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The DAOA gene is associated with schizophrenia in the Taiwanese population

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ABSTRACT

The gene *D-amino acid oxidase activator* (*DAOA*), which has a former name of *G72*, and the *D-amino acid oxidase (DAO)* gene have been suggested as candidate genes of schizophrenia. However, association studies have so far yielded equivocal results. We analyzed one single nucleotide polymorphism (SNP) for *DAO* (rs3741775) and seven SNPs for *G72* (rs3916956, rs2391191, rs9558562, rs947267, rs778292, rs3918342, and rs1421292) in this study enrolling 248 schizophrenia cases and 267 controls in the Taiwanese samples. In SNP-based single locus association analyses, the rs778292 in the *DAOA* gene showed significant association with schizophrenia. The rs3741775 in the *DAO* gene could not withstand statistically significant after multiple corrections. Additionally, a three-SNP haplotype (rs778292-rs3918342-rs1421292) in the *DAOA* gene were observed to be significantly associated with schizophrenia. Among them, the TCT haplotype presented higher prevalence in controls than in cases whereas the TTT and CTT haplotype were significantly more frequent in cases than in controls. The study also provides significant evidence for epistatic interactions among *DAOA* and *DAO* gene in the development of schizophrenia. These results provide additional evidence and indicate that the *DAOA* gene and *DAOA-DAO* gene-gene interactions might play a role for schizophrenia in a Taiwanese sample.

1. Introduction

Schizophrenia is a common debilitating mental disorder that affects approximately 0.7% of world population (McGrath et al., 2008). Understanding the etiology and pathogenesis of schizophrenia is one of the most important challenges in psychiatry. Although genetic factors are known to be important in the etiology of schizophrenia, the causative genes remain elusive. One of the explanations is that there are multiple susceptibility genes, each with small effect, which interact with one another and with environmental factors to influence susceptibility to the disorder (Harrison, 2015).

The gene *D-amino acid oxidase activator* (*DAOA*), which has a former name of *G72*, was shown to be significantly associated with schizophrenia in a case-control study involving French-Canadian samples and Russian cohort of samples (Chumakov et al., 2002). Interestingly, in expression and functional studies, the DAOA protein interact the gene for D-amino acid oxidase (*DAO*) protein to regulate glutamatergic signaling through the N-methyl-D-aspartate (NMDA) receptor pathway which was thought to be involved in the pathogenesis model of schizophrenia (Tsai and Coyle, 2002). Previous studies showed association between schizophrenia and *DAO* (Liu et al., 2004; Yang et al., 2013; Sacchetti et al., 2013), *DAOA* genes (Wang et al., 2004; Korostishevsky et al., 2004; Hong et al., 2006; Addington et al., 2004; Shinkai et al., 2007; Bass et al., 2009), or both (Schumacher et al., 2004; Chumakov et al., 2002; Corvin et al., 2007). However, other reports demonstrated negative associations for these finding in their family-based studies (Mulle et al., 2005; Liu et al., 2006). In addition, previous well-known studies showed that rs3741775 of the *DAO* alleles contributed elevated risk of schizophrenia in Western countries of France, Russia (Chumakov et al., 2002), Germany (Schumacher et al., 2004), and Italy (Sacchetti et al., 2013). But inconsistent findings existed in Asian regions. A significant association between rs3741775 and schizophrenia was found in one study from Taiwan (Liu et al., 2004), but not replicated in other studies

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from Taiwan (Liu et al., 2006) and Japan (Yamada et al., 2005).

To further investigate the role of the *DAOA* and *DAO* genes in schizophrenia susceptibility, we have performed an association study in these two genes and schizophrenia using a cohort of the Taiwanese Han case-control samples.

2. Materials and methods

2.1. Sample

Our sample of 248 Taiwanese Han schizophrenic patients (139 men, 109 women; mean age ± SD: 39.22 ± 10.64) was recruited from three clinical sites in Taiwan: Kaohsiung Veterans General Hospital, Long-Cyuan Veterans Hospital, and Jianan Mental Hospital. The diagnosis of schizophrenia (n = 210) or schizoaffective disorder (n = 38) was defined by the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision criteria (DSM-IV-TR) (APA (American Psychiatric Association), 2000). The Operational Criteria Checklist for Psychotic Illness (OPCRIT) program was also used to assess patients (McGuffin and Farmer, 2001; McGuffin et al., 1991). The age of onset of the disorders was 24.79 ± 8.09, and the mean of years of education was 11.19 ± 3.55 years. Subjects were excluded if psychosis was occurring solely in relation to alcohol or substance abuse, or psychosis only secondary to medical illness or medication.

A total of 267 control participants (218 men, 49 women; mean age ± SD: 36.13 ± 11.95), screened for lifetime absence of psychiatric disorder, was recruited from the blood donation center in southern Taiwan. The average years of education were 13.46 ± 2.85 years. Control subjects were excluded if they or a first-degree relative ever fulfilled criteria for schizophrenia, schizoaffective disorder, bipolar disorder, or major depressive disorder. All control subjects were also of Han ancestry.

The study was approved by the Institutional Review Boards of Kaohsiung Veterans General Hospital and Jianan Mental Hospital, and written informed consent was obtained from all the participants.

2.2. Genotyping

Blood samples were obtained from all patients and controls. Genomic DNA was extracted using QIAamp® DNA Blood Mini Kit according to the manufacturer's protocol provided by Qiagen®. One SNP, rs3741775, located in intron 4 of the *DAO* gene, and a selection of 9 SNPs spanning the *DAOA* gene: rs3916956, rs12584489, rs2391191, rs9558562, rs1935062, rs947267, rs778292, rs3918342, and rs1421292 (covering ~95 kb region, including the 5' and 3' flanking regions of the DAOA locus, constituting the region of interest for linkage disequilibrium (LD) mapping) were investigated. Reason for selection of SNPs for *DAOA* were that these SNPs were significantly associated with schizophrenia, as reported by (Chumakov et al., 2002; Bass et al., 2009; Shinkai et al., 2007; Schumacher et al., 2004). Rs3741775 of the *DAO* was selected because of inconsistent findings in Asian countries, as mentioned above in the introduction.

Genotyping of the ten SNPs was carried out using the SNPlex™ Genotyping System (Applied Biosystems, Foster City, CA). The system uses multiplex oligonucleotide ligation (OLA), polymerase chain reaction (PCR), and capillary electrophoresis to analyze bi-allelic SNP genotypes.

Genotyping was performed blind with regard to all phenotypic information. Analysis of the raw data was performed using GeneMapper Software v3.7 and Microsoft Office Excel 2003. The following quality control criteria were applied: (1) SNPs were omitted from analysis if poor genotype clustering prevented GeneMapper from making calls, (2) individual genotypes were omitted if their peak heights were < 20% of the average for that genotypic group across the entire sample to avoid a false heterozygosity assignment because of background noise in poor-quality samples, (3) markers were omitted if the call rate after the earlier exclusions was < 80% as low call rates may indicate inaccurate genotyping. Therefore, we omitted two SNPs (rs12584489 and rs1935062) due to missing data (MD) > 20%.

2.3. Statistics

To calculate the power of our case-control sample, the Power and Sample Size Calculations (PS) program (Dupont and Plummer, 1990) was used.

To test for deviation from the Hardy-Weinberg Equilibrium (HWE), the computer program FINETTI (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) was used to perform exact statistics, and cases and controls were considered separately.

Genotype and allele frequencies were assessed for an association with schizophrenia using standard contingency table analyses incorporating chi-squared tests of independence, producing a χ^2-statistic with 1 or 2 degrees of freedom depending on the number of parameters and corresponding p-values for allele and genotype distributions, respectively. Multiple logistic regressions with age and sex as covariables were conducted to assess the associations between different genotypes, including dominant and recessive models of the allele, and schizophrenia. Additionally, gene-gene interactions were examined using binary logistic regression. Pairwise interactions were tested by introducing interaction terms into the two-factor logistic regression models using forward selection strategy. Odds ratios (OR) with 95% confidence intervals (CI) and *p* values were assigned.

Haploview 4.2 program was used to perform linkage disequilibrium (LD) analysis of all SNPs and to predict haplotype frequencies for two- and three-SNP sliding windows for patients with schizophrenia and control individuals (Barrett et al., 2005). Samples with call rates less than 50% were excluded from single SNP analyses and haplotype analyses. Haplotypes were estimated using SNPs that showed a significant association with the phenotypes of interest in the case samples, using the expectation-maximization (EM) algorithm to obtain the maximum likelihood estimates of haplotype frequencies in each sample. The criteria for the selected SNPs to construct haplotype blocks is that all SNPs in one region must be in strong LD for which the one-sided upper 95% confidence bound on D' was greater than 0.98 and lower bound was greater than 0.7 according to the Gabriel's method (Gabriel et al., 2002).

Given the potential impact on gender-specific effect for the studies genes (Kim et al., 2010; Sacchetti et al., 2013), a stratification based on sex and a forward logistic regression with sex and age as covariates was conducted to examination the different genetic effect between the two sexes.

To correct for multiple testing in the single marker comparisons for both genotypic and allelic association, we applied the false discovery rate (FDR) method (Benjamini et al., 2001). Permutation tests were used for multiple testing corrections of the haplotype analyses (n = 10,000). The significance level for all statistical tests was two-tailed ($p < 0.05$).

All of these analyses were performed using SPSS version 15.0. The single SNP association analyses and haplotype association were tested by Haploview 4.2.

3. Results

The study included 248 schizophrenia cases and 267 controls in the Taiwanese samples. The power of the current study is modest. With a specified power level of 80% and a type I error rate of 0.05, these samples could detect the alternative hypothesis in a range of odd ratios smaller than 0.61 or larger than 1.65 in the cases versus controls.

Allele frequencies and genotype distributions for each SNP in patients and controls are listed in Table 1. Markers rs12584489 and rs1935062 were excluded from further analyses as the percentage of

Table 1
Single-marker association analyses of *DAOA* and *DAO* genes in schizophrenia.

SNP ID	Position[a]	Location	Allele[b]	MD%	Patient Genotype (%)	Control Genotype (%)	Case MAF	Control MAF	Genotypic χ^2 (*p*-value)	Allelic χ^2 (*p*-value)
DAOA	13q33.2									
rs3916965(M12)	104901361	5'UTR	A/G	19.2			42.3	44.0	3.442 (0.179)	0.166 (0.684)
Genotype					N=215	N=235				
AA					29.3	32.3				
AG					56.7	47.2				
GG					14.0	20.5				
Allele										
A					57.6	55.9				
G					42.4	44.1				
rs2391191(M15)	104917447	Exon 2	A/G	8.2			41.5	39.5	2.196 (0.334)	0.931 (0.335)
Genotype					N=224	N=253				
AA					31.4	36.4				
GA					54.4	48.2				
GG					14.2	15.4				
Allele										
A					31.3	36.4				
G					68.7	63.6				
rs9558562	104922938	Exon 3	A/G	18.2			6.6	9.6	0.607 (0.344)	0.573 (0.449)
Genotype					N=226	N=254				
AA					87.1	86.5				
GA					12.3	8.0				
GG					0.6	5.5				
Allele										
A					93.3	89.7				
G					6.7	10.3				
rs947267(M18)	104937663	Intron 3	A/C	8.8			43.4	39.0	3.266 (0.195)	1.361 (0.243)
Genotype					N=221	N=251				
AA					31.4	39.5				
CA					50.6	43.0				
CC					18.0	17.5				
Allele										
A					56.5	60.9				
C					43.5	39.1				
rs778292	104966952	3'UTR	C/T	7.6			33.1	25.1	**12.333 (0.002)**	**9.110 (0.003)**
Genotype					N=225	N=254				
CC					12.8	5.2				
CT					40.4	40.1				
TT					46.8	54.7				
Allele										
C					33.2	25.2				
T					66.8	74.8				
rs3918342(M23)	104983750	3'UTR	C/T	9.8			43.6	45.9	5.016 (0.081)	1.404 (0.236)
Genotype					N=221	N=246				
CC					16.2	23.1				
CT					54.7	45.5				
TT					29.1	31.4				
Allele										
C					43.6	45.9				
T					56.4	54.1				
Rs1421292 (M24)	104996236	3`UTR	A/T	9.0			40.8	43.1	1.860 (0.394)	0.606 (0.436)
Genotype					N=217	N=245				
AA					13.8	19.1				
AT					53.9	47.8				
TT					32.3	33.1				
Allele										
A					40.8	43.1				
T					59.2	56.9				
DAO	12q24									
rs3741775(MDAAO–6)	107807732	Intron 4	G/T	8.0			47.0	41.3	**9.803 (0.007)**	**4.747 (0.029)**
Genotype					N = 224	N = 253				
GG					19.1	16.9				
GT					55.8	48.6				
TT					25.1	34.5				
Allele										
G					47.1	41.4				
T					52.9	58.6				

Abbreviations: *DAOA*, D-amino acid oxidase activator gene; *DAO*, D-amino oxidase gene; HW: Hardy-Weinberg; MAF: Minor Allele Frequency; MD, missing data.
P-values are shown prior to correction for multiple testing (for corrected p-values see "Results" Section).
Bold values represent significant *p*-values.
[a] Position according to the HapMap database (http://www.hapmap.org).
[b] Minor alleles are underlined.

Table 2
Pair-wise comparisons (linkage disequilibrium) of the SNPs investigated in the *DAOA* gene.

SNP	rs3916965	rs2391191	rs9558562	rs947267	rs778292	rs3918342	rs1421292
rs3916965	/	**0.980**	**1.000**	0.686	0.452	0.119	0.224
rs2391191	**0.942**	/	0.821	0.683	0.430	0.150	0.202
rs9558562	0.076	0.054	/	**1.000**	0.536	0.085	0.121
rs947267	0.462	0.458	0.027	/	0.326	0.062	0.034
rs778292	0.103	0.094	0.005	0.053	/	0.427	**1.000**
rs3918342	0.009	0.014	0.000	0.002	0.059	/	0.891
rs1421292	0.025	0.020	0.001	0.001	0.257	0.620	/

D' values are shown in the upper right triangle, r^2 values are shown in the lower left triangle. (**Bold number** = significant LD).

MD was >20%. Genotypic frequencies and Hardy-Weinberg equilibrium (HWE) were assessed for each SNP. The observed genotypes distribution of each SNP in patients and control group accorded with the Hardy-Weinberg equilibrium ($p > 0.05$).

In Table 1, single-marker association analyses showed rs778292 polymorphism, located at the 3'UTR of *DAOA,* was associated with schizophrenia before and after correction for multiple testing (genotypic $\chi^2 = 12.333$, df = 2, $p = 0.002$, FDR q-value = 0.032; allelic $\chi^2 = 9.110$, df = 1, $p = 0.003$, FDR q-value = 0.032).

For *DAO*, there was a significant genotypic ($p = 0.007$) and allelic (p=0.029) association observed with marker rs3741775 and schizophrenia. However, this just failed to withstand multiple testing corrections (genotypic χ^2 =9.803, df =2, p=0.007, FDR q-value =0.059; allelic $\chi^2 = 4.747$, df = 1, $p = 0.029$, FDR q-value = 0.203).

The linkage disequilibrium pattern between the analyzed SNPs was shown in Table 2 and Fig. 1. The strongest LD was observed between rs3916965 and rs2391191, rs3916965 and rs9558562, rs9558562 and rs947267 as well as rs778292 and rs1421292.

As shown in Table 3.1., in comparison with subjects with *DAOA* rs778292 CC genotype carriers, CT and TT genotype carriers exhibited a lower risk of schizophrenia (CT genotype, OR = 0.42, 95% CI = 0.20–0.86, $p = 0.018$; TT genotype, OR = 0.36, 95% CI = 0.18–0.73, $p = 0.005$) after adjustment for age and sex. Moreover, the TT genotype

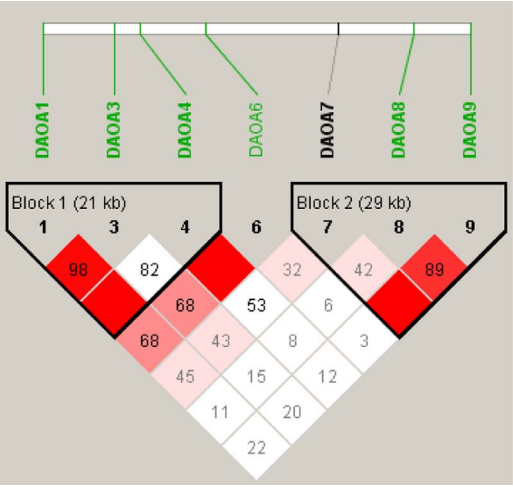

Fig. 1. Haplotype block structure of the *DAOA* gene. Genomic organization and linkage disequilibrium (LD) structure of the *DAOA* gene are shown. Exons are shown as boxes. Shades of red represent extent of LD (darker red denotes D' =1). Numbers in squares give r2 values multiplied by 100.

Table 3.1
Association between *DAO* rs3741775 and *DAOA* rs778292 polymorphisms and risk of schizophrenia.

DAO		
rs3741775(MDAAO–6)	Adjusted OR (95% CI)	*p* value[a]
GG	1.0 (reference)	
GT	0.96 (0.58–1.58)	0.857
TT	0.64 (0.37–1.10)	0.108
Dominate (TT vs GT+GG)	0.82 (0.51–1.33)	0.42
Recessive (GT+TT vs GG)	0.66 (0.44–0.99)	**0.044**
Allele		
G	1.34 (1.03–1.75)	0.203
T		
DAOA		
rs778292		
CC	1.0 (reference)	
CT	0.42 (0.20–0.86)	**0.018**
TT	0.36 (0.18–0.73)	**0.005**
Dominate (TT vs TC+CC)	0.38 (0.19–0.77)	**0.007**
Recessive (TC+TT vs CC)	0.74 (0.51–1.07)	0.112
Allele		
C	1.56 (1.17–2.08)	**0.032**
T		

Abbreviations: *DAOA*, D-amino acid oxidase activator gene; *DAO*, D-amino oxidase gene; OR, odd ratios; CI, confidence interval.
Bold values represent significant *p*-values.
[a] Adjusted for age and gender.

was associated with a reduced risk of schizophrenia compared to the TC+CC genotype in a dominant model (OR = 0.38, 95% CI = 0.19–0.77, $p = 0.007$). Increased risks of schizophrenia was found among subjects with allele C (OR = 1.56, 95% CI = 1.17–2.08, $p = 0.032$). For *DAO* rs3741775, no significant associations between the genotype and allele distributions and schizophrenia were observed. However, the GT+TT genotype was associated with a reduced risk of schizophrenia compared to the GG genotype in a recessive model (OR = 0.66, 95% CI = 0.44–0.99, $p = 0.044$). For *DAOA* and *DAO* gene, we did not find any significant gender-specific effect on schizophrenia after adjusting sex in logistic regression models. In addition, as shown in Table 3.2., we identified evidence for epistatic interaction between the DAO rs3741775 and DAOA rs778292 in contributing to schizophrenia risk (OR = 1.04, 95% CI = 1.11–1.06).

As shown in Table 4, three-marker haplotype analyses across the *DAOA* gene showed positive associations before multiple testing

Table 3.2
The gene-gene interaction between *DAO* and *DAOA* genes.

rs3741775 (*DAO*)	rs778292 (*DAOA*)	Case %	Control %	OR (95% CI)[a]
GG	CC	7.1	7.1	1
	CT+TT	92.9	92.9	0.81 (0.15–4.42)
GT+TT	CC	14.4	4.8	1
	CT+TT	85.6	95.2	1.04 (1.11–1.06)

Abbreviations: *DAOA*, D-amino acid oxidase activator gene; *DAO*, D-amino oxidase gene; OR, odd ratios; CI, confidence interval.
[a] Adjust for age, gender.

corrections, with T-C-T under-transmitted ($p = 0.008$) in the schizophrenia group and C-T-T and T-T-T over-transmitted ($p = 0.042$ and 0.006, respectively). After permutation-based methods following 10,000 permutations, the T-T-T haplotype was still significantly associated with schizophrenia ($p = 0.041$) whilst a trend was observed in the T-C-T haplotype ($p = 0.057$). The T-T-T haplotype was risk factors for schizophrenia (OR = 3.16, 95% CI = 1.31–5.09). The T-C-T haplotype seems to be protective factors for schizophrenia (OR = 0.68, 95% CI = 0.35–1.25) with a marginal *p*-value of 0.057.

4. Discussion

We have performed a case-control study to investigate the association between *DAOA* and *DAO* polymorphisms and schizophrenia. Genotyping a selection of 7 SNPs spanning the *DAOA* gene and 1 SNP in *DAO* gene in a sample of 248 patients with a diagnosis of schizophrenia and 267 control subjects from three clinical sites in Taiwan (Kaohsiung, Long-Cyuan, and Jianan). We found a statistically significant genotype and allele distributions differences in schizophrenia were observed on one marker: *DAOA* rs778292. The frequency of the C allele of this SNP was significantly higher in the patient sample than the controls. In the haplotype analysis, the haplotype of rs778292–rs3918342–rs1421292 was significantly different between schizophrenia and control groups, and the haplotype frequencies of TTT were significantly higher in the schizophrenia group. No genotypic and allelic associations with schizophrenia were observed in *DAO* gene after multiple testing corrections. The study also provides significant evidence for epistatic interactions among *DAOA* and *DAO* gene in the development of schizophrenia. These indicate that the *DAOA* gene and *DAOA–DAO* interactions may play a vital role in the etiology of schizophrenia in the Taiwanese population.

In our study, we have significantly demonstrated the evidence of an association between schizophrenia and rs778292, a gene *DAOA* SNP marker that had not been reported to be associated with schizophrenia in previous studies. The results of single-marker analyses showed significant allelic and genotypic associations and both the allelic and genotypic *p*-values withstood correction for multiple testing applying the FDR. To the best of our knowledge, there is only one publication reporting a negative association between rs778292 and bipolar disorder (Gaysina et al., 2010); therefore, this is the first work publishing a positive association between rs778292 and psychiatric disorder, especially schizophrenia.

The three-marker haplotype covering components rs778292, rs3918342, and rs1421292 of *DAOA* were also observed to be significantly associated with schizophrenia in this study. Amongst the haplotype, the SNP marker rs3918342 was one of the most associated markers in both French Canadian and Russian populations presented by Chumakov et al. (2002). In our haplotype analysis, the TCT haplotype presented higher occurrence in controls than in cases and might implicate a variant that was likely to be protective against schizophrenia. Contrarily, the haplotype TTT and CTT were significantly more frequent in cases than in controls. The T alleles at both rs3918342 and rs1421292 were found to be associated with schizophrenia in the original report of Chumakov et al. (2002). Our haplotypic association supported their findings. In the present study, we have achieved an independent replication of association between *DAOA* and schizophrenia in a cohort of the Taiwanese sample.

Although no robust evidence showing the analyzed SNPs are functional or have a putative effect on gene expression and protein availability, some clinical studies reported genetic variations were associated with alternations of brain activation and CSF biomarkers. For example, Hall et al. found that genetic variation in both rs3918342 and rs1421292 was associated with both decreased hippocampal/parahippocampal activation and increased prefrontal activation in their fMRI study in subjects at high risk of schizophrenia (Hall et al., 2008). Krug et al. identified that brain activation in the right middle temporal gyrus (BA 39) and the right precuneus (BA 7) was positively correlated with the rs1421292 risk allele in the *DAOA* gene during a verbal fluency task in healthy individuals. These findings show that *DAOA* is not only associated with schizophrenia but also modulates verbal fluency performance via its neural correlates (Krug et al., 2011). One study by Andreou and colleagues enrolled healthy Caucasians and found that the rs3918342 and rs1421292 in the *DAOA* gene were associated with CSF homovanillic acid (HVA) concentrations. These results implicated that dopamine turnover could be not only altered via *DAOA* gene variation but also as a possible pathophysiological mechanism behind schizophrenia in humans (Andreou et al., 2012).

In *DAO* gene, the SNP rs3741775 was reported to be the most associated marker (MDAAO-6, $p = 0.001$) in the French Canadian population (Chumakov et al., 2002). Liu et al. also replicated the finding at the same SNP rs3741775 in the Chinese population (Liu et al., 2004). However, we just found nominal allelic and genotypic associations of the SNP rs3741775 (MDAAO-6) with schizophrenia

Table 4
Haplotype analyses within the *DAOA* genes in schizophrenia.

SNPs	Haplotype	Control freq	Case freq	χ^2	Individual *p*-value	Adjusted *p*-value[a]	OR (95% CI)
1-3-4	A-A-A	0.58	0.59	0.014	0.905	1.000	1.02 (0.59–1.83)
	G-T-A	0.31	0.33	0.137	0.711	1.000	1.07 (0.61–1.99)
	G-T-T	0.03	0.02	0.565	0.452	0.999	0.71 (0.11–4.04)
	G-A-A	0.03	0.02	0.946	0.331	0.996	0.71 (0.11–4.04)
	A-A-T	0.01	0.01	0.319	0.572	0.999	0.70 (0.06–16.21)
7-8-9	T-T-A	0.41	0.37	1.517	0.218	0.995	0.87 (0.48–1.49)
	T-C-T	0.30	0.22	7.123	**0.008**	0.057	0.68 (0.35–1.25)
	C-C-T	0.18	0.21	1.339	0.247	0.921	1.24 (0.60–2.44)
	C-T-T	0.07	0.11	4.122	**0.042**	0.293	1.65 (0.79–7.03)
	T-T-T	0.02	0.05	7.604	**0.006**	**0.041**[a]	**3.16 (1.31–5.09)**

Haplotypes with *p*-value < 0.50 are presented, *p*-values < 0.05 are in bold.
SNP 1= rs3916965, SNP 3= rs2391191, and SNP 4= rs9558562, SNP 7= rs778292, SNP 8= rs3918342, and SNP 9= rs1421292.
[a] For permutation-based methods, p-values were based on up to 10,000 permutations.

that failed to withstand multiple testing corrections in our Taiwanese samples. The differences between results from our study and those from Chumakov et al. and Liu et al. may be explained as follows: (1) Schizophrenia could be heterogeneous, with different loci influencing liability in different populations. (2) Individual SNPs in different ethnic groups have different informational content and are easier than haplotypes to give unstable frequencies among different populations in association studies of complex disorders.

DAO was mentioned to interact with *DAOA* in the regulation of glutamatergic signaling through the NMDA pathway, which may be especially involved in the pathogenesis model of schizophrenia (Tsai and Coyle, 2002; Harrison et al., 2003). Chumakov et al. also observed the interaction between *DAOA* and *DAO*; they showed the synergic effect of these two genes increasing the risk for the disorder is higher than the effect of each single factor per se. Our results suggest that only *DAOA* gene is likely to be major susceptibility genes for schizophrenia, but not *DAO* gene. *DAO* rs3741775 showed no association with schizophrenia in genotype and allele distributions, except in a recessive model. Even though, there are significant epistatic interactions between *DAOA* and *DAO* gene in the development of schizophrenia. The importance of the *DAO* rs3741775 only be highlighted by interacts with *DAOA* markers, or else to analyze more *DAO* markers.

Evidence regarding the functions of *DAOA* and *DAO* also support their involvement in the pathogenesis of schizophrenia (Liu et al., 2004; Wang et al., 2004; Korostishevsky et al., 2004; Schumacher et al., 2004; Hong et al., 2006; Yang et al., 2013). D-amino acid oxidase activator (DAOA) protein regulates the function of D-amino acid oxidase (DAO), a flavoenzyme that catalyzes the oxidative deamination of D-3,4-dihydroxyphenylalanine (D-DOPA) and notably an endogenous N-methyl-D-aspartate receptor (NMDAR) co-agonist, D-serine. D-DOPA is converted to L-3,4-DOPA, a precursor of dopamine, whereas D-serine is a potent activator of the NMDAR and the overall effect of activation of DAO is to decrease glutamatergic neurotransmission at NMDAR. As such, this provides an important potential pathogenic link between DAOA, DAO, and the glutamate hypofunction hypothesis of schizophrenia (Coyle, 2006).

Some limitations should be addressed. First, one major limitation of the current study is that the sample size is relatively small, which may decline the power of the study and increase the possibility of a type II error (i.e. false negative), but our results are positive nevertheless. Second, our samples were not sex-matched: there were 81% males of controls and 56% males of cases, which might have an impact on the gender-specific genetic effect (Kim et al., 2010). Third, only one SNP (rs3741775) was selected in the present study. It is hard to conclude whether the *DAO* gene is linked to schizophrenia or not, as single SNP is not representative for this gene. Studies enrolling more SNPs in *DAO* gene are warranted. Lastly, genome-wide association studies (GWAS) of schizophrenia do not further implicate *DAOA* or *DAO*, both of the genes are not on the top list of GWAS hits, however, as we know, the GWAS may have many false negatives because of stringent *p* values (Bass et al., 2009). Failure to detect association signal in GWAS does not provide conclusive exclusion of any given genes identified so far. Replication of these findings in more independent samples is essential to help in clarifying the underlying pathophysiological mechanisms of schizophrenia.

In conclusion, the study provides support for the association of *DAOA* with schizophrenia in a different ethnic group. Besides, *DAOA* gene and *DAOA–DAO* gene interactions might likely play a part for schizophrenia in this Taiwanese population. More studies are warranted to confirm the role of *DAO* gene in the development of schizophrenia.

Conflict of interest

All authors declare no conflict of interest.

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