PLOS ONE

RESEARCH ARTICLE

Impacts of *GRIN3A*, *GRM6* and *TPH2* genetic polymorphisms on quality of life in methadone maintenance therapy population

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Citation: Wang R-Y, Chen H-J, Huang C-L, Wang J-Y, Lee T-E, Lee H-Y, et al. (2018) Impacts of *GRIN3A*, *GRM6* and *TPH2* genetic polymorphisms on quality of life in methadone maintenance therapy population. PLoS ONE 13(7): e0201408. https://doi.org/10.1371/journal.pone.0201408

Editor: Weihua YUE, Peking University, Institute of Mental Health, CHINA

Received: March 15, 2018

Accepted: July 14, 2018

Published: July 30, 2018

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This work was supported by Ministry of Health and Welfare, Taiwan (MOHW107-TDU-B-212-123004)(CCH), China Medical University and Asia University (CMU105-ASIA-24)(CCH), China Medical University (CMU105-S-16)(CCH) and China Medical University Hospital (1 JWO)(RYW). The funders had no role in study design, data

Abstract

Opioid addiction is a major public health issue worldwide. Methadone maintenance treatment (MMT) is used to detoxify users of illicit opiates, but drug relapse is common and associated with poor quality of life (QoL). This study investigated the associations between the *GRIN3A*, *GRM6*, and *TPH2* genetic variants and QoL in the MMT population. A total of 319 participants were included in the study, and genotyping of *GRIN3A*, *GRM6*, and *TPH2* genes was performed using the Sequenom iPLEX. Associations between genotypes and the domains of QoL were examined through posthoc analysis with LSMEANS syntax using SAS 9.1.3. The single nucleotide polymorphisms rs9325202 and rs1487275 in the *TPH2* gene were significantly associated with the QoL domain of physical functioning. The least absolute shrinkage and selection operator regression model revealed that the risk allele rs1487275-G was significantly correlated with the domain of physical functioning when clinical characteristics were considered as covariates. The results of the present study illuminate the importance of the genetic basis of QoL in the MMT population, and suggest that genotypes should be considered as a potential QoL indicator.

Introduction

The health burden associated with heroin use is well documented, but the quality of life (QoL) of patients undergoing methadone maintenance treatment (MMT) to overcome a drug habit has been less well studied. In medical research, QoL is a key index for evaluating health status and identifying the principal problems faced by people in various phases of life [1]. Opioid addiction is a major public health issue worldwide [2]. MMT reduces illicit opiate abuse,

collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

decreases the incidence of risky behavior that can result in the transmission of the human immunodeficiency virus (HIV), and extends the lives of injection drug users; however, relapse is common among those participating in MMT programs [3]. One study reported that poor QoL was associated with drug relapse among opioid users in an MMT program [4]. However, few studies have identified the major factors associated with QoL in the MMT population.

Evaluating QoL on the basis of physical, mental, and social well-being may provide clinicians with a holistic perspective of an individual's condition [5, 6]. Sex, education, occupation, income, and HIV status have been reported to be key factors potentially related to QoL scores in the MMT population [7–9], but research has not yet examined whether genetic factors are associated with QoL scores or are determinants of individual QoL items. Several studies have suggested that numerous genetic variants may play influential roles in the etiology of heroin addiction [10–12]. The *N*-methyl-D-aspartate (NMDA) receptor was revealed to be related to the development of neuropsychiatric disorders such as drug addiction [13]. Inhibition of NMDA neurotransmission has been determined to not only block the development of morphine dependence but also reduce the occurrence of withdrawal symptoms [14, 15]. The components of the NMDA receptor are two NR1 subunits and two subunits from the NR2 and NR3 families. The NR3 subunits have two subtypes, NR3A and NR3B, and have been associated with a neuroprotective function and cocaine-induced addiction [16–19]. The NR3A subtype is encoded by *GRIN3A*, which is located on chromosome 9q31.1 with proximity to 170 kb. NR3A expression has been reported to be correlated with heroin withdrawal symptoms and cocaine-induced glutamatergic transmission [19, 20]. In addition, the genetic variants in NR3 subunits may influence human brain function [19, 21, 22]. In light of these findings, additional studies that focus on whether the QoL of the MMT population is affected by the genetic variants in NR3A and whether the genetic variants are associated with drug relapse in opioid users in MMT programs are warranted.

Glutamate receptor metabotropic 6 (*GRM6*) and tryptophan hydroxylase 2 (*TPH2*) have also been reported to be involved in heroin dependence [19, 20, 23, 24]. *GRM6* encodes a glutamate receptor subunit and is related to the pathophysiology of visual function, autism, mood disorders, and addiction [25, 26]. Glutamate homeostasis is critical for mood and addiction behavior, and alterations in the expression or function of glutamate receptors may contribute to opiate addiction [27, 28]. Tryptophan hydroxylase is the rate-limiting enzyme involved in serotonin biosynthesis [29]. Serotonin plays a crucial role in the regulation of multiple aspects of mood and impulsivity [30], and thus the factors that influence serotonin expression may also affect—and possibly result in a deficit of—impulse control [31, 32]. *TPH* has two isoforms, *TPH1* and *TPH2*, which are mainly expressed in the pineal gland and raphe nuclei in the brain, respectively [33–35]. The genetic variants in *TPH1* and *TPH2* have been reported to be associated with alcoholism, nicotine dependence, and heroin addiction [24, 36–38]. Therefore, the genetic variants that influence the function or expression of glutamate receptors and the biosynthesis of serotonin could be associated with drug relapse in the MMT population. However, no reports have been published on correlations between the genetic variants in *GRM6*, *TPH1*, and *TPH2* and QoL or drug relapse in the MMT population.

The objective of the present study was to explore the integrative effects of NMDA and glutamatergic and serotoninergic neurotransmission-related genes on QoL in the MMT population. The Medical Outcome Studies 36-Item Short-Form Health Survey, a standard questionnaire used to evaluate QoL, was employed in the present study to assess clinical characteristics, social status, and multiple genetic variants in the study participants in order to investigate possible correlations between the genetic variants and QoL in the MMT population.

Materials and methods

Subjects

The study protocol was reviewed and approved by the institutional review board of China Medical University Hospital (DMR98-IRB-166) and was in compliance with the Declaration of Helsinki.

Han Chinese participants were enrolled with the following inclusion criteria: (1) with heroin dependence and under methadone maintenance treatment in China Medical University Hospital; (2) signed the written informed consent; (3) within normal EKG; (4) not using concurrent medications which may affect methadone metabolism. The following clinical information was recorded for each patient: gender, weight (kg), height (cm), liver function, comorbidities and the daily dose of methadone. The Medical Outcome Studies 36-item Short-Form Health Survey (SF-36) was used to evaluate the QoL of the participants either by a trained nurse or self-reported. There are 36 items in the SF-36 and these items are divided into eight domains of physical health (Physical Function, Role-Physical, Bodily Pain and General Health) and mental health (Vitality, Social Functioning, Role-Emotional and Mental Health) (S1 Table). The score of each item would be calculated according to the manual.

Candidate variants selection and genotyping

It is known that several variants in *GRIN3A*, *GRM6* and *TPH2* genes may be associated with expression and function of these enzymes or receptors. Among these variants, it has been shown that rs7030238, rs1983812, rs942142, rs10512285, and rs3983721 in *GRIN3A* gene, rs17078853, rs2071247, rs17078877, rs11746675, rs2067011 in *GRM6* gene, and rs2129575, rs1386493, rs2171363, rs7305115, rs10506645, rs4760820, rs9325202 and rs1487275 in *TPH2* gene were the eighteen single-nucleotide polymorphisms (SNPs) with minor allele frequency more than 5% in Asia population according to NCBI database. Hence, these eighteen variants were selected for investigation in the present study.

DNA was extracted from 3–10 ml of whole blood by using the QIAamp DNA Blood mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. The genotyping procedure was performed at the National Center for Genome Medicine, Taiwan by the Sequenom iPLEX matrix-assisted laser desorption/ionization time-of-flight mass-spectrometry technology. We randomly selected 20 duplicates samples for quality control and the concordance rate was >0.99 for all SNPs assayed.

Statistical analysis

Each SNP genotype frequency distribution was examined for Hardy-Weinberg equilibrium (HWE) by using the chi-square one-degree of freedom goodness-of-fit test. Demographic characteristics and clinical parameters were evaluated with a chi-squared contingency table for categorical variables and Wilcoxon's U test for continuous variables among the groups. We calculated Cohen's *d* by each group n, mean, and standard deviation at given two-tailed alpha = 0.05 for power level = 0.80 (80%) in SNPs reached significant difference between domain of Physical functioning or domain of Role-Physical of QoL to estimate the sample size.

Linkage disequilibrium (LD) plot was generated by a commonly used bioinformatics software, Haploview (version 4.2), computing pairwise LD statistics for SNPs within a certain distance of each other and analyzing the patterns of SNPs in the present study subjects[39]. Furthermore, we also performed LDlink, a web-based applications designed to interrogate linkage disequilibrium in population groups, to illustrate the LD plots as well[40]. The CHB

(Han Chinese in Beijing, China) genotypes data was used to generate pairwise LD plots from Phase 3 (Version 5) of 1000 Genomes Project and variant rs numbers were indexed based on dbSNP build 142 with LDlink. The R^2 values range from 0 to 1 and higher values indicate higher degree of correlation. The post hoc analysis with LSMEANS syntax was performed in individual SNP and haplotypes for domains of Physical Functioning and Role-Physical[41]. The multiple correction related to multiple SNPs was handled by SAS automatically.

The LASSO (least absolute shrinkage and selection operator) regression method was used for linear model (domains of QoL = demographic characteristics + methadone treatment dose + SNPs). It is a powerful penalty-based method used in predictor selection to avoid model overfitting and a more robust statistical methodology than standard variable selection methods (forward, backward or stepwise). The LASSO model uses PROC GLMSELECT, selection SBC (the Schwarz Bayesian Information Criterion) based on likelihood function and evaluates for all models obtained by deleting an effect from the current model or by adding an effect to this model[42, 43].

The ancestry of population in the present study is Han Chinese, with similar genetic background. Data analyses were conducted using SAS and SAS/Genetics software, version 9.1.3 (SAS Institute, Cary, NC). A two-sided *P*-value <0.05 was considered statistically significant.

Results

Subjects

A total of 319 participants (253 men/66 women) were included and further divided into three groups based on their maximum stabilized methadone daily doses: less than 55 mg/day, between 56 and 99 mg/day, and more than 100 mg/day. Due to the fact that the observed methadone dose have a tri-modal distribution, the methadone dose groups was decided according to the distribution of the dosage of included participants. The basic characteristics of participants among the three methadone dosage groups were not significantly different (Table 1).

The associations between genotypes and domains of QoL

The genotype frequencies of the *GRIN3A*, *GRM6* and *TPH2* polymorphic loci and the score of each item of SF-36 of participants were listed in S2 Table. The genotypic distribution of each genotype was consistent with Hardy-Weinberg equilibrium proportions (S3 Table). The associations between genotypes and domains of QoL were examined by the post hoc analysis with LSMEANS syntax and there were two SNPs, rs9325202 and rs1487275 in *TPH2* gene, significantly associated with domain of Physical Functioning of QoL (Table 2). The sample size power was estimated by the Cohen's *d* method and in domain of Physical functioning, the rs942142 was genotyped in 119 subjects and the power was calculated at 77.8%. The rs9325202 was genotyped in 249 subjects and the power was calculated at 88%. The rs1487275 was genotyped in 249 subjects and the power was calculated at 88%. In domain of Role-Physical, the rs942142 was genotyped in 120 subjects and the power was calculated at 96.1%. The rs17078853 was genotyped in 251 subjects and the power was calculated at 65.9%. The rs11746675 was genotyped in 247 subjects and the power was calculated less than 25%. Therefore, the powers of comparisons in the domain of Physical functioning reached 80%, while some of the comparisons in the domain of Role-Physical did not.

The genomic locations and linkage disequilibrium patterns of the *GRIN3A*, *GRM6* and *TPH2* genetic polymorphisms were generated by LDlink in CHB database (S1, S2 and S3 Figs) and by Haploview in the present study subjects (Figs 1, 2 and 3) as well. There are no significant discrepancies between LD structures of CHB population and the study population of the present study.

Table 1. Demographic data of included subjects of methadone maintenance therapy.

		Maxdose≦55 mg		56 mg<Maxdose<99 mg		Maxdose≧100 mg		*P*-value
Variable		**N**	**Mean±SD**	**N**	**Mean±SD**	**N**	**Mean±SD**	
Gender								
	Male	77	(30.43%)	119	(47.04%)	57	(22.53%)	0.3726
	Female	22	(33.33%)	25	(37.88%)	19	(28.79%)	
Education level								
	Elementary school or less	5	(23.81%)	12	(57.14%)	4	(19.05%)	0.5707
	Junior high school	48	(33.57%)	65	(45.45%)	30	(20.98%)	
	Senior high school	46	(29.68%)	67	(43.23%)	42	(27.10%)	
Marital status								
	Never-married	55	(30.39%)	78	(43.09%)	48	(26.52%)	0.6488
	Married	24	(35.29%)	31	(45.59%)	13	(19.12%)	
	Divorce	19	(29.69%)	32	(50.00%)	13	(20.31%)	
Age		99	42.23±7.13	144	42.88±7.52	76	40.38±7.15	0.0554
SGOT		93	40.43±38.73	140	42.75±29.25	74	46.89±59.15	0.5967
SGPT		93	48.25±43.48	139	55.02±52.59	74	57.16±74.35	0.5425
rGT		92	45.96±61.84	135	37.46±28.35	74	37.64±51.24	0.3524
BMI		91	22.90±3.15	133	22.85±2.90	72	22.34±2.40	0.4003
Items of SF-36								
Physical Function		92	25.04±5.30	136	24.42±4.71	73	24.34±4.38	0.5560
Role-Physical		91	5.66±1.69	135	5.24±1.48	73	5.26±1.61	0.1142
Bodily Pain		92	9.30±1.76	139	8.85±2.12	73	9.01±1.69	0.2101
General Health		91	14.41±3.38	137	14.19±3.72	72	13.16±3.32	0.0575
Vitality		90	13.70±3.19	136	13.35±2.89	72	13.08±3.26	0.4370
Social Functioning		91	6.60±1.65	138	6.34±1.41	72	6.56±1.48	0.3718
Role-Emotional		91	4.08±1.22	135	3.82±1.16	73	4.07±1.33	0.2105
Mental Health		90	18.17±3.74	136	17.36±3.14	72	17.58±4.21	0.2542

Note: Data in parentheses are shown in percentage.

SGOT: serum glutamic oxaloacetic transaminase; SGPT: serum glutamic-pyruvic transaminase; rGT: r-glutamyl transferase; BMI: body mass index

https://doi.org/10.1371/journal.pone.0201408.t001

There were two LD blocks (rs7030238 and rs1983812; rs942142 and rs10512285) in *GRIN3A* gene were generated from five SNPs in both CHB database and the present study subjects (Fig 1 and S1 Fig). One LD block (rs17078853, rs2071247 and rs17078877) in *GRM6* gene was generated from five SNPs in both CHB database and present study subjects. In addition, another LD block (rs11746675 and rs2067011) was only shown in CHB database but not in present study subjects (Fig 2 and S2 Fig). There was one LD block (rs2171363, rs7305115, rs10506645, and rs4760820) in *TPH2* gene generated from eight SNPs in the present study subjects, while two LD blocks (rs2171363 and rs7305115, rs9325202 and rs1487275) were generated in CHB database (Fig 3 and S3 Fig). Further haplotype analysis by post hoc analysis with LSMEANS syntax demonstrated that no haplotype in *GRIN3A* or *TPH2* gene was associated with performance of domains of Physical Functioning or Role-Physical (Table 3).

Regression model analysis

The demographic characteristics, age, categories of methadone treatment dose, and SNPs were independent variables in the LASSO regression model to predict the dependent variable QoL.

Table 2. Associations between *TPH2*, *GRIN3A*, and *GRM6* genotypes and QoL of participants.

Gene	SNP	Allele	Physical Functioning			P-value	Role-Phusical			P-value
			LSMEANS	Lower CL	Upper CL		LSMEANS	Lower CL	Upper CL	
GRIN3A	rs7030238	A vs C	1.01	-2.22	4.25	0.6557	0.28	-0.78	1.34	0.7828
	rs1983812	G vs A	0.38	-2.06	2.82	0.7803	0.73	-0.06	1.52	**0.0459**
	rs942142	C vs A	2.97	-1.34	7.28	0.2368	1.34	-0.02	2.70	0.0520
	rs10512285	G vs A	2.67	-0.82	6.16	0.1454	1.08	-0.08	2.24	0.0605
	rs3983721	C vs T	0.77	-1.32	2.85	0.5851	0.48	-0.21	1.16	0.1773
GRM6	rs17078853	T vs G	0.97	-3.61	5.55	0.5025	0.39	-1.09	1.88	**0.0409**
	rs2071247	A vs G	0.63	-1.60	2.87	0.7841	0.27	-0.46	1.00	0.6663
	rs17078877	A vs G	1.03	-3.46	5.51	0.3988	0.40	-1.09	1.89	0.0695
	rs11746675	T vs C	0.57	-1.85	2.99	0.7604	0.19	-0.60	0.98	0.0614
	rs2067011	T vs C	0.39	-2.12	2.90	0.8880	0.18	-0.60	0.97	0.4310
TPH2	rs2071247	A vs G	0.63	-1.60	2.87	0.2411	0.27	-0.46	1.00	0.8325
	rs1386493	T vs C	0.28	-3.74	4.31	0.3198	0.70	-0.63	2.04	0.3598
	rs2171363	C vs T	0.62	-1.50	2.74	0.2991	0.11	-0.60	0.83	0.7634
	rs7305115	G vs A	0.62	-1.55	2.79	0.3148	0.22	-0.50	0.94	0.4773
	rs10506645	C vs T	1.98	-0.34	4.30	0.1077	0.35	-0.42	1.12	0.3524
	rs4760820	G vs C	0.51	-7.97	9.00	0.9845	2.13	-0.62	4.88	0.1508
	rs9325202	G vs A*	2.32	0.17	4.46	**0.0260**	0.51	-0.20	1.22	0.1044
	rs1487275	T vs G*	2.64	0.03	5.26	**0.0430**	0.60	-0.26	1.46	0.1593

*$p<0.05$ denoted statistical significance.

https://doi.org/10.1371/journal.pone.0201408.t002

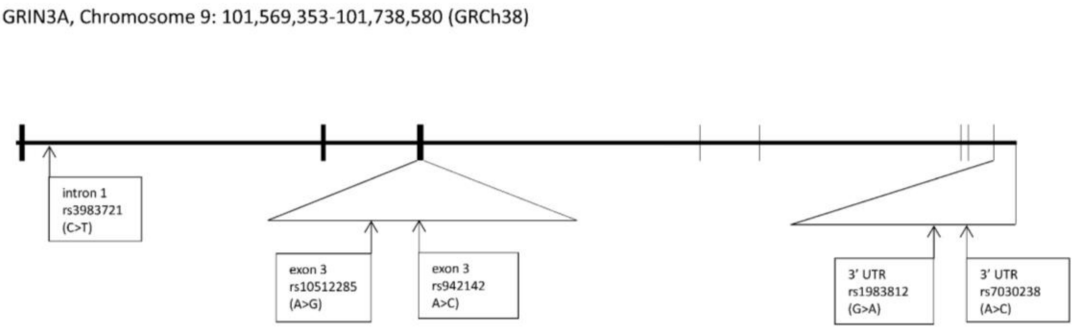
There was only one factor, a risk allele rs1487275-G, was selected by the LASSO selection method to predict the domain of Physical Functioning (Fig 4A) and the coefficient estimate of rs1487275_G is close to 0 (- 1.347569). There was no variable selected by the LASSO selection method to predict the domain of Role-Physical (Fig 4B).

Discussion

The present study identified several genetic risk factors associated with the QoL of the MMT population. We found that the scores obtained by patients undergoing MMT in the domain of Physical Functioning were significantly influenced by *TPH2* rs9325202 G>A and rs 1487275 T>G. In addition, the *TPH2* rs1487275-G was identified as risk allele by the LASSO regression model.

Factors associated with the QoL of the MMT population that have been previously reported were mainly focused on social status[7–9]. The QoL of the MMT population has been related to marital status, main source of income, sexual behaviours, HIV infection, and social support [9–12]. After the confounders of age, gender, education, and municipality were controlled for, HIV infection was still significantly related to lower QoL scores in the MMT population[8]. In addition to these socioeconomic factors, biological pathways and neurotransmitters such as serotonergic and glutamatergic pathways have been identified as related to various QoL domains, including pain, fatigue, and emotional and social functioning[44–47]. Since it was proposed in 2004, the hypothesis that QoL has a genetic basis has been supported by several studies[48, 49]. In the present study, we discovered that *TPH2* rs1487275-G was significantly associated with the QoL of the MMT population after adjustment of clinical and socioeconomic factors, supported the hypothesis that QoL has a genetic basis.

(a)



(b)

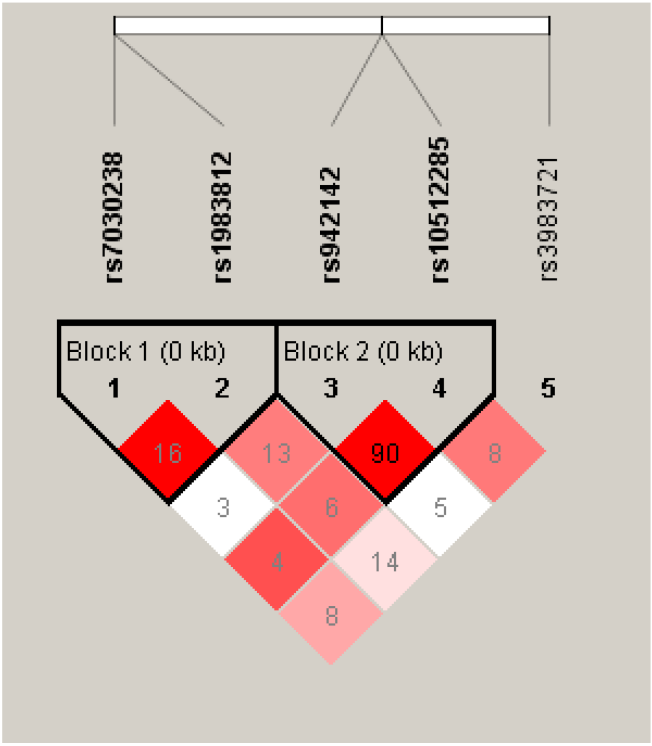


Fig 1. The genomic location and linkage disequilibrium pattern of the *GRIN3A* genetic polymorphisms included in this study. Genomic locations of the genetic polymorphisms on chromosome 9. Haploview 4.2 software was used to estimate the linkage disequilibrium blocks. The R^2 values were shown in squares; range from 0.03 to 0.90 and higher values indicate higher degree of correlation.

https://doi.org/10.1371/journal.pone.0201408.g001

Serotonin (5-HT) is a monoamine that functions as a neurotransmitter or peripheral hormone. TPH isoform 2, encoded by *TPH2*, catalyses the rate-limiting step in the synthesis of 5-HT. Genetic polymorphisms of *TPH2* have been demonstrated to be related to altered TPH2 expression in the central nervous system, symptoms of depression, hopelessness, cocaine addiction, and heroin addiction[24, 50, 51]. The influence of TPH2 expression on brain function and susceptibility to depression was supported in the transgenic animal model[52]. Thus, TPH2 activation may be regarded as a new prospective for neuropsychiatric diseases related to brain 5-HT levels. *TPH2* rs4290270 A>T polymorphism was related to the efficacy of

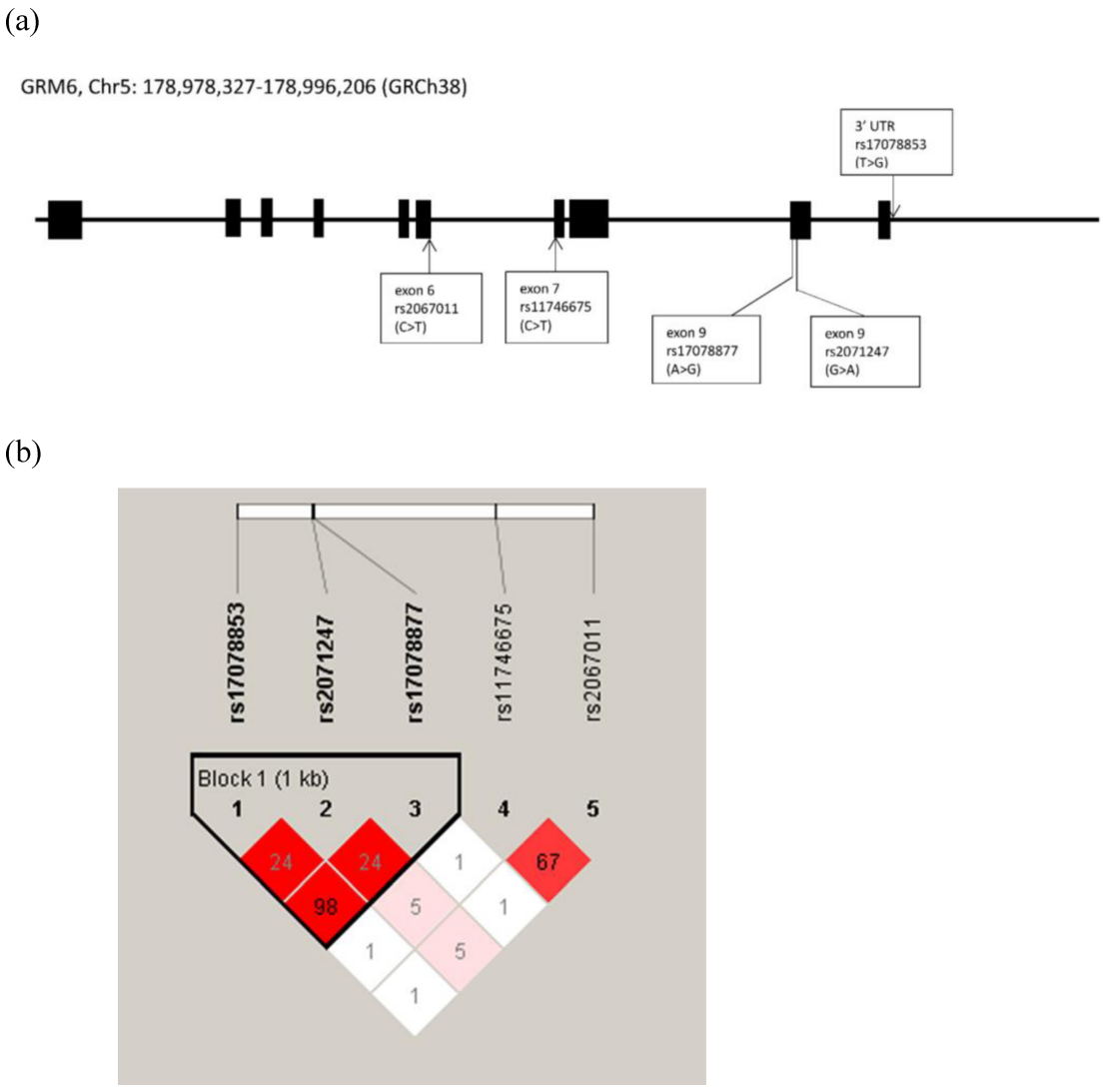


Fig 2. The genomic location and linkage disequilibrium pattern of the *GRM6* genetic polymorphisms included in this study. Genomic locations of the genetic polymorphisms on chromosome 5. Haploview 4.2 software was used to estimate the linkage disequilibrium blocks. The R^2 values were shown in squares; range from 0.01 to 0.98 and higher values indicate higher degree of correlation.

https://doi.org/10.1371/journal.pone.0201408.g002

disulfiram treatment for cocaine addiction. Carriers of the *TPH2* rs4290270 A allele may respond more favourably to disulfiram than a placebo[51]. Conversely, haplotypes of *TPH2* (rs4570625, rs7963720, rs4760816, rs7305115, rs4290270, and rs17110747) were associated with heroin addiction[24]. In the present study, *TPH2* rs9325202 G>A and rs 1487275 T>G polymorphisms were demonstrated to be associated with performance of domain of Physical Functioning. The genetic polymorphisms detected in the present study are not the same as those identified in other studies because the minor allele frequencies are influenced by ethnicity. These results support the influence of variants in genes involved in the serotonergic synapse on subject-reported QoL in the MMT population.

Regarding the variants in genes involved in the glutamatergic synapse, *GRIN3A* and *GRM6* were two candidate genes related to heroin addiction and response to MMT, respectively. Glutamate is one of the excitatory neurotransmitters in the human brain, and the glutamatergic

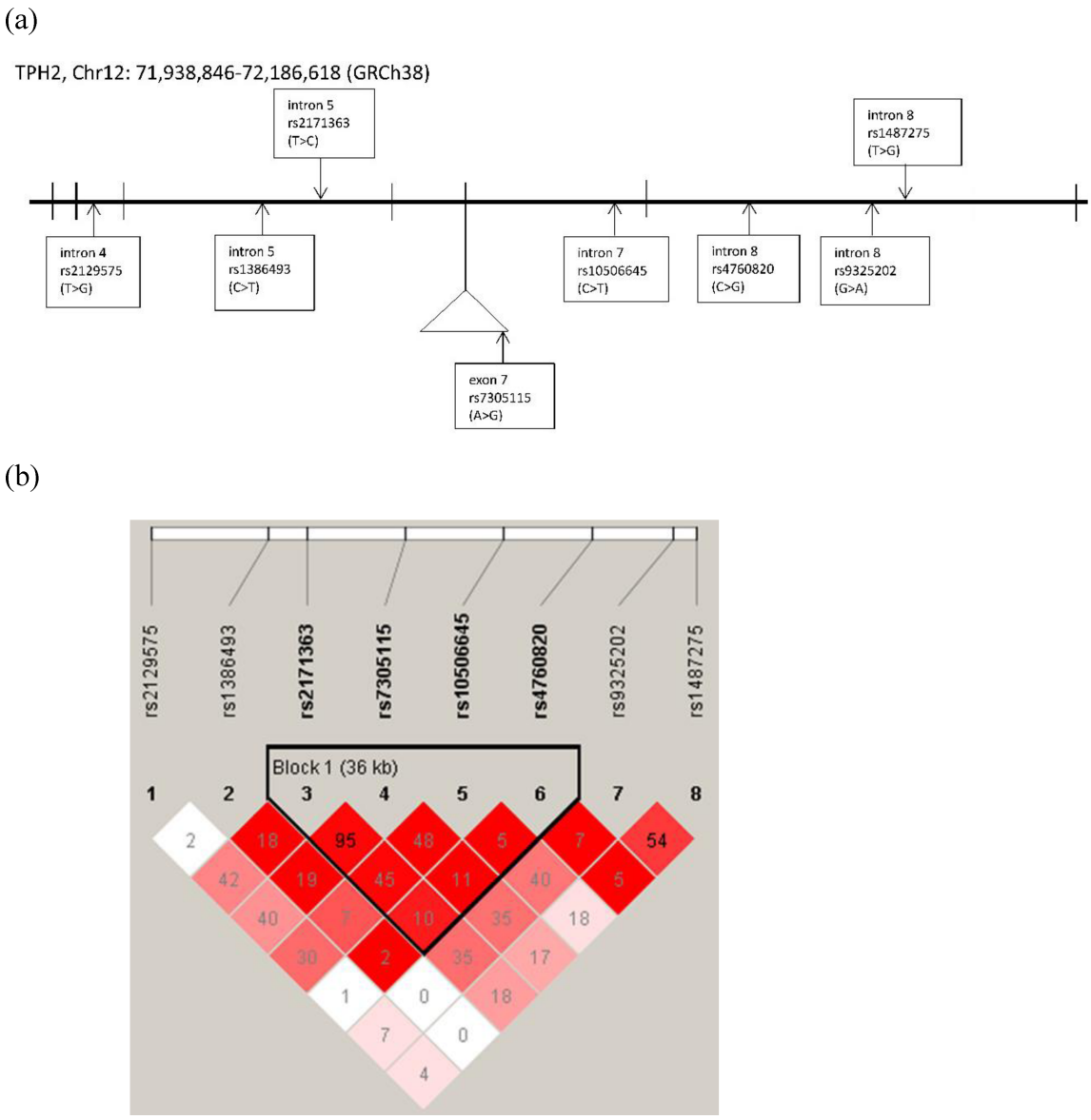


Fig 3. The genomic location and linkage disequilibrium pattern of the *TPH2* genetic polymorphisms included in this study. Genomic locations of the genetic polymorphisms on chromosome 12. Haploview 4.2 software was used to estimate the linkage disequilibrium blocks. The R^2 values were shown in squares; range from 0 to 0.95 and higher values indicate higher degree of correlation.

https://doi.org/10.1371/journal.pone.0201408.g003

projection circuit was discovered to contribute to the development of an addiction[53, 54]. Several genetic variations in the glutamatergic pathway have been reported to be associated with susceptibility to drug addiction[10–12]. The haplotypes of *GRIN3A* rs4807399 C>T and rs2240158 C>T were associated with vulnerability to drug addiction[55]. In terms of *GRM6*, carriers of the AG genotype at rs953741 A>G were found to be at increased risk of being non-responsive to MMT[23]. However, in the present study, no *GRIN3A* or *GRM6* genetic polymorphism was associated with the QoL of the MMT population. These results imply that glutamatergic synapse alteration may be related to the pathophysiology of mood disorders and addiction, but may not be related to the self-reported QoL of those undergoing MMT.

Table 3. Associations between *GRIN3A* and *TPH2* haplotypes and QoL of participants.

Gene	haplotype	haplotype	Physical Functioning			Role-Physical		
			LSMEANS	Lower CL	Upper CL	LSMEANS	Lower CL	Upper CL
GRIN3A	rs942142-	C-G/C-G vs A-A/C-A	0.83	-5.90	7.56	0.92	-1.30	3.13
	rs10512285	C-G/C-G vs A-A/C-G	2.32	-1.88	6.53	1.15	-0.23	2.53
		C-G/C-G vs A-A/A-A	2.74	-1.29	6.76	1.09	-0.24	2.41
		A-A/C-A vs A-A/C-G	1.49	-4.23	7.21	0.24	-1.64	2.12
		A-A/C-A vs A-A/A-A	1.90	-3.69	7.50	0.17	-1.67	2.01
		A-A/C-G vs A-A/A-A	0.41	-1.50	2.33	0.07	-0.56	0.69
TPH2	rs9325202-	G-T/G-T vs A-T/G-T	0.21	-3.94	4.37	-0.51	-1.88	0.86
	rs1487275	G-T/G-T vs A-G/G-T	0.44	-2.35	3.23	0.08	-0.85	1.00
		G-T/G-T vs A-G/A-G	2.00	-2.21	6.22	0.28	-1.10	1.67
		G-T/G-T vs G-G/G-T	2.36	-4.00	8.73	0.23	-1.87	2.32
		G-T/G-T vs A-G/A-T	2.49	-1.95	6.93	0.64	-0.82	2.10
		G-T/G-T vs A-T/A-T	4.25	-13.96	22.46	1.45	-4.55	7.45
		G-T/G-T vs A-G/G-G	5.92	-4.72	16.56	0.78	-2.72	4.29
		A-T/G-T vs G-T/G-T	-0.21	-4.37	3.94	0.51	-0.86	1.88
		A-T/G-T vs A-G/G-T	0.23	-3.86	4.32	0.59	-0.77	1.94
		A-T/G-T vs A-G/A-G	1.79	-3.38	6.96	0.79	-0.91	2.50
		A-T/G-T vs G-G/G-T	2.15	-4.88	9.19	0.74	-1.58	3.05
		A-T/G-T vs A-G/A-T	2.28	-3.08	7.63	1.15	-0.61	2.91
		A-T/G-T vs A-T/A-T	4.04	-14.41	22.49	1.96	-4.12	8.04
		A-T/G-T vs A-G/G-G	5.71	-5.35	16.76	1.29	-2.35	4.93
		A-G/G-T vs G-T/G-T	-0.44	-3.23	2.35	-0.08	-1.00	0.85
		A-G/G-T vs A-T/G-T	-0.23	-4.32	3.86	-0.59	-1.94	0.77
		A-G/G-T vs A-G/A-G	1.56	-2.60	5.72	0.21	-1.16	1.58
		A-G/G-T vs G-G/G-T	1.92	-4.40	8.25	0.15	-1.93	2.24
		A-G/G-T vs A-G/A-T	2.05	-2.34	6.43	0.57	-0.88	2.01
		A-G/G-T vs A-T/A-T	3.81	-14.39	22.01	1.38	-4.62	7.37
		A-G/G-T vs A-G/G-G	5.48	-5.14	16.10	0.71	-2.79	4.21
		A-G/A-G vs G-T/G-T	-2.00	-6.22	2.21	-0.28	-1.67	1.10
		A-G/A-G vs A-T/G-T	-1.79	-6.96	3.38	-0.79	-2.50	0.91
		A-G/A-G vs A-G/G-T	-1.56	-5.72	2.60	-0.21	-1.58	1.16
		A-G/A-G vs G-G/G-T	0.36	-6.71	7.43	-0.06	-2.38	2.27
		A-G/A-G vs A-G/A-T	0.49	-4.92	5.90	0.36	-1.42	2.14
		A-G/A-G vs A-T/A-T	2.25	-16.22	20.72	1.17	-4.92	7.25
		A-G/A-G vs A-G/G-G	3.92	-7.16	15.00	0.50	-3.15	4.15
		G-G/G-T vs G-T/G-T	-2.36	-8.73	4.00	-0.23	-2.32	1.87
		G-G/G-T vs A-T/G-T	-2.15	-9.19	4.88	-0.74	-3.05	1.58
		G-G/G-T vs A-G/G-T	-1.92	-8.25	4.40	-0.15	-2.24	1.93
		G-G/G-T vs A-G/A-G	-0.36	-7.43	6.71	0.06	-2.27	2.38
		G-G/G-T vs A-G/A-T	0.13	-7.08	7.34	0.41	-1.96	2.79
		G-G/G-T vs A-T/A-T	1.89	-17.19	20.96	1.22	-5.06	7.50
		G-G/G-T vs A-G/G-G	3.56	-8.51	15.62	0.56	-3.42	4.53
		A-G/A-T vs G-T/G-T	-2.49	-6.93	1.95	-0.64	-2.10	0.82

https://doi.org/10.1371/journal.pone.0201408.t003

The strength of the present study is that by adjusting for clinical confounders, the effects of genetic variants on the QoL of the MMT population could be evaluated fairly. Additionally, the participants had been receiving MMT for at least 3 months prior to the study; thus, the

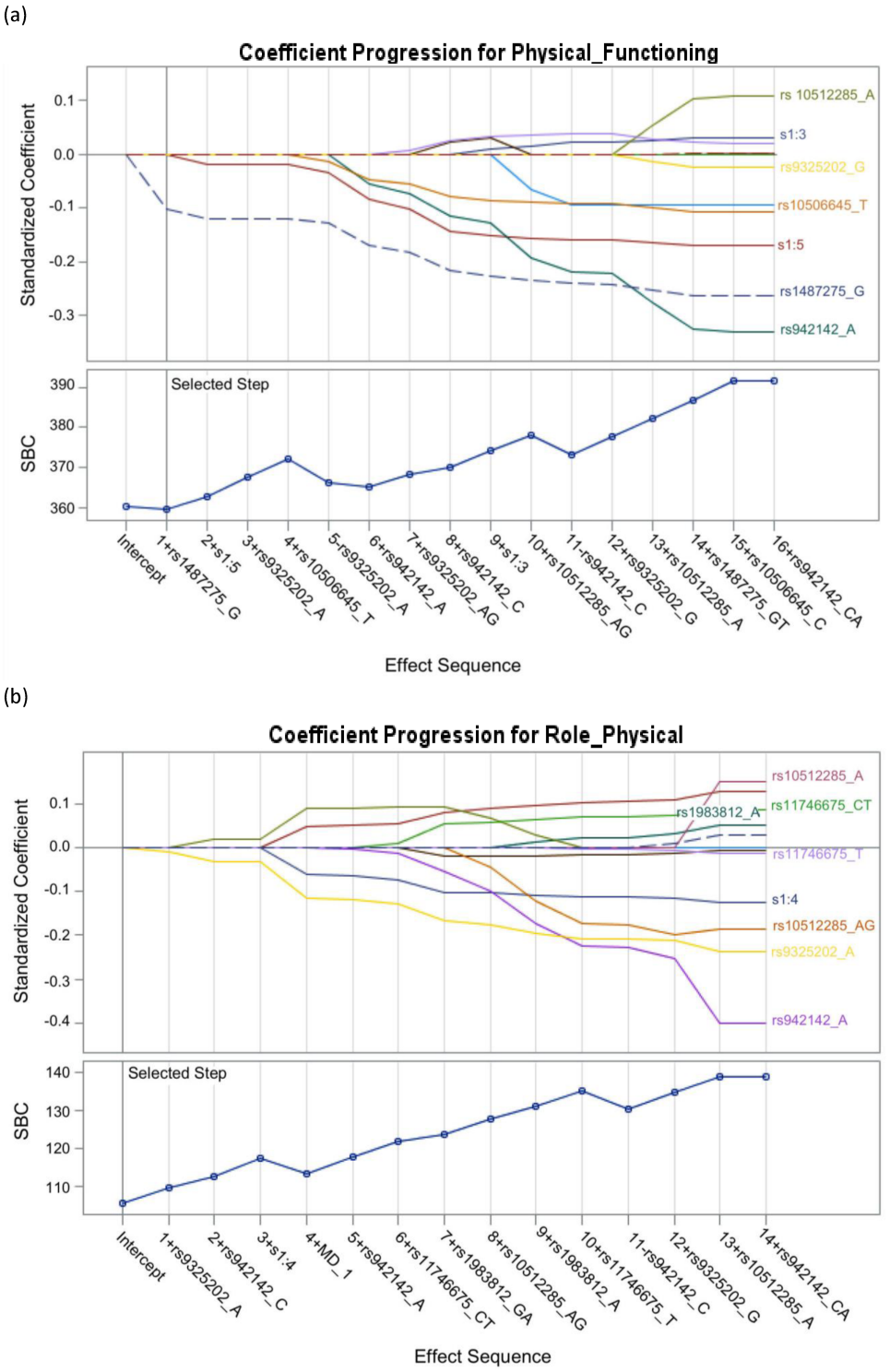


Fig 4. The LASSO regression model. (a) A risk factor rs1487275_G was selected in the LASSO regression model. (b) No risk factor was selected in the LASSO regression model to predict Role-Physical of QoL.

https://doi.org/10.1371/journal.pone.0201408.g004

results of this study may identify genetic markers affecting the QoL of stable individuals undergoing MMT. However, the present study does have some limitations: the sample size and the

characteristics of the participants. Because of the limited sample size, the results should be carefully interpreted when considering the general MMT population. Regarding the characteristics of the participants, we did not enrol patients with HIV infection; thus, we could not detect the influence of HIV infection on the QoL of the MMT population. Furthermore, relapse rate is an indicator of poor QoL. We did not record the relapse rate in the included MMT population; therefore, the associations between genetic variants and relapse rate could not be detected in the present study.

In conclusion, rs 1487275 T>G polymorphism in *TPH2* gene was significantly associated with the domain of Physical functioning of QoL in subjects undergoing MMT. The results of the present study may shed light on the importance of the genetic basis of QoL and provide future directions of clinical MMT practice improvements.

Supporting information

S1 Table. SF-36 measurement model.
(PDF)

S2 Table. Genotype frequencies and score of each item of SF-36 of the participants.
(PDF)

S3 Table. Genotypes of *TPH2*, *GRM6*, *GRIN3A* in included participants.
(PDF)

S1 Fig. LD plot of SNPs in *GRIN3A* from CHB data in 1000 Genome project.
(PDF)

S2 Fig. LD plot of SNPs in *GRM6* from CHB data in 1000 Genome project.
(PDF)

S3 Fig. LD plot of SNPs in *TPH2* from CHB data in 1000 Genome project.
(PDF)

Acknowledgments

We thank the National Center for Genome Medicine for the technical/ bioinformatics/statistics support. This manuscript was edited by Wallace Academic Editing.

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