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Genetic association study of *PDLIM5* and *HTR2A* variants in Malaysian subjects diagnosed with bipolar disorder; a genetic modelling approach

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Abstract: Genetic hereditary has been implicated in bipolar disorder pathogenesis. The *PDLIM5* and *HTR2A* genes have been investigated for its association with bipolar disorder in various populations, however, the results have been conflicting. In this study, we investigate the association between bipolar disorder and the two genes of interest, *PDLIM5* and *HTR2A* genes. We recruited 253 bipolar disorder patients (75 Malays, 104 Chinese, and 74 Indians) and 505 control individuals (198 Malays, 155 Chinese, and 152 Indians) from three ethnic groups within Malaysian population. We genotyped for 3 SNPs of the *PDLIM5* (rs2433320, rs2433322 and rs2438146) and 3 SNPs of the *HTR2A* (rs6313, rs2070040 and rs6311). Significant associations between bipolar disorder and each of the 3 SNPs of *PDLIM5* in Malays, Indians and pooled samples. However, only rs2438146 remains significant in the Malays as co-dominant (T/T vs. C/C, p=0.004, OR=0.128, 95%CI=0.031-0.524) and recessive genetic models (T/T vs. C/T+C/C, p=0.003, OR=0.122, 95%CI=0.030-0.494) after applying conservative Bonferroni correction. Haplotype analysis of 3 SNPs of *PDLIM5* also showed a significant association with bipolar disorder. No association was observed between bipolar disorder and each of the 3 SNPs of *HTR2A* in any of the ethnicities. We conclude that *PDLIM5* polymorphisms are associated with bipolar disorder in the pooled analysis. After stratification to different ethnic groups, the association remains significant in the Malay and Indian groups. The association is also supported by the significant association in haplotype analysis of *PDLIM5*. We also conclude there is no association between the *HTR2A* polymorphisms in the Malaysian population.

Keywords: *PDLIM5*; *HTR2A*; polymorphism; bipolar disorder

1. Introduction

Bipolar disorder (BPD), also known as manic-depressive illness, is a mental disorder characterized by typical symptoms of mania and depression which manifested as an elevated mood, hyperactive, unusual talkativeness, unusual thought patterns, extreme sadness or hopelessness and in severe cases, psychosis. The symptoms could be severe and may result in damaged relationships, career failure and even suicide. Family, twin and adoption studies suggest a strong genetic aetiology with an estimated genetic hereditary of 63% [1].

The *PDLIM5* gene, or also known as PDZ and LIM domains, encodes the enigma homologue (ENH) which comprises of one *PDZ* domain located on the N-terminal and 1-3 domains located on the C-terminal. The *PDLIM5* lies on chromosome 4q22, found to be linked with BPD [2], major depression [3] and schizophrenia [4,5] through several linkage studies. The *PDLIM5* gene is ubiquitously expressed in the brain such as in the hippocampus, thalamus, hypothalamus, cortex, and amygdala and its cellular localization is identical with Synapsin I, which is known to be involved in neurotransmitter release [6]. While the *PDZ* domain of the *PDZ-LIM* protein has been demonstrated to be associated with the actin cytoskeleton [7], the *LIM* domain of *PDZ-LIM* proteins is known to be associated with the kinase protein [8,9]. Furthermore, the *LIM* domain was also found able to regulate the protein kinase C (PKC) activities in a PKC isoform-specific manner [10] and acts as an adaptor to PKC and N-type calcium channel [6]. Abnormalities in PKC activity had been suggested to be involved in the pathophysiology of BPD [11].

Previous gene expression studies revealed dysregulation of *PDLIM5* mRNA expression in the post-mortem brain tissues of BPD patients. It was reported that the expression level of *LIM* mRNA was significantly increased in the post-mortem brain tissues BPD patient [12]. Interestingly, PKC activities have also been found to be increased in post-mortem brain tissues of patient with BPD [13]. Besides, *PDLIM5* were found to be involved in the regulation of dendritic spine morphogenesis which was associated with BPD [14]. Recent information from animal behavioural study also showed that the *PDLIM5* was implicated in the development of psychiatric symptoms, including mood disorder [15]. The involvement of *PDLIM5* in mental disorders was supported by genetic association studies that relate several polymorphisms on *PDLIM5* with mental disorders. For examples, the single nucleotide polymorphism (SNP) rs2433320 is associated with schizophrenia [16], major depression [17] and BPD [18-20]. Moreover, *PDLIM5* SNP rs2433322 has been found associated with schizophrenia [17] whereas another SNP, rs2438146 is associated with BPD in its allelic frequencies but not genotype frequencies [18]. In addition, a previous study showed that rs2433322 and rs2438146 did not associate with BPD in its own, but the haplotypes constructed from three SNPs of rs2433320-rs2438146-rs2433322 were significantly associated with BPD [20]. There were also several studies failed to replicate the significant association found in schizophrenia [21], major depression [22] and BPD [23].

A serotonin or 5-hydroxytryptamine (5-HT), is a neurotransmitter in the brain that involved in many physiological roles including developmental, cardiovascular, gastrointestinal, and endocrine function, sensory perception, behaviours such as memory and learning, mood, sexual desires, sleep and cognition [24]. Serotonin-2A receptor (*HTR2A*) is a subtype of serotonin gene families and is located on chromosome 13q14-q21. The *HTR2A* has been associated with neuropsychiatric disorder like schizophrenia, BPD, obsessive compulsive disorder, anorexia and major depressive disorder, and

cardiovascular disorders such as atherosclerosis and hypertension [25]. In gene expression study, the *HTR2A* mRNA was reported to be upregulated in the peripheral blood of major depression patients and correlated with severity and duration of depression illness [26]. In BPD particularly, studies based on post-mortem brain, cerebrospinal fluid (CSF), neuroendocrine, genetic, platelet and psychopharmacological studies have shown that serotonin plays a pivotal role in the pathophysiology of BPD [26]. However, inconsistent results have been observed in previous genetic association studies; even though in general more studies failed to find a significant association between *HTR2A* polymorphism and bipolar disorder [27-29]. Inconsistency in the results reported for both genes of *PDLIM5* and *HTR2A* in genetic association studies of BPD may be attributed to genetic heterogeneity in certain populations or methodological diversity among studies.

In the present study, we genotyped three SNPs of *PDLIM5* (rs2433320, rs2433322 and rs2438146) and three SNPs of *HTR2A* (rs6313, rs2070040 and rs6311) which previously reported to have conflicting result in their association with BPD. We performed a case-control study involving Malaysian subjects diagnosed with BPD and analysed the data based on four different genetic models which are multiplicative (allelic), co-dominant, recessive and dominant models. Besides, we also performed haplotype analysis to identify the effects of multiple SNPs of *PDLIM5* and *HTR2A* in BPD.

2. Materials and Methods

2.1. Samples recruitment

The study population consisted of 253 bipolar disorder patients (male=131, female=122, mean age 43.25±12.35 years), and 505 control individuals (male=298, female=207, mean age 33.81±12.29). The number of patients after stratification into ethnicities were 75 Malays (mean age 39.93±12.33), 104 Chinese (mean age 43.47±12.35) and 74 Indians (mean age 46.52±11.61) with bipolar disorder, whereas the number of control participants were 198 Malays (mean age 31.97±11.41), 155 Chinese (mean age 33.94±13.03) and 152 Indians (mean age 36.11±12.31) each.

Recruitment of study cases was carried out in the inpatient and outpatient psychiatric clinics of two medical centres, namely, the Universiti Malaya Medical Centre (UMMC), and the Universiti Kebangsaan Malaysia Medical Centre. All patients fulfilled the criteria of Diagnostic and Statistical Manual of Mental Disorders, 4th ed. for bipolar disorder. Control individuals were recruited from among the staff and students of the University of Malaya and from the general medical clinics of UMMC who had been confirmed to be free from chronic diseases. The protocol used in this study was approved by the Medical Ethics Committee of UMMC, and this study was conducted in accordance with the Declaration of Helsinki. All the participants signed a consent form before participating in the study and an absence of inter-ethnic marriage for at least three previous generations was self-reported. These individuals were also checked for an absence of concurrent chronic diseases and/or alcoholism and substance abuse. Furthermore, all cases and controls were interviewed to obtain socio-demographic details including age, sex, marital status, ethnicity and occupation.

2.2. SNPs selection and genotyping

There were three SNPs of *PDLIM5* (rs2433320, rs2433322 and rs2438146) and three SNPs of *HTR2A* (rs6313, rs2070040 and rs6311) which were genotyped using Real-Time PCR (RT-PCR). The SNPs information based on dbSNP database of National Centre for Biotechnology Information (NCBI) was assessed on 19 Jun 2018 [30] and was summarized in Table 1. Four genetic models were tested; multiplicative (A vs. B), co-dominant (AA vs. BB; AB vs. BB), recessive (AA vs. BB + AB) and dominant (AA + AB vs. BB) models – A and B are alleles for variant and wild-type, respectively. The DNA of the patients and control individuals were extracted from the blood samples using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) and followed the standard protocols provided by the manufacturer.

Table 1: Basic information of three polymorphisms of *PDLIM5* and *HTR2A* genes

Gene	SNPs	Gene position	Heterozygosity	MAF*	Functions	mRNA position	Maj/Min alleles
PDLIM5	Rs2433320	Upstream	ND[a]	0.272	-	-	G/A
	Rs2438146	Intron	0.406	0.277	-	-	C/T
	Rs2433322	Intron	0.406	0.272	-	-	A/G
HTR2A	Rs6313	Intron	0.486	0.441	Synonymous	791	G/A
	Rs2070040	Intron	0.479	0.334	-	-	G/A
	Rs6311	Promoter	ND[a]	0.444	-	-	C/T

[a] ND = not determined; * MAF = Minor Allele Frequency; Maj/Min = Major/Minor

Genotyping by RT-PCR was performed by following the manufacturer's protocol and the probe of the SNPs used were commercially available (rs2433320, C_16015055_20; rs2438146, C_2095071_10; rs2433322, C_16015054_10; rs6313, C_3042197_1; rs2070040, C_16285134_10; rs6311, C_8695278_10) (Applied Biosystem, CA, USA).

2.3. Statistical analysis

The output of the genotype data was analysed using Microsoft Excel (Microsoft, Redmond, Washington, USA) and SPSS Statistical Software (SPSS Inc., Chicago, Illinois, USA). The Hardy–Weinberg equilibrium (HWE) values for both SNPs were calculated using calculator online tools (Court, 2005). The odds ratio (OR) with 95% confidence interval (CI) were adjusted for age at study and sex through binary logistic regression analysis in all ethnic groups, while, the OR with 95% CI were adjusted for ethnicity, age at study and sex in the pooled population. Two-sided tests of statistical significance were used to determine statistically significant p-values ($p<0.05$), followed by Bonferroni correction for multiple tests of the six SNPs in patients and controls ($p=0.05/6$ SNPs; $p=0.0083$). To avoid increasing Type II error rate, the Bonferroni correction was corrected for only the number of SNPs as it was an actual variable tested in the present study. The power of this study was determined and calculated to estimate the sample size by using Power for Association with Error online tools (Rockefeller University, New York, New York, USA) [31]. Haplotype and linkage disequilibrium (LD) analysis for both SNPs of *PDLIM5* was carried out using SHEsis and SHEsisPlus online tools (SHEsis; Bio-X Life Science Research Center, Shanghai, China) [32-34]. The p-value for haplotype analysis was adjusted according to Benjamini-Hochberg false-discovery rate (FDR) BH procedure.

3. Results

3.1. BPD relationship *with PDLIM5 and HTR2A SNPs*

Genotypic and allelic distributions of both *PDLIM5* and *HTR2A* polymorphisms are summarized in Table 2. The genetic models of both *PDLIM5* and *HTR2A* polymorphisms in the Malay, Chinese, Indian and pooled population are presented in Table 3. The genotype distributions for all controls in both *PDLIM5* and *HTR2A* polymorphisms were in Hardy-Weinberg equilibrium.

In the present study, we observed no association between the allelic frequencies of the six SNPs and BPD in every group (Table 3). In the Malays, there was a significant difference between case and control subjects in co-dominant and recessive models of rs2433320 (A/A vs. GG; A/A vs. G/A+G/G) and rs2438146 (T/T vs. C/C; T/T vs. C/T + C/C) and rs2433322 (A/G vs. A/A; G/G + A/G vs. A/A) polymorphisms. While, no association was found between any *PDLIM5* polymorphisms and BPD in the Chinese, the genotypes for co-dominant and recessive models of rs2438146 (T/T vs. C/C; T/T vs. C/T+C/C) and rs2433322 (G/G vs. A/A; G/G vs. A/G + A/A) polymorphisms were significantly associated with BPD in the Indians. There was also a trend towards association in SNP rs2070040 under co-dominant (G/A

vs. G/G; p=0.06, OR=0.712, 95% CI= 0.500-1.013) model in the pooled subjects. After correction for multiple SNPs by using conservative Bonferroni correction, only SNP rs2438146 remains significant under co-dominant (T/T vs. C/C, p=0.004, OR=0.128, 95%CI=0.031-0.524) and recessive (T/T vs. C/T+C/C, p=0.003, OR=0.122, 95%CI=0.030-0.494) models in the Malays (p=0.0042 after Bonferroni correction).

Table 2: Genotype and allele frequencies of *PDLIM5* and *HTR2A* polymorphisms of case and control in three ethnic groups of the Malaysian population

*Allele/	Malay		Chinese		Indian		Pooled	
Genotype	Case (%)	Control (%)	Case (%)	Control (%)	Case (%)	Control (%)	Case (%)	Control (%)
PDLIM5								
rs2433320								
G	155 (77)	327 (83)	167 (80)	246 (79)	116 (80)	245 (81)	398 (79)	818 (81)
A	35 (23)	69 (17)	41 (20)	64 (21)	30 (20)	57 (19)	106 (21)	190 (19)
GG	47 (63)	132 (67)	67 (64)	100 (64)	47 (64)	100 (66)	161 (64)	332 (66)
AG	21 (28)	63 (32)	33 (32)	46 (30)	22 (30)	45 (30)	76 (30)	154 (31)
AA	7 (9)	3 (1)	4 (4)	9 (6)	4 (6)	6 (4)	15 (6)	18 (3)
rs2438146								
C	113 (75)	327 (83)	174 (84)	246 (79)	107 (72)	245 (81)	394 (78)	818 (81)
T	37 (25)	69 (17)	32 (16)	64 (21)	41 (28)	57 (19)	110 (22)	190 (19)
CC	47 (63)	132 (67)	74 (72)	101 (65)	43 (58)	100 (66)	164 (65)	333 (66)
CT	19 (25)	63 (32)	26 (25)	44 (28)	21 (28)	45 (30)	66 (26)	152 (30)
TT	9 (12)	3 (1)	3 (3)	10 (7)	10 (14)	6 (4)	22 (9)	19 (4)
rs2433322								
A	115 (77)	327(83)	170 (82)	240 (77)	106 (72)	245 (81)	391 (77)	812 (81)
G	35 (23)	69 (17)	38 (18)	70 (23)	42 (28)	57 (19)	115 (23)	196 (19)
AA	48 (64)	132 (67)	70 (67)	96 (62)	42 (57)	100 (66)	160 (63)	328 (65)
AG	19 (25)	63 (32)	30 (29)	48 (31)	22 (30)	45 (30)	71 (28)	156 (31)
GG	8 (11)	3 (1)	4 (4)	11 (7)	10 (14)	6 (4)	22 (9)	20 (4)
HTR2A								
rs6313								
G	53 (35)	146 (37)	71 (34)	122 (39)	77 (52)	153 (51)	201 (40)	421 (42)
A	97 (65)	250 (63)	137 (66)	188 (61)	71 (48)	145 (49)	305 (60)	583 (58)
GG	11 (15)	35 (18)	11 (11)	21 (13)	19 (26)	43 (29)	41 (16)	99 (20)
GA	31 (41)	76 (38)	49 (47)	80 (52)	39 (52)	67 (45)	119 (47)	223 (44)
AA	33 (44)	87 (44)	44 (42)	54 (35)	16 (22)	39 (26)	93 (37)	180 (36)
rs2070040								
G	104 (69)	286 (72)	138 (66)	215 (70)	97 (66)	202 (67)	167 (33)	303 (30)
A	46 (31)	110 (28)	70 (34)	93 (30)	51 (34)	100 (33)	339 (67)	703 (70)
GG	37 (49)	111 (56)	43 (41)	73 (47)	30 (40)	68 (45)	110 (44)	252 (50)
AG	30 (40)	64 (32)	52 (50)	69 (45)	37 (50)	66 (44)	119 (47)	199 (40)
AA	8 (11)	23 (12)	9 (9)	12 (8)	7 (10)	17 (11)	24 (10)	52 (10)
rs6311								
C	52 (35)	143 (36)	73 (35)	120 (40)	76 (52)	146 (49)	201 (40)	409 (41)
T	96 (65)	253 (64)	135 (65)	184 (60)	70 (48)	154 (51)	301 (60)	591 (59)
CC	12 (16)	33 (17)	11 (11)	21 (14)	19 (26)	37 (25)	42 (17)	91 (18)
CT	28 (39)	77 (39)	51 (49)	78 (52)	38 (52)	72 (48)	117 (47)	227 (46)
TT	34 (46)	88 (44)	42 (40)	53 (35)	16 (22)	41 (27)	92 (37)	182 (36)

* Few samples failed to be genotyped which resulted a slightly difference in the number of sample and number of genotypes.

Table 3: Genotype and allelic frequencies of *PDLIM5* and *HTR2A* polymorphisms in case and control subjects in total population and in the three ethnic subgroups under alternative genetic models.

Genotype	Ethnicity												Pooled			
	Malay				Chinese				Indian							
	Cs	Ctl	*p	[a]OR (95% CI)	Cs	Ctl	*p	[a]OR (95% CI)	Cs	Ctl	*p	[a]OR (95% CI)	Cs	Ctl	*p	[a]OR (95% CI)
rs2433320																
A vs. G	35	69	0.19	0.168 (0.038-0.744)	41	64	0.30	2.390 (0.463-12.326)	30	57	0.34	0.460 (0.099-2.144)	106	190	0.24	0.615 (0.272-1.390)
G/A vs. G/G	47	132	0.83	1.067 (0.588-1.939)	33	46	0.56	0.835 (0.452-1.541)	22	45	0.78	1.107 (0.548-2.223)	76	154	0.74	0.939 (0.651-1.356)
A/A vs. G/G	7	3	0.01	0.155 (0.038-0.624)	4	9	0.34	2.256 (0.432-11.783)	4	6	0.35	0.475 (0.100-2.245)	15	18	0.23	0.603 (0.264-1.375)
A/A vs. G/A+G/G	7	3	0.02	0.168 (0.038-0.744)	4	9	0.30	2.390 (0.463-12.326)	4	6	0.32	0.460 (0.099-2.144)	15	18	0.24	0.615 (0.272-1.390)
G/A+A/A vs. G/G	28	65	0.43	0.786 (0.435-1.420)	37	55	0.81	0.928 (0.514-1.675)	26	51	1.00	1.002 (0.514-1.954)	91	172	0.52	0.890 (0.627-1.264)
rs2438146																
T vs. C	37	69	0.53	0.827 (0.458-1.490)	32	64	0.26	1.415 (0.772-2.592)	41	57	0.48	0.788 (0.410-1.514)	110	190	0.88	0.973 (0.685-1.381)
C/T vs. C/C	19	63	0.65	1.161 (0.606-2.224)	26	44	0.45	1.281 (0.675-2.429)	21	45	0.79	1.104 (0.536-2.273)	66	152	0.01	2.664 (1.261-5.627)
T/T vs. C/C	9	3	**<0.01**	**0.128 (0.031-0.524)**	3	10	0.21	2.474 (0.603-10.151)	10	6	0.01	0.191 (0.054-0.677)	22	19	0.02	2.310 (1.139-4.683)
T/T vs. C/T+C/C	9	3	**<0.01**	**0.122 (0.030-0.494)**	3	10	0.24	2.308 (0.569-9.363)	10	6	0.01	0.185 (0.053-0.645)	22	19	0.01	2.410 (1.199-4.844)
T/T+C/T vs. C/C	28	66	0.53	0.827 (0.458-1.490)	29	54	0.26	1.415 (0.772-2.592)	31	51	0.48	0.788 (0.410-1.514)	88	171	0.88	1.028 (0.724-1.459)
rs2433322																
G vs. A	35	69	0.54	0.829 (0.458-1.502)	38	70	0.46	1.251 (0.695-2.254)	42	57	0.73	3.727 (0.379-1.394)	115	196	0.65	0.923 (0.652-1.306)
A/G vs. A/A	19	63	0.01	0.144 (0.034-0.606)	30	48	0.17	2.490 (0.673-9.212)	22	10	0.99	0.993 (0.486-2.030)	71	156	0.79	1.052 (0.724-1.528)
G/G vs. A/A	8	3	0.74	1.116 (0.583-2.137)	4	11	0.77	1.097 (0.588-2.047)	10	6	0.01	0.185 (0.052-0.658)	22	328	0.04	0.485 (0.240-0.981)
G/G vs. A/G+A/A	8	3	0.01	0.139 (0.033-0.579)	4	11	0.18	2.421 (0.663-8.834)	10	6	0.01	0.185 (0.053-0.645)	22	328	0.04	0.478 (0.238-0.957)
G/G+A/G vs. A/A	27	66	0.54	0.829 (0.458-1.502)	34	59	0.46	1.251 (0.695-2.254)	32	16	0.34	0.727 (0.379-1.394)	93	484	0.65	0.923 (0.652-1.306)
rs6313																
A vs. G	97	250	0.15	0.553 (0.246-1.247)	137	188	0.41	0.690 (0.283-1.678)	71	145	0.86	0.936 (0.458-1.916)	305	583	0.10	0.691 (0.444-1.077)
G/A vs. G/G	31	76	0.20	0.572 (0.241-1.356)	49	80	0.21	0.541 (0.206-1.422)	39	67	0.50	0.769 (0.361-1.639)	119	223	0.11	0.678 (0.424-1.086)
A/A vs. G/G	33	87	0.16	0.533 (0.222-1.279)	44	54	0.65	0.806 (0.320-2.032)	16	39	0.44	1.442 (0.573-3.631)	93	180	0.17	0.709 (0.433-1.162)
A/A vs. G/A+G/G	33	87	0.70	0.895 (0.506-1.584)	44	54	0.15	0.645 (0.356-1.170)	16	39	0.18	1.712 (0.786-3.726)	93	180	0.72	0.923 (0.659-1.335)
A/A+G/A vs. G/G	64	163	0.15	0.553 (0.246-1.247)	93	134	0.41	0.69 (0.283-1.678)	55	106	0.86	0.936 (0.458-1.916)	212	403	0.10	0.691 (0.444-1.077)
rs2070040																
A vs. G	46	110	0.40	1.497 (0.582-3.847)	70	93	0.94	1.043 (0.371-2.937)	51	100	0.69	1.312 (0.427-4.032)	339	703	0.33	1.331 (0.746-2.372)
G/A vs. G/G	30	64	0.24	0.695 (0.379-1.276)	52	69	0.25	0.708 (0.392-1.281)	37	66	0.52	0.803 (0.413-1.561)	119	199	0.06	0.712 (0.500-1.013)
A/A vs. G/G	8	23	0.61	1.293 (0.486-3.438)	9	12	0.81	0.875 (0.297-2.579)	7	17	0.80	1.165 (0.358-3.793)	110	252	0.69	1.131 (0.618-2.068)
A/A vs. G/A+G/G	8	23	0.40	1.497 (0.582-3.847)	9	12	0.94	1.043 (0.371-2.937)	7	17	0.64	1.312 (0.427-4.032)	110	252	0.33	1.331 (0.746-2.372)
A/A+G/A vs. G/G	38	87	0.45	0.803 (0.455-1.414)	61	81	0.28	0.731 (0.413-1.294)	44	83	0.62	0.851 (0.448-1.618)	229	451	0.14	0.775 (0.553-1.084)
rs6311																
T vs. C	96	253	0.61	0.860 (0.485-1.525)	135	184	0.49	0.813 (0.451-1.465)	70	154	0.20	1.649 (0.766-3.550)	301	591	0.95	0.989 (0.695-1.407)
C/T vs. C/C	28	77	0.34	0.654 (0.273-1.563)	51	78	0.52	0.737 (0.295-1.845)	38	72	0.75	1.137 (0.524-2.464)	117	227	0.37	0.805 (0.501-1.291)
T/T vs. C/C	34	88	0.30	0.640 (0.275-1.490)	42	53	0.35	0.634 (0.244-1.649)	16	41	0.22	1.801 (0.708-4.578)	92	182	0.50	0.845 (0.515-1.385)
T/T vs. C/T+C/C	34	88	0.61	0.860 (0.485-1.525)	42	53	0.49	0.813 (0.451-1.465)	16	41	0.20	1.649 (0.766-3.550)	92	182	0.95	0.989 (0.695-1.407)
T/T+C/T vs. C/C	62	165	0.28	0.646 (0.291-1.436)	93	131	0.42	0.694 (0.287-1.679)	54	113	0.47	1.311 (0.627-2.739)	209	409	0.39	0.821 (0.526-1.282)

Continued from previous page
Cs, Case: Ctl, Control.
[a] estimated odds ratio by binary logistic regression after adjustment to age at study, gender and ethnicity in pooled population, while the odds ratio was adjusted to age at study and gender in each ethnicity.
* p-value was rounded to two decimals point. Only SNP rs2438146 in Malay remains significant (bold) after Bonferroni correction was applied p<0.0083.

3.2. Linkage disequilibrium (LD) and haplotype analysis

Pairwise LD coefficients r^2 between *PDLIM5* SNPs rs2433320-rs2438146-rs2433322 in the pooled population was less than 0.90. The LD coefficients r^2 at 0.90 and above was set as in LD. The LD for rs2438146 and rs2433322 was at r^2=0.86. Moreover, the r^2 between *HTR2A* SNPs rs6313, rs2070040 and rs6311 in the pooled population did not reach 0.90.

For haplotype analysis, we analysed only the common haplotypes (frequency> 0.03) in the pooled population. We summarized haplotype analysis results in Table 4 and Table 5, with the column for case and control frequencies in both tables that were presented in percentage unit with zero decimal. In Table 4, we showed that G-C-A haplotype constructed from *PDLIM5* SNPs rs2433320, rs2438146 and rs2433322 was the most frequent SNPs in case (0.74) and control (0.72) subjects. However, only A-C-A showed significantly associated after adjusted with FDR correction. The ratio of this wild-type haplotype (G-C-A) to the mutant haplotype (A-C-A) frequency was 24.67 in case subject. While, in table 5, there was no association observed in any haplotype constructed between *HTR2A* SNPs rs6313, rs2070040 and rs6311.

Table 4: Haplotype frequencies of rs2433320, rs2438146 and rs2433322 polymorphisms of PDLIM5 gene within case and control subjects in the pooled population.

#Haplotypes	Pooled Population			
	Case (Freq %)	Control (Freq %)	p-value (*FDR q-value)	OR (CI 95%)
G-C-A	370 (0.74)	712 (0.72)	0.285 (0.326)	1.138 (0.896-1.445)
A-T-G	82 (0.16)	150 (0.15)	0.489 (0.489)	1.108 (0.826-1.486)
A-C-A	17 (0.03)	74 (0.08)	**0.002 (0.003)**	0.439 (0.256-0.753)
G-T-G	22 (0.04)	28 (0.03)	0.105 (0.14)	1.594 (0.902-2.815)

* The FDR q-value < 0.05 is significant (bold). # Haplotypes with frequency <0.03 are ignored.

Table 5: Haplotype frequencies of rs6313, rs2070040 and rs6311 polymorphisms of *HTR2A* gene within case and control subjects in the pooled population.

#Haplotypes	Pooled Population			
	Case (Freq %)	Control (Freq %)	p-value (*FDR q-value)	OR (CI 95%)
A-G-C	277 (0.551)	531 (0.534)	0.424 (0.543)	1.091 (0.88-1.351)
G-A-T	143 (0.284)	268 (0.269)	0.475 (0.543)	1.09 (0.859-1.384)
G-G-T	53 (0.105)	98 (0.098)	0.636 (0.636)	1.088 (0.765-1.549)

* The FDR q-value < 0.05 is significant. # Haplotypes with frequency <0.03 are ignored.

4. Discussion

The present study found a significant association between *PDLIM5* SNPs (rs2433320, rs2438146 and rs2433322) and BPD in Malays, Indians and pooled Malaysian subjects. However, after performing conservative Bonferroni correction, only rs2438146 remains associated with BPD in the Malays. In addition, the haplotype analysis that we performed also supported the result of association found between *PDLIM5* and BPD in the Malays. While we observed a significant association between *PDLIM5* and BPD, there was no association observed between *HTR2A* and BPD in any groups of the population tested in single SNP association and haplotype studies.

Previous genetic association studies of *PDLIM5* SNP rs2433320 showed significant association in BPD [18], schizophrenia [16] and major depression [17].

However, there was a study that reported an absence of association between rs2433320 and BPD in the Han Chinese population [20], which is comparable with our result found in the present study with the Chinese group. The A allele of rs2433320 has been suggested to associate with higher expression of *PDLIM5* in the post-mortem brain of schizophrenia [16], while in another study, the G allele of rs2433320 has been suggested to have a protective role in major depression [17]. In the present study, the co-dominant model of rs2433320 was significantly increased in BPD of the Malays group, however, did not survive after strict Bonferroni correction. Our results suggest that the co-dominant model of rs2433320 may have a small effect size to show a true association.

Kato *et al*. (2005) and Zhao *et al*. (2009) reported significant associations between two SNPs of *PDLIM5*; rs2438146 and rs2433322, with BPD in Japanese (independent sample sets) and Han Chinese, respectively [18,20]. Zhao *et al*. (2009) [20] suggested that the G allele of rs2433322 might be a risk factor in BPD as the allele appears to be in higher frequency in cases compared to control subjects. While, our result in Chinese did not support a significant association of rs2433322 and BPD [20], we did find a significant association in recessive model of rs2433322 and BPD in Malays and Indians. We also observed that the G allele of rs2433322 appeared more in the case than in control subjects and thus supported previous suggestions that the G allele is a risk factor for BPD. The risk, however, is likely to be small (OR<1). In addition, we also observed the co-dominant (T/T vs. CC) and recessive (T/T vs. C/T+C/C) models of SNP rs2438146 remains significant even after Bonferroni correction. Our results may explain previous results which found no association observed in allelic (T vs. C) and genotype (T/T vs. T/C vs. C/C) frequencies between SNP rs2438146 and BPD; the discrepancies between the present and the previous study suggested that allele T of SNP rs2438146 carries more effect in the homozygous form of T/T, and loses its effect in the heterozygous form of C/T as no significant observed under dominant (T/T+C/T vs. C/C) and co-dominant (C/T vs. C/C) models.

In the haplotype analysis, after corrected for FDR, we observed a significant association between A-C-A haplotype constructed from *PDLIM5* SNPs rs2433320, rs2438146 and rs2433322, and BPD. The odds ratio produced was 0.439 at 95% confidence interval of 0.256-0.753, indicates protective nature of the haplotype.

In the present study, there are several limitations that need to be taken into consideration. Firstly, the sample size in this study after stratification into ethnicities did not achieve 80% of power of study. For example, for the SNP rs2438146 to achieve 80% power of study, it required a minimal sample size of 182 cases and 480 controls for Malay; 559 cases and 833 controls for Chinese; and 203 cases and 426 controls for Indian. Thus, we cannot exclude a false positive error from the analysis for each ethnic group. However, it must be noted that the present results are not conflicting with previous studies. Secondly, the SNPs rs2438146 and rs2433322 are in the deviation of HWE in the Malays and Indians cases (but not in controls). However, we did not find any genotyping errors via manual checks as suggested by Lewis (2002) [35]. It was explained that the cases would not be in HWE for a SNP with a true genetic effect that is not controlled by a multiplicative model. However, if the cases were in HWE, the data can be analysed by allele counting (multiplicative model) [35,36].

5. Conclusions

In conclusion, we showed a significant association between polymorphisms of the *PDLIM5* and BPD in Malays, Indians and pooled subjects. However, only the SNP rs2438146 remains significant in Malays after performing the correction for multiple tests. The significant association was strengthened by results of haplotype analysis of the *PDLIM5* polymorphism. We did not detect any significant association between the screened SNPs or haplotypes of the *HTR2A* with BPD.

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