ORIGINAL ARTICLE

Allelic Diversity of the Hemochromatosis Gene (*HFE*) in Malays, Chinese and Indians

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ABSTRACT

Introduction: Hereditary hemochromatosis (HH) is an autosomal recessive disorder that causes accumulation of iron in circulating blood and organs. The disease is associated with H63D, S65C and C282Y variants of the haemochromatosis (*HFE*) gene and, if not treated can cause organ damage and may prove fatal. The main objectives of the present survey were to screen these genetic variants and establish risk profiles for developing HH in Malays, Chinese and Indians. **Methods:** A total of two hundred and twenty-two unrelated and healthy individuals together representing Malay, Chinese and Indian ethnicities in Malaysia were scored for the H63D, S65C and C282Y variants using a polymerase chain reaction-restriction fragment length polymorphism technique. **Results:** There are clear differences in H63D, S65C and C282Y allele and genotype frequency distributions between Malays, Chinese and Indians. In particular, H63D is more common in Chinese (5.19%) and Indians (7.29%), while S65C is more common in Malays (1.03%) and Chinese (1.04%). In addition, a susceptibility genotype for HH (the compound heterozygote for C282Y and H63D) was only detected in Indians (0.02%). **Conclusion:** Overall, our study is the first to provide data on the prevalence of H63D, S65C, and C282Y genetic variants and HH risk profiles for Malays, Chinese and Indians.

Keywords: Hereditary hemochromatosis, H63D, S65C, C282Y, polymorphism, Malays, Chinese, Indians

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INTRODUCTION

Hereditary hemochromatosis (HH) is a genetic disease associated with iron metabolism disorder. The disease leads to accumulation of iron in the blood and if not treated can cause the failure of organs that are essential for maintaining the normal physiological functions of the digestive and circulatory systems (1, 2). Other commonly observed clinical symptoms include changes in skin pigmentation (bronze to dark), joint pain and sexual dysfunction (3, 4).

Susceptibility to HH has been linked to three genetic variants in the *HFE* gene located on the short arm of chromosome 6. Two of these genetic variants are nucleotide changes in exon 2 at position 187 from cytosine to guanine (designated as H63D: aspartic acid to histidine at amino acid position 63) and at position 193 from adenine to thymine (designated as S65C: serine to cysteine at amino acid position 65). The third genetic variant is caused by guanine to adenine substitution (G845A) in exon 4 which leads to a single amino acid change at codon 282 (designated as C282Y) from cysteine to tyrosine (5). The *HFE* gene codes for three α-domains (assigned as α1, α2 and α3) which non-covalently linked to one β-2-microglobulin chain encoded by B2M gene to form *HFE* protein that are important in regulating transferrin-bound iron intake (6). Functional studies showed that the C282Y mutation disrupts the association between α3 and β-2-microglobulin of *HFE* protein while the H63D mutation

affects tertiary structure of the *HFE* protein and both lead to iron overloading (7). In contrast, the S65C mutation which is located in close proximity to the site of H63D has little effect on overall *HFE* protein activity and only contributes to mild iron overload symptoms (8-10). The H63D mutation is more widespread in worldwide populations compared with C282Y and S65C (11, 12). However, most patients are found to be HH homozygous for C282Y and/or compound heterozygotes for the C282Y and H63D (13).

The *HFE* population data (i.e. H63D, S65C and C282Y frequencies) have mostly been collected from Europeans, who are most affected by HH (14). Among Asian populations, data are only available for those from Indonesia, China and India, but limited to H63D and C282Y (11). To our knowledge, the prevalence of HH and the H63D, S65C and C282Y alleles and their genotype frequency distributions have never been reported for any Malaysian subjects or patients. Therefore, the present survey was conducted with the aim to score H63D, S65C and C282Y genetic variants and risk profiles for HH among the three Malaysian ethnicities (Malay, Chinese and Indian).

MATERIALS AND METHODS

Subjects

This study was approved by the Human Research Ethics Committee, Universiti Sains Malaysia (USM/JEPeM/16050191) and the Medical Research and Ethics Committee, Ministry of Health, Malaysia (NMRR-16-1399-31311-IIR). Blood samples were taken with informed consent from two hundred and twenty-two individuals registered as blood donors at three blood transfusion units; Hospital Universiti Sains Malaysia, Kelantan, Hospital Seberang Jaya, Pulau Pinang and Hospital Temerloh, Pahang (15- 18). The volunteers for this study were determined as healthy, unrelated and unadmixed via questionnaire and family tree analyses (i.e. family members were excluded and only those with no history of any diseases and intermarriage with other ethnicities for at least 3 generations were recruited) and belonging to three main ethnicities in Peninsular Malaysia; 97 Malays, 77 Chinese and 48 Indians (15-18). Samples were collected through the collecting pouch of the blood donation bag and kept in ethylenediaminetetraacetic acid (EDTA) tubes.

Genotyping

Genomic DNA templates for H63D, S65C and C282Y typing were extracted from whole blood (50µl) using Invisorb® Spin Forensic Kit (STRATEC Molecular, Berlin, Germany) as described elsewhere (15-18). The C282Y, H63D and S65C variants were typed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods as previously described by Mura et al. (8), Merryweather et al. (11) and Spнnola et al. (12) with some small modifications to the PCR reaction mixture; a 25µl mixture containing >20ng of genomic DNA, 0.2µM of forward and reverse oligonucleotide primers for C282Y, H63D and S65C and 12.5 µl of Taq 2X Master Mix (New England BioLabs, Ipswich, USA). The region containing C282Y variant was amplified using a pair of forward (5'-CAAGTGCCTCCTTTGGTGAAGGTGACACAT-3') and reverse (5'-CTCAGGCACTCCTCTCAACC-3') primers and amplified products were then digested using RsaI restriction enzyme. PCR amplification of DNA region containing H63D and S65C variants were both amplified using forward 5'-ACATGGTTAAGGCCTGTTGC-3' and reverse 5'-ACATGGTTAAGGCCTGTTGC-3' primers and were digested with *HinfI* and *MboI* restriction enzyme, respectively (8, 11, 12).

Statistical Analysis

Allele and genotype frequencies were determined by direct counting. Deviations from Hardy-Weinberg equilibrium (HWE) was tested based on chi-square goodness-of-fit test (19) and estimated using an online calculator (16). The test was considered significant at p-value of <0.05 (19, 20).

RESULTS

Genotype and allele frequencies of H63D, S65C and C282Y variants observed in Malays, Chinese and Indians in Peninsular Malaysia are shown in Tables I and II, respectively. Statistical analyses on the H63D, S65C and C282Y genotypic data show that Malays, Chinese and Indians are in HWE. There were 1 Malay, 2 Chinese and 1 Indian who were homozygous for H63D and no individual homozygous for both S56C and C282Y. Our data show differences in distributions of H63D, S65C and C282Y allele frequencies in Malays, Chinese and Indians (Table II). In particular, H63D is relatively common in Chinese (5.19%) and Indians (7.29%) while S65C is most frequent in Malays (1.03%) and Chinese (1.04%). Furthermore, the C282Y allele was only detected in Indians (1.04%).

DISCUSSION

Genetics and molecular biology techniques are now increasingly used in modern medicine. This includes searching for markers of diseases susceptibility; e.g. human leukocyte antigen (*HLA*) gene for Type 1 Diabetes (21), methylenetetrahydrofolate reductase (*MTHFR*) gene for vascular diseases (22) and nuclear receptor subfamily 4 group A member 2 (*NR4A2*) gene for Parkinson disease (23) using Sanger sequencing, PCR with sequence specific primers and next generation sequencing techniques (22, 23). All these molecular techniques are also widely adopted for diseases diagnostic (e.g. detection of severe acute respiratory syndrome coronavirus 2) and for testing between donors and recipients for transfusion and transplantation (23-26).

Table I: Genotype frequencies of H63D, S65C and C282Y in Malays, Chinese and Indians

Genotypes			Malays		
H63D	**S65C**	**C282Y**	**Observed**	**Expected**	***p*-value* (chi-square)**
-/-	-/-	-/-	89	87.387	
+/-	-/-	-/-	5	5.680	
+/+	-/-	-/-	1	1.748	
-/-	+/-	-/-	2	1.748	
+/-	+/-	-/-	0	0.000	0.870 (0.712)
+/-	-/-	+/-	0	0.437	
-/-	-/-	+/-	0	0.000	
-/-	+/+	-/-	0	0.000	
-/-	-/-	+/+	0	0.000	
Total			97		
Genotypes			**Chinese**		
H63D	**S65C**	**C282Y**	**Observed**	**Expected**	***p*-value* (chi-square)**
-/-	-/-	-/-	70	69.369	
+/-	-/-	-/-	4	4.509	
+/+	-/-	-/-	2	1.387	
-/-	+/-	-/-	1	1.387	
+/-	+/-	-/-	0	0.000	0.753 (1.200)
+/-	-/-	+/-	0	0.347	
-/-	-/-	+/-	0	0.000	
-/-	+/+	-/-	0	0.000	
-/-	-/-	+/+	0	0.000	
Total			77		
Genotypes			**Indians**		
H63D	**S65C**	**C282Y**	**Observed**	**Expected**	***p*-value* (chi-square)**
-/-	-/-	-/-	41	43.243	
+/-	-/-	-/-	4	2.811	
+/+	-/-	-/-	1	0.865	
-/-	+/-	-/-	1	0.865	
+/-	+/-	-/-	0	0.000	0.980 (0.426)
+/-	-/-	+/-	1	0.216	
-/-	-/-	+/-	0	0.000	
-/-	+/+	-/-	0	0.000	
-/-	-/-	+/+	0	0.000	
Total			48		

* *p*-value; level of significance (<0.05) for deviations from Hardy-Weinberg equilibrium (HWE)

The susceptibility marker for HH in *HFE* gene (C282Y and H63D) was first discovered by Feder et al. in 1996 by screening 178 patients using PCR-RFLP (27). This was followed by identification of *HFE* S65C variant by Barton et al. (28) in 20 patients using sequencing method. Patients of these earlier studies are Europeans

Table II: Allele frequencies of H63D, S65C, and C282Y *HFE* gene variants in Malays, Chinese and Indians

HFE alleles	Malays N= 97/A=194	Chinese N=77/A=154	Indians N=48/A=96	Mean
H63D	7 (3.60%)	8 (5.19%)	7 (7.29%)	5.36%
S65C	2 (1.03%)	1 (0.65%)	1 (1.04%)	0.91%
C282Y	0 (0.00)	0 (0.00%)	1 (1.04%)	0.35%

*N-number of subjects; A-number of chromosome tested

which followed by extensive screening of C282Y, H63D and S65C genetic variants in their general population (29-34). The *HFE* population data has now emerged for non-Europeans including Africans (11) and Middle Eastern (11, 35). However, very limited population data currently available for Asian (11) and none for the Malaysians.

In this study, the susceptibility genotype to HH (compound heterozygote for C282Y and H63D) was only detected in Indians (Table Ic). However, the risk is low as compared with Europeans (Table III) that are more affected by HH and have higher frequencies of these *HFE* variants including for individuals homozygous for C282Y (36). These frequency data support a unique repertoire of H63D, S65C and C282Y variants between people of unrelated origins; refer (8, 11, 12, 29-35, 37-40) and Table III. An important caveat is that our inferences are based on small sample sizes and limited to just three population groups in Malaysia. Therefore, we cannot rule out the possibility that these HH susceptibility markers may be at higher frequency elsewhere in Malaysia or in other groups. Thus future studies should use larger sample sizes for more accurate estimation of H63D, S65C and C282Y frequency spectra in these population groups.

Statistically significant differences between the Malays, Chinese, Indians and other population groups have also been recently demonstrated in other medically relevant population datasets (blood group, human platelet antigen, major histocompatibility complex and human neutrophil antigen) and are attributed to both, ancestral origins and local selection forces (15-18, 41-46). Therefore, a study should also be conducted in other population groups in Malaysia including Orang Asli and ethnic groups of Sabah and Sarawak (47-51) to better capture overall population susceptibility to HH in Malaysia (47). In a larger context, the distributions of H63D, S65C and C282Y variants in Malays, Chinese and Indians are more similar to those found in data collected from other Asian populations, rather than ones obtained from Europeans and Africans (Table III). In general, *HFE* data from Asian populations including from various putative ancestors (e.g., Taiwanese aboriginals and Indo-China populations) of the population groups in Malaysia are still too limited to provide reliable ancestry determination (42, 51).

Table III: Allele frequencies of *HFE* variants in Malays, Chinese and Indians compared with reference populations

Population	Allelic frequencies %			References
	H63D	S65C	C282Y	
Malays	3.60	1.03	0.00	Present study
Chinese	5.19	0.65	0.00	Present study
Indians	7.29	1.04	1.04	Present study
Lithuania	15.90	1.90	2.60	(37)
Bulgaria	23.00	NA	0.00	(38)
Croatia	14.50	1.80	3.30	(29)
Denmark	12.80	1.80	5.60	(30)
Italy	13.40	1.30	3.40	(31)
Finland	9.80	2.30	4.60	(32)
France	14.00	1.95	7.70	(8)
Portugal	20.50	1.00	0.33	(12)
Russia	13.30	1.70	3.70	(33)
Spain	20.00	1.00	3.00	(34)
Sweden	11.40	1.60	6.20	(9)
Jordan	11.25	0.11	0.00	(39)
Saudi Arabia	8.5-17.70	NA	0.00	(11, 35)
Gambians	1.30	NA	0.00	(11)
Nigeria	1.90	NA	0.00	(11)
Tunisia	17.50	NA	0.50	(11)
USA, Caucasian	15.20	1.60	6.80	(11)
USA, Hispanic	12.40	0.60	2.70	(11)
USA, African	5.10	1.70	1.10	(11)
Ecuador	3.50	4.00	0.00	(11)
Sri Lanka	9.20	NA	0.00	(11)
India	7.50	NA	0.50	(11)
China	2.80	NA	0.00	(11)
Indonesia	2.80	NA	0.00	(11)
Mexico	6.50	NA	0.00	(11)
Kazakhstan	0.08	NA	0.01	(40)
Uzbekistan	0.06	NA	0.00	(40)

*NA-not available in original article

CONCLUSION

Our study is the first to provide the prevalence of H63D, S65C, and C282Y genetic variants in the three Malaysian ethnicities (Malay, Chinese and Indian). Further study and analysis of other as yet uncharacterized population groups with larger sample sizes are needed for better understanding of population structure and risk profiles for developing HH in Malaysia.

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