NEUROSCIENCE RESEARCH NOTES

OPEN ACCESS | RESEARCH NOTES ISSN: 2576-828X

Dopamine transporter 1 (DAT1) rs40184 single nucleotide polymorphism is not associated with the Malaysian major depressive disorder subjects

Asraa Faris Aldoghachi [1], Pike-See Cheah [2], Normala Ibrahim [3], Munn Sann Lye [4] and King-Hwa Ling [1]*

[1] Department of Biomedical Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

[2] Department of Human Anatomy, University Putra Malaysia, Serdang, Selangor, Malaysia.

[3] Department of Psychiatry, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

[4] Department of Community Health, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

* Correspondence: lkh@upm.edu.my; Tel.: +603-89472564

Received: 18 July 2019; **Accepted:** 11 November 2019; **Published:** 4 December 2019

Edited by: Narisorn Kitiyanant (Mahidol University, Thailand)

Reviewed by: Azlina Ahmad Annuar (Universiti Malaya, Malaysia); Banthit Chetsawang (Mahidol University, Thailand); Meow-Keong Thong (Universiti Malaya, Malaysia)

https://doi.org/10.31117/neuroscirn.v2i4.36

Abstract: Major depressive disorder (MDD) is a serious mental illness with a multifactorial aetiology that was shown to influence behaviour and affect cognition. Previous research has favoured the involvement of dopamine in the aetiology of the disorder, and since one of the critical regulators of the dopamine levels and activity in the brain is DAT1, the present study investigated the association of a single nucleotide polymorphism in the *DAT1* gene (rs40184) and MDD in the Malaysian population. A total of 300 cases and 300 matched controls were recruited from four Klang valley hospitals and were screened for *DAT1* rs40184 using high resolution melting assays. The allele and genotype frequencies were analysed by using Chi-square. Hardy Weinberg equilibrium for the distribution of alleles and genotypes was tested by using Chi-square. Determination of the association between rs40184 and MDD was achieved by conditional logistic regression using SPSS. In the present study, no significant association was obtained between *DAT1* and MDD in the Malaysian population.

Keywords: Major depressive disorder; DAT1; rs40184; high-resolution melting; polymerase chain reaction

1.0 INTRODUCTION

Sadness is a normal emotion that is experienced by everyone at one point of life due to sickness, stressful life events and loss of a loved one. However, when this sadness occur due to no possible reason and lasts for two or more constitutive weeks and is accompanied by

not only emotional symptoms (depressed mood) but also a range of somatic (dizziness, tiredness and fatigue), cognitive (inability to concentrate and feelings of guilt and worthlessness) and motivational symptoms (changes in eating and sleeping habits and lack of interest in once pleasurable activities and an intention to suicide), then it is called major depressive disorder (MDD) [1]. Not only does MDD affect the emotional functioning of an individual but also, it affects the occupational and social functioning and can lead to suicidal acts.

Worldwide, major depressive disorder is the fourth disabling disorder, and it is expected to be the first by the year 2030 [2]. The disorder was reported to be twice as common in females as compared to males with a lifetime prevalence of 16.2% [3]. To date, no established mechanism can thoroughly explain the disorder, but there are many factors exerting small effects such as nutrition, neurotransmitters imbalance, lifestyle and genetics. In a study by Nazree et al. (2013), the tryptophan hydroxylase 2 (TPH2) rs7305115, rs1386494 and rs1386495 variants were screened in 265 Malaysian MDD case and 332 Malaysian controls. The study reported no association between the variants and MDD [4]. Likewise, in a study conducted on 265 MDD patients and 332 healthy subjects from the three Malaysian ethnicities (Malay, Indian and Chinese), no association was obtained between the variants rs1800532 and rs1799913 of the Tryptophan Hydroxylase 1 (TPH1) gene and MDD. However, results for the haplotype analysis in the same study suggested that TPH1 may be a risk factor for MDD in the Indian ethnicity but not the Malay and the Chinese ethnicities [5]. On the other hand, in a study on 300 MDD case and 300 controls matched for age, gender and ethnicity, BDNF rs6265 was shown to increase the risk of developing MDD by 2.05 folds (95% CI = 1.48–3.65) [6]. In a preliminary study by Tiong et al., 2013, the association of the serotonin transporter reporter gene and response to the antidepressant escitalopram was investigated among 29 MDD Malaysian patients. The study reported a better response rate among the SS genotype of the 5-HTTLPR gene as compared to the LS and the LL genotypes (P=0.04), but no association was obtained between the 5-HTTLPR and the adverse effect of the antidepressant among MDD patients [7].

Since the reports in 1970 on the antidepressants behavioural, biochemical and other clinical effects, the function of dopamine in the aetiology of MDD has gained a huge interest [8]. Dopamine is a neurotransmitter that regulates the sense of pleasure, motivation and concentration in the central nervous system and these functions were found to be impaired in depressed patients. Hence, several recent studies have focused on the role of dopamine in the aetiology of mental illnesses among which is MDD [9-13]. Clinically, several studies have noted an alteration in the activity of dopamine upon antidepressant administration [8]. Recent research has focused on the molecular role of genes responsible for the dopaminergic activity and MDD among which is the dopamine transporter, DAT1 [14]. DAT1 is the primary regulator of the dopamine level in the brain and was shown to control the concentration of dopamine between the dopaminergic neurons in the synaptic cleft by dopamine reuptake from the presynaptic terminals, hence terminating the dopaminergic activity and, in the human brain, the distribution was mainly found in the basal ganglia [15]. DAT1 consists of 15 exons expressing in all the dopaminergic neurons and was found to be located at chromosome 5q35.1 [16-18].

Despite the availability of research on the association of dopamine with MDD, there have been few studies linking DAT1 and MDD. In a review conducted by Gatt et al. (2015) on 157 meta-analysis studies to analyse the genes associated with mental illnesses such as schizophrenia, anxiety disorder, attention deficit hyperactivity disorder, bipolar disorder, and MDD, DAT1 was found to be associated with MDD [10]. One of the studied SNPs in *DAT1* is the rs40184 (C/T) that is located on the 14th intron of the gene. In a gene-environment study by Haeffel et al., (2008), the association between style of maternal parenting and *DAT1* rs40184, rs6347 and rs2652511, and major depressive disorder was studied in 176 Russian male adolescents. In the study,

only rs40184 was found to moderate the maternal rejection that was found to elevate the risk of depression among individuals that carried the TT genotype as compared to CC and carriers of the CT genotypes (p=0.003) [19]. In another study conducted on 178 MDD patients and 205 healthy controls in Thailand, no significant difference in the allele and genotype distribution was observed. However, the C allele carriers were observed more in the controls as compared to the cases whereas a significant difference in the rs40184 allele frequencies between the Caucasian, African and Asian healthy subjects was observed [20]. Due to the crucial role of dopamine in regulating mood and vital role of DAT1 in controlling its level, the purpose of this study was to determine the association between *DAT1* rs40184 and major depressive disorder in the Malaysian population.

2.0 MATERIALS AND METHODS

2.1 Study design and sample recruitment

A total of 300 matched controls and 300 cases were recruited from four hospitals at the Klang Valley region. The cases were diagnosed as MDD by psychiatrists using the DSMV criteria and were of the Malay, Chinese or Indian ethnicity between the age of 18-65. Cases diagnosed with other neuropsychiatric illnesses were excluded from the study. The controls were recruited from the ear, nose and throat clinic and ophthalmology clinic of the same four hospitals and were also recruited from Universiti Putra Malaysia and were matched to the cases by ethnicity, gender and age in a ratio of 1:1. The subject recruitment lasted for 4 years starting from 2014 until 2018, and all the study subjects were asked to fill a written consent after being given a detailed introduction of the study nature and the expected outcome. Subjects were also informed about the confidential and the voluntary nature of joining the study and were allowed to ask questions related to the study and withdraw from the study whenever they wanted to. All the procedures used in the study complied with Helsinki's declaration and were approved by the National Medical Research and Ethics Committee (reference number: NMRR-14-688-19696 (IIR)). The overview of the study and the definition of the cases and the controls was the same as the one used by [6].

2.2 Screening of *DAT1* rs40184

Following the manufacturer's protocol, the genomic DNA was extracted from the buffy coat using the QIAamp® DNA Mini Kit (QIAGEN). All the samples were of high quality (A260/A280 absorbance of 1.7-1.9). The integrity of the samples was measured by running the samples on gel electrophoresis for 40 minutes at 100V on 1% (w/v) agarose gel. Following the extraction of the DNA, samples were screened for the rs40184 SNP in the *DAT1* gene by high resolution melting using LightCycler® 480 (Roche, Switzerland) with CACAGTCTCGCGGCTTTT as a forward primer and CACAGTCTCGCGGCTTTT as the reverse primer. Both the primers were designed using Primer3Plus following the same criteria used by Faris et al., (2018) [21]. The HRM mixture consisted of DNA (15ng), forward and reverse primers (0.35µmol), PCR grade water, MgCl2 (2mM) and 1X LightCycler® 480 High-Resolution Melting Master (Roche, Switzerland) to achieve a final reaction volume of 10µl. The amplification of the DNA was based on the following program: After the initial denaturation at 95°C for 10 min, each cycle (45) consisted of 95°C amplification for 10s, 61°C annealing for 15s and 72°C extension for 10s. The HRM was then carried out at temperature range of 65°C-95°C with 25 acquisitions per every 1°C increment. The different genotypes were identified based on the difference in the melting profiles. 10% of the samples with different melting profiles as observed by the HRM were sent to First BASE Laboratories Sdn Bhd, Malaysia, for sequencing and confirmation of their genotypes and were then used as controls for the subsequent runs. The sequencing results were assembled and analysed using SNAPGENE software (GSL Biotech; available at snapgene.com), and only sequences with a Phred score of >20 were considered good quality.

2.3 Statistical analysis

The deviation of the genotypes from Hardy Weinberg equilibrium was tested using Chi-square. The allele and genotype frequencies were analysed using SNPStats.

Several inheritance models such as the dominant, recessive, codominant and the over-dominant models were analysed by logistic regression using SNPStats. Finally, the association between *DAT1* rs40184 and MDD was studied by conditional logistic regression using SPSS upon adjusting for gender, ethnicity, age, religion, income, educational level, job status, alcohol consumption, family history of neuropsychiatric disorders (anxiety, depression, schizophrenia) alcohol consumption, and chronic disease (stroke, heart disease, diabetes, asthma, arthritis, hypertension, cancer).

3.0 RESULTS

3.1 Screening and validation of *DAT1* rs40184

Screening of *DAT1* rs40184 of 300 matched controls and 300 cases was carried out using High resolution melting (HRM). From the result, three different melting peaks were obtained denoting the three genotypes (GG, GA, AA). The heterozygous genotype was identified by the difference in the shape of the melting curve, whereas the wildtype and the mutant genotypes were identified by the variation in the melting temperature (Figure 1 A and B).

The HRM analysis showed that 101 of the screened cases had the wildtype genotype (G/G), 139 had the heterozygous genotype (G/A) while 60 cases had the mutant genotype (A/A). On the other hand, 124 of the screened controls had the wildtype genotype (G/G), 118 were carriers of the heterozygous genotype (G/A), and 58 subjects had the mutant genotype (A/A). To validate the findings of the HRM, 23 G/G (10%), 25 G/A (10%) and 12 A/A (10%) samples were sent for validation by sequencing. The sequencing results were 100% in concordance with the screening results suggesting that the assay was accurate. Figure 1 C, D and E displays the sequencing result of *DAT1* rs40184.

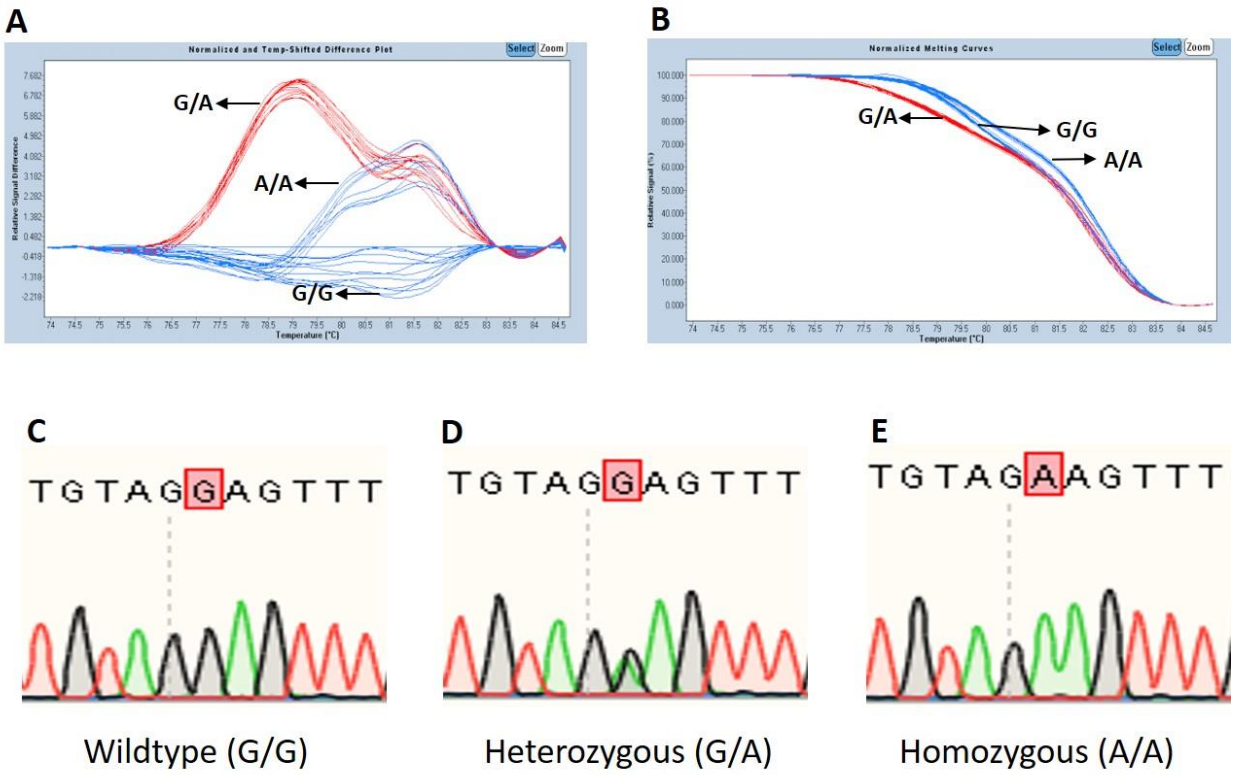

Figure 1. A representative result of **(A)** the temperature shifted difference plot of *DAT1* rs40184 and **(B)** the normalised melting curves of DAT1 rs40184. **(C)** A representative result of the sequencing of DAT1 rs40184 for the wildtype genotype G/G, **(D)** the heterozygous genotype G/A and **(E)** the homozygous genotype A/A.

Table 1. Stratification of the genotypes of the cases and controls among females and males.

Gender	Case			Control			*p*-value
	Genotype			Genotype			
	Wild type	Mutant	Heterozygous	Wild type	Mutant	Heterozygous	
Male	29 (29.9)	21 (21.6)	47 (48.5)	43 (43.3)	20 (20.6)	35 (36.1)	0.125
Female	72 (35.5)	39 (19.2)	92 (45.3)	82 (40.4)	38 (18.7)	83 (40.9)	0.570

Values in parenthesis are percentages.

Table 2. Stratification of the genotypes of the cases and controls among the different Malaysian ethnicities.

Ethnicity	Case			Control			*p*-value
	Genotype			Genotype			
	Wild type	Mutant	Heterozygous	Wild type	Mutant	Heterozygous	
Malay	51 (34.7)	27 (18.4)	69 (46.9)	57 (38.5)	29 (19.6)	62 (41.9)	0.679
Chinese	36 (40.0)	14 (15.6)	40 (44.4)	41 (45.6)	15 (16.7)	34 (37.8)	0.655
Indian	13 (22.0)	17 (28.8)	29 (49.2)	26 (44.8)	12 (20.7)	20 (34.5)	0.033

Values in parenthesis are percentages.

No significant difference was obtained upon stratifying the genotypes of the cases and controls among both the males and females (p>0.05) (Table 1). Stratification of the genotypes of the cases and controls among the three Malaysian ethnicities namely, Malay, Chinese and Indian resulted in a significant difference among only the Indian ethnicity (p=0.038) but not the Malay and Chinese ethnicities (Table 2). Rare ethnicities were removed from the analysis.

3.2 The distribution of the alleles and genotypes among the different study subjects

Chi-square was used to measure the allelic and genotypic distribution. Of the 300 cases, 57% had the G allele, and 43% had the A allele whereby for the controls, 61% had the G allele, and 39% had the A allele. As for the distribution of the genotypes among the cases and controls, 34% of the cases had the G/G wildtype genotype, 46% had the G/A heterozygous genotype and 20% had the A/A genotype. As for the controls, 41% had the G/G wildtype genotype, 39% had the G/A heterozygous genotype, and 19% had the A/A mutant genotype. The allelic and genotypic distribution among the cases and the controls are listed in table 3. The genotypic distribution was in Hardy-Weinberg equilibrium for the cases but not for the controls (P=0.0035).

3.3 Association of *DAT1* to the development of MDD

Table 4 shows the results obtained by logistic regression to determine the association of *DAT1* rs40184 and MDD. Based on the p-value, a significant association was only obtained for the dominant model (p<0.05) with an odds ratio of 1.39 (95% CI 1.00-1.93). Upon performing multivariable conditional logistic regression adjusting for gender, age, ethnicity, income, job, religion, education level, alcohol consumption, family history of neuropsychiatric disorders, schizophrenia, anxiety, depression, stroke, chronic disease, hypertension, arthritis, heart disease, cancer, asthma and diabetes, all the models showed no significant association between the *DAT1* rs40184 and MDD (Table 5).

Table 3. The distribution of the alleles and genotypes among the study subjects.

Status	Allele frequency		Genotype frequency			Hardy-Weinberg
	G	A	Wild Type (G/G)	Heterozygous(G/A)	Mutant (A/A)	equilibrium (*p*-value)
Cases	341 (57)	259 (43)	101 (34)	139 (46)	60 (20)	0.35
Controls	366 (61)	234 (39)	124 (41)	118 (39)	58 (19)	0.0035

Values in parenthesis are percentages.

Table 4. Association of *DAT1* rs40184 to MDD with crude odds ratio by considering four different inheritance models.

Model	Genotype	Case	Control	OR (95%CI)	*p* value	AIC
Codominant	G/G	101 (33.7%)	124 (41.3%)	1.00	0.13	833.7
	G/A	139 (46.3%)	118 (39.3%)	1.45 (1.01-2.07)		
	A/A	60 (20%)	58 (19.3%)	1.27 (0.81-1.98)		
Dominant	G/G	101 (33.7%)	124 (41.3%)	1.00	0.05	832
	G/A-A/A	199 (66.3%)	176 (58.7%)	1.39 (1.00 -1.93)		
Recessive	G/G-G/A	240 (80%)	242 (80.7%)	1.00	0.84	835.7
	A/A	60 (20%)	58 (19.3%)	1.04 (0.70-1.56)		
Overdominant	G/G-A/A	161 (53.7%)	182 (60.7%)	1.00	0.083	832.8
	G/A	139 (46.3%)	118 (39.3%)	1.33(0.96-1.84)		

Table 5. The association of *DAT1* rs40184 to MDD upon adjusting the odds ratio.

Model	Genotype	Case	Control	OR (95%CI)	*p* value	AIC
Codominant	G/G	101 (33.8%)	124 (41.3%)	1.00	0.33	687.2
	G/A	138 (46.1%)	118 (39.3%)	1.59 (0.78-3.23)		
	A/A	60 (20.1%)	58 (19.3%)	1.48 (0.83-2.65)		
Dominant	G/G	101 (33.8%)	124(41.3%)	1.00	0.14	685.2
	G/A-A/A	198 (66.2%)	176 (58.7%)	1.51 (0.87-2.63)		
Recessive	G/G-G/A	239 (79.9%)	242 (80.7%)	1.00	0.50	687.2
	A/A	60 (20.1%)	58 (19.3%)	1.23 (0.68-2.25)		
Overdominant	G/G-A/A	161 (53.9%)	182 (60.7%)	1.00	0.44	686.4
	G/A	138 (46.1%)	118 (39.3%)	1.21(0.75-1.96)		

4.0 DISCUSSION

One of the symptoms experienced by depressed subjects is anhedonia or failure to experience pleasure, and that has been mainly associated with the reward system, specifically dopamine [22]. A study by Bonhomme and Esosito (1998) reported alterations in the activity of the dopaminergic and serotonergic neurons in MDD patients undergoing long term antidepressant treatments [8]. Also, several antidepressants were shown to affect the dopaminergic level indicating a possible role of dopamine in the aetiology of major depressive disorder [23]. One of the critical regulators of the dopaminergic activity is the DAT1 protein that controls the presynaptic dopaminergic reuptake. To date, the number of studies exploring the association of single nucleotide polymorphisms in the DAT1 gene and MDD has been scarce. The rs40184 is one of the SNPs in the *DAT1* gene

that was shown to play a role in the aetiology of bipolar disorder [24] and attention deficit hyperactivity disorder [25]. Despite the lack of its functional significance, the rs40184 is hypothesised to alter the function of the *DAT1* gene by altering the splicing, localisation and the stability of the mRNA and by producing small RNA [20]. The current study aimed to determine the association of this SNP with the aetiology of major depressive disorder in the Malaysian subjects. Hence, 300 cases and 300 controls matched for age, gender and ethnicity were screened for *DAT1* rs40184 using high resolution melting. The distribution of the genotypes was in Hardy-Weinberg equilibrium for the cases but not for the controls (P=0.0035). Among the possible causes is the effect of assortative mating as Malaysia is a multiracial country where it is common to practice same race marriage contradicting one of Hardy Weinberg's assumption (selection). This can result in deviation in the Hardy Weinberg equilibrium [26]. In addition, assortative mating can lead to the Wahlund effect (increase in homozygotes and either increase or decrease in heterozygotes) that may result in linkage disequilibrium or allelic association between the variant loci in the overall individual sample [27]. Findings from the logistic regression revealed a positive association between the rs40184 and the disorder as indicated by the significant difference obtained for the dominant model (p=0.05) with an odds ratio of 1.39 (95% CI 1.00-1.93) indicating that having a single copy of the T allele is sufficient to modify the risk of developing MDD by approximately 1.39 folds.

Major depressive disorder is a multifactorial disorder that is caused by several factors such as stressful life events [28], nutrition [29], lifestyle [30] and genetics [31]. Conditional logistic regression was used to determine the role of the SNP alone by adjusting for other potential confounders. Upon adjusting for other confounding variables, no significant difference was obtained for any of the models implying a possible role of other factors in the aetiology of the disorder. Findings from this study were in concordance with the only case-control study available to date to determine the role of the genotypes of *DAT1* rs40184 in the aetiology of major depressive disorder in the north-eastern Thai population, whereby in their study, 178 case and 205 controls were screened for *DAT1* rs40184 polymorphism, and although more controls carried the C allele as compared to the cases, no significant difference was obtained in the distribution of the alleles and genotypes [20].

In order to confirm the findings, it is advisable to replicate the study using a larger sample size. Also, future studies with variable genes are required to study the plausible interaction between genetics and the pathogenesis of MDD. Major depressive disorder is a multifactorial disorder, in addition to the genetics, environmental and psychosocial factors are also involved in the aetiology of the disorder, and there may be an interaction among these factors that warrant further investigation.

5.0 CONCLUSIONS

Findings from the study did not provide any evidence for an association between *DAT1* rs40184 and major depressive disorder in the Malaysian population. To develop a clearer understanding of the role of the dopamine transporter in the aetiology of the disorder, future studies must be conducted to screen for the role of other SNPs in the gene using larger sample size both at the individual and haplotype level and determination of the gene × environment interaction needs to be further investigated.

Acknowledgements: The study was supported by the fund of the UPM Geran Putra IPB (Interdisciplinary) (GP-IPB/2013/9415701) grant awarded to NI and KHL.

Author Contributions: KHL, PSC, NI, AF, MSL designed and conceived the experiment. AF, PSC, KHL, drafted the manuscript. PSC, KHL, MSL, AF, analysed the data. NI, PSC, KHL, MSL, revised and approved the manuscript.

Conflicts of Interest: The author declares no conflict of interest.

References

1. Diagnostic and Statistical Manual of Mental Disorders (DSM-5®). Fifth edition. American Psychiatric Publishing, Incorporated; 2013. pp. 991, ISBN:978-0-89042-554-1.
2. Vos T, Allen C, Arora M, Barber RM, Bhutta ZA, Brown A, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet*. 2016;388(10053):1545-1602. https://doi.org/10.1016/S0140-6736(16)31678-6
3. Kessler RC, Merikangas KR, Wang PS. Prevalence, comorbidity, and service utilization for mood disorders in the United States at the beginning of the twenty-first century. *Annu Rev Clin Psychol*. 2007;3:137-158. https://doi.org/10.1146/annurev.clinpsy.3.022806.091444
4. Nazree NE, Loke AC, Zainal NZ, & Mohamed Z. Lack of association between TPH2 gene polymorphisms with major depressive disorder in multiethnic Malaysian population. *Asia Pac Psychiatry*. 2015;7(1):72-77. https://doi.org/10.1111/appy.12118
5. Lian LH, Loke AC, Zainal NZ, Mohamed Z. Association study of the tryptophan hydroxylase 1 gene with major depressive disorder in three ethnic groups of the Malaysian population. *Asian Biomed*. 2013;7(1):105-112. https://doi.org/10.5372/1905-7415.0701.156
6. Aldoghachi AF, Tor YS, Redzun SZ, Lokman KA, Razaq NA, Shahbudin AF, et al. Screening of brain-derived neurotrophic factor (BDNF) single nucleotide polymorphisms and plasma BDNF levels among Malaysian major depressive disorder patients. *PloS One*. 2019;14(1):e0211241. https://doi.org/10.1371/journal.pone.0211241
7. Tiong CP, Loke AC, Mohamed Z, Zainal NZ. Serotonin transporter gene polymorphism is associated with antidepressant response to escitalopram in multiethnic Malaysians with major depressive disorder: a preliminary study. *Malay J Psychiatr*. 2013;22(2):59-71.
8. Bonhomme N, Esposito E. Involvement of serotonin and dopamine in the mechanism of action of novel antidepressant drugs: a review. *J Clin Psychopharmacol*. 1998;18(6):447-454. https://www.ncbi.nlm.nih.gov/pubmed/9864076
9. Davis C, Loxton NJ, Levitan RD, Kaplan AS, Carter JC, Kennedy JL. 'Food addiction' and its association with a dopaminergic multilocus genetic profile. *Physiol Behav*. 2013;118:63-69. https://doi.org/10.1016/j.physbeh.2013.05.014
10. Gatt JM, Burton KL, Williams LM, Schofield PR. Specific and common genes implicated across major mental disorders: a review of meta-analysis studies. *J Psychiatr Res*. 2015;60:1-13. https://doi.org/10.1016/j.jpsychires.2014.09.014
11. Huang CC, Lu RB, Shih MC, Yen CH, Huang SY. Association study of the dopamine transporter gene with personality traits and major depressive disorder in the Han Chinese population. *Pharmacogenet Genom*. 2011;21(2):94-97. https://doi.org/10.1097/FPC.0b013e3283424d94
12. Joyce PR, Stephenson J, Kennedy MA, Mulder RT, McHugh PC. The presence of both serotonin 1A receptor (HTR1A) and dopamine transporter (DAT1) gene variants increase the risk of borderline personality disorder. *Front Genet*. 2014;4:313. https://doi.org/10.3389/fgene.2013.00313
13. Willner P. The mesolimbic dopamine system as a target for rapid antidepressant action. *Int Clin Psychopharmacol*. 1997; 12(Suppl 3):S7-S14. http://dx.doi.org/10.1097/00004850-199707003-00002
14. Dunlop BW, Nemeroff CB. The role of dopamine in the pathophysiology of depression. *Arch Gen Psychiatry*. 2007;64(3):327-337. https://doi.org/10.1001/archpsyc.64.3.327
15. Hall H, Halldin C, Guilloteau D, Chalon S, Emond P, Besnard JC, et al. Visualization of the dopamine transporter in the human brain postmortem with the new selective ligand [125I]PE2I. *Neuroimage*. 1999;9(1):108-116. https://doi.org/10.1006/nimg.1998.0366
16. Giros BR, el Mestikawy SA, Godinot NA, Zheng KE, Han HO, Yang-Feng TE, et al. Cloning, pharmacological characterization, and chromosome assignment of the human dopamine transporter. *Mol Pharmacol*. 1992;42(3):383-390. https://www.ncbi.nlm.nih.gov/pubmed/1406597

17. Vandenbergh DJ, Persico AM, Hawkins AL, Griffin CA, Li X, Jabs EW, et al. Human dopamine transporter gene (DAT1) maps to chromosome 5p15.3 and displays a VNTR. *Genomics*. 1992;14(4):1104-1106. https://doi.org/10.1016/S0888-7543(05)80138-7
18. Ciliax BJ, Heilman C, Demchyshyn LL, Pristupa ZB, Ince E, Hersch SM, et al. The dopamine transporter: immunochemical characterization and localization in brain. *J Neurosci*. 1995;15(3):1714-1723. https://doi.org/10.1523/JNEUROSCI.15-03-01714.1995
19. Haeffel GJ, Getchell M, Koposov RA, Yrigollen CM, De Young CG, Klinteberg BA, et al. Association between polymorphisms in the dopamine transporter gene and depression: evidence for a gene-environment interaction in a sample of juvenile detainees. *Psychol Sci*. 2008;19(1):62-69. https://doi.org/10.1111/j.1467-9280.2008.02047.x
20. Pattarachotanant N, Sritharathikhun T, Suttirat S, Tencomnao T. Association of C/T polymorphism in intron 14 of the dopamine transporter gene (rs40184) with major depression in a northeastern Thai population. *Genet Mol Res*. 2010;9(1):565-572. https://doi.org/10.4238/vol9-1gmr757
21. Faris A, Yusof HH, Abidin SZ, Habib O, Cheah PS, Stanslas J, et al. Development and validation of high resolution melting assays for high-throughput screening of BDNF rs6265 and DAT1 rs40184. *Malay J Med Health Sci*. 2018;14(SP1):64-71.
22. Der-Avakian A, Markou A. The neurobiology of anhedonia and other reward-related deficits. *Trends Neurosci*. 2012;35(1):68-77. https://doi.org/10.1016/j.tins.2011.11.005
23. Donald SR, Robinson MD. The role of dopamine and norepinephrine in depression. *Prim Psychiatry*. 2007;14(5):21-23.
24. Mick E, Kim JW, Biederman J, Wozniak J, Wilens T, Spencer T, et al. Family based association study of pediatric bipolar disorder and the dopamine transporter gene (SLC6A3). *Am J Med Genet*. 2008;147B(7):1182-1185. https://doi.org/10.1002/ajmg.b.30745
25. Zhou K, Chen W, Buitelaar J, Banaschewski T, Oades RD, Franke B, et al. Genetic heterogeneity in ADHD: DAT1 gene only affects probands without CD. *Am J Med Genet*. 2008;147B(8):1481-1487. https://doi.org/10.1002/ajmg.b.30644
26. Hardy GH. Mendelian proportions in a mixed population. *Science*. 1908;28(706):49-50. https://doi.org/10.1126/science.28.706.49
27. Garnier-Géré P, Chikhi L. Population Subdivision, Hardy–Weinberg Equilibrium and the Wahlund Effect. In eLS, John Wiley & Sons, Ltd (Ed.). 2013. https://doi.org/10.1002/9780470015902.a0005446.pub3
28. Otte C, Gold SM, Penninx BW, Pariante CM, Etkin A, Fava M, et al. Major depressive disorder. *Nat Rev Dis Primers*. 2016;2:16065. https://doi.org/10.1038/nrdp.2016.65
29. Popa TA, Ladea M. Nutrition and depression at the forefront of progress. *J Med Life*. 2012;5(4):414-419.
30. Sarris J, O'Neil A, Coulson CE, Schweitzer I, Berk M. Lifestyle medicine for depression. *BMC Psychiatry*. 2014;14(1):107. https://doi.org/10.1186/1471-244X-14-107
31. Levinson DF. The genetics of depression: a review. *Biol Psychiatry*. 2006;60(2):84-92. https://doi.org/10.1016/j.biopsych.2005.08.024