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The prevalence of *TPH1* and *TPH2* genetic polymorphisms susceptible to irritable bowel syndrome among unrelated, healthy Malays in Malaysia

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Abstract

Tryptophan hydroxylase (TPH) gene which encodes the first rate-limiting enzyme in the serotonin biosynthesis pathway is one of the leading candidate genes in the etiology of the most common gastrointestinal (GI) disease, the irritable bowel syndrome (IBS). SNPs in the gene would distract the serotonergic function which led to the susceptibility to the syndrome. This study is aimed to determine the genotype distributions and allele frequencies of the three SNPs from two TPH genes, TPH1 and TPH2 genes among healthy, unrelated Malays in Malaysia. Nested-multiplex-allele specific PCR (NMAS-PCR) was subjected to 404 archived Malays' DNA to genotype rs211105, rs4537731 and rs4570625 variants following the validation of the obtained genotyping results through the direct Sanger sequencing. Results showed that the genotype frequencies of AA in rs211105 and rs4537731 among Malays were 59.2 and 51.5%. The heterozygous of GT was found to be slightly higher than GG with 47.5 to 43.3% in rs4570625. Meanwhile, the mutant allele frequencies of rs211105, G and rs4537731, T were relatively comparable with 30.3 to 33.0% accordingly. Concurrently, no departure of HWE was detected except in rs4537731. This study described low frequencies of *TPH1* and *TPH2* SNPs mutant variants associated with the IBS among unrelated, healthy Malays. Data generated from this study is important to enhance our knowledge of the association between IBS pharmacogenetic profiles and ethnic differences. Future studies on Malaysian IBS patients are recommended to determine the influence of rs211105, rs4537731 and rs4570625 on the syndrome locally.

Keywords: irritable bowel syndrome, rs211105, rs4537731, rs4570625, Malay, Malaysia

1. Introduction

Genetic polymorphism is defined as the inheritance of a trait controlled by a single genetic locus with two alleles in which the least common allele has a frequency of about 1% or greater. A genetic polymorphism would inscribe a difference in the DNA sequence among individuals, groups or populations. Understanding the functions of single nucleotide polymorphism (SNP) will significantly help to comprehend the variations of human phenotype in the genetic basis of complex human health and diseases (1). Therefore, local data on the types and frequencies of genetic polymorphisms of the putative genes among individuals or ethnicities is important as it can serve as a baseline for future studies on the associated health problems.

Serotonin (5-hydroxy tryptophan; 5-HT) is one of the most abundant neurotransmitter molecules in the gastrointestinal (GI) tract and has been considered an important enteric nervous system with essential roles in the gut (2). The biosynthetic pathway of serotonin is firstly underlying by the rate-limiting tryptophan hydroxylase enzyme (TPH) prior to its secretion in the GI motility and sensation (3). Due to that, any predisposition in the serotonergic system caused by the disruption of the enzyme will lead to the pathophysiology of the GI disorders including the most common, the irritable bowel syndrome (IBS) (4,5).

There are two isoforms of the TPH enzyme identified which associated with the clinical manifestations of IBS namely TPH1 and TPH2, encoded by the two different genes, *TPH1* and *TPH2*. The TPH1 gene is located on the chromosome 11p15.1, spanning more than 24 kbp, consisting of 10 exons and mainly expressed in the gut and peripheral organs. Meanwhile, the *TPH2* is located on the chromosome 12q21.1, spanning more than 247 kbp which composed of 13 exons and expressed mainly in the central nervous system and peripheral neurons. The genetic polymorphisms facilitating in the genes could alter the expression of the enzyme which cause to the susceptibility of the IBS in the individual (2,6). The

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SNPs of *TPH1* and *TPH2* genes such as rs211105, rs4537731 and rs4570625 are among the most extensively studied which relates to the IBS (7,8,9). The *TPH1* rs211105 polymorphism (known as 11:g.18033757T>G) which located within intron 3 showed some associations with the daily reporting of gastrointestinal symptoms including diarrhea, bloating and loose stools among women with the IBS (7) while rs4537731 polymorphism (known as −6526A/G, 11:g.18047335T>C) located at the upstream region (−6.5 kbp) where the homozygous minor allele (CC) carriers in the IBS patients were reported to experience severe diarrhea, bloating and a trend of more watery stool (9). These findings indicated that the SNPs might disrupt the function and level of 5-HT biosynthesis. On the other hand, previous studies also showed possible associations between a *TPH2* gene SNP in the promoter region, rs4570625 and the stool characteristics (7). The homozygous genotype of the minor allele (TT) of rs4570625 carriers experienced longer period of either with very hard or watery stools compared to the other genotype groups; GG and GT (7).

Even though the IBS may not be a life-threatening issue, the consequences have impacted the quality of life and become an economic burden to the societies (10,11). Among Malaysians, the prevalence of *TPH* gene variations of rs211105, rs4537731 and rs4570625 have not yet been studied. Therefore, this study will determine the types and frequencies of rs211105, rs4537731 and rs4570625 among unrelated healthy Malays of Malaysia. The findings from this study can be used as a database for future genetic association research on the IBS and other significant TPH-related medical diseases that involve with population- or geographic-specific analyses.

2. Materials and Methods

2.1. Ethics and general information

This study is an observational and comparative genotyping population research involving the largest major ethnic in Malaysia, the Malay. A total of 404 archived blood samples of healthy, unrelated Malays from a previous study; Development of Ethno-pharmacogenetics Relatedness and Personalised (Grant no. 1001/PSK/8620013), were used to investigate the SNPs of rs211105, rs4537731 and rs4570625. The subjects were categorized as healthy according to the blood donor's criteria. History of symptoms related to the IBS was not recorded in the study. The human ethical approvals were obtained from the Human Research Ethics Committee (HREC), Universiti Sains Malaysia (USM), Kelantan, Malaysia (Reference number: USM/JEPeM/19020149) and the Universiti Sultan Zainal Abidin (UniSZA) Human Research Ethics Committee (UHREC), Terengganu, Malaysia (Reference number: UniSZA.C/2/UHREC/628-2/73).

2.2. Genotyping

Genomic DNA was isolated from 200µL of -20ºC EDTA blood using QIAamp® DNA Blood Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. A two-steps polymerase chain reaction (PCR); PCR1 and PCR2, combining the nested, multiplex and allele-specific techniques, was performed to determine the SNPs. The use of NMAS-PCR would provide 100% sensitivity, robust and reproducible genotyping result of the SNPs. Primers used in the study are listed in the Table 1 while the PCR1 and 2 thermocycler settings are listed in the Table 2. The DNA yield and PCR products were stained using a non-mutagenic DNA staining reagent and were electrophoresed in 1.5% agarose gel before examined under the UV light. The gel image was analyzed using a digital imaging and analysis system (Alphaimager, CA). Sanger sequencing was performed on a few samples for genotyping result validation using an Applied Biosystems 3130xl Genetic Analyzer (Applied Biosystems, USA) according to the manufacturer's recommendations.

Table 1. List of the primers used to genotype rs211105, rs4537731 and rs4570625 through NMAS-PCR. For ease of reference, primers have been appended with 'FW' and 'RV', referring to forward and reverse sequences for particular gene/SNP. Each set of PCR2 primers consisted of a PCR1 primer sequence and a newly designed primer to exhibit a specific allele variant either wild or variant type (bold)

SNP	PCR1		PCR2	
	Primer sequence	Product size (bp)	Primer sequence	Product size (bp)
rs211105	*TPH1a_FW* AAC CAA GGA ACA GTT TCC ATA CCT *TPH1a_RV* AAA CAG AAG GGT AGG GTG GG	571	*TPH1a_FW* AAC CAA GGA ACA GTT TCC ATA CCT *rs211105A_RV (Wild type)* GAT TTC TAA GAT CTT TTC CAT CGG C**A** *rs211105C_RV (Variant type)* GAT TTC TAA GAT CTT TTC CAT CGG C**C**	430
rs4537731	*TPH1b_FW* AGG ACT GTA CAC ATA ACG AAG TAT *TPH1b_RV* TGA AAG GTC TCT CCC TGA CCA	664	*rs4537731T_FW (Wild type)* TGG ATG TAC TTT AAA GCT CAG GA**T** *rs4537731C_FW (Variant type)* TGG ATG TAC TTT AAA GCT CAG GA**C** *TPH1b_RV* TGA AAG GTC TCT CCC TGA CCA	251
rs4570625	*TPH2_FW* GCT TTC TCC TCA CCA CAT AAC G *TPH2_RV* CTG GCA AGT TAA CCT CAG TCT	801	*TPH2_FW* GCT TTC TCC TCA CCA CAT AAC G *rs4570625C_RV (Wild type)* AGC TTT TTC TGA CTT GAC ATA TT**C** *rs4570625A_RV (Variant type)* AGC TTT TTC TGA CTT GAC ATA TT**A**	390

Table 2. Thermal cycling conditions for PCR1 and 2. The amplifications product of PCR1 were used as template in the PCR2. PCR2A was used to detect rs211105 variants and PCR2B for the detection of rs4537731 and rs4570625 variants concurrently. The lid of the thermal cycler was set at 105°C

PCR profile	PCR1			PCR2					
				A			B		
	Temp. (°C)	Time	Cycles	Temp. (°C)	Time	Cycles	Temp. (°C)	Time	Cycles
Pre-denaturation	95	5 min	1	95	2 min	1	95	2 min	1
Denaturation	95	30 sec	34	95	30 sec	20	95	30 sec	20
Annealing	65	30 sec		68	30 sec		62	30 sec	
Extension	72	1 min		72	30 sec		72	30 sec	
Final extension	72	5 min	1	72	5 min	1	72	5 min	1
Hold	12	∞	-	12	∞	-	12	∞	-

2.3. Statistical analysis

The data of the SNPs derived from this study were analyzed using descriptive statistics. The observed genotype distributions were expressed in count and percentage and were used to calculate the percentage of the allele frequencies. Possible deviation from Hardy–Weinberg equilibrium (HWE) was tested for statistical significance by comparing the observed and expected genotypes frequency using the chi-square (χ^2) test with one degree of freedom. *P*-value of <0.05 was considered statistically significant. All statistical calculations were performed using SPSS ver. 20 Windows (SPSS Inc., Chicago, IL).

3. Results

This study employed a total of 404 DNA samples from an unrelated, healthy Malay ethnic of Malaysia. The male subjects were 328 while the females were 76. Their ages were between 19 and 55 years with the average being 29 years old. All SNPs were successfully genotyped in all DNA samples. Table 3 shows the genotype distributions and allelic frequencies of *TPH1* (rs211105 and rs4537731) and *TPH2* (rs4570625) polymorphisms among Malays in Malaysia. All variants of the three SNPs; rs211105 (AA, AC, CC), rs4537731 (AA, AG, GG) and rs4570625 (GG, GT, TT) were presented in the Malay subjects of study. However, it only demonstrated considerably low frequencies of allele mutant variants associated with the IBS; rs211105 (C- 24.0%), rs4537731 (G- 30.3%) and rs4570625 (T- 33.0%). The carriers of the mutant variant among Malays were also low with 7.1% in rs211105, 12.1% in rs4537731 and 9.2% in rs4570625. The genotype frequencies of AA in rs211105 and rs4537731 in Malays were 59.2 and 51.5% accordingly. Meanwhile, in rs4570625, the heterozygous GT was found to be slightly higher than GG with 47.5 to 43.3%. No departure of Hardy-Weinberg equilibrium was detected suggesting no unexpected genetic drift or sampling bias occurred except for the rs4537731 ($\chi^2 = 7.79$; $P < 0.005$). To the best of our knowledge, the current study was the first to publish on the polymorphisms of rs211105, rs4537731 and rs4570625 among Malaysian ethnics.

Table 3. Genotype distributions and allele frequencies of *TPH1* and *TPH2* polymorphisms (rs211105, rs4537731 and rs4570625) among Malays

SNP	Observed genotypes		Predicted genotypes		χ^2 test	*P*-value	Allele frequencies (%)	
	n	%	*n*	%				
rs211105								
TT	239	59.2	233	57.7			A	76.0
TG	136	33.7	148	36.5	2.42	0.119	C	24.0
GG	29	7.1	23	5.8				
rs4537731								
TT	208	51.5	196	48.6			A	69.7
TC	147	36.4	171	42.3	7.79	0.005	G	30.3
CC	49	12.1	37	9.2				
rs4570625								
GG	175	43.3	182	45.0			G	67.0
GT	192	47.5	178	44.1	2.33	0.126	T	33.0
TT	37	9.2	44	10.9				

Abbreviation: *n* – number of samples

4. Discussion

Tables 4, 5 and 6 exhibit the comparisons of the rs211105, rs4537731 and rs4570625 variants between the Malay from this study and other ethnics around the world. The ethnics tabulated in the Tables 4-6 were depicted from the healthy, control group of comparatives, case-controlled studies related to the SNPs. According to the Table 4, the rs211105 TT genotype was the most frequent genotype detected in all ethnicities; 46.5-71.9%, with the allele frequencies were ranging between 68.5 to 85.9%. Japanese was detected to derive the least of the G variant allele frequency with 14.1% (8). Apparently, the findings from the table were in concordance with the genotype distributions obtained from 1000 Genomes Project Phase 3 (www.internationalgenome.org) data where AA is also the major genotype in many ethnics worldwide for instances, among African Caribbean (91.7%), Pakistani (69.8%), Mexican (53.1%) and Vietnamese Kinh (50.5%). Meanwhile,

an epidemiology study showed that the IBS occurred less frequently among African Americans than the Whites (12). The finding was in agreement with the 1000 Genomes Project Phase 3 data where the frequency of the G minor allele was very low (less than 5%) among Africans compared to the Europeans; above 20%. This suggests that the rs211105 variant may a common genetic cause or pathophysiology of the TPH-related diseases including the IBS. More studies should be performed to solidify the findings.

Table 4. Comparisons of genotype distributions and allele frequencies of rs211105 in Malays and other reported ethnics worldwide

Ethnic	*n* (*M/F*)	Mean age	Observed genotypes, *n* (%)			Allele frequencies (%)		Reference
			TT	TG	GG	T	G	
Malay	404 *(328/76)*	29	239 (59.2)	136 (33.7)	29 (7.1)	76.0	24.0	Present study
Caucasian	79 (*0/79*)	36	48 (60.8)	28 (35.4)	3 (3.8)	78.5	21.5	(7)
Japanese	64 *(nr)*	nr	46 (71.9)	18 (28.1)	0 (0)	85.9	14.1	(8)
Han Taiwanese	84 *(0/84)*	29.7	39 (46.5)	38 (45.2)	7 (8.3)	68.5	31.5	(19)
Caucasian (Olsztyn)	91 (*66/25*)	44.2	54 (59.3)	34 (7.1)	3 (3.3)	78.0	22.0	(21)
Scandinavian	1473 (*812/661*)	44.1	839 (57.4)	520 (35.6)	102 (7.0)	75.2	24.8	(22)
Swedish	132 (*78/54*)	27	76 (58.0)	50 (38.2)	5 (3.8)	77.0	23.0	(27)

Abbreviations: *n* – number of samples, M – male, F – female, nr – not reported.

With respect to the rs4537731 (Table 5), Caucasians of the Toronto were noted to possess higher allele frequency of C, almost two-fold than the T's (35%) in the population (13). On the other hand, the substitution of T to C of the SNP only appeared in heterozygous genotypes where no GG carrier was detected among Japanese population (8). Data of rs4537731 allele frequency derived from the Allele Frequency Aggregator (ALFA) project (https://www.ncbi.nlm.nih.gov/snp) also recorded multiple trends among different ethnicities and geographical. The GG genotype carriers were the dominant among Africans of Kenya, Nigeria, Gambia and Sierra Leone; 68.1-73.2%. While among East Asians, for instances Chinese, Vietnamese and Japanese, AA carriers are the biggest proportion in the populations with the average frequency was 77.3% (12). According to a study conducted in Caucasian females of IBS patients, the homozygous of the minor allele C carriers experienced more severe diarrhea symptoms than the other two genotype groups of the patients (7). However, Katsumata et al. (8) have found no link between the *TPH1* rs4537731 SNP and the GI symptoms among Japanese people.

Table 5. Comparisons of genotype distributions and allele frequencies of rs4537731 in Malays and other reported ethnics worldwide

Ethnic	*n* (*M/F*)	Mean age	Observed genotypes, *n* (%)			Allele frequencies (%)		Reference
			TT	TC	CC	T	C	
Malay	404 (*328/76*)	29	208 (51.5)	147 (36.4)	49 (12.1)	69.7	30.3	Present study
Caucasian	79 (*0/79*)	36	29 (36.7)	41 (51.9)	9 (11.4)	62.7	37.3	(7)
Japanese	66 *(nr)*	nr	39 (59.0)	27 (41.0)	0 (0)	79.5	20.5	(8)
Caucasian (Toronto)	30 (*0/30*)	nr	4 (13.3)	13 (43.3)	13 (43.3)	35.0	65.0	(13)
Swedish	132 (*78/54*)	27	49 (37.1)	68 (51.5)	15 (11.4)	62.9	37.1	(20)
Scandinavian	1473 (*812/661*)	44.1	470 (32.0)	725 (49.3)	275 (18.7)	56.6	43.4	(22)

Abbreviations: *n* – number of samples, M – male, F – female, nr – not reported.

In the Table 6, the allele frequencies of the SNP from chromosome 12, rs4570625, seemed to have unique patterns in the observed genotype distributions among ethnicities. Korean exhibited equal carriers of G and T variants in the population according to the studies of Han et al. (14) and Serretti et al. (15), but Kim et al. (16) manifested that the ethnic derived higher minor allele carriers of T, 57.5% in the population. In contrast, Germany demonstrated the observable vice versa genotype distributions in two different findings where Baehne et al. (17) reported the population consisted of high homozygous G carriers, 67.9% while Reuter et al. (18) proved that Germany was high as homozygous T carriers, 57.4%. Interestingly, it was found that the East Asian communities were the only populations who have T allele frequencies of more than 50% while the rest of the populations in the world for examples American, African, South Asian and European, exhibited of less than 35% of the allele according to the 1000 Genomes database. Overall, based on the presented findings in

this study, studies cited above and the databases, it is understandable that the rs211105, rs4537731 and rs4570625 polymorphisms are commonly found in the individual or ethnics.

Table 6. Comparisons of genotype distributions and allele frequencies of rs4570625 in Malays and other reported ethnics worldwide

Ethnic	*n* (*M/F*)	Mean age	Observed genotypes, *n* (%)			Allele frequencies (%)		Reference
			GG	GT	TT	G	T	
Malay	404 (*328/76*)	29	175 (43.3)	192 (47.5)	37 (9.2)	67.0	33.0	Present study
Caucasian	79 (*0/79*)	36	46 (58.2)	25 (31.6)	8 (10.1)	74.0	26.0	(7)
Korean	86 (*59/27*)	nr	30 (34.9)	40 (46.5)	16 (18.6)	58.1	41.9	(14)
Korean	170 (*105/65*)	38.8	57 (33.5)	77 (45.3)	36 (21.2)	56.2	43.8	(15)
Korean	247 (*125/122*)	39.4	45 (18.2)	120 (48.6)	82 (33.2)	42.5	57.5	(16)
Germany	84 (*44/40*)	34.8	57 (67.9)	24 (28.6)	3 (3.6)	82.1	17.9	(17)
Germany	404 *(113/291)*	23.7	19 (4.7)	153 (37.9)	232 (57.4)	23.6	76.4	(18)
Polish	230 (*114/116*)	53.2	48 (20.9)	179 (77.8)	3 (1.3)	59.8	40.2	(28)
Chinese Han	244 (*126/118*)	38.5	97 (39.7)	110 (45.1)	37 (15.2)	62.3	37.7	(29)

Abbreviations: *n* – number of samples, M – male, F – female, nr – not reported.

The polymorphisms of rs211105, rs4537731 and rs4570625 also were documented to have significant correlations with other etiology of pathophysiology and clinical manifestations of depression and anxiety (19), psychosis (20), acute pancreatitis (21), schizophrenia (22) and many more. The SNPs implicate the alterations of protein expression of the TPH enzyme that can cause serotonin deficiency and distract the serotonergic functions (23,24). The lack of serotonin will lead to many problems including abdominal symptoms, visceral hypersensitivity, functional dyspepsia, unpredictable or violent behaviors and other neurological disorders (25,26). Eventually, this would lead to a higher susceptibility to exhibit the IBS and other related health problems. Therefore, research on the prevalence of the SNPs such as from this study is crucial in order to enhance a better understanding of the ethnic differences to the most common functional GI disorders, the IBS and provide some clues for the other health problems.

The investigation of the SNPs in the populations provides wider insight and some keys to the human phenotype variations individually or ethnically, especially when it relates to the health issues and diseases. This study described the prevalence of *TPH1* and *TPH2* SNPs susceptible to the IBS among unrelated, healthy Malays in Malaysia. The study proved only low frequencies of the mutant alleles of the SNPs among the Malays. The diverse trends observed in the distributions of genotypes and alleles frequencies of rs211105, rs4537731 and rs4570625 in different ethnic groups worldwide would play a decisive role in the IBS cohorts. Data generated from this study is beneficial to enhance our knowledge of the association between IBS pharmacogenetic profiles and the ethnic differences. While many researchers continue to investigate various possible factors to overcome and combat the symptoms of the IBS, data reported in the present study has provided a genetic hint of the *TPH* polymorphisms' roles on the pathophysiology. Future studies on Malaysian IBS patients are recommended to determine the susceptibility of rs211105, rs4537731 and rs4570625 to the IBS syndrome locally.

Conflict of interest

All authors declared having no conflict of interest.

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Authors' contributions

Concept: Z.Z., K.B.Y., M.A., Design: Z.Z., M.K.Z.J., Data Collection or Processing: N.M., Z.Z., M.K.Z.J., Analysis or Interpretation: N.M., R.A.R., Literature Search: R.A.R., M.A., Writing: R.A.R., N.M.

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