

Gene Polymorphisms and Expression of *NRG1*, *DAOA*, and *DISC1* Genes in a Chinese Han Population with an Ultra-High Risk for Psychosis

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Purpose: Although there is previous evidence supporting that ultra-high risk (UHR) for psychosis transformation is associated with *NRG1*, *DAOA*, and *DISC1* genes, there have been no relevant studies in the Chinese population. The objective of the current study was to explore the gene polymorphism and expression of *NRG1*, *DAOA*, and *DISC1* genes in a Han population with UHR for psychosis in China.

Methods: Eighteen UHR individuals, 61 first-degree relatives of patients with schizophrenia (FDR), 55 first-episode psychosis individuals (FEP), and 61 healthy controls (HC) were enrolled in the study. The genotypes at four loci of the *NRG1* gene, four loci of the *DAOA* gene, and two loci of the *DISC1* gene were tested for all subjects, and mRNAs of *NRG1* and *DISC1* were examined and analyzed in a pairwise comparison among the four groups. Statistical analysis of genetics was performed using snpStats software. For the case-control association analysis, a single site association study, epistatic effect analysis, and haplotype analysis were used to explore the association of the above genes.

Results: This study found that rs3918341 in the *DAOA* gene was associated with susceptibility to UHR by single site association analysis. Epistatic effect analysis results showed that the *NRG1* gene interacted with the *DAOA* gene and *DISC1* gene in the susceptibility to UHR. Haplotype association analysis showed that all haplotypes were not significantly associated with UHR. *NRG1* mRNA was significantly downregulated in the UHR group compared with the HC group as well as the FEP group.

Conclusion: Our preliminary results show that *NRG1*, *DAOA*, and *DISC1* genes may play a role in psychosis onset, opening the way to the identification of prognostic biomarkers.

Keywords: UHR, genetic susceptibility, *NRG1*, *DAOA*, *DISC1*

Introduction

Ultra-high risk (UHR) for psychosis is a special condition existing before the full-blown onset of psychosis, such as schizophrenia.¹ Identification of the UHR population and early intervention are extremely important to prevent or even delay the onset of disorders such as schizophrenia.^{2,3} A recent meta-study showed a conversion rate of UHR to psychosis was 20% in 2 years.⁴ However, 80% of the UHR population remains unconverted, so how to more accurately identify and predict the conversion of UHR has become a research focus. However, current diagnosis of UHR often depends on self-report by interview-based subjective assessment, and thus there is an urgent need for biomarkers to determine the diagnosis and prognosis of UHR.

Previous studies have shown that both genome-wide association studies and DNA methylation studies indicate an important role for genetics in the development of schizophrenia.^{5,6} Hypofunction of NMDA receptors may lead to decreased dopamine activity in the mesocortical pathway, which may manifest as negative symptoms and cognitive dysfunction in schizophrenia.⁷ Glutamate NMDA receptor hypofunction, generated early in brain development, is regulated by genetic

factors acting together with environmental factors to create abnormal fixed loop circuits in the development of the brain's nervous system, causing very early-onset schizophrenia with minimal or no trigger in adulthood. Reduced NMDA receptor function in schizophrenia may be partly explained by increased d-aminoacid oxidase (DAO) activity regulated by the d-aminoacid oxidase activator (DAOA) leading to a decrease in D-serine, a co-agonist of NMDA receptors.⁷ Neuregulin 1 (NRG1) function is mediated by binding to the receptor tyrosine kinases ErbB (ErbB3 and ErbB4), and altered NRG1/ErbB4 signaling is thought to contribute to NMDA receptor hypofunction.^{8,9} Namba et al¹⁰ found that DISC1 is a downstream molecule of the NMDA receptor signaling pathway, and DISC1 can be exogenously expressed by lentiviral mediation, which can then improve the transitional migration of newborn neurons triggered by the use of NMDA receptor antagonists. On the other hand, DISC1 plays a key role in dopamine (DA) neurotransmission.¹¹ Previous studies have also shown that the addition of drugs that selectively block DA receptors can improve cognitive performance and psychiatric symptoms in clozapine-resistant treatment-refractory schizophrenia.¹²

The NRG1, DAOA, and DISC1 genes are therefore all-important candidates for the development of schizophrenia, and similarly it has been shown that polymorphisms or expression characteristics of these genes change at the UHR stage. NRG1 polymorphisms were also shown to predict UHR conversion to schizophrenia.^{13,14} Two other studies have found that the level of NRG1 mRNA was also able to predict UHR conversion as well as to influence the functional outcome of UHR.^{15,16} Moreover, it has also been found that polymorphisms in DAOA predict UHR conversion.¹⁷ Another study has shown differential expression of the DISC1 gene in different stages of schizophrenia.¹⁸ As mentioned above, NRG1, DAOA, and DISC1 may also be promising predictive biomarkers of UHR and conversion to psychosis.

Therefore, the present study aims to examine and compare the polymorphisms and expression in NRG1, DAOA, and DISC1 genes in subjects at different stages of schizophrenia, including healthy controls (HC), first-degree relatives (FDR) of patients with schizophrenia, UHR individuals, and first episode patients with experiences of schizophrenia (FEP).

Methods

Ethical Considerations

The study was conducted by Guangdong Mental Health Center of Guangdong Provincial Peoples' Hospital, Xinhui Third People's Hospital and the Luoding Psychiatric Hospital in China. The protocol was approved by the Clinical Research Ethics Committee of Guangdong Provincial Peoples' Hospital. All participants and their legal guardians (participants less than 18 years old) gave written informed consent. The study was conducted in accordance with the revised Helsinki declaration criteria.

Study Participants

A total of 195 individuals were enrolled in the study, including the UHR group (n=18), FDR group (n=61), FEP group (n=55), and HC group (n=61). The specific operation process is as follows. Patients with schizophrenia were identified in outpatient clinics, wards, or in the community according to ICD-10 criteria, and if any first-degree relatives (parents, children, siblings) of the patient were between the ages of 15 and 45, they were contacted by the investigator, and if the family members of these schizophrenics agreed to participate in the study, the investigator interviewed these family members. All enrollees were evaluated at the first meeting using the Structured Inventory of Prodromal Syndrome (SIPS)¹⁹ to determine if they met the criteria for prodromal syndrome (COPS). Those who met COPS as assessed by the SIPS were defined as having a psychiatric risk syndrome for inclusion in the study. In short, 38 subjects satisfied the SIPS criterion for attenuated positive symptom states (APS), two subjects met the SIPS Genetic Risk and Exacerbation Status (GRDS) requirements, and no subjects met the Brief Intermittent Psychotic States (BIPS) criteria. All UHR group patients were medication-naïve. Participants in the FDR group were also recruited from first-degree relatives (siblings, parents, or children) of patients with schizophrenia who did not meet the diagnostic criteria for the UHR.

FEP group were patients who met the ICD-10²⁰ diagnostic criteria for schizophrenia with a first episode, and disease duration within 1 year. The HC group was recruited by posting advertisements in the community, and the diagnosis by the psychiatrist ruled out a history of diagnosis or treatment history of psychiatric disorders and had no positive psychiatric family history.

All participants were aged 15–45 years. They were able to sign an informed consent form and were compliant with the study arrangements. They could understand the content of the questionnaire and cooperate to complete all the test examinations. Participants were excluded from this study if they had severe or unstable physical diseases, patients with epilepsy, cerebrovascular disease, or other central nervous system organic diseases, and they had drug dependence or abuse.

Assessments

Data on socio-demographic variables, such as gender, age, marital status, educational level, and employment status were assessed using a form designed for this study by two psychiatrists with at least 3 years of clinical and research experience.

Symptomatology was determined using the Chinese version of positive and negative syndrome scale (PANSS).²¹ Depressive symptoms were assessed using the Chinese version of the Montgomery-Åsberg Depression Rating Scale (MADRS).^{22,23} Overall function was evaluated using the global assessment of functioning scale (GAF).²⁴

Single-Nucleotide Polymorphism Selection

SNPs (single nucleotide polymorphisms) of the NRG1, DAOA, and DISC1 genes were determined through the NCBI (www.ncbi.nlm.nih.gov/SNP) database on the basis of references to relevant literature. Inclusion criteria for SNPs were: (1) positive loci previously reported in the literature to be associated with UHR transformation; (2) selected functional SNPs that are in exon regions (part of the 5'UTR region, 3'UTR region, sites of nonsynonymous mutations in introns); (3) minimum allele frequency $\geq 5\%$; (4) trying to select tag SNPs as much as possible; and tag SNPs were determined using the following methods including locate the genotype data of the East Asian population in the 1000 Genomes browser database, applying the solid spine module in haploview software (<http://www.Haploview.com>) to determine the linkage disequilibrium (LD) regions of genes and selecting one SNPs with a minimum minor allele frequency (MAF) $\geq 5\%$ in the linkage disequilibrium (LD) region as tag SNPs. Finally, 10 loci were included in this study (NRG1: rs6994992, rs4281084, rs12155594, rs3924999; DAOA:rs778294, rs1341402, rs2391191, rs3918341, DISC1:rs821616, rs3738402).

Genotyping and Gene Expression

Genomic DNA was extracted from whole blood samples of all participants using a standard phenol/chloroform method. Gene expression was performed in four steps, the first being extraction of total RNA, according to the hipure liquid RNA/miRNA kit Pdf instructions operation, the second is to perform RT-PCR, the third is qPCR system and procedure, and the fourth is to run the gum validation.

Statistical Analyses

The SPSS21.0 package was used to analyze the data. The demographic and clinical characteristics of the FEP, UHR, FDR, and HC groups were analyzed using Pearson chi-square analyses or one-way analysis of variance (ANOVA).

SnStats (online software) was used for analysis of allele frequency, genotype frequency, and Hardy-Weinberg test.²⁵ SNPAssoc was employed for correlation analysis on SNP obtained from the different samples. Hardy-Weinberg equilibrium at SNP sites was evaluated with Chi-squared test to assess the potential association between the polymorphism and sample by designing the contingency table. Logistic regression method was used to measure the correlation between genotype and the distinct groups, and the correlation was quantified by calculating OR (odds ratio) of every genotype compared to the reference genome. The optimized SNP model was selected by comparing Akaike's Information Criterion (AIC). A lower *P*-value suggests a lower AIC value with higher level of confidence. Haploview was employed to calculate haplotype on those SNP loci satisfying Hardy-Weinberg equilibrium and located on the same chromosome. The haplotype frequency was investigated by using the Chi-squared test to measure the differences between groups. In this study, genetic polymorphism loci were compared using two-by-two way, and three comparisons were conducted, namely UHR group vs FEP group, UHR group vs FDR group, and UHR group vs HC group.

The gene expression was quantified with the $2^{-\Delta\Delta ct}$ method to measure the group mean value and statistically examined by *T* test to get the *P*-value. All tests were two-sided and considered statistically significant at $P < 0.05$.

Results

Demographic and Clinical Characteristics of the Four Groups

There were no statistically significant differences among the four groups of FEP, UHR, FDR, and HC, regarding gender, but statistically significant differences regarding age, marital status, employment status, and educational level. In the FEP, UHR, and FDR groups, these three groups showed a gradual decline in the PANSS total score, PANSS positive subscale score, PANSS negative subscale score, as well as the PANSS general psychopathology subscale. Additionally, the UHR group was significantly higher than the FDR and HC groups on the SIPS total score as well as on the various subscales. GAF total score increased gradually in the FEP, UHR, FDR, and HC groups, while the MADRS total score decreased gradually ([Supplement S1](#)).

Testing for Hardy Weinberg Equilibrium at SNP Loci

All eight polymorphic loci were subjected to Hardy-Weinberg equilibrium test, except Locus rs12155594 of NRG1 and Locus rs1341402 of DAOA, where only one genotype was present and hence it was not appropriate for Hardy-Weinberg test. The sampling results showed that Locus rs3918341 of DAOA did not fit H-W equilibrium on the FEP group and UHR group, and Locus rs3738402 of DISC1 did not fit H-W equilibrium on the FDR group. All other groups could fit H-W equilibrium on all the loci ([Supplement S2](#)).

UHR Group vs FEP Group

Analysis of SNP Loci Genotypes between FEP and UHR Groups

SNP rs12155594, SNP rs1341402, and SNP rs778294 were eliminated as they contained only one or two genotypes and could not be investigated by association analysis. The results demonstrated that Locus rs3918341 located in DAOA gene included the genotypes GG, AG, and AA, and distribution in the UHR and FEP groups was not identical. The frequencies showed by these genotypes in UHR group were 22.2%, 77.8%, and 0%, respectively, and in FEP group were 36.4%, 34.5%, and 29.1%, respectively. Hence, the frequency of genotype AG in the UHR group was significantly higher in comparison to that in the FEP group. Under the co-dominant model, genotype frequency distribution of SNP rs3918341 showed a significant difference between the groups (OR=3.68, 95% CI=1.03–13.02, $P < 0.001$). These results demonstrated that genotype AG at Locus rs3918341 of the DAOA gene was a susceptibility gene of UHR. Interestingly, SNP rs2391191 of DAOA was found with a missing value, thereby showing no significant difference between the groups despite a low *P*-value (0.0088). However, other SNPs showed no distinct differences ([Table 1](#)).

Interactions between SNPs of the NRG1, DAOA, and DISC1 Genes Family and UHR Risk

In this study, the log-likelihood ratio test was employed to clarify epistatic interaction among the SNPs susceptible to UHR. As indicated, Locus rs2391191 of DAOA ($P = 0.0177$) exhibited the major effect on the UHR risk (recessive model). In addition, log-likelihood ratio test analysis confirmed epistatic interaction of Locus rs6994992 of NRG1 and Locus rs778294 of DAOA (dominant model $P_{\text{interaction}} = 0.045$), and another epistatic interaction between Locus rs6994991 of NRG1 and Locus rs821616 of DISC1 (over-dominant model, $P_{\text{interaction}} = 0.04$), which suggested that interactions between the various candidate genes may be involved in UHR ([Table 2](#)).

Analysis of Haplotypes between SNPs

Haploid analysis using the EM algorithm displayed no significant difference between the FEP group and UHR group on haplotype ([Supplement S3](#)).

Table 1 Genotype for SNPs of NRG1, DAOA, and DISC1 Genes Family in the UHR and FEP Groups

Gene.site	Model	Genotype	FEP	UHR	OR (95% CI)	P-value	AIC
NRG1.rs6994992	Codominant	T/T	20 (36.4%)	3 (16.7%)	1	0.26	84.8
		C/T	27 (49.1%)	11 (61.1%)	2.72 (0.67–11.03)		
		C/C	8 (14.6%)	4 (22.2%)	3.33 (0.60–18.37)		
NRG1.rs4281084	Dominant	G/G	30 (54.5%)	8 (44.4%)	1	0.46	85
		A/G-A/A	25 (45.5%)	10 (55.6%)	1.50 (0.51–4.38)		
NRG1.rs3924999	Recessive	T/T-C/T	51 (92.7%)	18 (100%)	1	0.13	83.2
		C/C	4 (7.3%)	0 (0%)	0.00 (0.00-NA)		
DAOA.rs2391191	Codominant	A/A	20 (36.4%)	7 (38.9%)	1	0.0088	78.1
		A/G	21 (38.2%)	11 (61.1%)	1.50 (0.48–4.63)		
		G/G	14 (25.4%)	0 (0%)	0.00 (0.00-NA)		
DAOA.rs3918341	Codominant	G/G	20 (36.4%)	4 (22.2%)	1	<0.001*	72.6
		A/G	19 (34.5%)	14 (77.8%)	3.68 (1.03–13.20)		
		A/A	16 (29.1%)	0 (0%)	0.00 (0.00–NA)		
DISC1.rs821616	Recessive	T/T-A/T	54 (98.2%)	18 (100%)	1	0.45	85
		A/A	1 (1.8%)	0 (0%)	0.00 (0.00–NA)		
DISC1.rs3738402	Overdominant	C/C-T/T	38 (69.1%)	9 (50%)	1	0.15	83.4
		C/T	17 (30.9%)	9 (50%)	2.24 (0.75–6.63)		

Note: * $P < 0.05$.

Abbreviations: AIC, Akaike information criterion; OR, odds ratio; 95% CI, 95% confidence interval; FEP, first episode schizophrenia; UHR, ultrahigh risk for psychosis; NA, missing value, does not meet the calculation rules, and cannot be calculated.

UHR Group vs FDR Group

Analysis of SNP Loci Genotypes between UHR and FDR Groups

SNP rs12155594, SNP rs1341402, and SNP rs778294 were not entered into the association analysis because they contained only one or two genotypes. The results demonstrated that Locus rs3918341 located in the DAOA gene included the genotypes GG, AG, and AA, and distribution in the UHR and FDR groups was not identical. The frequencies shown by these genotypes in the UHR group were 22.2%, 77.8%, and 0%, respectively, and in the FEP group were 18%, 55.7%, and 44.3%, respectively. Hence, the frequency of genotype AG in the UHR group was significantly higher in comparison to that in the FDR group. Under an over-dominant model, genotype frequency distribution of SNP rs3918341 showed a significant difference between the groups (OR=4.41, 95% CI=1.30–14.94, $P=0.01$). These results demonstrated that genotype AG at Locus rs3918341 of DAOA gene was a susceptibility gene of UHR. Interestingly, SNP rs2391191 of DAOA was found to have a missing value, thereby showing no significant difference between the groups despite low P -value (0.013). However, other SNPs showed no distinct differences (Table 3).

Interactions between SNPs of NRG1, DAOA, and DISC1 Genes Family and UHR Risk

Log-likelihood ratio test analysis confirmed epistatic interaction of Locus rs6994992 of NRG1 and Locus rs2391191 of DAOA (over-dominant model $P_{\text{interaction}}=0.0169$), which suggested that interactions between the various candidate genes may be involved in UHR (Table 4).

Analysis of Haplotypes between SNPs

Haplotype analysis showed no significant difference between the UHR group and FDR group (Supplement S4).

UHR Group vs HC Group

Analysis of SNP Loci Genotypes between UHR and HC Groups

For the same reason as above, SNP rs12155594, SNP rs1341402, and SNP rs778294 were excluded in the association analysis. The results did not reveal which locus had a genotype that differed between the two groups in the UHR group versus the HC group (Table 5).

Table 2 Epistatic Interactions between NRGI, DAOA, and DISC1 Genes Family in the UHR and FEP Groups

	Genetic_models	S1.rs6994992	S2.rs4281084	S4.rs3924999	S5.rs778294	S7.rs2391191	S9.rs821616	S10.rs3738402
S1.rs6994992	Codominant	0.296085692	0.104479923	0.943238934	0.107720577	0.554787003	0.120292222	0.624368258
	Dominant	0.124569179	NA	0.377296043	0.045489905*	0.575786212	0.321391197	0.436305056
	Recessive	0.455913677	NA	0.999951896	NA	0.999940836	NA	NA
	Overdominant	0.386530899	0.051613928	0.442643523	0.066381846	0.116291804	0.040142683*	0.36239849
S2.rs4281084	Codominant	0.904619376	0.769433133	0.803986946	0.293953748	0.314435124	0.201986289	0.719959618
	Dominant	0.737187642	0.467445338	0.477017228	0.168676429	0.882043068	0.774308468	0.796710228
	Recessive	0.679657547	0.798952331	0.999970987	NA	0.999968488	NA	NA
	Overdominant	0.986702526	0.549426784	0.854670564	0.122203441	0.132752119	0.092471532	0.58232823
S4.rs3924999	Codominant	0.196014552	0.677179826	0.305079545	0.851527825	0.929496494	0.352305386	0.287183241
	Dominant	0.120649076	0.384512228	0.176297552	0.592779834	0.48752257	0.427613876	0.249596159
	Recessive	0.409064522	0.690655437	0.244952583	NA	0.999782492	NA	NA
	Overdominant	0.361221744	0.499514912	0.397469858	0.551224204	0.448365646	0.319889563	0.862737478
S5.rs778294	Codominant	0.91604993	0.744950228	0.733179439	0.740734382	0.313558448	0.997762914	0.395942129
	Dominant	0.875511489	0.703160317	0.743811539	0.740734382	0.11400354	0.922683707	0.544185315
	Recessive	NA	NA	NA	NA	NA	NA	NA
	Overdominant	0.709887913	0.670327855	0.74643179	0.740734382	0.579925894	0.972667033	0.309547476
S7.rs2391191	Codominant	0.276627961	0.747548017	0.194995294	0.572352865	0.045428277*	0.571049827	0.371420037
	Dominant	0.653673781	0.746273506	0.864602173	0.750793222	0.84578781	0.392985415	0.744448317
	Recessive	0.336962056	0.638650526	0.21784917	NA	0.01775501*	NA	NA
	Overdominant	0.612464296	0.768498234	0.372613302	0.960725582	0.091255292	0.2592723	0.576018806
S9.rs821616	Codominant	0.754185049	0.778567061	0.521515612	0.62556101	0.403691307	0.727708287	0.118449385
	Dominant	0.553961312	0.457114156	0.382981162	0.672050307	0.902439825	0.492141446	0.086297353
	Recessive	0.477491446	0.804810697	0.442527301	NA	0.391045747	0.562701128	NA
	Overdominant	0.609589561	0.582527615	0.566799931	0.667182335	0.380614466	0.598574154	0.124403466
S10.rs3738402	Codominant	0.529161694	0.885096513	0.404946416	0.368688137	0.476700015	0.624898168	0.353822737
	Dominant	0.249824396	0.50676449	0.214675859	0.948103873	0.926581159	0.468405044	0.201644121
	Recessive	0.870201199	0.818996231	0.730595968	NA	0.575133193	0.791410063	0.809122749
	Overdominant	0.436107553	0.665251596	0.408809199	0.977810783	0.158892922	0.491975043	0.148255257

Notes: * $P < 0.05$; The upper triangle in matrix from this function contains the P -values for the interaction (epistasis); The diagonal contains the P -values from LRT for the crude effect of each SNP; The lower triangle contains the P -values from LRT comparing the two-SNP additive likelihood to the best of the single-SNP models. Log-likelihood ratio test.

Abbreviations: NA, missing value, does not meet the calculation rules, and cannot be calculated; FEP, first episode schizophrenia; UHR, ultrahigh risk for psychosis.

Table 3 Genotype for SNPs of NRG1, DAOA, and DISC1 Genes Family in the UHR and FDR Groups

	Model	Genotype	FDR	UHR	OR (95% CI)	P	AIC
S1.rs6994992	Dominant	T/T	18 (29.5%)	3 (16.7%)	1	0.26	87.5
		C/T-C/C	43 (70.5%)	15 (83.3%)	2.09 (0.54–8.12)		
S2.rs4281084	Recessive	G/G-A/G	55 (90.2%)	16 (88.9%)	1	0.88	88.8
		A/A	6 (9.8%)	2 (11.1%)	1.15 (0.21–6.24)		
S4.rs3924999	Dominant	T/T	36 (59%)	14 (77.8%)	1	0.14	86.6
		C/T-C/C	25 (41%)	4 (22.2%)	0.41 (0.12–1.40)		
S5.rs778294	Codominant	G/G	51 (83.6%)	12 (66.7%)	1	0.12	86.5
		A/G	8 (13.1%)	6 (33.3%)	3.19 (0.93–10.92)		
		A/A	2 (3.3%)	0 (0%)	0.00 (0.00–NA)		
S7.rs2391191	Recessive	A/A-A/G	50 (82%)	18 (100%)	1	0.013	82.6
		G/G	11 (18%)	0 (0%)	0.00 (0.00–NA)		
S8.rs3918341	Overdominant	G/G-A/A	34 (55.7%)	4 (22.2%)	1	0.01*	82.2
		A/G	27 (44.3%)	14 (77.8%)	4.41 (1.30–14.94)		
S10.rs3738402	Recessive	C/C-C/T	60 (98.4%)	17 (94.4%)	1	0.39	88.1
		T/T	1 (1.6%)	1 (5.6%)	3.53 (0.21–59.43)		

Note: B* $P < 0.05$.

Abbreviations: AIC, Akaike information criterion; OR, odds ratio; 95% CI, 95% confidence interval; FDR, first-degree relatives of psychosis; UHR, ultrahigh risk for psychosis; NA, missing value, does not meet the calculation rules, and cannot be calculated.

Table 4 Epistatic Interactions between NRG1, DAOA, and DISC1 Genes Family in the UHR and FDR Groups

	Genetic_Models	S1.rs6994992	S2.rs4281084	S4.rs3924999	S5.rs778294	S7.rs2391191
S1.rs6994992	Codominant	0.657071581	0.79554588	0.824699616	0.38814628	0.155999706
	Dominant	0.359191643	NA	0.468367436	0.206019416	0.276505517
	Recessive	0.832206985	NA	NA	NA	NA
	Overdominant	0.525045663	0.791668826	0.391203858	0.221587393	0.016936026*
S2.rs4281084	Codominant	0.797500902	0.994757124	0.860365204	0.426877983	0.133081534
	Dominant	0.503154622	0.942516736	0.86282948	0.180079347	0.835726941
	Recessive	0.944588845	0.92675785	NA	NA	NA
	Overdominant	0.674174499	0.986822317	0.775307735	0.097261992	0.08533906
S4.rs3924999	Codominant	0.590865452	0.993575002	0.304586365	0.409989094	0.807692405
	Dominant	0.294193485	0.95986376	0.163595056	0.246162729	0.37368588
	Recessive	0.912254949	0.984403677	0.313476856	NA	NA
	Overdominant	0.467005052	0.981431866	0.309653056	0.1630864	0.386528286
S5.rs778294	Codominant	0.482323963	0.983437789	0.18224628	0.139138594	0.679500366
	Dominant	0.310806128	0.879244593	0.101060104	0.105635781	0.929978911
	Recessive	0.866436153	0.947312505	0.378091515	0.567997812	NA
	Overdominant	0.365031709	0.861388883	0.180235189	0.052103292	0.214214432
S7.rs2391191	Codominant	0.714144059	0.991602512	0.162006243	0.0792311	0.123482029
	Dominant	0.853647596	0.971405702	0.755199199	0.887671636	0.819793773
	Recessive	0.873781193	0.877568328	0.154873302	0.999978528	0.063371897
	Overdominant	0.58364572	0.830158982	0.263377284	0.088906531	0.130961889

Notes: * $P < 0.05$; The upper triangle in matrix from this function contains the P -values for the interaction (epistasis); The diagonal contains the P -values from LRT for the crude effect of each SNP; The lower triangle contains the P -values from LRT comparing the two-SNP additive likelihood to the best of the single-SNP models. Log-likelihood ratio test.

Abbreviations: NA, missing value, does not meet the calculation rules, and cannot be calculated; FDR, first-degree relatives of psychosis; UHR, ultrahigh risk for psychosis.

Interactions between SNPs of NRG1, DAOA, and DISC1 Genes Family and UHR Risk

Log-likelihood ratio test analysis confirmed epistatic interaction of Locus rs6994992 of NRG1 and Locus rs2391191 of DAOA (over-dominant model $P_{\text{interaction}} = 0.011$), and another epistatic interaction between Locus rs4281084 of NRG1

Table 5 Genotype for SNPs of NRGI, DAOA, and DISCI Genes Family in the UHR and HC Groups

	Model	Genotype	HC	UHR	OR (95% CI)	P	AIC
S1.rs6994992	Dominant	T/T	16 (26.2%)	3 (16.7%)	1	0.39	88.1
		C/T-C/C	45 (73.8%)	15 (83.3%)	1.78 (0.45–6.96)		
S2.rs4281084	Dominant	G/G	26 (42.6%)	8 (44.4%)	1	0.89	88.8
		A/G-A/A	35 (57.4%)	10 (55.6%)	0.93 (0.32–2.68)		
S4.rs3924999	Recessive	T/T-C/T	58 (95.1%)	18 (100%)	1	0.21	87.2
		C/C	3 (4.9%)	0 (0%)	0.00 (0.00–NA)		
S5.rs778294	Overdominant	G/G-A/A	49 (80.3%)	12 (66.7%)	1	0.24	87.4
		A/G	12 (19.7%)	6 (33.3%)	2.04 (0.64–6.55)		
S7.rs2391191	Recessive	A/A-A/G	57 (93.4%)	18 (100%)	1	0.14	86.7
		G/G	4 (6.6%)	0 (0%)	0.00 (0.00–NA)		
S8.rs3918341	Codominant	G/G	23 (37.7%)	4 (22.2%)	1	0.061	85.2
		A/G	32 (52.5%)	14 (77.8%)	2.52 (0.73–8.64)		
		A/A	6 (9.8%)	0 (0%)	0.00 (0.00–NA)		
S10.rs3738402	Recessive	C/C-C/T	61 (100%)	17 (94.4%)	1	0.083	85.8
		T/T	0 (0%)	1 (5.6%)	NA (0.00–NA)		

Abbreviations: AIC, Akaike information criterion; OR, odds ratio; 95% CI, 95% confidence interval; HC, health control; UHR, ultrahigh risk for psychosis; NA, missing value, does not meet the calculation rules, and cannot be calculated.

and Locus rs2391191 of DAOA (over-dominant model, $P_{\text{interaction}}=0.033$) which suggested that interactions between the various candidate genes may be involved in UHR (Table 6).

Analysis of Haplotypes and Linkage Disequilibrium between SNPs

Haploid analysis displayed no significant difference between UHR group and HC group on haplotype (Supplement S5).

Results of Gene Expression of NRGI and DISCI, in Four Groups

The expression content of the DAOA gene could not be measured because the expression level of the DAOA gene in tissues and blood was very low in the gene expression experiments and could not be amplified by qPCR. Only gene

Table 6 Epistatic Interactions between NRGI, DAOA, and DISCI Genes Family in the UHR and HC Groups

	Genetic_Models	S1. rs6994992	S2. rs4281084	S4. rs3924999	S5. rs778294	S7. rs2391191	S10. rs3738402
S1.rs6994992	Codominant	0.567234689	0.206196538	0.581475691	0.378045331	0.080009129	0.166843624
	Dominant	0.359677546	NA	0.443005192	0.878559745	0.331675127	0.776924573
	Recessive	0.448254017	NA	NA	NA	0.99992494	NA
	Overdominant	0.824888216	0.464359742	0.206558107	0.230868201	0.011519149*	0.335838202
S2.rs4281084	Codominant	0.353594688	0.992033238	0.841196063	0.535263751	0.148753006	0.886594169
	Dominant	0.441570512	0.981100195	0.799639036	0.798487757	0.455486144	0.899028701
	Recessive	0.224077878	0.90001464	NA	NA	0.999972959	NA
	Overdominant	0.924405991	0.954401149	0.499818698	0.282629197	0.033184111*	0.80193205
S4.rs3924999	Codominant	0.58740846	0.957487504	0.462364906	0.916597652	0.683051809	0.761353664
	Dominant	0.380045258	0.905489645	0.426745762	0.795314855	0.292336725	0.371923728
	Recessive	0.513924161	0.820238966	0.248532411	NA	NA	NA
	Overdominant	0.783506788	0.974628427	0.741483886	0.657991111	0.450125827	0.76861049
S5.rs778294	Codominant	0.525425052	0.982147396	0.308128396	0.394638211	0.999874758	0.91501062
	Dominant	0.228266479	0.845087657	0.486535276	0.374818342	0.957984194	0.890852941
	Recessive	0.484520355	0.863687781	0.297583762	0.454246733	NA	NA
	Overdominant	0.687541179	0.788054094	0.881060256	0.228958753	0.845707566	0.446257575

(Continued)

Table 6 (Continued).

	Genetic_Models	S1. rs6994992	S2. rs4281084	S4. rs3924999	S5. rs778294	S7. rs2391191	S10. rs3738402
S7.rs2391191	Codominant	0.459988589	0.99619912	0.265936087	0.300483381	0.51458046	0.526437512
	Dominant	0.879475609	0.97146078	0.771847696	0.884575927	0.83863067	0.477103719
	Recessive	0.385555187	0.981880654	0.146359663	0.296275404	0.282528584	NA
	Overdominant	0.829930793	0.991362193	0.715657821	0.533079504	0.500164959	0.130546037
S10.rs3738402	Codominant	0.304283915	0.993036223	0.306967206	0.501939716	0.206699574	0.132892983
	Dominant	0.307004215	0.925450751	0.382427239	0.502146275	0.674409837	0.219680387
	Recessive	0.397340667	0.96643978	0.173909739	0.321899289	0.158137809	0.081055276
	Overdominant	0.861421409	0.950289158	0.689998104	0.48618151	0.386064637	0.401590696

Notes: * $P < 0.05$; The upper triangle in matrix from this function contains the P -values for the interaction (epistasis); The diagonal contains the P -values from LRT for the crude effect of each SNP; The lower triangle contains the P -values from LRT comparing the two-SNP additive likelihood to the best of the single-SNP models. Log-likelihood ratio test.

Abbreviations: NA, missing value, does not meet the calculation rules, and cannot be calculated; HC, health control; UHR, ultrahigh risk for psychosis.

expression studies of the NRG1 gene as well as the DISC1 gene were performed. It can be seen from the table as well as the figure that, compared with the UHR group versus the HC group as well as the FEP group, NRG1 mRNA was significantly down regulated with statistical significance. There was no statistically significant difference in the expression amount of DISC1 gene among the four groups (Figure 1 and Supplement S6).

Discussion

In this study, four loci in the NRG1 gene (rs699492, rs4281084, rs12155594, rs3924999), four loci in the DAOA gene (rs778294, rs1341402, rs2391191, rs3918341), and two loci in the DISC1 gene (rs821616, rs3738402) were compared between the four groups for differences in genotype distribution in the above 10 loci to understand the information of UHR genetics. At the same time, we measured the mRNA expression of these three genes, but, in the course of the experiment, DAOA gene expression could not be measured, so only the NRG1 gene and DISC1 gene expression were analyzed in the four groups.

The sampling results showed that Locus rs3918341 of DAOA did not fit H-W equilibrium on the FEP group and UHR group, and Locus rs3738402 of DISC1 did not fit H-W equilibrium on the FDR group. The reasons may be as follows, first, the

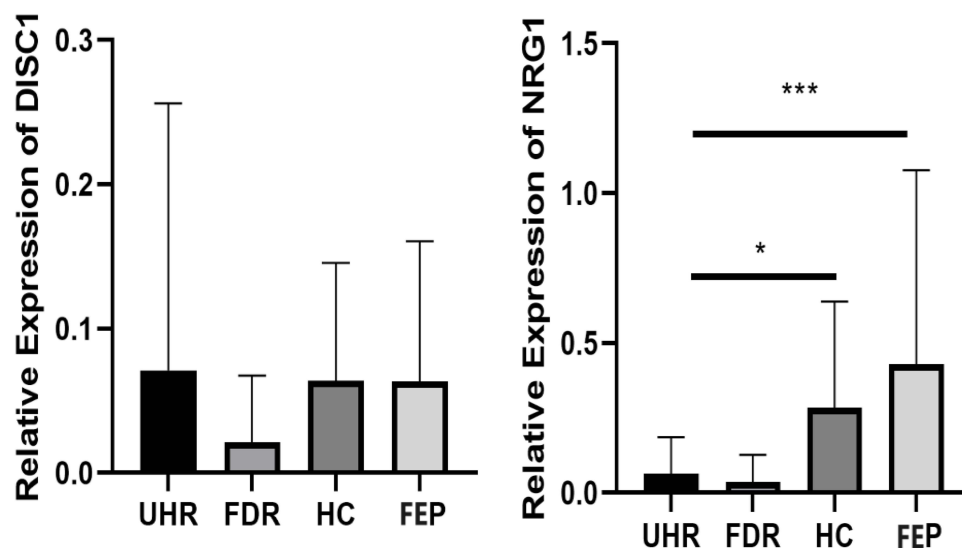


Figure 1 Analysis of the relative expression of NRG1 gene and DISC1 gene; * represents less than 0.05, *** represents less than 0.001. The expression amounts of genes were obtained by the $2^{-\Delta\Delta Ct}$ method using the T test for P -values in pairwise comparisons between the UHR group and the other groups.

H-W test could be vulnerable to the sample size, which implied that fewer samples or uneven sampling could lead to uneven distribution of the genotypes. This locus is primarily distributed in Asians with a G allele gene frequency of $G = 0.6$ and an A allele gene frequency = 0.4, which corresponds with the gene frequency in the UHR-FEP group in the present analysis. Thus, the results can be affected if genotype AA in the UHR group was zero during the sampling. On the other hand, previous studies have also shown that the case group may not conform to the H-W equilibrium because sites that are functionally subject to selective pressure (disease also counts as a selective pressure) and H-W disequilibrium is normal.²⁶

The results of the gene polymorphism showed that locus rs3918341 of the DAOA genotype AG was a potential risk factor of the UHR group. The study of epistatic effects found that epistatic interaction of rs6994992 of NRG1 and rs778294 of DAOA; rs6994992 of NRG1 and rs821616 of DISC1; rs6994992 of NRG1 and rs2391191 of DAOA, as well as the epistatic interaction of rs4281084 of NRG1 and rs2391191 of DAOA caused susceptibility of UHR. Haploid analysis displayed no significant difference between the UHR group and other groups on haplotype. This is somewhat inconsistent with previous studies. Primary analysis between the genetic polymorphism and UHR was conducted in 2006 by Hall et al,¹³ where all 12 high-risk patients with genotype TT of Locus rs6994992 of NRG1 converted to the psychotic disorder, significantly more than those with genotype TC and CC. Similarly, Kéri et al²⁷ found that all individuals with genotype TT of Locus rs6994992 of NRG1 developed psychotic disorder after 1 year of follow-up analysis in a population with ultra-high risk of psychosis. In 2013, Bousman et al²⁸ conducted polymorphism analyses on 225 UHR individuals, but no UHR transformation was found to be predicted by polymorphism of Locus rs6994992. However, rs12155594 and rs4281084 of NRG1 were able to predict UHR transformation, and haplotypes of them were also functional. Mössner et al¹⁷ followed the UHR population for 2 years and reported that all individuals with the DAOA gene rs1341402 genotype CC exhibited psychiatric disorders, with a relative risk ratio 4.58 times higher than that of genotype TT or CT and the conversion rate of individuals with genotype AA at locus rs778294 was 50%. These studies showed that rs6994992, rs12155594, and rs4281084 of the NRG1 gene and rs1341402 and rs778294 of the DAOA gene were associated with the transformation or symptoms of UHR. However, the above loci were not found to be associated with the status of UHR in the analysis of gene polymorphisms in this study. There may be the following reasons for the inconsistent results. First, Locus rs12155594 of NRG1 and Locus rs1341402 of DAOA were found to be linked with one genotype only and eliminated in following analysis. This phenomenon demonstrated large variation of genotype distribution among the different races, thus paving way for more analyses among the different races. In addition, the results of this study are consistent with the view that differences between SNPs identified across studies that confer risk are on expected lines rather than the exception. Indeed, meta-analysis indicated that the specific NRG risk allele may vary among the different populations,^{29,30} and some researchers have pointed out that risk increased as a result of NRG1 sequence which may not be driven by single SNP but accumulated by diversification of the nucleotide polymorphism.³¹

In the study we found that genotype AG of Locus rs3918341 of DAOA showed significant higher frequency in UHR population than the FDR group and FEP group, concomitant with a higher frequency than the HC group (not statistically significant). It has been reported that Locus rs3918341 of DAOA displayed different genotype distribution in patients with major depression or bipolar disorder compared with the HC group.^{32,33} In addition, two meta-analyses have also reported that loci in DAOA is involved in schizophrenia,^{34,35} which is demonstrated by over-expression of DAOA in the dorsolateral prefrontal cortex in patients with schizophrenia.³⁶ Additionally, the results of this study revealed that the distribution of genotypes varies considerably among the different ethnic groups, and genotype AG of Locus rs3918341 of DAOA is a potential risk genotype in the Chinese UHR population.

Epistatic interaction of Locus rs6994992 of NRG1 and Locus rs2391191 of DAOA were detected with effect on the UHR group upon comparison with the FEP group and HC group. Previous studies have demonstrated that risk allele, Locus rs6994992, constitutes a major part of primitive haplotype, altering three TF-binding loci in the NRG1 promotor region, and has been related to over-expression of IV type transcripts in autopsy tissues.^{37,38} In addition, another study demonstrated that patients with schizophrenia (with risk genotype T/T on Locus rs6994992) can express more unusual ideas when in conflicts, compared to those with type C carriers (C/T or C/C). Overall, significant mutual genotype-environment interaction of rs6994992 of NRG1 and social psychological stress has been observed.¹⁴

Locus rs2391191 of DAOA is a variant coding for DAOA protein, whose mutation can lead to lysine/arginine alteration. Recent analysis has demonstrated that this locus (rs2391191) is related to impaired verbal memory and

reduced cortical thickness in patients with schizophrenia, whereas another research showed that polymorphism at Locus rs2391191 of DAOA in the NMDA pathway was most closely related to schizophrenia.^{39,40} It has been demonstrated that Locus rs2391191 (A) is a risk allele involved in the age of first onset of schizophrenia, especially during early-onset schizophrenia.⁴¹ Overall, loci rs6994992 and rs2391191 were potentially found to be related to schizophrenia, and this study indicated that epistatic interaction between them is a possible risk factor in UHR group. More future research is required to understand the mechanisms through which this mutation can increase the risk of schizophrenia.

In this study, we found a significant downregulation of NRG1 mRNA in the UHR group relative to the HC group as well as the FEP group. Previous conclusions regarding the influence of the expression content of the NRG1 gene on UHR are controversial. In 2012, Kiss et al¹⁵ examined the expression of NRG1 gene in 97 patients with UHR as well as 50 normal controls, 31 patients with UHR transformation after 1 year, and the expression level of NRG1 gene in peripheral blood was significantly lower in the UHR transformed group than in the UHR untransformed and normal groups, and there was no difference between the UHR untransformed and normal groups. Whereas a 2018 study of 185 high-risk as well as ultrahigh risk individuals with 12 months of follow-up tested for NRG1 gene expression levels at baseline, and distinguished their functional outcomes at 12 months, dividing them into a good functional outcome (GFO) group and a poor functional outcome (PFO) group, which showed that patients with PFO had significantly higher NRG1 mRNA levels than those with GFO. The results suggest that NRG1 may serve as a predictor of functional outcome in individuals at high risk for psychosis.¹⁶ Also with somewhat negative results, a 2017 study by Jagannath et al found no association between gene expression of NRG1 and transformation by UHR.⁴² Larger sample sizes and long-range follow-up studies may be needed in the future to explore the effect of the NRG1 gene on UHR recognition and transformation.

In this study, the amount of DISC1 gene expression in the UHR group was not found to be different from that in the other groups. The upregulation of the DISC1 gene was shown in a 2015 Santoro et al⁴³ study comparing the UHR group with the FEP group, but statistical significance was lost when controlling for age as a covariate.

Limitations and Strengths

Limitations should be considered when interpreting these findings. First, this was a cross-sectional analysis, and as such we were not able to reveal the rate of UHR individuals' conversion to schizophrenia, which prevented us from investigating the potential predictive value of polymorphisms and expression in NRG1, DAOA, and DISC1 genes with respect to FEP onset. Second, we investigated a limited number of SNPs of NRG1, DAOA, and DISC1 genes. Besides, such as the complex disease etiology of psychotic disorders, which is more suitable to do genome-wide association studies, the results of individual genes are limited. Finally, the sample size of this study was relatively small, especially in the UHR group, due to the small sample size, several groups differed in demographic comparisons, thereby limiting the generalizability of the findings. Therefore, additional sample sizes as well as longitudinal study methods need to be included in future studies to track the role of genes in transformation to UHR.

This study has some worthy strengths, including the few to explore the biological basis of UHR, expecting that the identification of UHR will be more precise. The second we examined whether the genetic basis of UHR differed between ethnicities by verifying previous positive genetic results regarding UHR transformation in the Chinese population. A further strength is the understanding of the NRG1, DAOA, and DISC1 genotypes and gene expression changes in the context of the different stages of schizophrenia, and a better understanding of the role that the NRG1, DAOA, and DISC1 genes play in the development of schizophrenia disorders.

Conclusion

Overall, this study preliminarily explored the roles of the NRG1 gene, DAOA gene, and DISC1 gene in the identification of UHR in a Chinese Han population. The results showed that these genes may be a little helpful in recognizing UHR. Future larger sample and longitudinal studies will be required to fully understand the roles of the above several genes in UHR recognition as well as transformation.

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Disclosure

The authors report no conflicts of interest in this work.

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