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ORIGINAL PAPER

Novelty seeking mediates the effect of *DRD3* variation on onset age of amphetamine dependence in Han Chinese population

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Abstract The dopamine receptor D3 (*DRD3*) gene, one of the candidate genes for amphetamine dependence (AD), is involved in the mesolimbic dopaminergic system, implicated as the underlying mechanism of addiction. Our case–control study aimed to investigate whether the *DRD3* gene is associated with the susceptibility to AD and specific personality traits in AD patients. A total of 1060 unrelated Han Chinese subjects (559 AD patients and 501 controls) were screened using the same assessment tool and genotyped for eight *DRD3* polymorphisms. All patients met the DSM-IV-TR criteria for AD, and personality traits of 539 were assessed using a Tridimensional Personality Questionnaire. Furthermore, AD individuals were divided into four clinical subgroups based on gender and psychosis status, to reduce the clinical heterogeneity. We found that the ATA haplotype combination for SNPs rs324029, rs6280, and rs9825563, respectively, was significantly associated with total AD patients ($p = 0.0003$ after 10,000 permutations). Similar results were observed in the both male and non-psychosis subgroup but not in other subgroups. In addition, *DRD3* rs9825563 may influence onset age of drug use, partially mediated by novelty seeking in the non-psychosis AD group. In conclusion, *DRD3* is a potential genetic factor in the susceptibility to AD and is associated with onset age of drug use through interaction with novelty seeking in a specific patient group in the Han Chinese population.

Keywords Amphetamine dependence · *DRD3* gene · Personality trait · Onset age

Abbreviations

AD	Amphetamine dependence
DAT	Dopamine transporter
DRD3	Dopamine receptor D3
DRD4	Dopamine receptor D4
DSM-IV-TR	Diagnostic and statistical manual for mental disorders, 4th edition, text revision
HA	Harm avoidance
LD	Linkage disequilibrium
MA	Methamphetamine
NS	Novelty seeking
RD	Reward dependence
SADS-L	Schedule of affective disorder and schizophrenia-lifetime
SNP	Single-nucleotide polymorphism
TPQ	Tridimensional personality questionnaire

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Introduction

Amphetamine is the primary drug of the amphetamine-type substances/stimulants class. It is the second most used illicit drug worldwide, and the primary illicit drug threat in Asia [1]. Amphetamine use is linked to significant public health, legal, and environmental problems, as well as medical, psychiatric, and cognitive deficits [2, 3]. Addiction to amphetamine causes a chronic relapsing brain disease, associated with genetic and sociocultural factors [4], with an estimated heritability of up to 68% [5].

Converging anatomical, pharmacological, genetic, and behavioral evidence has implicated *DRD3* in the mechanisms of drug reward and drug-seeking behavior. Therefore, *DRD3* is a potential candidate gene for amphetamine dependence (AD). The mesocorticolimbic dopamine system may play a key role in drug reward and is thought to contribute to the development of substance dependence [6–8]. The dopamine receptor D3 (*DRD3*) is predominantly expressed in the ventral tegmental area and mesolimbic dopamine system, implicated in drug reward pathways [9]. Pharmacogenetic studies have indicated that central *DRD3* influences drug reward, drug taking, and prime-, stress-, and cue-induced reinstatement of drug-seeking behavior [10–12]. DRD3 may also be involved in the molecular mechanisms underlying the reward-related incentive learning [13, 14].

DRD3 spans approximately 50.3 kb on the chromosomal locus 3q13.3 [15]. *DRD3* sequence variation could result in subtle changes in receptor structure, or expression, and lead to different phenotypes [16]. The *DRD3* rs6280 polymorphism causes a serine to glycine (Ser9Gly) change, followed by a thymine (T) to cytosine (C) substitution, in the extracellular N terminus of the D3 receptor. Diverse genotypes at this variant position have been associated with different dopamine affinities and differential activity in downstream signaling pathways [17, 18].

The association between *DRD3* and psychostimulant addiction [19–23] has been examined previously, but indicative of controversial findings; however, few studies explored comprehensively the association between *DRD3* variants and the development of AD.

The initiation of drug abuse is an important factor to consider when investigating prevention strategies. Early age at first use is a risk factor for drug-use disorders with increased severity and complexity [24–26]. In addition, onset age of drug abuse is influenced by genetic and multifarious factors such as gender, personality traits, and childhood psychopathology [25, 27, 28]. The temperament and character of individuals may play a role in the susceptibility to substance dependence and onset age of drug use [29, 30]. Novelty seeking personality traits may contribute to the risk of using drugs earlier and for longer [31–33], and mediate the genetic effect on onset age of drug use [34].

Moreover, prior studies [35–39] suggested that *DRD3* affects personality traits, which may contribute to the risk of AD, and we further investigated specific personality traits in patients with AD using a Chinese version of the Tridimensional Personality Questionnaire (TPQ) to determine possible gene–personality interaction in pathogenesis of AD.

Therefore, we hypothesize that *DRD3* variants may be associated with onset age of drug use, partially mediated by specific personality traits. The aim of our study was to examine whether *DRD3* polymorphisms influence the development of AD in a Han Chinese population and to explore whether specific personality traits mediate the association between *DRD3* variants and onset age of drug abuse.

Materials and Methods

Participants

This study was performed in accordance with the 1994 Declaration of Helsinki (ethical laws pertaining to the medical profession), and its research protocol was approved by the Institutional Review Board for the Protection of Human Subjects at the Tri-Service General Hospital (TSGH; a medical teaching hospital belonging to the National Defense Medical Center in Taipei, Taiwan). The procedures of the study were fully explained to all participants before given written informed consent. To minimize the effect of ethnic differences in genetic distribution, the study participants selected from the Han Chinese population were also unrelated, born and living in Taiwan, and all their biological grandparents were of Han Chinese ancestry.

The patient group consisted of **559** AD patients recruited from drug rehabilitation clinics and one general hospital in Northern Taiwan. Each patient was interviewed by a well-trained psychologist using a Chinese version of the modified Schedule of Affective Disorder and Schizophrenia-Lifetime (SADS-L) [40] after initially evaluated by a psychiatrist. The diagnosis of AD was confirmed based on the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, Text Revision (DSM-IV-TR). All patients met the DSM-IV-TR criteria for AD based on interviews and all available information. Those with a history of psychosis prior to amphetamine use and those where psychosis was closely related to other psychoactive drugs were excluded. Occasional amphetamine users who did not experience psychosis were also excluded. There were 73 individuals

with amphetamine-induced psychosis (AD group with psychosis) and 473 AD patients without psychosis (non-psychosis AD group). Thirteen were judged to have perceptual disturbance due to amphetamine intoxication but not amphetamine-induced psychosis.

The control group consisted of 501 healthy volunteers enrolled from the community. We used the Chinese version of the SADS-L to exclude psychiatric conditions in the control group, to ensure they were free of past or present major and minor mental illnesses such as affective disorders, schizophrenia, anxiety disorders, personality disorders, and substance abuse disorders. In addition, there was no family history of psychiatric disorder or substance use disorder in the first-degree relatives of the control subjects.

SNP selection and genotyping methods for *DRD3* gene

Genomic DNA was extracted from peripheral blood leukocytes, using a commercial kit (DNAzol; Invitrogen, Carlsbad, CA, USA). Eight SNPs with minor allele frequencies of more than 0.1 to cover a region of 50.3 kb in the *DRD3* gene were randomly selected one the basis of the human *DRD3* polymorphisms listed in the NCBI SNP database (www.ncbi.nlm.nih.gov/projects/SNP/), the International Hap-Map Project database (www.hapmap.org) and a review of the literature: SNP rs9825563 in the promoter region, SNP rs6280 in exon 2, SNP rs324029 in intron 2, SNPs rs2630351 and rs9880168 in intron 3, SNP rs963468 in intron 4, SNP rs2134655 in intron 5, and SNP rs2046496 in the 3′ UTR. The positions of these polymorphisms within the gene are shown in Supplemental Fig. 1.

DRD3 variants were genotyped by TaqMan assays (Applied Biosystems, Foster City, CA, USA) employing FAM™ and VIC® dyes. The Applied Biosystems STEPONE™ software and STEPONEPLUS™ real-time PCR systems were used for thermocycling and data collection. To ensure the accuracy and quality control of our genotyping, we randomly selected 50 samples for blind duplicate sequencing, as described previously [41].

Assessment of specific personality traits in AD

A Chinese version of the TPQ [42] was used to assess specific personality traits. We excluded the reward dependence (RD) dimension due to low inter-reliability among the Han Chinese population in Taiwan (RD, 34 items, Cronbach's $\alpha = 0.54$). Novelty seeking (NS, 32 items, Cronbach's $\alpha = 0.70$) and harm avoidance (HA, 34 items, Cronbach's $\alpha = 0.87$) dimensions were analyzed. AD patients were assessed immediately following diagnosis and before or after the withdrawal period, to avoid confounding effects of amphetamine withdrawal symptoms.

Statistical analysis

Continuous variables were presented as mean ± standard deviation (SD) and analyzed by the independent samples *t* test. Categorical variables were determined by χ^2 test and expressed as frequency in percent (%). Allele and genotype frequencies for each polymorphism were compared between patients with AD and controls by a two-tailed Pearson χ^2 test (with Fisher's exact test when the sample size was smaller than expected). To assess the influence of age, gender, and *DRD3* variants on the incidence of AD, we conducted a logistic regression, using age, gender, and each SNP as covariates, and patient/control group as the binominal dependent variable. Independent *t* test was performed to examine whether different genotypes are associated with NS, HA scores, and onset age in patients with AD. SPSS (version 17, SPSS, Taipei, Taiwan) software was used for all analyses, and $p < 0.05$ was considered statistically significant.

The linkage disequilibrium (LD) coefficients (D'), haplotype frequency, haplotype block, haplotype association, and Hardy–Weinberg equilibrium for each variant were assessed using HAPLOVIEW software (version 4.2, Broad Institute, Cambridge, MA, USA) [43]. We defined a haplotype block as a set of contiguous SNPs with an average D' greater than 0.9 [44]. All tests were two-tailed, and α was set at 0.05. Power analysis was performed using G-POWER 3.1 software [45]. Our total sample size ($n = 1060$) had a power of approximately 0.85 to detect a small effect (effect size = 0.1), and 1.00 to detect medium (effect size = 0.3) and large effects (effect size = 0.5) of genotype distributions. This study had a power of 1.00 to detect small, medium, and large effects in the allele frequencies of these eight polymorphisms.

The mediation analysis model [46] was used to test whether NS mediates the effect of *DRD3* genotypes on clinical characteristics of AD. The structural equation model (SEM) was used to analyze the mediation effect, and standardized path coefficients were calculated by Amos 6.0 software (SPSS Inc, Chicago, IL, USA). The predictor variable DRD3 *rs9825563* variant G/G genotype is recoded as '0' and A-allele carrier as '1'. The outcome variable consisted of continuous variable, and is recoded as onset age of amphetamine abuse. For this model, the NS scores served as mediator variables and were tested by the Sobel *z* test (Sobel, 1982, 1987); if $|Z| > 1.96$, then the variable is considered a mediator [47].

Results

Demographic data

Mean age and gender were significantly different between patients and controls. AD patients were significantly

younger than controls (34.09 ± 7.42 vs. 40.15 ± 12.44, $t = 9.468$, $p < 0.001$), and there were more males in the patient group (male/female: 503/56 vs. 348/153, $\chi^2 = 70.287$, $df = 1$, $p < 0.001$). AD patients also had significantly lower education level (years) than controls (9.84 ± 1.80 vs. 14.21 ± 3.47, $t = -5.267$, $p < 0.001$). There were 279 patients with AD only, 170 patients comorbid other substance use disorder (SU), and 110 patients with non-SU psychiatric disorder.

Single-marker analysis of *DRD3* gene

All variants were in Hardy–Weinberg equilibrium in both groups. Allele and genotype frequencies of all SNPs of AD patients and controls are shown in Table 1. rs2134655 demonstrated a weak association between the AD patients and controls ($p = 0.056$; Table 1). Although genotype analysis showed no association with AD in the total cohort, genotypes of rs324029 were weakly associated with the development of AD in the male and non-psychosis subgroups ($\chi^2 = 5.399$, $df = 2$, $p = 0.067$ and $\chi^2 = 5.115$, $df = 2$, $p = 0.077$, respectively). However, all weak allelic or genotypic associations were insignificant after Bonferroni correction (Table 1). To assess the influence of each *DRD3* variant on AD, we performed logistic regression analyses using age as a covariate (Table 2). Of the eight markers, the odds ratio (OR) for the G-allele carrier of rs324029 was 0.482 (95% confidence interval [CI]: 0.236–0.985, p = 0.045), compared with the A/A genotype in the male subgroup. No significant associations were found in the total cohort or other subgroups ($p > 0.05$; Table 2).

Haplotype analysis of *DRD3* gene

An LD map and block structure of the investigated *DRD3* polymorphisms, and D' values (for all variants) were determined following haplotype analysis (Fig. 1) Two haplotype blocks were identified in our Han Chinese population under the confidence interval algorithm of haploview. Block 1 included three SNPs (rs2134655, rs963468, and rs9880168) and covered 8 kb from intron 3 to intron 5. Block 2 included three SNPs (rs324029, rs6280, and rs9825563) and covered 18 kb from the promoter region to intron 2 (Fig. 1). The block 2 ATA haplotype was significantly associated with total AD patients ($\chi^2 = 16.201$, $p = 0.0003$ after 10,000 permutations, Table 3). The block 2 ATA haplotype was also more frequent in the male AD and non-psychosis subgroups than in the control group ($\chi^2 = 14.006$, $p = 0.0007$; $\chi^2 = 19.767$, $p < 0.0001$, respectively, after 10,000 permutations). Neither female nor female non-psychosis subgroups showed an association with either haplotype block ($p > 0.05$; data not shown).

Associations of *DRD3* gene with NS and onset age for drug use

Of the AD patients, 539 (485 male; 54 female) completed personality assessment with the Chinese version of the TPQ. The mean NS scores and onset age were 13.26 ± 4.52 and 24.66 ± 7.27, respectively. Two *DRD3* gene variants (rs6280 and 9825563) were significantly associated with NS subscale scores ($t = 2.588$, $df = 433$, $p = 0.010$; $t = -2.712$, $df = 446$, $p = 0.007$, respectively), and *DRD3* gene variant rs9825563 had a significant effect on onset age of amphetamine use ($t = 2.159$, $df = 444$, $p = 0.031$) in non-psychosis AD subjects (Table 4). Similar results were also found in total cohort and male subgroup (Supplemental Table 1, 2). No significant association was found between personality traits and drug-use onset age and the *DRD3* gene SNPs in the other subgroups ($p > 0.05$; data not shown).

NS partially mediates the *DRD3* variant effect on onset age of drug use

NS personality traits were negatively correlated with onset age of drug use ($r = -0.188$, $p < 0.001$), indicating that AD subjects with higher NS scores had earlier drug-use onset age. Furthermore, *DRD3* variants at rs9825563 were associated with NS subscale scores and drug-use onset age. SEM was used to analyze the mediation effect. The direct path from *DRD3* rs9825563 to NS (raw regression coefficient $a = 2.073$, with standard error of a (s_a) = 0.772, $p = 0.007$) and from NS to onset age of drug use (raw regression coefficient $b = -0.295$, with standard error of b (s_b) = 0.076, $p < 0.001$) was significant in non-psychosis subgroup. The direct path from *DRD3* rs9825563 to onset age of drug use was less significant (raw regression coefficient $c' = -1.672$, with standard error of $c' = 1.277$, $p = 0.095$). The Sobel test [46]: z value = $a*b$/SQRT $\left(b^2 * s_a^2 + a^2 * s_b^2\right) = 2.208 > 1.96$, supported our hypothesis that NS was the mediation path. A partially mediated model containing the mediator (NS) and direct paths from *DRD3* rs9825563 to the onset age was tested. The standardized total effect of *DRD3* rs9825563 to onset age through NS was −0.101, while the standardized direct effect of *DRD3* rs9825563 was −0.078, and the indirect effect of *DRD3* rs9825563 through NS was (0.126) * (−0.178) = −0. 022 (Fig. 2).

Discussion

We investigated the association of eight *DRD3* SNPs with the development of AD. We identified one intronic polymorphism, rs324029, with a weak association with male

Table 1 Gene location, allele and genotype frequencies of the investigated *DRD3* gene polymorphisms among patients with amphetamine dependence (AD) ($n = 559$) and controls ($n = 501$)

Variants	Loci	Position reference dbSNP	MAF		p[a]	Allele[b]		Genotype (n, %)[c]						p[d]
			Case	Control		1	2	Controls ($n = 501$)			Total AD ($n = 559$)			
								1/1	1/2	2/2	1/1	1/2	2/2	
rs2046496	3′ UTR	115,317,621	0.408	0.430	0.308	***G***	C	92(18.4)	247(49.3)	162(32.3)	86(15.6)	277(50.4)	187(34.0)	0.489
rs2134655	Intron 5	115,340,891	0.230	0.265	0.056	***T***	C	34(6.8)	198(39.5)	269(53.7)	23(4.2)	206(37.5)	320(58.3)	0.105
rs963468	Intron 4	115,345,577	0.407	0.381	0.236	***A***	G	64(12.8)	254(50.7)	183(36.5)	83(15.3)	274(50.6)	184(34.0)	0.431
rs9880168	Intron 3	115,349,241	0.126	0.127	0.954	***G***	A	6(1.2)	115(23.0)	380(75.8)	9(1.6)	121(21.9)	422(76.4)	0.785
rs2630351	Intron 3	115,357,749	0.116	0.110	0.638	***A***	G	5(1.0)	100(20.0)	396(79.0)	10(1.8)	107(19.6)	429(78.6)	0.524
rs324029	Intron 2	115,364,313	0.287	0.254	0.091	***A***	G	20(4.0)	215(42.9)	266(53.1)	39(7.0)	243(43.5)	277(49.6)	0.087
rs6280	Exon 2	115,373,505	0.306	0.291	0.464	***C***	T	41(8.2)	210(41.9)	250(49.9)	49(9.2)	229(42.9)	256(47.9)	0.758
rs9825563	Promoter	115,382,910	0.291	0.271	0.315	***G***	A	34(6.8)	204(40.7)	263(52.5)	44(8.2)	226(41.9)	269(49.9)	0.580
Variants	Loci	Position reference dbSNP	Genotype, (n, %)			p[e]		Genotype, n (%)						p[f]
			Non-P AD ($n = 473$)					Male controls ($n = 348$)			Male AD($n = 503$)			
			1/1	1/2	2/2			1/1	1/2	2/2	1/1	1/2	2/2	
rs2046496	3′ UTR	115,317,621	69(14.9)	231(49.8)	164(35.3)	0.298		64 (18.4)	171 (49.1)	113 (32.5)	80 (16.2)	256 (51.7)	159 (32.3)	0.647
rs2134655	Intron 5	115,340,891	20(4.3)	177(38.0)	269(57.7)	0.170		25 (7.2)	135 (38.8)	188 (54.0)	21 (4.3)	187 (37.9)	285 (57.8)	0.153
rs963468	Intron 4	115,345,577	71(15.5)	229(49.9)	159(34.6)	0.471		47 (13.5)	175 (50.3)	126 (36.2)	74 (15.1)	242 (49.8)	173 (35.4)	0.804
rs9880168	Intron 3	115,349,241	8(1.7)	101(21.6)	359(76.7)	0.716		4 (1.1)	81 (23.3)	263 (75.6)	8 (1.6)	108 (21.8)	380 (76.6)	0.762
rs2630351	Intron 3	115,357,749	8(1.7)	84(18.2)	369(80.0)	0.503		3 (0.9)	74 (21.3)	271 (77.9)	9 (1.8)	96 (19.6)	385 (78.6)	0.440
rs324029	Intron 2	115,364,313	34(7.2)	205(43.3)	234(49.5)	0.077		12 (3.4)	157 (45.1)	179 (51.4)	36 (7.2)	223 (44.3)	244 (48.5)	**0.067**
rs6280	Exon 2	115,373,505	42(9.3)	191(42.2)	220(48.6)	0.813		25 (7.2)	158 (45.4)	165 (47.4)	45 (9.6)	199 (42.5)	224 (47.9)	0.414
rs9825563	Promoter	115,382,910	36(7.9)	191(42.0)	228(50.1)	0.681		21 (6.0)	154 (44.3)	173 (49.7)	41 (8.5)	201 (42.7)	241 (49.9)	0.375

[a] Minor allele frequency (MAF) in AD patients compared with the control group using Pearson χ^2 test

[b] Allele 1 that is italicized and bold indicates the minor allele, and only alleles with frequency higher than 1% are showed

[c] The genotyping completing rate ranged from 97.6 to 100% in a total of 1060 samples

[d] Genotype frequencies in AD patients compared with the controls using Pearson χ^2 test

[e] Genotype frequencies in non-psychosis AD (Non-P AD) patients compared with the controls using Pearson χ^2 test

[f] Genotype frequencies in male AD patients compared with the male controls using Pearson χ^2 test

*A p value < 0.002 (0.05/24) was considered significant after Bonferroni correction

Table 2 A logistic regression analysis of *DRD3* gene polymorphisms as risk factors for amphetamine dependence (AD)

Variants (reference)			Male AD ($n = 503$)			Non-P AD ($n = 473$)		
			Odds ratio	95% CI	p [b]	Odds ratio	95% CI	p [b]
rs2046496	C/G and C/C	(G/G) [a]	1.112	0.763–1.618	0.581	1.195	0.836–1.708	0.329
rs2134655	C/T and C/C	(T/T) [a]	1.706	0.922–3.157	0.089	1.439	0.799–2.593	0.226
rs963468	A/G and G/G	(A/A) [a]	0.920	0.608–1.392	0.692	0.864	0.589–1.267	0.454
rs9880168	A/G and A/A	(G/G) [a]	0.941	0.270–3.282	0.924	1.270	0.419–3.853	0.673
rs2630351	A/G and G/G	(A/A) [a]	0.523	0.136–2.014	0.346	0.639	0.201–2.029	0.448
rs324029	A/G and G/G	(A/A) [a]	0.482	0.236–0.985	0.045	0.576	0.309–1.074	0.082
rs6280	C/T and T/T	(C/C) [a]	0.681	0.400–1.160	0.157	0.818	0.508–1.316	0.407
rs9825563	A/G and A/A	(G/G) [a]	0.605	0.344–1.063	0.081	0.752	0.453–1.248	0.271

[a] Genotype within parenthesis indicates the reference group of genotype

[b] Odds ratio is given with 95% confidence intervals (95% CI) after using a logistic regression analysis with age as covariates

*A p value < 0.003 (0.05/16) was considered significant after Bonferroni correction

AD patients and non-psychosis AD patients, but not with the total cohort or other subgroups. The borderline association was insignificant after correction for multiple testing and by logistic regression analyses adjusting for age and sex. Haplotype analysis revealed significant differences between total patients and normal controls, indicating that the rare ATA haplotype of rs324029–rs6280–rs9825563 was associated with a higher vulnerability for development of AD. Similar results were obtained in the male patient group and non-psychosis patient group. Compared to previous studies which consistently showed no association between the *DRD3* rs6280 functional polymorphism and AD [21–23], our results indicate that the haplotype block rs324029–rs6280–rs9825563, not a single polymorphism, may play an important role in AD, especially among Han Chinese patients. As discussed in our earlier report [33], ethnic differences may lead to diverse allele frequencies in genetic polymorphisms, which may account for the contrary findings.

The frequency of the ATA haplotype of rs324029, rs6280, rs9825563 was significantly higher in patients than in controls ($p = 0.0003$ after 10,000 permutations; Table 3). This was also observed in the male AD and AD without psychosis subgroups, compared to the control group. In contrast, haplotypes of the female AD group and AD with psychosis group were not different from the control group. This suggests that the haplotype block between the promoter region and intron 2 (rs324029, rs6280, rs9825563) may increase individual susceptibility to the development of AD specifically in the male and non-psychosis subgroups. Positive finding of our genetic association study may benefit from some potential factors: Case–control populations were chosen solely from Taiwan, resulting in increased genetic homogeneity [48] and reduced population stratification bias [49]. Additionally, we used well-screened controls, from unrelated Han Chinese community members, and excluded psychopathologies after psychiatrist screening using the SADS-L. Furthermore, the statistical power of our study was increased by expanding sample size and using haplotype analyses of eight *DRD3* SNPs [50]. Finally, our results suggest that rs324029, rs6280, and rs9825563 SNPs may be associated with AD. The relationship between these SNPs and the development of AD may be in high LD with the functional rs6280 and/or a nearby regulatory region, possibly associated with an alternative splicing site of messenger RNA or the DNA binding site of transcription factors.

D3 receptors function differently in men and women, and men express greater striatal dopamine release following exposure to amphetamine [51]. Sex-related differences in the link between regional DA release and affect and cognitive function, which may influence drug dependence, have been demonstrated in vivo [52]. Furthermore, D2/D3 receptor availability is more strongly associated with positive symptoms of schizophrenia in men [53] and may also increase risk of nicotine dependence in men compared to women [54]. Furthermore, the *DRD3* functional polymorphism has been associated with alcohol dependence among men [55]. Recently, a sex-specific link between *DRD3* hypermethylation and schizophrenia risk was identified [56]. These lines of evidence support the difference between the male subgroup and controls found in this study. We should cautiously interpret the negative result in the female group, which may be attributed to type I error due to small sample size. Future investigation in larger female samples with adequate power to detect potential sex-specific effect would provide a more definitive result.

An in vivo study [57] found the reduced hemodynamic changes in the bilateral ventrolateral prefrontal cortex,

Table 3 Haplotype analysis of *DRD3* gene in AD patients and normal controls

Haplotype block 1			Frequency		χ^2	*p*	Frequency		χ^2	*p*	Frequency		χ^2	*p*
rs2134655	rs963468	rs9880168	Total AD	Total NC			Male AD	Male NC			Non-P AD	Total NC		
C	A	A	0.400	0.367	2.492	0.736	0.393	0.366	1.214	0.976	0.397	0.367	1.886	0.841
C	G	A	0.243	0.241	0.009	1.000	0.248	0.240	0.154	1.000	0.244	0.241	0.016	1.000
T	G	A	0.229	0.241	2.502	0.735	0.232	0.256	1.341	0.965	0.233	0.259	1.788	0.862
C	G	G	0.122	0.119	0.058	1.000	0.122	0.117	0.088	1.000	0.121	0.119	0.018	1.000
Haplotype block 1			Frequency		χ^2	*p*	Frequency		χ^2	*p*	Frequency		χ^2	*p*
rs2134655	rs963468	rs9880168	Total AD	Total NC			Male AD	Male NC			Non-P AD	Total NC		
G	T	A	0.660	0.704	4.719	0.255	0.655	0.696	3.177	0.570	0.656	0.704	5.060	0.203
A	C	G	0.237	0.231	0.097	1.000	0.238	0.239	0.003	1.000	0.232	0.231	0.005	1.000
G	C	G	0.040	0.041	0.084	1.000	0.041	0.040	0.016	1.000	0.043	0.039	0.260	1.000
A	C	A	0.023	0.021	0.122	1.000	0.025	0.019	0.666	0.999	0.024	0.021	0.231	1.000
A	T	A	0.023	0.003	16.201	0.0003*	0.025	0.002	14.006	0.0007*	0.027	0.003	19.767	<0.0001*

NC normal controls, *Non-P AD* AD patients without drug-induced psychosis Haplotype frequencies in controls were >0.001

* $p < 0.001$ after 10,000 permutations for multiple comparisons

suggesting a common underlying pathophysiology in methamphetamine (MA)-induced psychosis and schizophrenia. Moreover, a genome-wide association study (GWAS) of MA-dependent patients [58] had found a host of MA-induced psychosis 'risk' SNPs is over-represented in schizophrenic patients, indicating a shared genetic risk between MA-induced psychosis and primary psychosis. Taken together, these results indicated that AD patients without psychosis seem to make distinction from those with psychosis, who may have more genetic similarity with schizophrenia patients than with AD patients without psychosis. The *DRD3* gene was also associated with susceptibility to schizophrenia (primary psychosis) in several meta-analyses, and the homozygosity of the Ser9Gly polymorphism was suggested to confer risk for schizophrenia [59, 60]. Subsequent genetic association studies failed to provide further evidence for the role of *DRD3* in the emergence of primary and MA-induced psychosis [21, 22], which may have resulted from ethnic differences [61]. Consistent with previous studies, we did not identify an association between *DRD3* gene variation and AD patients with psychosis. In contrast, our haplotype analysis found a significant association with SNPs rs324029, rs6280, and rs9825563 and the non-psychosis subgroup as well as the total group, compared to the controls. The influence of rs324029, rs6280, and rs9825563 SNPs on a specific subgroup may help elucidate the role of *DRD3* variants in the phenotypes of complex disorders such as amphetamine use with/without psychotic disorder and schizophrenia.

Mesolimbic dopaminergic neurotransmission plays a crucial role in the novelty seeking system. A recent neuroimaging study also indicated that NS scores were positively correlated with left striatal D2/D3 receptor availability in healthy subjects [62], consistent with Cloninger's theory concerning personality and character [63]. Our gene–personality study examining eight *DRD3* variants found a borderline significant association with specific personality traits in AD patients without psychosis, even after conservative Bonferroni correction. Prior studies exploring the relationship between *DRD3* and NS personality traits have had mixed results. Our results that the rs6280 polymorphism was not found associated with NS and HA personality traits in the total group are similar to those in healthy Caucasian and Japanese volunteers [39, 60, 64]. On the contrary, inconsistent finding from the study by Staner et al. [35] found that the Ser9Gly polymorphism (rs6280) was associated with the NS scores in bipolar patients. However, the correlation of the *DRD3* rs6280 gene with NS was not replicated in another Han Chinese population [65]. Previous studies evaluated gene–personality interaction using the Temperament and Character Inventory or revised NEO personality inventory, whereas we used a TPQ to assess AD patients. The conflicting findings should be interpreted with

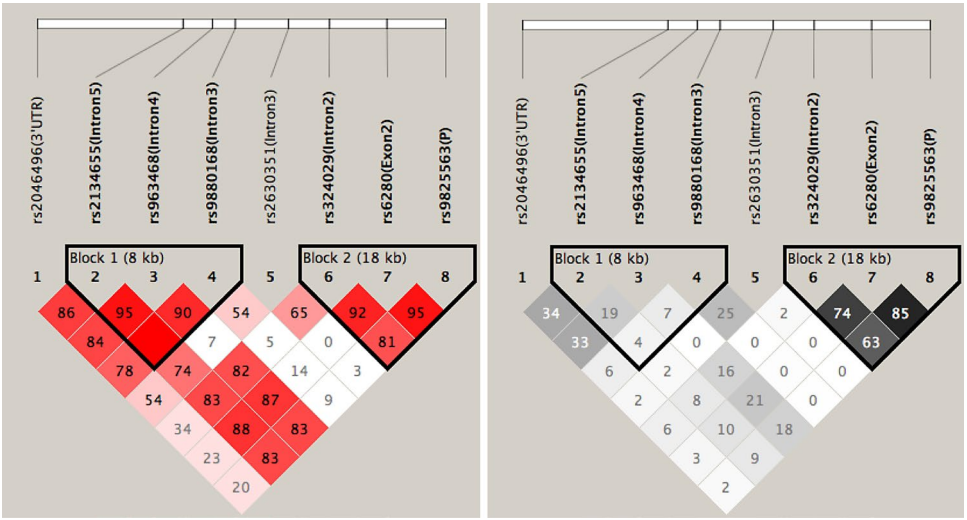

Fig. 1 LD structure between eight polymorphisms in *DRD3* gene is presented. The *upper panel* shows the location of eight polymorphisms in *DRD3* gene, and the *lower panel* shows the output of HAPLOVIEW version 4.2. *D*' value (left LD map) and r^2 value (right LD map) shown within the each square represent a pairwise LD relationship between the two polymorphisms. *Red squares* indicate statistically significant LD between the pair of polymorphisms. Darker colors of *red* indicate higher values of *D*' up to a maximum of 1, and *white squares* indicate pairwise *D*' values with no statistically significant difference of LD

caution, owing to the amount of variability across studies. To our knowledge, this is the first report of a correlation between the other seven *DRD3* SNPs and specific personality traits in AD patients in a Han Chinese population. Our results indicate that *DRD3* expression may affect specific personality traits, including NS, in specific subtyped AD patients.

Dopamine pathways project from the ventral tegmental area to the nucleus accumbens and the frontal cortex and are major components of reward processes [66]. Gene-knockout studies indicated that *DRD3*-deficient mice exhibited supersensitivity to amphetamines and cocaine [67], whereas subsequently, Le Foll et al. [14] suggested that *DRD3* knockout mice also displayed hyperactivity to drug-related stimuli by increasing dopaminergic tone. They also proposed that *DRD3* up-regulation in rat brain after drug exposure is involved in behavioral sensitization, mediating the persistence, and relapse of drug-seeking behavior. These results are in line with recent evidence indicating a direct link between deficient D2/D3 receptor availability and the vulnerability to relapse among stimulant users despite knowledge of consequent negative consequences [68]. Barrus, Winstanley [69] examined cued tasks and DRD3-mediated neurotransmission and identified a role for D3 receptors in mediating the facilitatory effects of cues in addiction. Furthermore, age-at-onset of drug abuse has often been reported to be a factor associated with similar genetic backgrounds [25], supporting our finding that carriers of the rs9825563 A-allele may have a trend to earlier onset age for AD. Taken together, we propose that those carrying *DRD3* risk genotype may have dysfunctional DRD3, leading to hyperdopaminergic tone, and increased sensitivity to drug abuse or drug-related environmental stimuli. This can increase the vulnerability to early initiation of drug abuse and/or facilitate the development of substance dependence.

Our results confirmed the relationship of the A-allele of *DRD3* rs9825563 with higher NS subscale scores and with earlier onset age of drug use. These results support the hypothesis that dopamine-related genes involved in drug use operate through interaction with specific personality traits that are also moderated by dopamine transmission [63, 70]. Consistent with previous studies, our results also imply that NS had a robust direct effect on age of onset of amphetamine use [29–32, 71]. Mediation analysis with SEM showed that DRD3 variants may determine onset age of drug abuse, and this effect is partially mediated by NS in subtyped AD patients. The indirect effect ratio through NS was $-0.022/-0.101 = 21.7\%$. Past studies also explored the interplay between dopamine-related genes, NS personality traits, and other substance abuse. For instance, studies have showed that the *DRD4* 7 repeat (7R) variable number tandem repeat is linked to higher NS scores and greater susceptibility to tobacco and alcohol use in young adulthood [72–74]. Another study suggested that the dopamine transporter (DAT) gene is associated with early tobacco and alcohol intake with more extent in adolescence [75]. Li et al. [34] indicated a role of NS as mediator of

Table 4 Comparison of NS subscale score and onset age of drug use in subtyped AD patients with different *DRD3* genotypes

Variants	Genotype		Non-Psychosis AD ($n = 473$)					
	1	2	Novelty seeking score		p [a]	Onset age of drug use		p [a]
			1	2		1	2	
rs2046496	C/G and C/C	(G/G)	13.18 (± 4.52)	12.60 (± 3.86)	0.324	24.70 (± 7.29)	24.74 (± 8.21)	0.974
rs2134655	C/T and C/C	(T/T)	13.13 (± 4.45)	13.15 (± 4.96)	0.982	24.56 (± 7.24)	26.05 (± 9.08)	0.375
rs963468	A/G and G/G	(A/A)	13.10 (± 4.35)	12.77 (± 4.83)	0.562	24.77 (± 7.54)	24.47 (± 6.47)	0.733
rs9880168	A/G and A/A	(G/G)	13.07 (± 4.43)	13.75 (± 5.65)	0.670	24.83 (± 7.42)	19.75 (± 4.77)	0.054
rs2630351	A/G and G/G	(A/A)	13.08 (± 4.47)	12.75 (± 4.95)	0.837	24.82 (± 7.43)	20.75 (± 5.01)	0.124
rs324029	A/G and G/G	(A/A)	13.16 (± 4.46)	11.63 (± 4.23)	0.084	24.64 (± 7.29)	25.93 (± 8.75)	0.379
rs6280	C/T and T/T	(C/C)	13.32 (± 4.47)	11.43 (± 3.96)	0.010	24.50 (± 7.16)	26.15 (± 8.87)	0.176
rs9825563	A/G and A/A	(G/G)	13.25 (± 4.46)	11.17 (± 4.04)	0.007	24.52 (± 7.19)	27.28 (± 8.92)	0.031

[a] Uncorrected p value; total 466 patients with non-psychosis amphetamine dependence completed the Tridimensional Personality Questionnaire (TPQ)

* A p value < 0.006 (0.05/8) was considered significant after Bonferroni correction

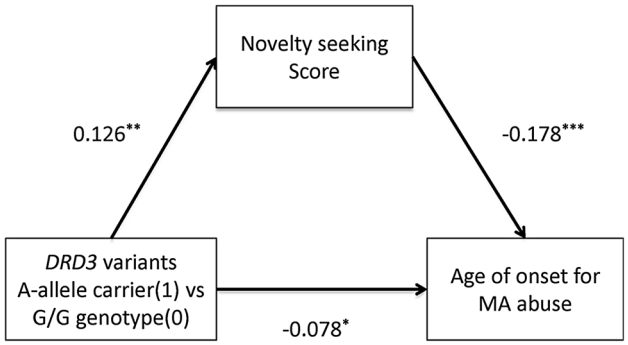

Fig. 2 Structural equation models with standardized coefficients for the *DRD3 rs9825563* variants and mediation model of novelty seeking in the prediction of onset age of drug use in non-psychosis subgroup. *Rectangles* represent observed measured variables. Values are standardized path coefficients. (*$p < 0.05$;**$p < 0.01$; ***$p < 0.001$)

the association between the COMT gene and early onset of drug use, which would heighten the vulnerability and severity of subsequent heroin dependence. In line with previous reports, we suggested that the *DRD3* risk genotype in amphetamine addicts is related to earlier onset of drug use, perhaps through the complex interaction of higher level of NS traits.

Limitations

Some points should be considered when interpreting our results. First, gene–gene interaction analysis showed that either *DRD3*×*DRD4*×*5HT2C* or *MAOA*×*COMT*×*DRD3* had a significant association with personality traits, without finding a significant association with these items in single-marker analysis of *DRD3* [38]. Gene–gene epistatic or modifying effects on personality traits should be considered when explaining our negative findings between other *DRD3* variants and personality scores. Second, the nature of the study means it is challenging to elucidate whether the 'transient' type (remitting a psychotic state instantly or few days after exposure) or 'prolonged' type of psychosis has more genetic similarity with schizophrenia [58]. To further examine this, longitudinal studies with appropriate sample sizes are required. Third, although our total cohort ($n = 1060$) was sufficiently large to detect an effect of *DRD3* variants in the development of AD, the number of individuals recruited in female or psychosis subgroups was relatively small and possibly reduces the power to detect an association. Fourth, we randomly selected eight SNPs to cover a 50.3 kb span of *DRD3*. These markers may not provide thorough coverage of *DRD3* because the *D*' value between some adjacent markers was less than 0.9. Although the promoter and exon SNPs may cause functional consequences, there are some intron variants with no available information for their function. In addition, our method using random criteria for SNP selection may have reduced power compared to a pairwise tagging program [76]. Fifth, environmental factors have been implicated in the pathogenesis of AD [4] in addition to DRD3 regulation of the effect of environmental stimuli on drug-seeking behavior [14]. However, our study did not analyze the confounding effects of estimates of shared environmental risk factors.

Conclusion

Our study presents evidence of an association between the ATA haplotype (rs324029, rs6280, rs9825563) of *DRD3*

and the risk of AD in the subtyped groups among a Han Chinese population. This study also indicates that novelty seeking mediated the relationship between *DRD3* rs9825563 and drug-use onset age in AD patients without psychosis. These results add to our understanding of the multifaceted mechanisms at the interplay between gene, temperament, and behavior. Prevention-related gene-association studies should take the NS personality trait into account, and determination of this status may be helpful to identify those vulnerable of becoming AD in the early stage. Replication of our results in other ethnic populations is warranted to verify these findings.

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Author contributions All authors contributed extensively to the work presented in this paper. SYH was the principal investigator for the study, conceived of the study, and helped to draft the manuscript. The authors SCK and SYH designed the study and wrote the protocol; SCK and YWY wrote the main manuscript text and data collection. SCK undertook the statistical analysis and wrote the first draft of the manuscript. The authors CYC, CCH, TYC, PSH, CSL, and CHY managed the literature searches and analyses. RBL supervised data collection and provided overall scientific supervision. All authors contributed to and have approved the final manuscript.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

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