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Genotype and Allele Frequencies of Irritable Bowel Syndrome (IBS)-associated Single Nucleotide Polymorphisms among Malays in Malaysia

Malezya'daki Malaylar Arasında İrritabl Bağırsak Sendromu (IBS) ile ilişkili Tek Nükleotid Polimorfizmlerinin Genotip ve Alel Frekansları

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ABSTRACT

Aim: Irritable bowel syndrome (IBS) defined by chronic or recurrent abdominal pain or discomfort and changes in bowel habits, is the most common functional gastrointestinal disorder. Studies proved that polymorphisms in the genes were one of the key roles in the underlying IBS. This study aimed to investigate the genotypes and allele frequencies of the IBS-associated single nucleotide polymorphism (SNP) from the genes GNB3 (rs54443) and SCN5A (rs8015124) in unrelated, healthy Malays of Malaysia.
Material and Methods: The genomic DNA of 404 subjects was set to nested, multiplex, and allele-specific PCR to determine the aforementioned SNPs. The PCR results were validated through the Sanger sequencing analysis.
Results: Malays possessed a slightly higher frequency of wild (C) than mutant (T) alleles in the rs5443 with 56.3 vs 43.7%. However, the frequencies of the alleles were equivalent in the subset of Malay females (C-50%, T-50%). For rs1805124, only 18.6% of Malays carried the mutant allele G with less than 10 subjects being homozygous mutant GG carriers. Concurrently, the Hardy-Weinberg equilibrium of the SNPs in the study was not deviated.
Conclusion: IBS is a common gastrointestinal problem that has significantly reduced the life quality of oneself and become an economic burden to societies. Though the mutant alleles were rather low, the IBS-associated polymorphisms, rs5443 and rs1805124 were noted to be commonly present in the Malays. Further research on the local IBS patients is recommended to affirm the association of rs5443 and rs1805124 polymorphisms and the syndrome.
Keywords: Genetic population; irritable bowel syndrome; Malay; rs5443; rs1805124.

ÖZ

Amaç: Kronik veya tekrarlayan karın ağrısı veya rahatsızlığı ve bağırsak alışkanlıklarında değişiklik ile tanımlanan irritabl bağırsak sendromu (irritable bowel syndrome, IBS), en sık görülen fonksiyonel gastrointestinal bozukluktur. Çalışmalar, genlerdeki polimorfizmlerin, IBS'de altta yatan anahtar rollerden biri olduğunu kanıtlamıştır. Bu çalışmada, Malezya'nın ilişkisiz, sağlıklı Malaylarında, GNB3 (rs54443) ve SCN5A (rs8015124) genlerinden, IBS ile ilişkili tek nükleotid polimorfizminin (single nucleotide polymorphism, SNP) genotiplerinin ve alel frekanslarının araştırılması amaçlanmıştır.
Gereç ve Yöntemler: Toplam 404 katılımcının genomik DNA'sı, yukarıda belirtilen SNP'leri belirlemek için yuvalanmış, multipleks ve alele özgü polimeraz zincir reaksiyonu (polymerase chain reaction, PCR) ile analiz edildi. PCR sonuçları, Sanger sıralama analizi ile doğrulandı.
Bulgular: Malaylar, rs5443'te %56,3'e karşı %43,7 ile mutant (T) alellerinden biraz daha yüksek bir yabanıl (C) alel frekansına sahipti. Bununla birlikte, alellerin frekansları Malay kadınlar alt grubunda eşitti (C-%50, T-%50). rs1805124 için, Malayların sadece %18,6'sı mutant alel G'yi taşıyordu ve 10'dan az katılımcı homozigot mutant GG taşıyıcılarıydı. Aynı zamanda, çalışmadaki SNP Hardy-Weinberg dengesinden de sapmamıştı.
Sonuç: İBS, kişinin yaşam kalitesini önemli ölçüde düşüren ve toplumlara ekonomik yük haline gelen yaygın bir gastrointestinal sorunudur. Mutant alellerin oldukça düşük olmasına rağmen, IBS ile ilişkili polimorfizmlerin, rs5443 ve rs1805124'ün Malaylarda yaygın olarak bulunduğu kaydedildi. rs5443 ve rs1805124 polimorfizmleri ile sendromun ilişkisini doğrulamak için yerel IBS hastaları üzerinde daha fazla araştırma yapılması önerilir.
Anahtar kelimeler: Genetik popülasyon; irritabl bağırsak sendromu; Malay; rs5443; rs1805124.

INTRODUCTION

Irritable bowel syndrome (IBS) which defines by the presence of chronic or recurrent abdominal pain or discomfort and changes in bowel habits, is the most common functional gastrointestinal disorder (FGID) in the world (1,2). The syndrome has affected global populations variously with a prevalence between 7-21% and significantly reduced the life quality of the patients and become an economic burden to the region (1). Other than the host factors of pathophysiological, environmental, and psychological, numerous findings have proved that genetic background also has set up a key role in the underlying of IBS (3-5). The mutations or polymorphisms in the gene might implicate the inflammation in the intestinal, alter the cytokine response, and increase permeability or microbiome which eventually leads to the IBS (3). Several genetic association studies have identified the single nucleotide polymorphism (SNP) from the genes of G-protein subunit (GNβ3); rs5443 and sodium channel protein type 5 subunit alpha (SCN5A); rs1805124 to be significantly associated with the etiology of IBS in different ethnic groups in the world (1,6,7).

GNβ3 is a gene located in chromosome 12p13 that encodes guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-3 which is responsible for various functions such as ion channel, hormones, and contraction and acts as a 'molecular switch' in the pathways of signal transduction (7). Due to its crucial role in many cell mechanisms, genetic abnormalities in G protein subunits have led to a higher chance of the etiology of a wide range of clinical problems including the IBS (6). A synonymous SNP in the gene, rs5443 (C825T) in exon 10 caused an exchange of amino acid cytosine by thymidine at position 825 and led to the deletion of 41 amino acids coding protein in the sequence (8,9). This resulted in an increase of intracellular signal transduction between receptors and effectors which caused the motoric or sensory abnormalities of the GI tracts and pathophysiologic mechanisms of the IBS. The rs5443 has given rise to three possible genotypes; CC, CT, and TT, where the T allele is associated with alternative splicing and formatting of a truncated but functionally active splice variant (10,11). Studies performed among South Korean showed that the TT genotype was associated with constipation-predominant IBS (12,13).

The gene of SCN5A resides on chromosome 3p22.2 in the human genome, spanning more than 101kbp and consisting of 29 exons. The SCN5A-encoded $Na_v1.5$ Na^+ channel is expressed in interstitial cells of Cajal and smooth muscle in the circular layer of the human intestine (14,15). The polymorphism in the SCN5A which interrupt the smooth muscle electrical waves and mechanical sensitivity suggesting that the event is a potential pathogenic to the IBS. Researchers have reported that the polymorphisms in the SCN5A gene namely rs1805124 (histidine-558-to-arginine, H558R) that influence the $Na_V1.5$ function would contribute to the IBS pathophysiology (12,13). The SNP was caused by a T to C transition in the sequence which exhibited the TT, TC, or CC genotype variants. It was reported that individuals with this gain-of-function SNP of SCN5A exhibited a higher risk of IBS (16). A genome-wide association study on SCN5A genetic polymorphisms in a few ethnics also revealed a significant link between its variants and the IBS (17).

Though many reports have indicated the prevalence of GNβ3 and SCN5A gene polymorphisms; rs5443 and rs1805124 in other populations around the globe, the data on the SNPs in the Southeast Asia populations are yet very limited. Such data is absolutely crucial in order to investigate the genetic risk, in particular ethnic towards the IBS susceptibility. Therefore, in this study, we will investigate the genotypes and allele frequencies of the rs54443 and rs8015124 among unrelated healthy Malays of Malaysia. The results from this study can be initiated as a database and a risk predictor on the IBS for Southeast Asia people especially Malays in future genetic association studies.

MATERIAL AND METHODS

Ethics and General information

This is a comparative and observational genotyping population study that involved the largest major ethnicity in Malaysia, the Malay. Subjects were 404 healthy, unrelated Malays (328 male, 76 female) who obtained and informed consent from a previous study, Development of Ethno-pharmacogenetics Relatedness and Personalized Medicine project (Grant no. 1001/PSK/8620013). The subjects were considered healthy as they were all blood donors including not having any critical illnesses, normal pulse and blood pressure, and unmedicated diabetes, anemia, or hypertension. This research was admitted by Universiti Sultan Zainal Abidin (UniSZA) Human Research Ethics Committee (UHREC), Terengganu, Malaysia (Reference no: UniSZA.C/2/UHREC/628-2/73, Date: 19.02.2019), and the Human Research Ethics Committee (HREC), Universiti Sains Malaysia (USM), Kelantan, Malaysia (Reference no: USM/JEPeM/19020149, Date: 08.04.2019).

Genotyping

All genomic DNA was extracted from 200 µL of -20º C EDTA whole blood using QIAamp® DNA Blood Mini Kit (QIAGEN, Hilden, Germany) according to the protocol. A two-step polymerase chain reaction (PCR) combining nested, multiplex, and allele-specific techniques was performed to determine the GNβ3 and SCN5A SNPs, rs5443 and rs1805124. These PCR techniques were used to ensure that the sensitivity, robustness, and reproducibility of the method would achieve 100% and the false-positive result could be avoided. The list of primers used in this study was shown in Table 1. The 1st PCR thermal cycler was begun with pre-denaturation at 95º C for five min followed by 30 cycles of denaturation (95º C) and annealing (65º C) for 30 sec, the extension (72º C) for 1 min and the final extension (72º C) for 5 min. For 2nd PCR thermal cycler, cycles were reduced to 20 while the time of extension to 30 sec. The annealing temperature was increased to 68º C. The DNA yield and the PCRs products were assessed using a non-mutagenic staining reagent on the 1.5% agarose gel electrophoresis. The gel image was viewed under UV light and analyzed through a digital imaging analysis system (Alphaimager, CA). Sanger sequencing was performed on the selected samples for result validation using an Applied Biosystems 3130xl Genetic

Analyzer (Applied Biosystems, USA) according to the manufacturer's recommendations. Gene polymorphism examination was performed via Chromas.

Statistical Analysis

Data of the SNPs from this study were analyzed using descriptive analysis. The observed genotype distributions were presented in count and percentage. The percentage of allele frequencies in the ethnic was deliberated from the figures. Hardy-Weinberg equilibrium (HWE) was calculated by comparing the observed and expected genotype frequencies using the chi-square test with one degree of freedom to determine possible deviation in the Malay and the genders. A p-value of <0.05 was considered significant. All statistical analyses were performed using IBM SPSS v.20 for Windows.

RESULTS

Participants' demographics, the genotype distributions, and allele frequencies of IBS-associated SNPs, rs5443 and rs1805124, were summarized in Table 2. The age range of subjects was 19-55 with a mean of 29.0±9.8 years (the mean age was 30.0±1.11 years for males, and 26.0±7.9 years for females). The majority of the subjects were male (n=328). The determination of wild type, heterozygous mutant, and homozygous mutant variants of all subjects was based on the amplification of the sequence from the 2nd PCR. Each DNA sample was subjected in parallel in two wells consisting of the wild and variant type primers set to determine their variant. For example, as can be seen in Figure 1B, the genotype of rs5443 and rs1805124 for S1 was pronounced as homozygous wild type since only the amplifications of wild type sequence occurred where the band only appeared in the wild primer well. Meanwhile, S4 possessed a heterozygous mutant genotype in rs5443 after the amplifications appeared in both wells but become homozygous mutant for rs1805124 due to the amplification that solely appeared in the mutant variant well as observed.

Table 2 exhibited the genotype distributions and allele frequencies of rs5443 and rs1805124 polymorphisms in the Malay population of Malaysia. The HWE for both SNPs did not deviate either in total or the gender subsets since their p-values were more than 0.05 suggesting no unexpected genetic drift or sampling bias occurred.

Table 1. List of primers used to the genotype-specific site of GNβ3 and SCN5A genes in the 1st PCR followed by the amplifications of rs5443 and rs1805124 in the 2nd PCR

SNP	1st PCR		2nd PCR	
	Sequence	Product size	Sequence	Product size
rs5443	*FW_GNB3* CTG ATC CCT GAC CCA CTT GC *RV_GNB3* AGT CCG AAA TGG GAG CTG A	349 bp	*FW_rs5443C (Wild type)* TCA TCT GCG GCA TCA CGT C**C** *FW_rs5443T (Variant type)* TCA TCT GCG GCA TCA CGT C**T** *RV_GNB3* AGT CCG AAA TGG GAG CTG A	208 bp
rs8105124	*FW_SCN5A* GGG TGC TCT AGC ATC ACA GG *RV_SCN5A* GAT GAA AAC AGC ACA GCG GG	245 bp	*FW_SCN5A* GGG TGC TCT AGC ATC ACA GG *RV_rs1805124A (Wild type)* GGA GAG CGA GAG CCA CC**A** *RV_rs1805124G (Variant type)* GGA GAG CGA GAG CCA CC**G**	225 bp

SNP: single nucleotide polymorphism, PCR: polymerase chain reaction, FW: forward, RV: reverse. The diluted amplicons of 1st PCR were subjected to the 2nd PCR. Every sample would underwent two sets of primers in the 2nd PCR, wild and variant types to determine the genotype variant. Band would appear on the gel only when the amplification took placed.

Table 2. Genotype distributions and allele frequencies of rs5443 and rs1805124 polymorphisms among Malays

	All (n=404)		Male (n=328)		Female (n=76)	
Age (years), mean±SD	29.0±9.8		30.0±1.1		26.0±7.9	
	rs5443	**rs1805124**	**rs5443**	**rs1805124**	**rs5443**	**rs1805124**
Observed genotype, n (%)						
WT	130 (32.2)	269 (66.6)	109 (33.2)	231 (70.4)	21 (27.6)	38 (50.0)
HM	195 (48.3)	120 (29.7)	161 (49.1)	88 (26.8)	34 (44.8)	32 (42.1)
MT	79 (19.5)	15 (3.7)	58 (17.7)	9 (2.8)	21 (27.6)	6 (7.9)
Allele frequency, (%)						
Wild	C- 56.3	A- 81.4	C- 57.8	A- 83.8	C- 50.0	A- 71.0
Mutant	T- 43.7	G- 18.6	T- 42.2	G- 16.2	T- 50.0	G- 29.0
Predicted genotype, n (%)						
WT	128 (31.7)	268 (66.3)	110 (33.4)	230 (70.2)	19 (25.0)	38 (50.0)
HM	199 (49.3)	122 (30.2)	160 (48.7)	89 (27.2)	38 (50.0)	31 (40.8)
MT	77 (19.0)	14 (3.5)	59 (17.9)	8 (2.6)	19 (25.0)	7 (9.2)
p	0.702	0.723	0.913	0.860	0.360	0.837

SD: standard deviation, SNP: single nucleotide polymorphism, WT: wild type, HM: heterozygous mutant, MT: mutant type

According to the table, the genotypes of rs5443; CC, CT, and TT, and rs1805124; AA, AG, and GG were found existed either in male or female Malays. For rs5443, the allele frequencies seemed to be slightly higher in the wild, C-56.3% (n=325) than in the mutant, T-43.7% (n=247) of Malays (p=0.702). However, Malay females derived equal frequencies (both n=55) for both alleles in rs5443, C-50.0%, T-50.0% (p=0.360). Meanwhile, the frequency of rs1805124 mutant allele G was low (18.6%, n=135, p=0.723) among Malays, which was found at 16.2% in the male subset (n=97, p=0.860) and 29.0% in the female subset (n=38, p=0.837). To the best of our knowledge, this study was the first to publish on the polymorphisms of rs5443 and rs1805124 among Malaysian ethnicities.

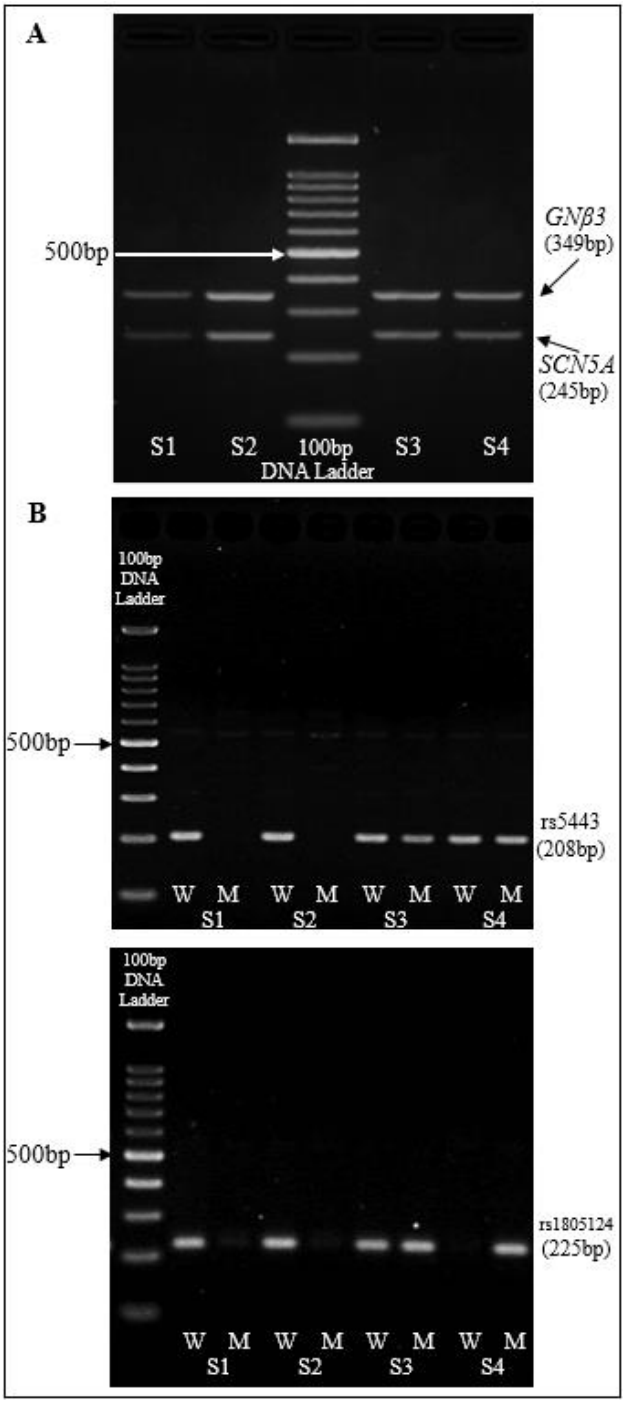

Figure 1. Band amplifications on the 1.5% gel electrophoresis from 1st and 2nd PCRs. **A)** The amplifications of GNβ3 and SCN5A interest regions from the 1st PCR. **B)** The amplifications of rs5443 and rs801524 sequences from the 2nd PCR.
Both SNPs were applying the same thermal condition, therefore they could be run simultaneously in a thermal cycler. W: well of wild type primer set, M: well of variant type primer set, Sn: subject number

DISCUSSION

This study has successfully genotyped the genes and their SNPs which susceptible to the IBS; GNβ3 (rs5443) and SCN5A (rs8015124) on 404 DNA samples of unrelated, healthy Malay of Malaysia. Tables 3 and 4 display the comparisons of the genotype and allele frequencies for rs5443 and rs1805124 in Malay from this study and other healthy ethnicities around the world. The data tabulated in the tables were depicted from the control group of comparatives, case-controlled studies related to the SNPs. According to the Table 3, the Quilombo and African-American dominated T allele more than C at the frequencies of 62.4% (n=184) and 71.6% (n=86) accordingly (18,19). The findings were aligned with the data from 1000 Genomes (1000genomes.org), Gambian Genome Variation (international genome.org/), and ALFA Allele Frequency (ncbi.nlm.nih.gov/snp/rs5443) projects where populations with African ancestors exhibited the T allele frequency higher with more than 70%.

The populations of Korean and Han Chinese reported similar prevalence of genotype and allele frequencies of rs5443 in two separate studies with CT genotype being the most common (Korean - 12,13 and Han Chinese - 20,21). On the other hand, Japanese are among the individuals who appeared to have comparable distributions of C and T allele frequencies in the populations (22), with C-50.7% (n=493) and T-49.3% (n=483), besides the Koreans (12,13) with average C-50.5% (n=441), T-49.6% (n=435). Meanwhile, Greek was the least to consist the frequency of mutant allele T among the populations, 13.8% (n=44) with only less than 5% (n=6) of them possessing TT in the individual (23). Notably, none of the ethnic groups from the 1000 Genomes or HapMap (ftp://ftp.ncbi.nlm.nih.gov/hapmap) or ALFA Allele Frequency databases has recorded the mutant allele T frequency as lowest as that observed in the Greek. Though the substitution of C to T in the rs5443 was pronounced to distract the intracellular signal transduction in the GI tract, the SNP may manifest a unique attribution in an ethnic or in the context of population gene heredity. However, more studies should be performed to solidify the findings.

According to the data presented in the Table 4, Taiwanese individuals exhibited the lowest frequency with a prevalence of only 2.6% (n=7) of the rs1805124 mutant allele, G (24). While Vietnamese population demonstrated the lowest occurrence of this allele among the populations in the ALFA Allele Frequency database, with a frequency of 8.7% (n=53). Ancient Sardinian also exhibited the least of mutant allele frequency as indicated by the database, 7.0% (n=2). However, the total subjects of the ethnic were very limited (n=30) making its statistical power of study reduced which could potentially affect the reliability and generalizability of the data. Simons Genome Diversity Project (ncbi.nlm.nih.gov/bioproject/PRJNA586841) documented that the African ancestors derived the highest allele mutant with 60.6% (n=131). Nevertheless, to the

best of our knowledge, till to date, no study has reported on the association of FGIDs or IBS prevalence and rs1805124 polymorphism in the population. On average, the ethnic groups displayed in the table seemed to exhibit lower frequencies (less than 23.5%) of the mutant allele rs1805124, G, compared to mutant allele rs5443, T, in the Table 3.

The polymorphisms of rs5433 and rs1805124 were also been documented to have significant correlations with the etiology of pathophysiology and clinical manifestations of functional dyspepsia (12,13,23), obesity (11,21,25), atrial fibrillation (26-28), sudden cardiac death (29-31) and many more. Due to the concern in the increasing cases of critical medicinal illnesses including the IBS, the SNP genotyping study is crucial to predict the DNA risk in the ethnic and find proper solutions for the health management and treatment inter-individually. However, compared to those monogenous hereditary diseases, it is undeniable that other non-genetic factors also play roles in causing related diseases in the individual which was not evaluated in this study. Furthermore, more than one gene has involved in IBS cases making the genetic factor is still

Table 3. Comparisons of genotype and allele frequencies of rs5443 in Malays and other reported healthy ethnics worldwide

Ethnic	n (M/F)	Age (years), mean±SD	Observed genotype, n (%)			Allele frequency (%)		Reference
			CC	CT	TT	C	T	
Malay	404 (328/76)	29.0±9.8	130 (32.2)	195 (48.3)	79 (19.5)	56.3	43.7	Present study
Caucasian (Israel&Spain)	340 (nd)	nd	132 (38.8)	169 (49.7)	39 (11.5)	63.7	36.3	(10)
Saudi	116 (nd)	nd	39 (33.6)	59 (50.9)	18 (15.5)	59.0	41.0	(11)
Korean	434 (167/267)	47.0±15.0	112 (25.8)	215 (49.5)	107 (24.7)	50.6	49.4	(12)
	148 (81/67)	10.8±3.9	35 (23.6)	79 (53.4)	34 (23.0)	50.3	49.7	(13)
Quilombo*	206 (98/108)	nd	22 (10.7)	111 (53.9)	73 (35.4)	37.6	62.4	(18)
African-American	95 (25/70)	20.5±2.7	9 (9.5)	36 (37.9)	50 (52.6)	28.4	71.6	(19)
Han Chinese	513 (187/326)	42.5±8.7	170 (33.1)	203 (39.6)	140 (27.3)	53.0	47.0	(20)
	130 (0/130)	nd	39 (30.0)	61 (46.9)	30 (23.1)	53.5	46.5	(21)
Japanese	649 (184/465)	nd	166 (25.6)	327 (50.4)	156 (24.0)	50.7	49.3	(22)
Greek	181 (68/113)	53.7±12.0	137 (75.7)	38 (21.0)	6 (3.3)	86.2	13.8	(23)
Taiwanese	505 (257/248)	39.7±12.3	89 (17.6)	263 (52.1)	153 (30.3)	43.7	56.3	(25)
West-Ukrainian	48 (18/30)	49.1±6.3	22 (45.8)	24 (50.0)	2 (4.2)	70.8	29.2	(33)
Caucasian (UK)$^{\alpha}$	427 (171/256)	50.0±16.0	208 (48.7)	171 (40.0)	48 (11.3)	68.7	31.3	(34)
Caucasian (Pisa)	225 (72/153)	42.2±11.0$^{\beta}$	110 (48.9)	99 (44.0)	16 (7.1)	70.9	29.1	(35)
Egyptian	222 (108/114)	38.5±12.4	70 (31.5)	118 (53.2)	34 (15.3)	58.1	41.9	(36)
Russian	200 (99/101)	36.0±8.2	95 (47.5)	90 (45.0)	15 (7.5)	70.0	30.0	(37)

SD: standard deviation, M/F: male/female; nd: not determined, *: isolated Brazilian populations of African ancestry, β: standard error of the mean; α: 96% of subjects were Caucasians

Table 4. Comparisons of genotype and allele frequencies of rs1805124 in Malays and other reported healthy ethnics worldwide

Ethnic	n (M/F)	Age (years), mean±SD	Observed genotype, n (%)			Allele frequency (%)		Reference
			TT	TC	CC	T	C	
Malay	404 (328/76)	29.0±9.8	269 (66.6)	120 (29.7)	15 (3.7)	81.4	18.6	Present study
Taiwanese	137 (46/91)	70.2±9.1	130 (94.9)	7 (5.1)	0 (0.0)	97.4	2.6	(24)
Chinese	296 (197/99)	58.4±10.8	281 (94.9)	11 (3.7)	4 (1.4)	96.8	3.2	(26)
	80 (18/62)	59.9±9.3	46 (57.5)	32 (40.0)	2 (2.5)	77.5	22.5	(31)
	81 (52/29)	62.5±7.6	60 (74.1)	20 (24.7)	1 (1.2)	86.4	13.6	(38)
Caucasian (Minnesota)	312 (236/76)	nd	197 (62.7)	103 (32.8)	12 (3.8)	79.7	20.3	(27)
Tunisian	106 (59/47)	64.0±13.0	69 (65.1)	32 (30.2)	5 (4.7)	80.2	19.8	(28)
Finnish	5043 (2352/2691)	51.4±14.1	3227 (64.0)	1613 (32.0)	201 (4.0)	80.0	20.0	(29)
Japanese	232 (135/97)	39.0±12.5	186 (80.2)	43 (18.5)	3 (1.3)	89.4	10.6	(30)
European-Caucasian	251 (185/66)	nd	170 (67.7)	70 (27.9)	11 (4.4)	81.7	18.3	(39)
Russian	411 (348/63)	37.2±17.1	253 (61.6)	143 (34.8)	15 (3.6)	78.9	21.1	(40)
Ihoba Chinese	100 (nd)	nd	77 (77.0)	23 (23.0)	0 (0.0)	88.5	11.5	(41)
Miao Chinese	98 (nd)	nd	71 (72.5)	25 (25.5)	2 (2.0)	85.2	14.8	(42)
Bai Chinese	200 (100/100)	nd	118 (59.0)	71 (35.5)	11 (5.5)	76.8	23.2	(43)
Jordanian	500 (320/180)	38.2±9.7	315 (63.0)	160 (32.0)	25 (5.0)	79.0	21.0	(44)

SD: standard deviation, M/F: male/female; nd: not determined

needed to be explored further. However, the investigation of the SNPs is worthy for the future benefits of society and should be proceeded through a genome-wide association approach and require multiple data of the ethnics to solidify the results.

CONCLUSION

IBS is a critical health concern as it can negatively affect the life value of oneself and increase the financial burden to the societies. This study has reported the genotype distributions and allele frequencies of rs5443 and rs1805124, the IBS-associated SNPs in the Malay population from Malaysia. Though pathophysiological, environmental, and psychological factors would also influence, the analysis of gene polymorphisms can always become the first hint to predict IBS susceptibility in individuals or in the ethnics. Based on the presented findings in the study and all cited studies above, it is understandable that the rs5443 and rs1805124 variants are diversified among populations and commonly present in healthy individuals and ethnicities. Nevertheless, the findings from this study have shed light on future research in order to explore more on the association of gene polymorphisms and the common health problem such as IBS and its causes. Future research should be conducted to determine the susceptibility of rs5443 and rs1805124 with the IBS-related causal and symptoms among local Malay patients to affirm the findings.

Ethics Committee Approval: This research was admitted by Universiti Sultan Zainal Abidin (UniSZA) Human Research Ethics Committee (UHREC), Terengganu, Malaysia (Reference number: UniSZA.C/2/UHREC/628-2/73; Date: 19/02/2019) and the Human Research Ethics Committee (HREC), Universiti Sains Malaysia (USM), Kelantan, Malaysia (Reference number: USM/JEPeM/ 19020149; Date: 08/04/2019). Subjects were 404 healthy, unrelated Malays obtained and informed consent from Development of Ethno-pharmacogenetics Relatedness and Personalised (Grant no. 1001/PSK/8620013).

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