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Ancestry Single Nucleotide Polymorphisms in Malayo-Polynesian Sub-Groups in the Malay Population: A Preliminary Study

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Abstract

Malay populations are sub-groups of the Malayo-Polynesian, with various sub-ethnic groups believed to have different ancestral origins based on their migration centuries ago. The variability in the genetic pattern within the Malay population might impose different risks and disease probabilities or certain phenotypes. This study aimed to analyze single-nucleotide polymorphisms (SNPs) in Malayo-Polynesian sub-groups of the Malay population in Southeast Asia. SNPs were genotyped through T-ARMS PCR in 52 unrelated individuals from three Malay sub-groups: Champa (n=16), Kelantan (n=25), and Bugis (n=11). Most (60%) of the SNP genotypes showed a similarity with all Malay sub-ethnic groups. The PCA plot showed that all Malay sub-ethnic groups were slightly separated but clustered together with Asian populations compared with population groups from other geographical regions. Overall, the SNP genotyping generated from this study provides essential knowledge of the genetic relationships within Malay sub-ethnic groups in Southeast Asia and other global populations. Additionally, these findings may be used for future illness research, drug response estimation, and the development of preventive and therapeutic management strategies toward more personalized or precision medicine.

Keywords: AIM-SNP, Malayo-Polynesian, ARMS-PCR, allele frequency

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INTRODUCTION

Malayo-Polynesian, also known as Austronesian, is one of a family of languages spoken by approximately 300 million people. It extends from Madagascar to the central Pacific, including the Malay Peninsula, Indonesia, New Guinea, the Philippines, Taiwan, the Melanesian, Micronesian, and Polynesian islands, and New Zealand [1]. As an ethnic group, Malays speak the Malayo-Polynesian language spoken mostly across Southeast Asia, such as Malaysia, Singapore, Brunei, Indonesia, southern Thailand, the southern Philippines, the Christmas and Cocos Islands of Australia, western Australia, Sri Lanka, and Cape Town in South Africa [2]. There are various sub-ethnic groups within the Malay population, such as Champa Malay, Kelantan Malay and Bugis Malay [3]. The Champa Malay, also known as the Cham people, live mainly in Vietnam and Cambodia, with smaller numbers living in Thailand and Malaysia. Most Cham in Vietnam live in the country's southern region, while in Cambodia they are concentrated in Kampong Cham, Kampot, and Phnom Penh. Here, they comprise between 1–2% of the total population [4]. Kelantan Malay reside in Kelantan, located northeast of Peninsular Malaysia, which is flanked to the north by Thailand, the northeast by the South China Sea, the east by Terengganu, the south by Pahang, and the west by Perak. Kelantan's geographical location allowed Kelantan Malays to come into contact with the northern half of the Malay Peninsula, as shown by the close genetic relationship between Kelantan Malay, Kedah Malay, and Thai Pattani [5, 6]. However, the Kelantan Malay are genetically distinct from the other Malay populations in the peninsula's western (Minang Malay) and southern (Jawa Malay and Bugis Malay) regions. The Bugis Malay originated on Celebes (now Sulawesi) and moved throughout Indonesia, encroaching on western Sulawesi (mainly Polewali Mandar, Mamuju, Central Mamuju, and North Mamuju), and Southeast Sulawesi, where the Bugis ruled several districts, including Kendari, Kolaka, North Kolaka, and East Kolaka [7, 8]. When Makassar was captured by the Dutch East India Company, the Bugis Malay migrated to the Malay Archipelago, where they constructed colonies along the banks of the Klang and Selangor rivers in the peninsula's southwest, establishing a Buginese state in the Selangor region during the 17th century. By 1722, the Bugis Malay had built a Buginese state in Riau, an area straddling the island of Sumatra's east-central portion [8].

The genetic ancestry of these Malay populations has been widely studied to determine the origin of the Malay population [5, 6, 9, 10]. These studies were conducted using several DNA markers such as single-nucleotide polymorphisms (SNPs), short tandem repeats (STRs), and insertion-deletion polymorphisms (INDELs) [11, 12]. Due to the availability of numerous high-throughput analysis technologies, SNPs are frequently used to investigate genetic differences between people and communities [12, 13]. The detection of SNPs can be carried out through tetra-primer amplification refractory mutation system polymerase chain reaction (T-ARMS PCR), which utilizes two outer primers and two allele-specific inner primers to amplify the allele-specific sequence in a single PCR reaction [14, 15, 16]. The outer primers serve as an internal control for the PCR, whereas the inner primers are positioned unequally from the corresponding outer primer to create amplicons of different sizes that can be distinguished by gel electrophoresis [17]. Therefore, the combinations of outer and specific inner primers produce allele-specific amplicons depending on the genotype [17].

Research on the genetic structure in Malay sub-ethnic groups in Southeast Asia is quite limited. Since the Malay population consists of various sub-ethnic groups that are believed to have different ancestral origins, it is important to analyse the ancestry of these sub-ethnic groups [18]. Therefore, this study focuses on ancestry SNPs in Malayo-Polynesian sub-groups of the Malay population in Southeast Asia.

MATERIALS AND METHODS

Subject recruitment

A total of 52 Malay individuals, consisting of 25 individuals from Kelantan Malay, 16 from

Champa Malay, and 11 from Bugis Malay, were recruited. Subject recruitment was carried out in Kelantan in Malaysia, Sulawesi in Indonesia, and Kampung Cham in Cambodia. Inclusion and exclusion criteria are listed in Table 1. Informed consent was obtained from all the recruited subjects. The study protocol was approved by the Universiti Sains Malaysia Human Ethics Committee.

Table 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Healthy adult, ≥18 years old, consented to the study	History of any self-reported systemic diseases like hypertension and diabetes; congenital abnormalities like spina bifida, Down's Syndrome and hemophilia
Known family tree and confirmed by other family members	Unclear family history
Descendants of the same ethnicity and no intermarriage with other ethnic groups for at least three generations	Descendants of other ethnic groups or mixed ethnicity
All three generations born and residing in similar states or provinces	
Ability to communicate in the local dialect	

SNPs selection

The SNP dataset used in this study was obtained from Padillah Yahya [10]. In the current study, ten SNPs were selected as they are reportedly present in the Malay population, according to the National Centre for Biotechnology Information (NCBI) Single Nucleotide Polymorphism Database (dbSNP) (https://www.ncbi.nlm.nih.gov/snp/?cmd=search). The chosen SNPs are listed in Table 2.

Table 2. SNPs dataset

SNP ID	Chromosome number	Allele
rs1407728	6	G>T
rs1250233	2	G>A
rs200354	14	G>T
rs9323786	14	C>T
rs2194530	14	G>A
rs10496298	2	T>C
rs10512333	9	A>C
rs2758988	10	A>T
rs4980285	10	C>A

DNA extraction

Following recruitment, 3.0 mL of blood was withdrawn from each subject for genotyping. DNA was extracted using MegaBio plus General Genomic DNA Purification Kit (Hangzhou Bioer Technology Co., Ltd. (BIOER) and the quality of extracted DNA was checked using A260/A280 ratio, with a preferred range of 1.7–2.1. Extracted DNA was kept at -20ºC until further use.

SNP genotyping

SNP genotyping was performed using T-ARMS PCR, which required four primers: a forward outer (FO), reverse outer (RO), forward inner (FI), and reverse inner (RI). The sequences of primers for each SNP and their respective melting temperatures (T_m) are listed in Table 3**.**

Table 3. List of primers and respective T_m

SNP ID	Primers	Sequences (5' – 3')	T_m (ºC)
rs1407728	FO	AATGTGGAAAGAGAAGATGATCAAGGA	65.0
	RO	GGTTCCATTTTTTTCCTTTAAACCCAGT	65.0
	FI	TATGGAAGATCTCCAGGAGTAGAATTGTT	65.0
	RI	TTCATAGGAAAGCTAAATCAAT	65.0
rs1250233	FO	AAACACTCTGAGATTCACATTCAGTGACT	63.0
	RO	ATTGATAATGCAGGTCCTAGGGAAAG	63.0
	FI	GGACAGAGCTGAAATTGAAATCAAGA	64.0
	RI	AAGAGCAAGAATTTTGTGTCAGGAATAC	63.0
rs200354	FO	GCAGATCACCACCCCAGGTGACAAATGG	75.0
	RO	TGCTCCGTGTTCTTTCCCTTTTGGGCAT	75.0
	FI	CTATGCACCATGGCCATTTGGAACTAGG	75.0
	RI	TCTACAATGGACAGGGCAGCCTCTCACCAA	75.0
rs9323786	FO	TGGACTCCTATTATGGCACTTGGAGTAT	61.0
	RO	GTTGCTATACCGCTATGTCTAGTGGTTG	61.0
	FI	GTAAATGTAATAGGTGTTCCATAAATAGTT	57.0
	RI	CACTGTTTTAGCGTTCCTTGATTCACAG	65.0
rs2194530	FO	ACTTGTTAAAGCCTACGCTTAACTTG	56.0
	RO	TTCTATGTGATCCAACACTCTATGG	56.0
	FI	GGTCAAGGTTTTGCTACTTCACCA	56.0
	RI	AGAAGAAAAGATCCAGGAAACTTAC	61.0
rs10496298	FO	CACTAATAATATCCCTGCCT	48.7
	RO	TAAGTTCAATAAGGTGAAAACTG	49.0
	FI	TCTGTAGATTGTAGTGAATCATAAT	49.5
	RI	TAAACTGGAGTAAATTAATTCG	51.0
rs10512333	FO	ATTATGCCTGCCAAAAGTTACGTGCGTAGT	70.0
	RO	CCCATTGGCTATGTGGTGTAAGCCGTAT	70.0
	FI	TCGCCTTCTTATGAGCAATTTCTGAAGAC	69.0
	RI	TGGGTTCACTGTCACCTTTAGCCACT	71.0
rs2758988	FO	GCTCAAGGCCACAGTTAGTCCTACCTAC	66.0
	RO	GAGCATATCTTTGGAATGAGGAGGACAT	66.0
	FI	TGCCCTGTACAAATGTGAATTCTACTCCT	66.0
	RI	ATTGCCGTTCTTTATCTGGTAGCTCCTT	66.0
rs4980285	FO	TTGCTATTTAAGGGATAAGAACATTTCCA	64.0
	RO	GCAAACAAATTTTGAGAATGCAAGACTA	64.0
	FI	GGTAAATGCCATGATGTAAATTTGTAAGA	64.0
	RI	CCTAAATTCCTTAATGTTCACACAACCTG	64.0
rs6955239	FO	ATGATTATGGGAGCAGATATTGTGAAAA	63.0
	RO	TTTCCTAGACAGAGCTTTTGATTTCCAC	63.0
	FI	TGACAAAATAGACTTGACATATAGCTTCG	63.0
	RI	ACCCTATTTCTCAAATACATATCTGCCAT	63.0

A total of 12.50 µL of master mix was prepared, consisting of 2.50 µL of 5X PCR buffer, 0.75 µL of 25 mM of magnesium chloride ($MgCl_2$), 0.20 µl of 10mM deoxynucleotide triphosphate (dNTP), 0.25 µL of 0.20 µL of each primer, 0.25 µL of 0.1 U/µL Taq polymerase, 1.00 µL of 11.5 ng/µL DNA template and 6.75 µL of nuclease-free water. The PCR protocol was set at 95ºC for the pre-denaturation step for five minutes, followed by

35 cycles of 94ºC denaturation for 30 seconds, annealing steps for 30 seconds, 72ºC extension for 25 seconds, and 72ºC final extension for three minutes. The annealing temperature (T_a) for each SNP is listed in Table 4. PCR products were loaded into 2% agarose gel and run at 90 V for 60 minutes.

Table 4. Annealing temperature (T_a) for each SNP

SNP ID	T_a (ºC)
rs1407728	55.9
rs1250233	49.0
rs200354	66.9
rs9323786	48.8
rs2194530	56.0
rs10496298	53.0
rs10512333	62.1
rs2758988	58.9
rs4980285	48.4
rs6955239	49.4

Data analysis

Hardy-Weinberg equilibrium (HWE) was determined using the HWE online calculator (https://wpcalc.com/en/equilibrium-hardy-weinberg/). The χ^2 value was 3.84 (degree of freedom, df=1; significance level, α=0.05). Any sub-groups with χ^2 value <3.84 were considered, as in HWE [19]. The principal component analysis (PCA) was constructed to map the genetic structure between the Malay sub-groups and other populations such as African, African American, Asian, East Asian, South Asian, Latin American, European, and others, using XLSTAT software. In the PCA analysis, the factor for the first two principal components, F1 and F2, was used to construct a PCA graph.

RESULTS

All DNA samples (n=52) obtained from the three Malay sub-ethnic individuals (Champa, Kelantan, and Bugis Malays) were successfully genotyped for the ten SNPs (rs1407728, rs1250233, rs200354, rs9323786, rs2194530, rs10496298, rs10512333, rs2758988, rs4980285, and rs6955239) using T-ARMS PCR. This technique required four primers: a forward outer (FO), reverse outer (RO), forward inner (FI), and reverse inner (RI). The FO/RO primer combination creates the SNP locus's outer fragment (OF) and serves as an internal control for the PCR. The FI/RO and FO/RI primer combinations produce allele-specific amplicons depending on the sample's genotype (Figures 1–3).

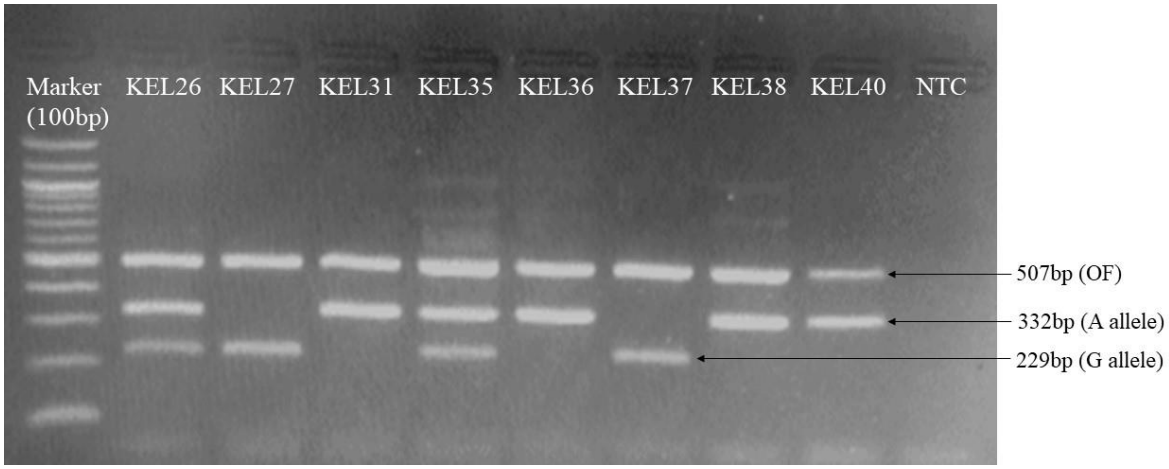

Figure 1. Agarose gel electrophoresis of the rs1250233 allele-specific products for Kelantan Malay. The allele-specific products for this SNP are A and G alleles. The A allele was detected at 332 bp while the G allele was at 229 bp. The outer fragment (OF) was observed at 507 bp, at the top of the allele-specific products

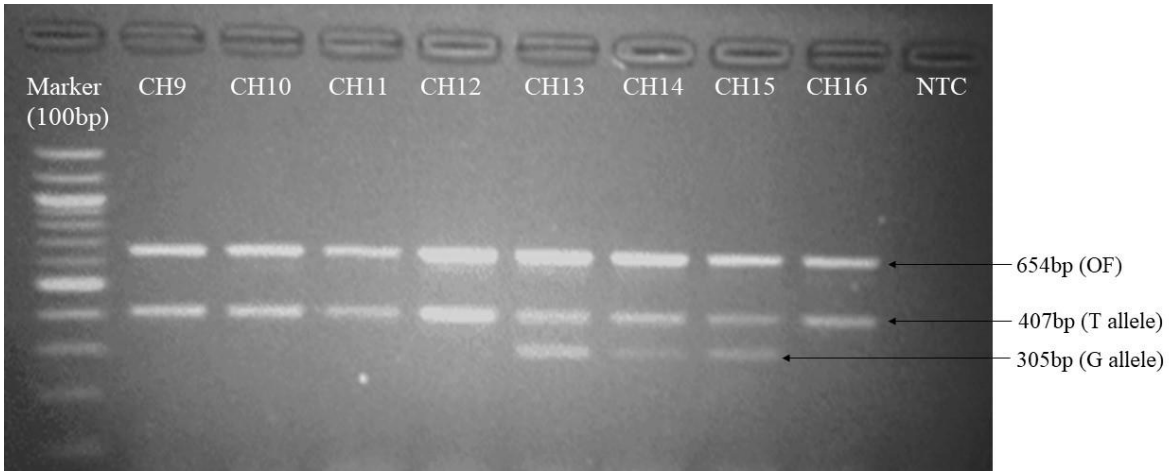

Figure 2. Agarose gel electrophoresis of the rs200354 allele-specific products for Champa Malay. The allele-specific products for this SNP are T and G alleles. The T allele was detected at 407 bp while the G allele was at 305 bp. The outer fragment (OF) was observed at 654 bp, at the top of the allele-specific products

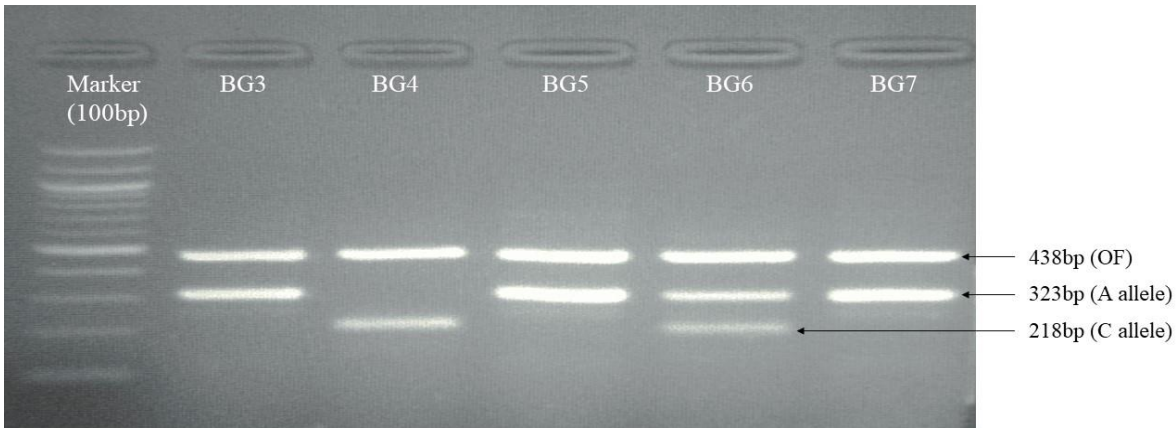

Figure 3. Agarose gel electrophoresis of the rs4980285 allele-specific products for Bugis Malay. The allele-specific products for this SNP are A and C alleles. The A allele was detected at 323 bp, while the C allele was at 218 bp. The outer fragment (OF) was observed at 483 bp, at the top of the allele-specific products

The dataset in Table 5 shows the genotype frequencies of SNPs in the studied Malay sub-ethnic groups. Six SNPs (rs200354, rs9323786, rs10496298, rs10512333, rs2758988, and rs6955239) showed a similarity with all Malay sub-ethnic groups. For SNP rs200354, all Malay sub-groups are predominantly homozygote genotype TT (Champa Malay=0.688, Kelantan Malay=0.640, and Bugis Malay=0.909), while for SNP rs9323786, homozygote genotype CC was predominant in all Malay sub-ethnic groups with a genotype frequency of 1.000. For SNP rs10496298, all Malay populations are observed to be predominantly heterozygote genotype CT (Champa Malay=0.939, Kelantan Malay=0.680, and Bugis Malay=0.909), whereas for SNP rs10512333, all the Malay sub-groups were predominantly heterozygote genotype AC (Champa Malay=0.875, Kelantan Malay=0.800, and Bugis Malay=0.727). For SNPs rs2758988 and rs6955239, heterozygote genotype TA and heterozygote genotype AG were observed to be predominantly in all the Malay sub-ethnic groups, respectively.

Table 5. SNP genotype frequencies

		rs1407728		
Population		Champa Malay (CH)	Kelantan Malay (KN/KEL)	Bugis Malay (BG)
No. of Samples		16	25	11
Genotype	GG	0.563	0.320	0.455
	GT	0.438	0.600	0.182
	TT	0.000	0.080	0.364

		rs1250233		
Population		Champa Malay (CH)	Kelantan Malay (KN/KEL)	Bugis Malay (BG)
No. of Samples		16	25	11
Genotype	GG	0.500	0.080	0.000
	AG	0.375	0.240	0.364
	AA	0.125	0.680	0.636

		rs200354		
Population		Champa Malay (CH)	Kelantan Malay (KN/KEL)	Bugis Malay (BG)
No. of Samples		16	25	11
Genotype	TT	0.688	0.640	0.909
	TG	0.250	0.240	0.000
	GG	0.063	0.120	0.091

		rs9323786		
Population		Champa Malay (CH)	Kelantan Malay (KN/KEL)	Bugis Malay (BG)
No. of Samples		16	25	11
Genotype	CC	1.000	1.000	1.000
	CT	0.000	0.000	0.000
	TT	0.000	0.000	0.000

		rs2194530		
Population		Champa Malay (CH)	Kelantan Malay (KN/KEL)	Bugis Malay (BG)
No. of Samples		16	25	11
Genotype	GG	0.375	0.360	0.636
	GA	0.625	0.640	0.364
	AA	0.000	0.000	0.000

		rs10496298		
Population		Champa Malay (CH)	Kelantan Malay (KN/KEL)	Bugis Malay (BG)
No. of Samples		16	25	11
Genotype	CC	0.000	0.000	0.000
	CT	0.938	0.680	0.909
	TT	0.063	0.320	0.091

		rs10512333		
Population		Champa Malay (CH)	Kelantan Malay (KN/KEL)	Bugis Malay (BG)
No. of Samples		16	25	11
Genotype	AA	0.125	0.080	0.273
	AC	0.875	0.800	0.727
	CC	0.000	0.120	0.000

		rs2758988		
Population		Champa Malay (CH)	Kelantan Malay (KN/KEL)	Bugis Malay (BG)
No. of Samples		16	25	11
Genotype	TT	0.000	0.080	0.000
	TA	0.938	0.920	1.000
	AA	0.063	0.000	0.000

		rs4980285		
Population		Champa Malay (CH)	Kelantan Malay (KN/KEL)	Bugis Malay (BG)
No. of Samples		16	25	11
Genotype	CC	0.063	0.120	0.091
	CA	0.563	0.520	0.273
	AA	0.375	0.360	0.636

		rs6955239		
Population		Champa Malay (CH)	Kelantan Malay (KN/KEL)	Bugis Malay (BG)
No. of Samples		16	25	11
Genotype	AA	0.250	0.040	0.091
	AG	0.500	0.880	0.909
	GG	0.250	0.080	0.000

The Hardy-Weinberg analysis of SNP genotyping (Tables 6–7) showed that all Malay sub-ethnic groups were in equilibrium for SNPs rs1250233, rs9323786, and rs4980285. However, SNPs rs1407728 and rs200354 were in a Hardy-Weinberg disequilibrium (HWD) among Bugis Malay only, while for SNP rs2194530, only the Kelantan Malay group deviated from HWE. On the other hand, for SNPs rs10512333 and rs6955239, the Malay sub-ethnic groups that followed the HWE were the Bugis Malay and Champa Malay, respectively. In addition, all the Malay sub-ethnic groups were not in equilibrium for SNPs rs10496298 and rs275898.

Table 6. Hardy-Weinberg (HW) analysis of the three Malay sub-ethnic groups

rs1407728									
	Champa (n=16)			Kelantan (n=25)			Bugis (n=11)		
Genotype	Observed	Expected	Chi-squared (χ^2)	Observed	Expected	Chi-squared (χ^2)	Observed	Expected	Chi-squared (χ^2)
GG	9	9	1.25*	8	10	1.87*	5	3	4.41
GT	7	5		15	12		2	5	
TT	0	1		2	4		4	2	

rs1250233									
	Champa (n=16)			Kelantan (n=25)			Bugis (n=11)		
Genotype	Observed	Expected	Chi-squared (χ^2)	Observed	Expected	Chi-squared (χ^2)	Observed	Expected	Chi-squared (χ^2)
GG	8	8	0.26*	2	1	1.56*	0	1	0.54*
GA	6	7		6	8		4	3	
AA	2	2		17	16		7	7	

rs200354									
	Champa (n=16)			Kelantan (n=25)			Bugis (n=11)		
Genotype	Observed	Expected	Chi-squared (χ^2)	Observed	Expected	Chi-squared (χ^2)	Observed	Expected	Chi-squared (χ^2)
TT	11	10	0.52*	16	14	2.93*	10	9	11.00
TG	4	5		6	9		0	2	
GG	1	1		3	1		1	0	

Table 6: Hardy-Weinberg (HW) analysis of Malay sub-ethnic groups (continued)

rs9323786									
	Champa (n=16)			Kelantan (n=25)			Bugis (n=11)		
Genotype	Observed	Expected	Chi-squared (χ^2)	Observed	Expected	Chi-squared (χ^2)	Observed	Expected	Chi-squared (χ^2)
CC	16	16	0*	25	25	0*	11	11	0*
CT	0	0		0	0		0	0	
TT	0	0		0	0		0	0	

rs2194530									
	Champa (n=16)			Kelantan (n=25)			Bugis (n=11)		
Genotype	Observed	Expected	Chi-squared (χ^2)	Observed	Expected	Chi-squared (χ^2)	Observed	Expected	Chi-squared (χ^2)
GG	6	7	3.31*	9	11	5.54	7	7	0.54*
GA	10	6		16	11		4	3	
AA	0	1		0	3		0	1	

rs10496298									
	Champa (n=16)			Kelantan (n=25)			Bugis (n=11)		
Genotype	Observed	Expected	Chi-squared (χ^2)	Observed	Expected	Chi-squared (χ^2)	Observed	Expected	Chi-squared (χ^2)
CC	0	3	12.46	0	2	6.64	0	2	7.64
CT	15	7		17	12		10	6	
TT	1	4		8	11		1	3	

rs10512333									
	Champa (n=16)			Kelantan (n=25)			Bugis (n=11)		
Genotype	Observed	Expected	Chi-squared (χ^2)	Observed	Expected	Chi-squared (χ^2)	Observed	Expected	Chi-squared (χ^2)
AA	2	5	9.68	2	6	9.08	3	5	3.59*
AC	14	8		20	12		8	5	
CC	0	3		3	7		0	1	

Table 6: Hardy-Weinberg (HW) analysis of Malay sub-ethnic groups (continued)

rs2758988									
	Champa (n=16)			Kelantan (n=25)			Bugis (n=11)		
Genotype	Observed	Expected	Chi-squared (χ^2)	Observed	Expected	Chi-squared (χ^2)	Observed	Expected	Chi-squared (χ^2)
TT	0	4	12.46	2	7	18.14	0	2	11.00
TA	15	8		23	12		11	6	
AA	1	4		0	5		0	3	

rs4980285									
	Champa (n=16)			Kelantan (n=25)			Bugis (n=11)		
Genotype	Observed	Expected	Chi-squared (χ^2)	Observed	Expected	Chi-squared (χ^2)	Observed	Expected	Chi-squared (χ^2)
CC	1	2	0.97*	3	4	0.27*	1	1	0.55*
CA	9	7		13	12		3	4	
AA	6	6		9	9		7	6	

rs6955239									
	Champa (n=16)			Kelantan (n=25)			Bugis (n=11)		
Genotype	Observed	Expected	Chi-squared (χ^2)	Observed	Expected	Chi-squared (χ^2)	Observed	Expected	Chi-squared (χ^2)
AA	4	4	0*	1	6	14.55	1	3	7.64
AG	8	8		22	12		10	5	
GG	4	4		2	7		0	2	

n=sample size, *=HWE, chi-squared, $\chi^2<3.84$ shows the sub-ethnic group followed the principle of HWE

Table 7. Summary of Hardy-Weinberg (HW) analysis

SNP ID	**Champa**	**Kelantan**	**Bugis**
rs1407728	HWE	HWE	HWD
rs1250233	HWE	HWE	HWE
rs200354	HWE	HWE	HWD
rs9323786	HWE	HWE	HWE
rs2194530	HWE	HWD	HWE
rs10496298	HWD	HWD	HWD
rs10512333	HWD	HWD	HWE
rs2758988	HWD	HWD	HWD
rs4980285	HWE	HWE	HWE
rs6955239	HWE	HWD	HWD

Abbreviation: HWE=Hardy-Weinberg equilibrium, HWD=Hardy-Weinberg disequilibrium

In general, the allele frequencies of SNPs in the three studied Malay sub-ethnic groups are most similar to those of Asian populations, and the genetic relationships were mapped using PCA (see Figure 4 and Table 8, respectively). In the PCA plot, Kelantan Malay, Bugis Malay, and Champa Malay, as plotted against East Asian, Asian, and other Asian populations, demonstrate some degree of genetic affinity with these populations. African, African American, and other African populations are plotted closer to each other, while South Asian, Latin American 1, European, and other populations are plotted approximately to each other. In contrast, Latin American 2 is well separated from other populations.

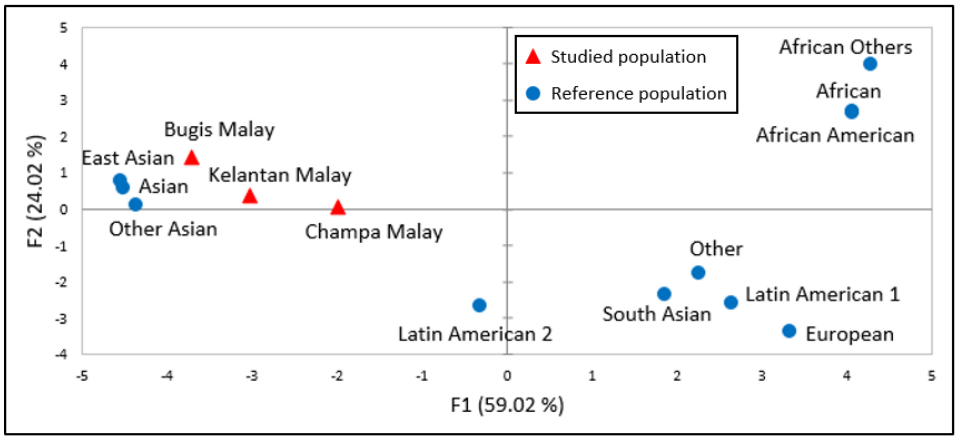

Figure 4. PCA plot of genetic relationship between the studied Malay sub-groups and reference populations (plot constructed using SNP allele frequencies listed in Table 8)

Table 8. SNP allele frequencies in the studied Malay sub-ethnic groups and reference populations

Population	rs1407728		rs1250233		rs9323786		rs200354		rs2194530	
	G	T	G	A	C	T	G	T	G	A
Champa Malay	0.781	0.219	0.688	0.313	1.000	0.000	0.188	0.813	0.688	0.313
Kelantan Malay	0.620	0.380	0.200	0.800	1.000	0.000	0.240	0.760	0.680	0.320
Bugis Malay	0.545	0.455	0.182	0.818	1.000	0.000	0.091	0.909	0.818	0.182
Europeans*	0.988	0.012	0.763	0.237	0.554	0.446	0.852	0.148	0.369	0.631
Africans*	0.917	0.083	0.711	0.289	0.449	0.551	0.413	0.587	0.840	0.160
Other Africans *	0.890	0.110	0.737	0.263	0.448	0.552	0.370	0.630	0.949	0.051
African American*	0.918	0.082	0.710	0.290	0.449	0.551	0.415	0.585	0.836	0.164
Asian*	0.511	0.489	0.095	0.905	0.968	0.032	0.265	0.735	0.589	0.411
East Asian*	0.448	0.552	0.084	0.916	0.971	0.029	0.267	0.733	0.580	0.420
Other Asians*	0.640	0.360	0.139	0.861	0.954	0.046	0.258	0.742	0.620	0.380
Latin Americans 1*	0.930	0.070	0.687	0.313	0.577	0.423	0.698	0.302	0.457	0.543
Latin Americans 2*	0.813	0.187	0.503	0.497	0.762	0.238	0.465	0.535	0.350	0.650
South Asians*	0.976	0.024	0.811	0.189	0.678	0.322	0.676	0.324	0.413	0.587
Others*	0.908	0.092	0.667	0.333	0.611	0.390	0.705	0.295	0.481	0.519

Table 8. SNP allele frequencies in the studied Malay sub-ethnic groups and reference populations (continued)

Population	rs10496298		rs10512333		rs2758988		rs4980285		rs6955239	
	T	C	A	C	A	T	C	A	A	G
Champa Malay	0.531	0.469	0.563	0.438	0.531	0.469	0.344	0.656	0.500	0.500
Kelantan Malay	0.660	0.340	0.480	0.520	0.460	0.540	0.380	0.620	0.480	0.520
Bugis Malay	0.545	0.455	0.636	0.364	0.500	0.500	0.227	0.773	0.545	0.455
Europeans*	0.749	0.251	0.794	0.206	0.221	0.779	0.581	0.419	0.574	0.426
Africans*	0.934	0.066	0.915	0.085	0.672	0.328	0.921	0.080	0.874	0.126
Other Africans *	0.972	0.028	0.941	0.059	0.798	0.202	0.997	0.003	0.940	0.060
African Americans*	0.932	0.068	0.914	0.086	0.667	0.333	0.918	0.082	0.872	0.128
Asians*	0.590	0.410	0.484	0.516	0.920	0.080	0.418	0.582	0.000	1.000
East Asians*	0.630	0.370	0.507	0.493	0.950	0.050	0.424	0.576	0.000	1.000
Other Asians*	0.540	0.460	0.400	0.600	0.810	0.190	0.394	0.606	0.000	1.000
Latin Americans 1*	0.829	0.171	0.831	0.169	0.356	0.644	0.714	0.286	0.000	1.000
Latin Americans 2*	0.590	0.410	0.803	0.197	0.439	0.561	0.515	0.485	0.000	1.000
South Asians*	0.940	0.060	0.716	0.284	0.660	0.340	0.435	0.565	0.050	0.950
Others*	0.709	0.292	0.796	0.204	0.409	0.591	0.601	0.399	0.587	0.413

*References SNPs obtained from NCBI Allele Frequency Aggregator (ALFA) dataset (refer to https://www.ncbi.nlm.nih.gov/snp/docs/gsr/alfa/)[20]

DISCUSSION

In this study, all samples from three Malay sub-ethnic groups residing in geographically different locations (Cambodia (Champa Malay), Peninsular were successfully genotyped using the T-ARMS PCR method. This technique has become the gold standard for SNP genotyping. T-ARMS PCR has proved suitable for SNP genotyping due to being rapid and inexpensive for typing many samples. In comparison to other approaches, T-ARMS PCR is a valuable tool for SNP genotyping due to the variable sensitivity of PCR-SSCP [21]. Sequencing is a time-consuming, technically demanding, and expensive procedure [22], and the likelihood of obtaining a restriction site for an enzyme is almost impossible for PCR-RFLP genotyping [23].

The genotype frequencies of six out of ten SNPs (60%) (rs200354, rs9323786, rs10496298, rs10512333, rs2758988, and rs6955239) showed a similarity with all Malay sub-ethnic groups. This is unsurprising because Champa, Kelantan, and Bugis Malay are Malayo-Polynesian sub-groups, and so there must be a similarity in their genetic structure. However, the remaining SNPs, including rs1407728, rs1250233, rs2194530 and rs4980285, only showed a similarity either between Champa Malay and Bugis Malay or Kelantan Malay and Bugis Malay, as well as between Champa Malay and Kelantan Malay. For SNP rs1407728, Champa Malay and Bugis Malay showed similarities. Most of these populations are homozygote genotype GG, while SNP rs1250233, Kelantan Malay and Bugis Malay are similar as most are homozygote genotype AA. In addition, Champa Malay and Kelantan Malay showed similarities for SNPs rs2194530 and rs4980285. For SNPs rs2194530, the heterozygote genotype GA is dominant in these groups, while for SNP rs4980285, both Champa and Kelantan Malay have a higher frequency of genotype CA (heterozygote). The differences in the SNPs genotype frequencies between these Malay sub-ethnic groups are expected to be due to their geographical location and interaction with the local population, such as mixed marriage. Kelantan Malays live in Kelantan state, northeast of Peninsular Malaysia, while Champa Malays reside in Cambodia. Bugis Malay, on the other hand, are in Sulawesi, Indonesia. Interaction with other populations, such as Chinese, Indians, Arabs, Europeans, and other Asian populations in ancient times may reflect the genetic mosaic in these Malay populations [9].

Despite the correlation due to Malayo-

Polynesian origins, Kelantan Malay also showed a relation with Champa Malay due to trade and religious activities. The Champa Kingdom has had established trading ties with both present-day Malaya, Pattani, Acheh, and Java since the 4th century [24]. Furthermore, for religious activities, Islamic missionaries from Kelantan have been visiting the Cham people in both Cambodia and Vietnam since at least the 16th century. At the same time, many Chams send their children to Kelantan for religious education [25]. For these reasons, we expect more genetic similarity between these two groups [26]. Hence, we observed no significant difference in genotype frequencies in 8 out of 10 SNPs (80%) genotyped between Kelantan Malay and Champa Malay.

HW analysis was carried out to determine whether the Malay sub-ethnic groups are in equilibrium or not. The test estimates the number of homozygous and heterozygous variant carriers based on their allele frequency in populations that are not evolving [27]. We postulate that evolution occurred within the population if the allele frequencies changed from the original frequencies after one round of random mating. Based on the HW analysis, all studied populations showed significant deviation from HWE for SNPs rs10496298 (Champa Malay, χ^2=12.46, Kelantan Malay, χ^2=6.64 and Bugis Malay, χ^2=7.64) and rs2758988 (Champa Malay, χ^2=12.46, Kelantan Malay, χ^2=18.14 and Bugis Malay, χ^2=11.00). In addition, for SNP rs10512333, Champa Malay (χ^2=9.68) and Kelantan Malay (χ^2=9.08) are not in HWE, while for SNP rs6955239, Kelantan Malay (χ^2=14.55) and Bugis Malay (χ^2=7.64) showed deviation from HWE. Moreover, Bugis Malay (χ^2=11.00) and Kelantan Malay (χ^2=5.54) are not in HWE for SNPs rs200354 and rs2194530, respectively.

Five factors might contribute to these HW disequilibria: (1) gene flow, (2) mutation, (3) genetic drift, (4) genetic recombination, and (5) natural selection. If one or more of these conditions are acting on the population, evolution will occur [27], resulting in changes in gene pool frequencies which we could observe in the Malay sub-ethnic populations. Departure from HWE can also be caused by genotyping error. In general, there is no genotyping method that is 100% accurate. As mentioned earlier, in this study, we used T-ARMS PCR. Even though many studies have reported the high accuracy of this method [28–31], it does have some pitfalls. Therefore, it is highly recommended to test the method's accuracy by introducing another genotyping technique (such as sequencing) and comparing the results with these two methods. Unfortunately, we did not validate the T-ARMS PCR method's accuracy in this study. Another potential factor contributing to observed HWE deviation in the Malay sub-groups is relatively small sample size. This is due to the limited number of individuals with 'pure' lineage of the sub-ethnic group. Most either have a mixed-marriage background or have assimilated into the other population. Furthermore, due to the COVID-19 pandemic, our movement was restricted, making the situation more challenging to collect more samples.

The distribution of SNP allele frequencies among Malay sub-groups was compared with population groups from other geographical regions using PCA. Based on the PCA plot (Figure 4), all Malay sub-ethnic groups are slightly separated from each other but are clustered together with Asian populations at the top left of the PCA plot, as most of the Asian population are Malayo-Polynesian speakers. They are well separated from European, Latin American, African, and African American populations. These findings are supported by Deng et al., who suggest that the Malay populations shared four significant components: East Asian, South Asian, Austronesian, and aboriginal Southeast Asian [9]. This finding is in accordance with others [19, 32, 33]. The slight separation observed between each Malay sub-ethnic group demonstrates their variations in genetic structure due to the reasons mentioned earlier.

In addition, African, African American and other African populations show a close genetic structure as they are plotted closer to each other. Similarly, South Asian, Latin American 1, European, and other populations are plotted

approximately to each other at the bottom right of the PCA plot. In contrast, Latin American 2 is well separated from other populations studied. Overall, we found that the PCA plot based on the SNP allele frequencies reflects the populations' geographical proximity and ancestral relationships. However, this finding should be interpreted with some caution as the PCA analysis was carried out using the first two factors (F1 and F2), as these captured most of the information. However, some information might be hidden in the following factors.

CONCLUSION

In this report, we can conclude that ten SNPs in three selected Malay sub-ethnic groups in Southeast Asia have been successfully genotyped using the T-ARMS PCR technique. T-ARMS PCR is an appropriate platform to genotype single-base mutations such as SNPs. The simplicity of this method makes it suitable for the analysis of a large number of samples. The SNP genotyping generated in this study provides essential knowledge on the genetic relationships within Malay sub-ethnic groups in Southeast Asia and other global populations. These Malay sub-ethnic groups show a closer genetic relationship between each other and with Asian people rather than with African and European populations. Even though there are some variations in their genetic structure, these variations reflect their geographical origin and interaction with other people. Furthermore, the comprehensive SNP genotype and allele frequencies information in this report has potential applications for the future study of diseases, estimating drug response, and designing preventive approaches and treatment management for personalized or precision medicine.

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تحليل تعدد أشكال النوكليوتيدات المنفردة (SNP) بين المجموعات الفرعية الملايو بولينيزية لسكان الملايو: دراسة أولية

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الملخص

الخلفية والأهداف: سكان الملايو هم إحدى المجموعات الفرعية للملايو البولينيزية. الملايو لديها مجموعات عرقية فرعية مختلفة يعتقد أن لها أصول أسلاف مختلفة بناءً على هجرتها منذ قرون. قد يفرض التباين في النمط الجيني داخل سكان الملايو مخاطر مختلفة واحتمالات مرضية أو أنماط ظاهرية معينة. هدفت هذه الدراسة إلى تحليل تعدد الأشكال بين المجموعات الفرعية الملايو بولينيزية من سكان الملايو في جنوب شرق آسيا.

منهجية الدراسة: تم التنميط الجيني SNPs من خلال T-ARMS PCR في 52 فردًا غير مرتبطين من 3 مجموعات فرعية من الملايو: Champa (n = 16)، Kelantan (n = 25) و Bugis (n = 11). يعتبر تفاعل البوليميراز المتسلسل T-ARMS مناسبًا للتنميط الجيني SNP نظرًا لكونه سريعًا وسريعًا وغير مكلف لتنميط عدد كبير من العينات.

النتائج : أظهرت الغالبية (60 ٪) من الأنماط الجينية للنيوكليوتيدات SNPs تشابهًا مع جميع المجموعات العرقية الفرعية للملايو. أظهرت مؤامرة PCA أن جميع المجموعات العرقية الفرعية للملايو تم فصلها قليلاً عن بعضها البعض ولكن تم تجميعها مع السكان الآسيويين مقارنةً بالمجموعات السكانية من المناطق الجغرافية الأخرى. بشكل عام ، يوفر التنميط الجيني SNPs

الاستنتاجات : الناتج عن هذه الدراسة المعرفة الأساسية حول العلاقات الجينية داخل المجموعات العرقية الملاوية في جنوب شرق آسيا وسكان العالم الآخرين. بالإضافة إلى ذلك ، يمكن استخدام النتائج الواردة في هذا التقرير لأبحاث المرض المستقبلية ، وتقدير الاستجابة للأدوية ، وتطوير استراتيجيات الإدارة الوقائية والعلاجية نحو الطب الأكثر تخصيصًا أو الدقة.

الكلمات الدالة: AIM-SNP، Malayo-Polynesian، ARMS-PCR، تواتر الأليل.