This scarcity is prevalent globally, especially in South East Asia where X-STR analysis is not widely used (1).

Here, the first two applications of X-STR analysis in Malaysia, involving (a) one case of disputed siblings and another on (b) deficiency maternity with the presence of maternal grandparents, are reported.

CASE REPORT

Case #1 involved disputed siblings, requested by the National Registration Department for amending the identity of a Malaysian Indian male (A) whom both parents were deceased. Blood specimens from two female siblings (B and C) purportedly fathered by the same man were analyzed for 24 autosomal STR markers. While half-sibling relationship between A and B were established, either paternal nor maternal relationship could be conclusively determined. The inference of likelihood ratio (LR) may appear presumptive and can be contested.

Case #2 involved a request for identifying the next of kin of a Malay newborn male by the Department of Social Welfare. The mother died 10 days postpartum, and the legality of parent's marriage was disputed (absence of a legal marriage certificate). The maternal grandmother possessed a forged identity document, belonging to a person whom passed away in 2021. Blood specimens from the alleged father, grandmother and grandfather were submitted for the autosomal STR analysis. The use of X-STR analysis in resolving complex kinship in Malaysia has been highlighted in these two cases, indicating its potential for forensic application.

The ethical approval was granted by the Human Research Ethics Committee of Universiti Sains Islam Malaysia (USIM/JKEP/2022-225) and the permission to publish the data was given by the Director of Department of Chemistry Malaysia (DCM), Johor State (JKJB: (P).2/272(123)). Each blood specimen (~2 mL) was collected in an EDTA blood tube by a registered government medical officer which later submitted to the Forensic DNA Section Southern Zone, DCM, Johor State Laboratory by the investigating officer. At the laboratory, each blood specimen was kept at -20 °C prior to analysis. The blood was directly stained onto a Whatman® FTA® classic cards, air-dried and punched (approximately 1.2 mm) using Harris Micro Punch™ before direct amplification. The punched blood specimen on FTA card was purified following the internal protocol established by DCM (JKM DNA-EX010), involving two washes with 200 µL of 10 mM NaOH followed by two additional washes with 200 µL of Tris-EDTA buffer. Autosomal STRs amplification was performed using the GlobalFiler™ Express PCR Amplification Kit (Thermo Fisher Scientific). The purified blood samples were amplified using the reduced PCR reaction volume of Qiagen Investigator® Argus X-12 QS Kit (3). The PCR products were separated using the Applied Biosystems 3500xL Genetic Analyzer (Thermo Fisher Scientific) and raw data were analyzed using the GeneMapper™ ID-X v1.5 software.

The LR for autosomal STRs was calculated using DNAVIEW version 37.56, based on arbitrary kinship scenarios. For paternity testing, the combined paternity index was computed by multiplying the paternity index values for each locus, while FamLinkX version 2.9.2 was used for calculating the same for X-STRs data. For inferring the LR support, the categorical definition of strengths provided by the literature (4) detailed below was used:

(a) 1: Uninformative
(b) 2-99: Limited support
(c) 100-9,999: Moderate support
(d) 10,000-999,999: Strong support
(e) ≥ 1,000,000: Very strong support

LR values for the X-STRs data were calculated using the Malaysian population data for Indians (Case #1) and Malays (Case #2).

DISCUSSION

Case #1

The autosomal STRs typing (Table I) indicated that A and B were half-siblings (LR= 5590), fell well within the 100-9,999 LR range, indicating moderate support for the relationship, which may not be sufficient for forensic cases (4). However, the autosomal STRs alone did not provide any information in proving whether the relationship was maternally or paternally related, necessitating the use of X-STR analysis for investigation. As for A and C, they were not biologically related as siblings or half-siblings (LR=0.0017).

Table I: Autosomal STR profiles for disputed siblings (Case 1) using the GlobalFiler™ Express PCR Amplification Kit

Locus	A	B	C
D3S1358	15	14,17	17,18
vWA	17,19	18,19	18,21
D16S539	11,13	11	9,10
CSF1PO	10,12	10,12	11
TPOX	8,11	8,11	9,12
Yindel	1	NM	NM
Amelogenin	XY	XX	XX
D8S1179	16	13,16	14
D21S11	29,30	30,31.2	29,33.2
D18S51	16	14,16	15,19
DYS391	9	NM	NM
D2S441	11,12	10,14	11
D19S433	15,15.2	13,14.2	13,14
TH01	9,9.3	6,9.3	6,9.3
FGA	21,26	21,22.2	20,25

CONTINUE

Table I: Autosomal STR profiles for disputed siblings (Case 1) using the GlobalFiler™ Express PCR Amplification Kit (CONT.)

Locus	A	B	C
D22S1045	13,16	13,15	11,14
D5S818	9,10	10,13	11
D13S317	11,12	8,11	10,12
D7S820	11	8,11	9,11
SE33	25.2,27.2	18,25.2	16,21
D10S1248	13,14	14	14
D1S1656	15	11,15	15,16
D12S391	17,19	17,22	18.3,22
D2S1338	19,23	19,22	22,23

X and Y represent the sex chromosome. (XX=female; XY=male)
NM refers to Non-male profile.

Table II represents the X-STRs profiles for the two disputed siblings, supporting that A and B were maternally related, as they shared 10 out of 12 alleles in the X-STRs analysis (LR= 2.497 x 106). The partial matching haplotypes inheritance can be attributable to the maternal recombinant events (5). Shall the two sisters (B and C) be fathered by the same man; the sisters would have inherited the exact paternal X-chromosome (1). However, the results of the X-STR analysis revealed no evidence of shared paternal X haplotype, disputing that both B and C were biological sisters. Interestingly, the fact that they were maternally related was also not supported since they only shared similarities in 5 out of 12 X-STRs loci (LR=0.5519), even lower than the value of 1.0 to indicate any possible maternal relationship. Notwithstanding, the possibility for other types of relationships (e.g. cousins) was not investigated in this specific case request by the National Registration Department. Therefore, owing to the unique inheritance pattern of X-STR, this particular case demonstrated for the first time the usefulness of such an analysis for revealing the paternal and maternal relationships among disputed siblings in the diverse Malaysian population. This fact can be of applied value for reconstructing the paternal profile of the putative father in cases of disputed siblings.

Table II: X-STRs profiles for disputed siblings (Case 1) using the Qiagen Investigator® Argus X-12 QS Kit

Locus	A	B	C
DXS10103	18*	16,18*	19,20
DXS8378	11*	10^,11*	10^,12
DXS10101	32.2	32,33	29.2,31.2
DXS10134	35*	35*,38	36,39
DXS10074	19	17,18	16
DXS7132	14*	13,14*^	14^,15
DXS10135	21*	21*,27	17,35

CONTINUE

Table II: X-STRs profiles for disputed siblings (Case 1) using the Qiagen Investigator® Argus X-12 QS Kit (CONT.)

Locus	A	B	C
DXS7423	17*	14^,17*	14^
DXS10146	30*	27,30*	26,42.2
DXS10079	22*	17,22*	20
HPRTB	14*	12^,14*	12^,13
DXS10148	26.1*	25.1^,26.1*	20,25.1^

* Refers to the shared alleles between A and B.
^ Refers to the shared alleles between B and C.

Case #2

In this case, the initial autosomal STRs results (Table III) supported that (a) the alleged father as being the biological father of the newborn male and (b) the biological association of the newborn male with both the maternal grandparents can be well established with high probabilities (99.9999%) for both scenarios. To simulate a scenario whereby the biological parents are missing, leaving only the child and the questioned grandparents, the X-STR analysis may prove useful. Table IV depicts the X-STRs profiles for the newborn male and the purported maternal grandmother. The results revealed a full matching haplotype at all the 12 loci (LR=2.472 x 108), indicating a very strong support of the relatedness (4). Hence, the proposition that the purported maternal grandmother as the biological maternal grandmother was highly supported.

Table III: Autosomal STR profiles for deficiency maternity cases with the presence of maternal grandparents (Case 2) using the GlobalFiler™ Express PCR Amplification Kit

Locus	Newborn Male	Alleged Father	Maternal Grandfather	Maternal Grandmother
D3S1358	16,17	16,18	16,17	16,17
vWA	14,17	17	14,20	14,17
D16S539	10,11	11,13	10,11	9,11
CSF1PO	11,12	11,13	11,12	10
TPOX	8,9	9,11	8,12	9,11
Yindel	1	1	1	NM
Amelo-genin	XY	XY	XY	XX
D8S1179	11	11	14,15	11,14
D21S11	29,32.2	29	29,32.2	32,33.2
D18S51	14,15	14	16,18	15
DYS391	11	11	11	NM
D2S441	11.3,12	11,11.3	12	10,11
D19S433	14,15	14,15	13.2,14	15
TH01	6,7	6,7	9,9.3	7,9
FGA	23	23	19,23	22,25
D22S1045	15,16	15	11,15	16,17
D5S818	10,11	11,12	9,13	10

CONTINUE

Table III: Autosomal STR profiles for deficiency maternity cases with the presence of maternal grandparents (Case 2) using the GlobalFiler™ Express PCR Amplification Kit (CONT.)

Locus	Newborn Male	Alleged Father	Maternal Grandfather	Maternal Grandmother
D13S317	9	9,12	12	9,12
D7S820	12	10,12	11,12	8,10
SE33	27.2,29.2	29.2,31.2	25.2,27.2	27.2,29.2
D10S1248	13,15	14,15	13	12,15
D1S1656	11,14	11,13	14,16	15,17
D12S391	19,20	19,22	22	20,23
D2S1338	23,24	22,23	24	21,24

X and Y represent the sex chromosome. (XX=female; XY=male)
NM refers to Non-male profile.

Table IV: X-STRs profiles for the newborn male and the purported maternal grandmother (Case 2) using the Qiagen Investigator® Argus X-12 QS Kit

Locus	Newborn Male	Maternal Grandmother
DXS10103	16*	16*,19
DXS8378	10*	10*,12
DXS10101	31*	30.2,31*
DXS10134	32*	32*,38
DXS10074	17*	17*,18
DXS7132	14*	14*,15
DXS10135	29*	27,29*
DXS7423	14*	14*
DXS10146	28*	24,28*
DXS10079	20*	20*
HPRTB	14*	13,14*
DXS10148	18*	18*,26.1

* Refers to the shared alleles between the newborn male and the maternal grandmother.

It has to be mentioned here that utilization of X-STRs for deficiency paternity testing involving paternal grandparents has been duly reported; the same remains scarce for the maternal grandparents (2). Because a grandchild has a 25% chance of obtaining a specific gene from the maternal grandmother (1), it is important to genotype the X-STR markers between them to determine their relatedness in the event that the mother is not present. A male child may or may not inherit the X chromosome from his mother that could be transmitted from her father (the child maternal grandfather). Additionally, due to recombination during meiosis, the child could inherit a mixture of alleles from both maternal grandparents (2). However, recombination probabilities would require the establishment of data set for determining the possible relationship, an avenue for further research. Therefore, this case demonstrated explicitly the usefulness of X-STR analysis for deficiency maternity cases with the presence of maternal grandparents.

CONCLUSION

The successful proving of half-siblings and maternal grandmother-child relationships in these cases supported the use of X-STR analysis in complex kinship investigations, an often-overlooked aspect in many forensic DNA laboratories. It may complement the autosomal and Y-STRs analyses for routine forensic DNA profiling.

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