No association between genetic polymorphisms of the *NDUFS7* gene and schizophrenia in Han Chinese

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Accumulating evidence suggests that mitochondrial dysfunction contributes toward the pathogenesis of psychiatric diseases. NADH dehydrogenase Fe-S protein 7 (NDUFS7), a subunit of respiratory chain complex I, has been reported recently to be associated with bipolar disorder. To test whether this gene can confer a wide variety of psychiatric disorders, we carried out a case–control association analysis of three tagging single-nucleotide polymorphisms (rs2074896, rs2074897, and rs2074898) in the *NDUFS7* gene by sequencing 330 Han Chinese patients with schizophrenia and 330 well-matched healthy controls. We found no significant difference in the frequency distributions of alleles, genotypes, and haplotypes between the cases and the controls, indicating no active role of this gene in schizophrenia. ***Psychiatr Genet*** **23:29–32 © 2013 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Schizophrenia is a chronic, severe, and disabling brain disorder that affects ~1% of the world's population. Mitochondrial dysfunction has been reported frequently in patients with schizophrenia and other psychiatric disorders (Shao *et al.*, 2008). As the largest complex of the respiratory chain, mitochondrial complex I plays a major role in controlling oxidative phosphorylation and its abnormality can lead to a variety of diseases associated with mitochondrial dysfunction (Distelmaier *et al.*, 2009). Hitherto, many studies have reported that both the nuclear-encoded and the mtDNA-encoded genes for mitochondrial complex I may be involved in schizophrenia and bipolar disorder (Karry *et al.*, 2004; Rollins *et al.*, 2009), although some of the previous conclusions were controversial (Bandelt *et al.*, 2007; Rollins *et al.*, 2009). In our recent study, we found no association between the *NDUFV2* gene (encoding a subunit of mitochondrial complex I) and schizophrenia (Zhang *et al.*, 2010); thus, it failed to verify the previous report for a positive association between this gene and schizophrenia (Washizuka *et al.*, 2006). However, whether other counterparts of the mitochondrial complex I would be involved in schizophrenia remains to be determined.

The NADH dehydrogenase Fe-S protein 7 (*NDUFS7*) gene, encoding a 20 kD subunit of complex I, has been reported to be associated with Leigh syndrome (Triepels *et al.*, 1999; Lebon *et al.*, 2007). A decreased level of NDUFS7, which might cause a decreased activity of complex I and a resultant resultant increase in protein oxidation and nitration, has been found in bipolar patients compared with healthy controls (Andreazza *et al.*, 2010). These lines of evidence indicated that *NDUFS7* might be a susceptible gene for psychiatric diseases. In this study, we aimed to investigate the association of *NDUFS7* gene polymorphisms with schizophrenia in a cohort of Han Chinese patients with schizophrenia and healthy controls from Hunan Province (China).

Materials and methods

Participants

A total of 330 unrelated patients with schizophrenia [mean age of onset 24.4±8.3 years old (range: 10–57); 70% men] and 330 matched healthy controls [mean age 42.9±15.3 years old (range: 17–74); 66% men], all of Han Chinese origin, were recruited from Hunan Province in South Central China. These patients were analyzed for the *NDUFV2* gene in our recent study (Zhang *et al.*, 2010). Schizophrenia patients were diagnosed independently by two psychiatrists according to the DSM-IV criteria for schizophrenia and had at least a 2-year history of schizophrenia. The controls were clinically diagnosed as having no psychiatric disorders or other diseases and were well matched in geographic origin and ethnicity with the schizophrenia patients. All participants or supervisors of patients signed informed consent. This study was approved by the institutional review boards of the Kunming Institute of Zoology and the 2nd Xiangya Hospital of the Central South University.

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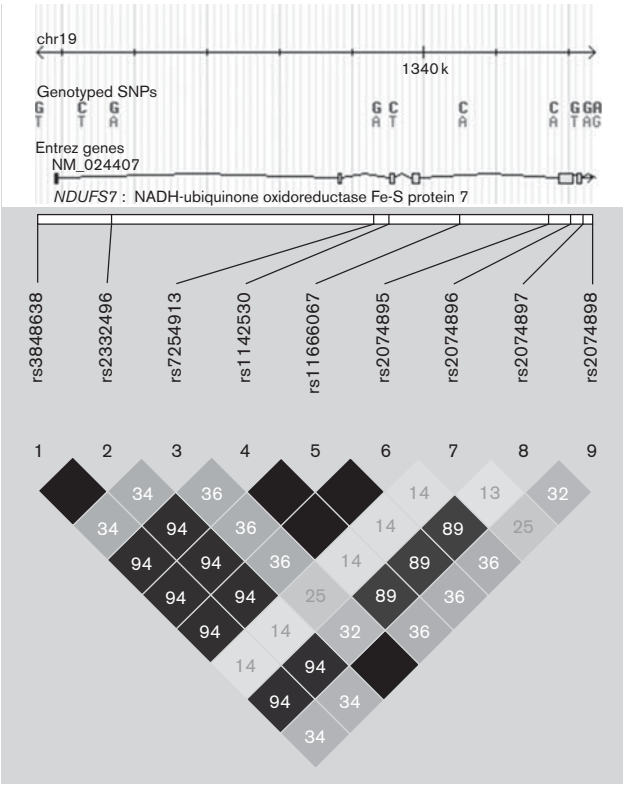
Selection of tagging single-nucleotide polymorphisms and genotyping

Genomic DNA was isolated from the peripheral blood of patients and controls according to the standard phenol–chloroform procedure. To set inclusion criteria for tagging single-nucleotide polymorphisms (SNPs), we retrieved the CHB data from the HapMap database (*http://hapmap.ncbi.nlm.nih.gov/*; HapMap III, release 2) and defined linkage disequilibrium (LD) blocks using the Haploview Program (version 4) (Barrett *et al.*, 2005). All SNPs listed in the entire *NDUFS7* gene region were included in the LD analysis (Fig. 1). Haplotype-tagging SNPs (htSNPs) were selected at the cutoff level of r^2 of at least 0.80 and a minor allele frequency (MAF) of at least 0.2 (Fig. 1). All selected htSNPs were amplified and genotyped by direct sequencing. Nested PCRs were carried out using three primers: for the first step PCR, the primer pair F1 (5′-ACGA CATGGACCGCTTTG-3′) and R1 (5′-AAACTAATGGCA AGTGAGAAGG-3′) was used; for the second step PCR, the primer pair F2 (5′-GGCGTCGCACTTGGTATGT-3′) and R1 was used. Amplification was carried out in a total volume of 25 µl. Purified PCR products were sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit on an ABI PRISM 3730 Genetic Analyzer (Applied Biosystems, Foster City, California, USA).

Fig. 1



Linkage disequilibrium (LD) pattern of the *NDUFS7* gene in CHB population [data were retrieved from HapMap III (release 2)]. Values are represented in a grayscale ranging from white (no LD) to black (high LD). The values in each square are $r^2 \times 100$.

Statistical analysis

Deviation from the Hardy–Weinberg equilibrium was calculated using the HWsim program (*http://krunch.med.yale.edu/hwsim/*). PHASE2.1.1 program (Stephens *et al.*, 2001) was used to reconstruct the haplotype of the three htSNPs. We compared the allele, genotype, and haplotype frequencies of the three htSNPs between the case and the control samples using the χ^2-test. A *P*-value less than 0.05 was considered as statistically significant.

Results and discussion

The role of mitochondria in psychiatric disorders has received increasingly more attention in recent years (Shao *et al.*, 2008; Rezin *et al.*, 2009). Genetic variants and abnormal gene expression of nuclear-encoded and mtDNA-encoded genes for the respiratory chain have been reported to be associated with a disturbance in energy metabolism and excessive oxidative stress, which were actively involved in schizophrenia, bipolar disorder, and other psychiatric disorders (Karry *et al.*, 2004; Rollins *et al.*, 2009; Clay *et al.*, 2011; Verge *et al.*, 2011). We hypothesize that genetic variants of the structural subunits of the respiratory chain complexes would confer susceptibility to schizophrenia. We have started a systematic analysis for the main subunits of the electron transport chain in Han Chinese with schizophrenia, with the aim of understanding energy impairment as a mechanism underlying the pathophysiology of schizophrenia.

In this study, we genotyped three htSNPs (rs2074896, rs2074897, and rs2074898) of the *NDUFS7* gene, which encodes a subunit of mitochondrial complex I, and was reported recently to be associated with bipolar disorders (Andreazza *et al.*, 2010), in 330 Han Chinese patients with schizophrenia and 330 matched normal individuals. These three htSNPs were selected for genotyping because they could capture all nine common variants of the *NDUFS7* gene according to the analysis of the CHB data in HapMap III dataset (release 2) (Fig. 1). No deviation from the Hardy–Weinberg equilibrium was observed for each of the three htSNPs in our control individuals or schizophrenia patients. There was no statistical difference for the three htSNPs in the allele and genotype frequencies between our case and control populations (Table 1). However, we observed a marked difference for allele frequencies and LD pattern of the nine SNPs in the *NDUFS7* gene (including the three htSNPs) between East Asian and European populations according to the HapMap data (Table S1 and Fig. S1, *http://links.lww.com/PG/A63*), which indicated a huge ethnic difference. A total of six haplotypes were reconstructed on the basis of the three htSNPs (rs2074896–rs2074897–rs2074898). Four main haplotypes (GGA, TGG, GAG, and GGG) were prevalent and accounted for ~99% of all

Table 1 Genotype and allele frequencies of three haplotype-tagging single-nucleotide polymorphisms in the *NDUFS7* gene in Han Chinese patients with schizophrenia and healthy controls from central China

SNP	Case ($n=330$)	Control ($n=330$)	*P*-value[a]	OR	95% CI
rs2074896					
GG	153	169	0.213	0.905	0.774–1.059
GT	138	129	0.475	1.070	0.889–1.288
TT	39	32	0.379	1.219	0.783–1.896
G	444	467	0.171	0.951	0.884–1.022
T	216	193	–	1.119	0.952–1.315
HWE[b]	0.498	0.461	–	–	–
rs2074897					
GG	179	181	0.876	0.989	0.718–1.326
GA	127	118	0.468	1.076	0.882–1.313
AA	24	31	0.324	0.774	0.465–1.290
G	485	480	0.756	1.010	0.946–1.079
A	175	180	–	0.972	0.814–1.162
HWE[b]	0.91	0.184	–	–	–
rs2074898					
GG	130	129	0.936	1.008	0.833–1.218
GA	152	139	0.308	1.094	0.921–1.299
AA	48	62	0.144	0.774	0.549–1.093
G	412	397	0.397	0.943	0.823–1.080
A	248	263	–	1.038	0.952–1.131
HWE[b]	0.847	0.074	–	–	–

CI, confidence interval; HWE, Hardy–Weinberg equilibrium; OR, odds ratio; SNP, single-nucleotide polymorphism.
[a]Two-tailed Pearson's χ^2-test.
[b]Hardy–Weinberg equilibrium was computed using the Monte Carlo permutation test (1 000 simulations).

Table 2 Haplotype of the three haplotype-tagging single-nucleotide polymorphisms in the *NDUFS7* gene

Haplotype[a]	Case	Control	*P*-value[b]	OR	95% CI
GGA	227	259	0.202	0.913	0.793–1.051
TGG	205	191	0.183	1.118	0.949–1.317
GAG	165	178	0.703	0.965	0.805–1.157
GGG	26	25	0.771	1.083	0.632–1.855
GAA and TGA	5	1	0.117	5.207	0.610–44.445

CI, confidence interval; OR, odds ratio.
[a]rs2074896–rs2074897–rs2074898.
[b]Haplotypes GGA, TGG, GAG, and GGG were calculated by Pearson's χ^2-test (two-tailed). Haplotypes GAA and TGA were combined together and Fisher's exact test (two-tailed) was used.

observations. However, we found no statistical difference in the haplotype distribution in the case and the control populations (Table 2).

The lack of association between the three htSNPs of the *NDUFS7* gene and schizophrenia in the current study is not completely surprising when considering the complex pathogenesis of this psychiatric disorder. The current observation, together with our negative result of the *NDUFV2* gene (Zhang *et al.*, 2010), could not completely reject a possibility of mitochondrial dysfunction as a cause for schizophrenia, because the mitochondrion was composed of 1000–1500 proteins (Pagliarini *et al.*, 2008), and each impaired counterpart might lead to dysfunction of the mitochondrion. Another limitation of our current study is the incompleteness of the clinic information available, which did not allow us to draw a conclusion on the association between the *NDUFS7* gene SNPs and schizophrenia subtypes. Furthermore, schizophrenia would more likely fit for the 'common disease-rare variants' hypothesis (McClellan *et al.*, 2007; International Schizophrenia Consortium, 2008; Sebat *et al.*, 2009); we could not rule out the possibility that rare variants in the *NDUFS7* gene would contribute to schizophrenia. We compared the matrilineal genetic component of our case and control samples and found that both populations were very similar (W. Zhang, J. Tang, A.-M. Zhang, M.-S. Peng, H.-B. Xie, L. Xu, Y.-P. Zhang, X. Chen, Y.-G. Yao, unpublished data), which indicated that the lack of association was unlikely caused by population stratification.

Conclusion

We genotyped three htSNPs of the *NDUFS7* gene in our patients with schizophrenia, but found no positive association. Replication studies in other independent populations with a large sample size would help to further define the potential role of the *NDUFS7* gene in schizophrenia. Although our analysis for multiple structural subunits of complex I encoded by nuclear genes, such as the *NDUFS7* gene and the *NDUFV2* gene (Zhang *et al.*, 2010), showed no association with schizophrenia, we would expect that other subunits of this complex may confer genetic susceptibility to schizophrenia. Our ongoing project to screen all mitochondrial complex I genes may provide information to answer this question.

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Conflicts of interest

There are no conflicts of interest.

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Table S1 Minor allele frequency of 9 SNPs in the *NDUFS7* gene

SNP	Position	Allele [a]	Minor allele frequency [b]												
			HNP	HNC	CHB	CHD	JPT	CEU	TSI	ASW	LWK	MKK	YRI	GIH	MEX
rs3848638	1334649	G/T	-	-	0.304	0.359	0.401	0.479	0.472	0.294	0.250	0.374	0.270	0.449	0.423
rs2332496	1335690	G/A	-	-	0.304	0.353	0.430	0.491	0.466	0.294	0.211	0.364	0.270	0.455	0.423
rs7254913	1339320	A/G	-	-	0.440	0.382	0.360	0.359	0.364	0.325	0.356	0.311	0.335	0.261	0.356
rs1142530	1339538	C/T	-	-	0.315	0.359	0.419	0.380	0.386	0.325	0.244	0.458	0.309	0.398	0.481
rs11666067	1340517	C/A	-	-	0.315	0.359	0.419	0.376	0.369	0.325	0.244	0.458	0.309	0.398	0.481
rs2074895	1341757	C/A	-	-	0.315	0.359	0.419	0.380	0.386	0.325	0.244	0.465	0.309	0.398	0.481
rs2074896	1342059	G/T	0.327	0.292	0.244	0.259	0.221	0.021	0.023	0.087	0.167	0.031	0.074	0.125	0.115
rs2074897	1342235	G/A	0.265	0.273	0.292	0.353	0.378	0.479	0.494	0.119	0.011	0.262	0.022	0.426	0.423
rs2074898	1342361	G/A	0.376	0.398	0.440	0.382	0.355	0.359	0.364	0.325	0.356	0.304	0.339	0.261	0.356

[a] Major allele / minor allele

[b] Data were retrieved from HapMap datasets (HapMap III release 2). Minor alleles of SNPs rs3848638 and rs2332496 in population TSI were all G, whereas in other populations the minor alleles were T and A, respectively. Minor alleles of SNPs rs1142530, rs11666067, and rs2074895 in populations TSI and CEU were all C, whereas in other populations the minor alleles were T, A and A, respectively.

HNP: Han Chinese with schizophrenia from Hunan, China; HNC: Normal controls from Hunan, China

CHB: Han Chinese in Beijing, China; CHD: Chinese in Metropolitan Denver, Colorado

JPT: Japanese in Tokyo, Japan; CEU: Utah residents with Northern and Western European ancestry from the CEPH collection

TSI: Tuscan in Italy; ASW: African ancestry in Southwest USA

LWK: Luhya in Webuye, Kenya; MKK: Maasai in Kinyawa, Kenya

YRI: Yoruban in Ibadan, Nigeria; GIH: Gujarati Indians in Houston, Texas

MEX: Mexican ancestry in Los Angeles, California

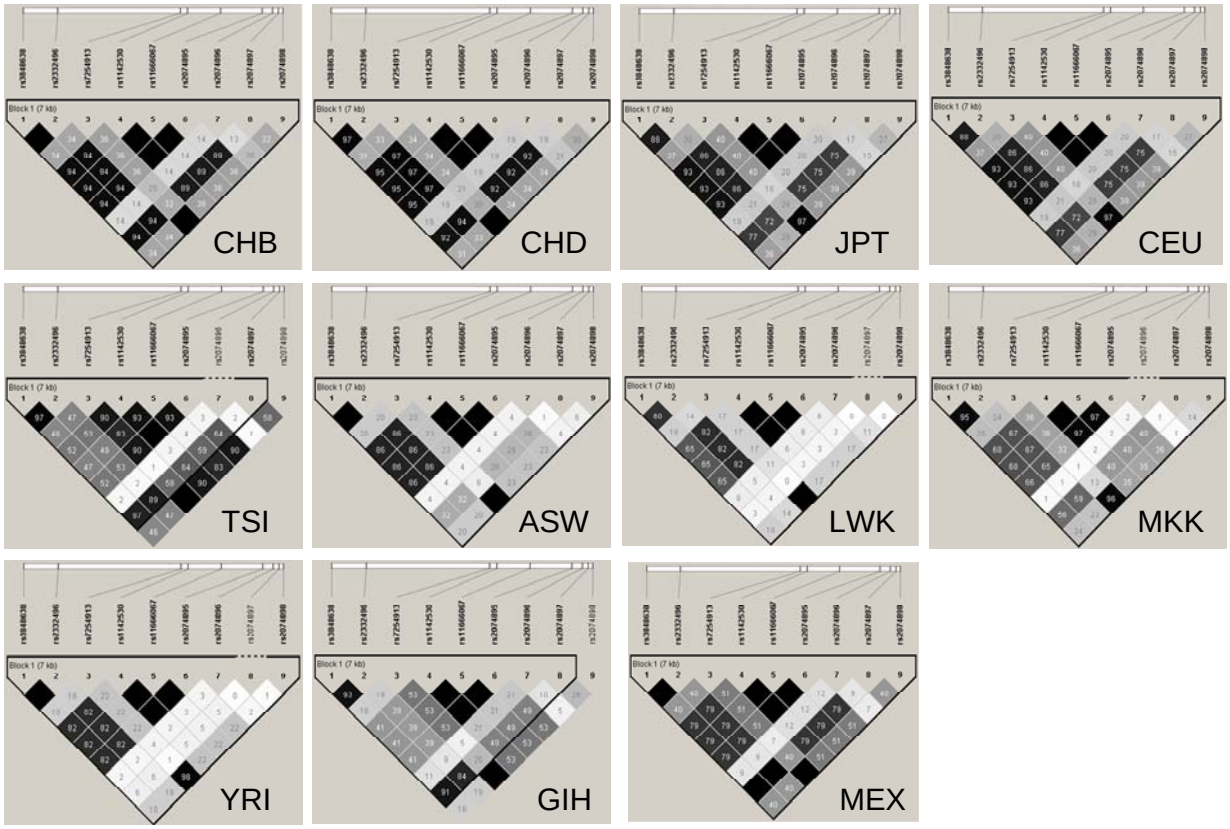

Figure S1. Linkage disequilibrium (LD) pattern of 9 SNPs in the *NDUFS7* gene. Data were retrieved from HapMap III release 2. Block was defined according to Gabriel et al. (2002).

Supplemental Reference

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