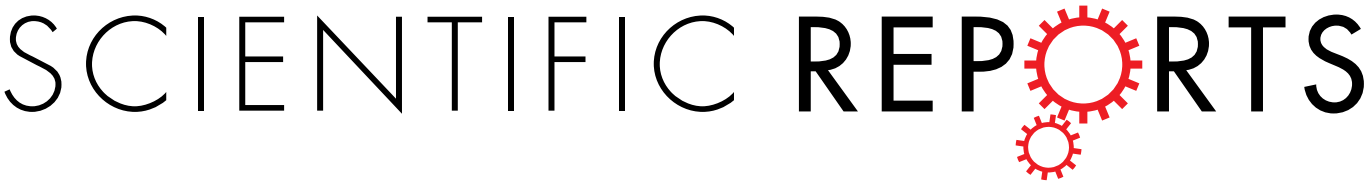

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Do nuclear-encoded core subunits of mitochondrial complex I confer genetic susceptibility to schizophrenia in Han Chinese populations?

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Xiao Li[1,3,*], Wen Zhang[1,*], Jinsong Tang[2], Liwen Tan[2], Xiong-jian Luo[1], Xiaogang Chen[2] & Yong-Gang Yao[1,4]

Schizophrenia is one of the most prevalent psychiatric disorders with complex genetic etiology. Accumulating evidence suggests that energy metabolism and oxidative stress play important roles in the pathophysiology of schizophrenia. Dysfunction of mitochondrial respiratory chain and altered expression of complex I subunits were frequently reported in schizophrenia. To investigate whether nuclear-encoded core subunit genes of mitochondrial complex I are associated with schizophrenia, we performed a genetic association study in Han Chinese. In total, 46 tag single nucleotide polymorphisms (SNPs) from 7 nuclear-encoded core genes of mitochondrial complex I were genotyped in 918 schizophrenia patients and 1042 healthy controls. We also analyzed these SNPs in a large sample mainly composed of Europeans through using the available GWAS datasets from the Psychiatric Genomics Consortium (PGC). No significant associations were detected between these SNPs and schizophrenia in Han Chinese and the PGC data set. However, we observed nominal significant associations of 2 SNPs in the *NDUFS1* gene and 4 SNPs in the *NDUFS2* gene with early onset schizophrenia (EOS), but none of these associations survived the Bonferroni correction. Taken together, our results suggested that common SNPs in the nuclear-encoded core subunit genes of mitochondrial complex I may not confer genetic susceptibility to schizophrenia.

Schizophrenia is one of the most common psychiatric disorders with a heritability as high as 80%[1]. Though significant progress has been made during the past decades, the etiology and pathophysiology of schizophrenia remains largely unknown. Accumulating evidence implies that mitochondrial dysfunction may play an important role in schizophrenia[2].

Human brain is the largest energy consumer among all organs (it consumes about 20% energy used by the human body)[3], which makes it more susceptible to disrupted cellular energy metabolism. Most of the energy used by the human body is produced by mitochondrion, a complex intracellular organelle with many components. As the energy factory, mitochondrion plays a key role in maintaining the normal

[1]Key Laboratory of Animal Models and Human Disease Mechanisms of the Chinese Academy of Sciences & Yunnan Province, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, China. [2]Institute of Mental Health, the Second Xiangya Hospital, Central South University, Changsha 410011, China. [3]Kunming College of Life Science, University of Chinese Academy of Sciences, Kunming 650223, China. [4]CAS Center for Excellence in Brain Science, Chinese Academy of Sciences, Shanghai, 200031, China. *These authors contributed equally to this work. Correspondence and requests for materials should be addressed to X.C. (email: chenxghn@163.com) or Y.G.Y. (email: yaoyg@mail.kiz.ac.cn)

function of brain and mitochondrial dysfunction has been frequently reported in patients with brain diseases, including neurodegenerative diseases and psychiatric disorders[2,4].

Several lines of evidence support the dysfunction of mitochondria in schizophrenia. First, defect of mitochondrial oxidative phosphorylation and altered expression of mitochondria-related genes were reported in brains from patients with schizophrenia[5–7]. Second, data from transcriptomics, proteomics and metabolomics revealed aberrant brain metabolism and oxidative stress in schizophrenia patients[8]. Third, perturbations in mitochondrial network dynamics and in complex I dependent cellular respiration were also reported in schizophrenia[9]. Besides, genetic variations of mitochondrial DNA (mtDNA) were also reported to be susceptible to schizophrenia and other neurological disorders[2,10,11], although with controversies[12–15]. Despite the significant progress in recent years, the role of mitochondria in the pathophysiology of schizophrenia remains elusive, and further studies using more rigorous methodological and statistical standards are necessary to avoid false positive results.

Among the mitochondrial complexes, NADH ubiquinone oxidoreductase (complex I) may have a role in schizophrenia. Human mitochondrial complex I, which contains 45 subunits, is the largest and most complicated component of the respiratory chain and plays a central role in electron transportation[16]. The 14 "core" subunits of complex I are conserved from bacteria to human and are sufficient for catalysis[17,18], suggesting their importance in energy metabolism. Seven of the 14 "core" subunits were nuclear-encoded which ligate the flavin mononucleotide and the iron-sulfur clusters[19]. Due to its pivotal role in energy metabolism, it has been speculated that mitochondrial complex I may be involved in the pathophysiology of schizophrenia. Consistent with this speculation, previous studies showed aberrant expression of mitochondrial complex I subunits or altered complex I activity in schizophrenia[6,20–22]. In addition, several "core" genes of mitochondrial complex I have been reported to be functionally related to schizophrenia[23–25].

To further explore the potential association between mitochondrial complex I and schizophrenia, we conducted a comprehensive genetic association study by genotyping 46 tag SNPs from seven nuclear-encoded genes of mitochondrial complex I in Han Chinese with and without schizophrenia and reanalysis of the Psychiatric Genetics Consortium (PGC) dataset[26]. In addition, we also performed stratified analyses to test if genetic variants of the seven nuclear-encoded genes of mitochondrial complex I were associated with early onset schizophrenia (EOS) in Han Chinese.

Results

In total, 46 tag SNPs were successfully genotyped in our samples. Considering an average population minor allele frequency (MAF) of 0.1, the power to detect an odds ratio as low as 1.5 for a risk allele/genotype/haplotype was above 90% for the comparison between schizophrenia patients and controls in our sample set (Supplementary Figure 1).

None of the 46 SNPs was deviated from HWE in both case and control samples (Supplementary Table 1). The linkage disequilibrium (LD) structures of the 46 SNPs showed high degrees of similarity between cases and controls (Fig. 1). Allelic and genotypic association analyses revealed no significant association between these 46 tag SNPs and schizophrenia (Table 1). In addition, haplotype-based analysis also showed no significant association between schizophrenia cases and healthy controls (Supplementary Table 2). Further stratified association analysis revealed that two SNPs (rs4147713, $P = 0.019$; and rs1044120, $P = 0.049$) in the *NDUFS1* gene and 4 SNPs (rs3924264, $P = 0.004$; rs1136224, $P = 0.013$; rs2070902, $P = 0.006$; and rs4233368, $P = 0.038$) in the *NDUFS2* gene were nominally associated with EOS (onset age $\leq$ 18 years) (Table 2). Nevertheless, none of the six SNPs survived for multiple testing corrections.

We further examined the genetic association between these 46 SNPs and schizophrenia in the PGC data[26]. Overall, 35,476 schizophrenia cases and 46,839 controls were included for association analysis. We observed marginally significant associations with schizophrenia of rs10908826, rs4656994, rs5085 and rs2307424 in the *NDUFS2* gene and rs12457810, rs12964485 and rs2377961 in the *NDUFV2* gene. However, none of these SNPs showed significant association after correcting for multiple testing (Table 1).

We further performed interaction analysis to test whether there is an interaction between SNPs in both case-control samples and the EOS-control samples. Our results showed that although there were many marginally significant interactions, none of them could survive the Bonferroni correction (Supplementary Tables 3-4).

Discussion

Mitochondrial dysfunction has been frequently reported in schizophrenia[8,27]. Previous studies have suggested that genes related to energy metabolism and oxidative stress may be responsible for mitochondrial dysfunction in schizophrenia[8]. As the largest component of the three membrane-bound enzymes, mitochondrial complex I plays a vital role in energy metabolism and altered activity of mitochondrial complex I was repeatedly reported in schizophrenia[20,22,28,29]. Though multiple studies have reported that mitochondrial dysfunction may be involved in schizophrenia pathogenesis, most of the conclusions were based on gene expression[6,21]. We previously analyzed the *NDUFS7* gene in Han Chinese with and without schizophrenia, with an intention to discern the effect of the complex I genes on this disorder[30]. In this study, we analyzed other complex I core subunit genes including *NDUFS1*, *NDUFS2*, *NDUFS3*, *NDUFS8*, *NDUFV1* and *NDUFV2*, as well as four more common SNPs of the *NDUFS7* gene in a larger sample set. We systematically investigated the potential association between these nuclear-encoded mitochondrial

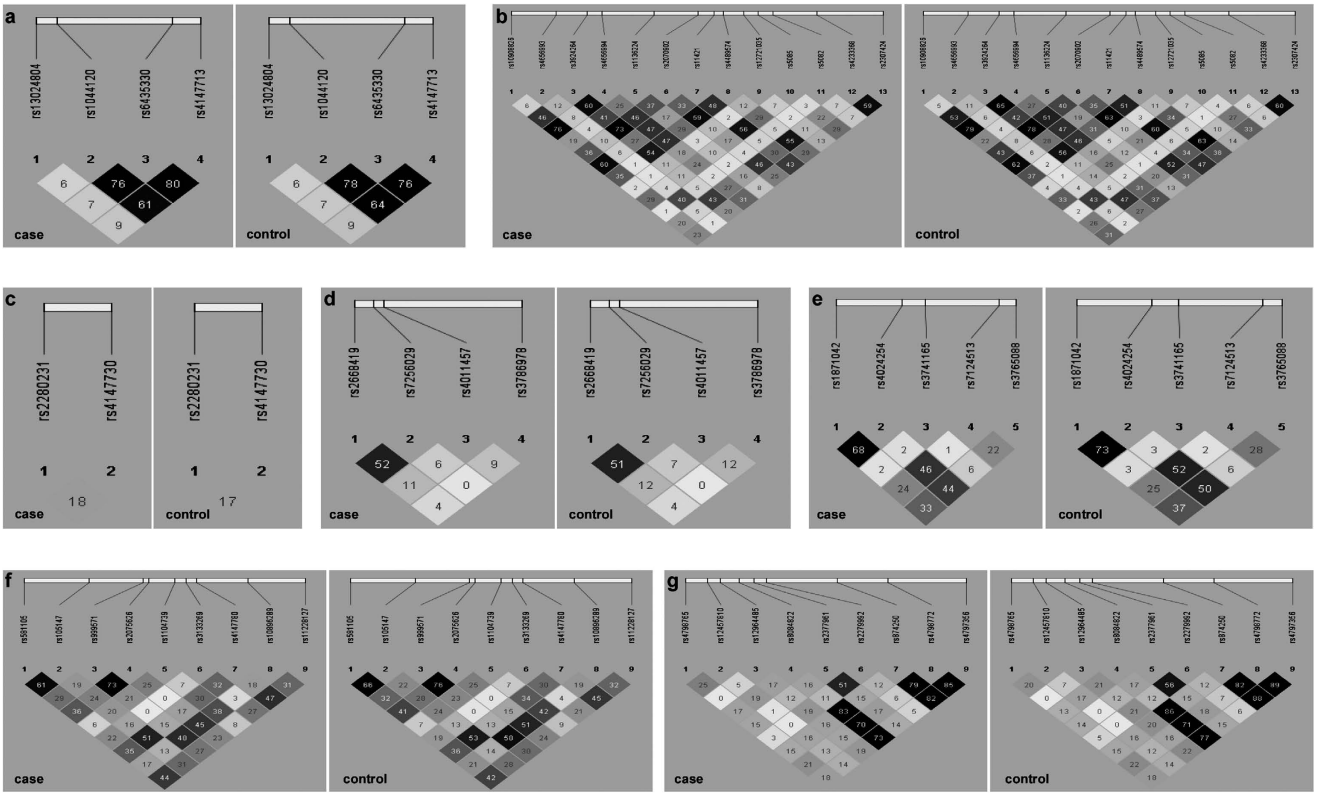

Figure 1. The linkage disequilibrium (LD) structures of the *NDUFS1* gene (a), the *NDUFS2* gene (b), the *NDUFS3* gene (c), the *NDUFS7* gene (d), the *NDUFV1* gene (e), the *NDUFS8* gene (f) and the *NDUFV2* gene (g) in Han Chinese with and without schizophrenia. The value in each square refers to $r^2 \times 100$. The blacker square represented the higher LD. The individual square showed the $r^2 \times 100$ value for each SNP pair.

complex I genes and schizophrenia in a Chinese case-control sample. Our results revealed no significant association between genetic variants from the seven selected genes and schizophrenia, suggesting that these genes are unlikely to confer risk of schizophrenia in Han Chinese population. Consistent with the finding in Han Chinese, we also found no robust association between these SNPs and schizophrenia in the PGC data[26]. It should be mentioned that if we did not consider the effect of multiple corrections, SNPs of the *NDUFS2* gene would show (marginally) significant association with schizophrenia in both Han Chinese and the PGC data[26].

It is interesting that we observed nominal significant association between genetic variants in the *NDUFS1* and *NDUFS2* genes and EOS in Han Chinese. The nominal significance would not survive from adjustment and we cannot rule out the possibility of false positive association caused by relatively small sample size of EOS used in the analyses. It should be mentioned that genetic variant in the *NDUFS1* gene was reported to be associated with schizophrenia and negative symptoms in Han Chinese from Eastern China, albeit the samples size was also relatively small[31]. We also noticed that comparing with whole samples (odds ratio [OR] > 1), 2 of the 6 nominal significant SNPs (rs4147713 and rs3924264) were associated with EOS in reverse style (OR < 1). This observation indicated population stratification may exist in the EOS-subpopulation.

Considering that mitochondrial dysfunction was repeatedly reported in schizophrenia, it is amazing that none of the selected SNPs showed significant association with schizophrenia. One of the possible explanations is that other genes but not the seven selected core genes of mitochondrial complex I contribute to schizophrenia susceptibility. Moreover, possibility of rare variant(s) in these genes contributing to schizophrenia susceptibility needs to be studied as well. In addition, given that schizophrenia has a strong genetic heterogeneity, it is also possible that these genes would have a strong effect in other populations but not in Han Chinese[23,24].

There are several limitations in this study. First, the sample size is relatively modest in this study. As a result, it may be difficult to detect a robust significant association. Second, we only analyzed seven nuclear-encoded genes in the mitochondrial complex I in this study, we could not exclude the possibility that other genes of complex I are associated with schizophrenia.

SNP ID	A_{12}[a]	Freq.[b]	*P*-value[c]		OR (s.e.)[d]	
			Hunan	PGC	Hunan	PGC
NDUFS1						
rs4147713	G/T	0.325	0.570	0.165	1.039 (0.068)	0.985 (0.011)
rs6435330	T/G	0.280	0.357	0.077	1.067 (0.071)	1.019 (0.011)
rs1044120	T/G	0.243	0.892	0.106	0.990 (0.075)	1.018 (0.011)
rs13024804	G/A	0.174	0.174	0.277	0.889 (0.086)	1.022 (0.020)
NDUFS2						
rs10908826	T/C	0.346	0.319	**0.028**	0.935 (0.068)	1.033 (0.015)
rs4656993	A/G	0.100	0.110	0.274	1.179 (0.103)	0.988 (0.011)
rs3924264	A/G	0.493	0.628	0.314	1.031 (0.064)	1.011 (0.011)
rs4656994	A/G	0.393	0.588	**0.016**	0.965 (0.066)	1.031 (0.013)
rs1136224	C/T	0.304	0.892	0.105	0.991 (0.070)	1.024 (0.015)
rs2070902	T/C	0.455	0.509	0.100	0.958 (0.064)	0.980 (0.012)
rs11421	C/T	0.384	0.312	0.059	0.935 (0.066)	0.973 (0.015)
rs4489574	T/C	0.474	0.826	0.057	1.014 (0.064)	0.979 (0.011)
rs12721035	A/G	0.146	0.579	0.406	1.051 (0.090)	1.018 (0.022)
rs5085	C/G	0.305	0.347	**0.015**	0.936 (0.070)	0.967 (0.014)
rs5082	C/T	0.088	0.149	0.585	1.171 (0.109)	1.006 (0.011)
rs4233368	A/C	0.398	0.306	0.066	0.935 (0.066)	0.978 (0.012)
rs2307424	C/T	0.491	0.885	**0.017**	0.991 (0.064)	1.027 (0.011)
NDUFS3						
rs2280231	T/C	0.278	0.846	0.167	0.986 (0.072)	0.984 (0.012)
rs4147730	A/G	0.318	0.721	0.311	1.025 (0.069)	1.015 (0.015)
NDUFS7						
rs2668419	A/G	0.438	0.873	0.383	1.010 (0.064)	1.017 (0.020)
rs7256029	G/A	0.406	0.881	0.354	0.990 (0.065)	1.017 (0.018)
rs4011457	C/G	0.145	0.987	0.143	1.002 (0.091)	0.960 (0.028)
rs3786978	C/T	0.395	0.836	0.196	1.014 (0.065)	1.044 (0.033)
NDUFS8						
rs581105	G/T	0.390	0.285	0.585	1.072 (0.065)	1.006 (0.011)
rs105147	C/T	0.465	0.462	0.865	1.049 (0.064)	1.002 (0.011)
rs999571	A/G	0.183	0.685	0.053	0.967 (0.083)	0.969 (0.017)
rs2075626	C/T	0.222	0.594	0.408	1.042 (0.077)	1.010 (0.013)
rs1104739	C/A	0.235	0.940	0.747	0.994 (0.076)	0.996 (0.011)
rs3133269	C/T	0.202	0.826	0.675	1.018 (0.080)	1.005 (0.012)
rs4147780	C/T	0.428	0.821	0.277	1.015 (0.065)	1.012 (0.011)
rs10896289	A/C	0.143	0.897	0.055	0.988 (0.092)	0.973 (0.015)
rs11228127	A/G	0.317	0.760	0.098	0.979 (0.069)	0.973 (0.016)
NDUFV1						
rs1871042	T/C	0.172	0.267	0.266	0.909 (0.086)	0.987 (0.012)
rs4024254	C/T	0.207	0.380	0.365	0.932 (0.080)	1.010 (0.011)
rs3741165	G/A	0.134	0.638	0.390	1.045 (0.093)	1.047 (0.053)
rs7124513	T/C	0.127	0.061	0.425	0.830 (0.100)	1.009 (0.012)
rs3765088	G/A	0.326	0.692	0.950	1.027 (0.068)	1.000 (0.011)
NDUFV2						
rs4798765	T/C	0.323	0.051	0.623	0.873 (0.069)	1.006 (0.011)
rs12457810	G/T	0.107	0.980	**0.035**	0.997 (0.104)	0.958 (0.021)
rs12964485	T/C	0.394	0.967	**0.019**	0.997 (0.066)	0.975 (0.011)
rs8084822	T/A	0.250	0.327	0.786	0.929 (0.075)	0.996 (0.014)

Continued

SNP ID	A_{12}[a]	Freq.[b]	*P*-value[c]		OR (s.e.)[d]	
			Hunan	PGC	Hunan	PGC
rs2377961	C/T	0.345	0.071	**0.019**	1.128 (0.067)	0.973 (0.012)
rs2279992	G/A	0.275	0.119	0.107	1.117 (0.071)	0.982 (0.011)
rs874250	A/G	0.261	0.250	0.538	0.919 (0.074)	1.008 (0.014)
rs4798772	G/A	0.299	0.342	0.507	0.935 (0.071)	1.008 (0.012)
rs4797356	A/T	0.283	0.497	0.163	0.953 (0.072)	1.015 (0.011)

Table 1. Association results of the 46 SNPs in Hunan sample and the PGC sample. [a]A_{12}, minor allele and major allele. [b]Minor allele frequency (Freq.) of control samples in Hunan. [c]*P*-value < 0.05 was marked in bold. [d]Odds ratio (OR) estimates and standard errors (s.e.).

In summary, we detected no significant association between the genetic polymorphisms of the nuclear-encoded core subunit genes of mitochondrial complex I and schizophrenia by using a rigorous statistical standard so as to avoid false positive results. Further work is needed to test if the expression and rare variants, but not common variants of these genes, contribute to schizophrenia.

Materials and methods

Subjects. A total of 1960 subjects, including 918 unrelated patients with schizophrenia (561 males: mean age $\pm$ SD, 38.5 $\pm$ 13.6 years; 357 females: 38.4 $\pm$ 16.8 years) and 1042 healthy controls (631 males: mean age $\pm$ SD, 38.5 $\pm$ 14.2 years; 411 females: 46.0 $\pm$ 8.2 years) were recruited. All of the individuals are of Han Chinese origin from Hunan Province of South Central China. Among the 918 patients, 189 were EOS (first age of onset $\leq$ 18 years old). The patients were clinically diagnosed according to Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) and had at least two-year history of schizophrenia. Diagnosis and review of schizophrenia cases were independently checked and verified by two senior psychiatrists prior to blood sample collection. The healthy controls were collected from local hospitals and assessed by experienced psychiatrists. Individuals with psychiatric history, alcohol dependence, drug abuse, or family history of psychiatric disorders were excluded. All of the schizophrenia patients and healthy controls have been reported in our previous studies[11,32]. Written informed consents conforming to the tenets of the Declaration of Helsinki were obtained from all participants or the appointed guardians of the patients (for those who were unable to provide informed consent at the time of blood collection) prior to this study. The experimental methods were carried out in accordance with the approved guidelines. All experimental protocols of this study were approved by the institutional review board / Ethics Committee of Kunming Institute of Zoology, Chinese Academy of Sciences.

SNP selection and genotyping. Genomic DNA of all participants was extracted from peripheral blood using the AxyPrep™ Blood Genomic DNA Miniprep Kit (Axygen, USA) according to the manufacturer's instruction. Seven nuclear-encoded genes of mitochondrial complex I (*NDUFS1*, *NDUFS2*, *NDUFS3*, *NDUFS7*, *NDUFS8*, *NDUFV1* and *NDUFV2*) were chosen in this study. To select the tag SNPs, we retrieved genotypic data of Han Chinese (CHB) from the HapMap database (http://hapmap.ncbi.nlm.nih.gov/) and defined LD blocks using the Haploview 4.2[33]. The gene region and potential regulatory sequences (20 kb of both upstream and downstream regions) were taken into consideration during the selection of tag SNPs. In total, 51 tag SNPs were selected based on the following criteria: minor allele frequency (MAF) $\geq$ 0.1 and $r^2 \geq 0.8$ (Supplementary Figures 2-5). Three tag SNPs (rs2074896, rs2074897 and rs2074898) in the *NDUFS7* gene were analyzed in our previous study[30], therefore were not included in this study. The remaining 48 tag SNPs were divided into four panels (12 SNPs for each panel) according to their compatibility in multiplex PCR. Genotyping of each panel was conducted by SNaPshot assays reported in our previous studies[34,35]. The GeneMarker software was utilized to read the genotyping results[36].

Power calculation and statistical analysis. Among the 48 tag SNPs, two (rs12798346 and rs3751084) were failed to be genotyped in our samples. Therefore, these two SNPs were excluded from our statistical analysis. The genotyping call rate of each SNP was above 99.0% in 1960 individuals. LD plot of the genotyped SNPs of each gene was constructed using Haploview 4.2 program (version 4.2). We tested deviation from the Hardy-Weinberg equilibrium (HWE), individual SNP association, haplotype comparison and SNP-SNP interaction by using PLINK[37]. Quanto software[38] was used for power analysis under the gene only hypothesis and log additive model and following parameters: risk allele frequency from 0.1 to 0.5 in increments of 0.1; overall disease risk in the general population = 0.01; sample size = 918 cases vs. 1042 controls; range of OR from 1.0 to 2.0 in increments of 0.1; two-sided type I error rate = 0.05.

SNP ID	A_{12}[a]	Frequency of minor allele		OR	95% CI	*P*-value[b]
		EOS	control			
NDUFS1						
rs4147713	G/T	0.265	0.325	0.746	0.583-0.954	**0.019**
rs6435330	T/G	0.246	0.280	0.838	0.651-1.079	0.171
rs1044120	T/G	0.196	0.243	0.760	0.579-0.999	**0.049**
rs13024804	G/A	0.204	0.174	1.213	0.922-1.596	0.168
NDUFS2						
rs10908826	T/C	0.373	0.346	1.122	0.894-1.409	0.319
rs4656993	A/G	0.130	0.100	1.336	0.958-1.863	0.087
rs3924264	A/G	0.426	0.507	0.721	0.578-0.899	**0.004**
rs4656994	A/G	0.434	0.393	1.184	0.948-1.477	0.136
rs1136224	C/T	0.241	0.304	0.727	0.564-0.937	**0.013**
rs2070902	T/C	0.378	0.455	0.729	0.582-0.913	**0.006**
rs11421	C/T	0.429	0.384	1.204	0.964-1.503	0.101
rs4489574	T/C	0.434	0.474	0.852	0.683-1.062	0.154
rs12721035	A/G	0.161	0.146	1.130	0.837-1.525	0.425
rs5085	C/G	0.336	0.305	1.152	0.913-1.454	0.234
rs5082	C/T	0.087	0.088	0.994	0.674-1.465	0.974
rs4233368	A/C	0.341	0.398	0.784	0.623-0.987	**0.038**
rs2307424	C/T	0.439	0.491	0.812	0.651-1.012	0.064
NDUFS3						
rs2280231	T/C	0.315	0.278	1.194	0.942-1.514	0.142
rs4147730	A/G	0.304	0.318	0.939	0.741-1.191	0.605
NDUFS7						
rs2668419	A/G	0.460	0.438	1.094	0.878-1.363	0.424
rs7256029	G/A	0.413	0.406	1.028	0.823-1.285	0.806
rs4011457	C/G	0.124	0.145	0.835	0.601-1.160	0.281
rs3786978	C/T	0.400	0.395	1.019	0.815-1.275	0.868
NDUFS8						
rs581105	G/T	0.378	0.390	0.951	0.759-1.192	0.665
rs105147	C/T	0.455	0.465	0.961	0.771-1.198	0.724
rs999571	A/G	0.156	0.183	0.824	0.611-1.111	0.204
rs2075626	C/T	0.201	0.222	0.881	0.671-1.156	0.361
rs1104739	C/A	0.217	0.235	0.903	0.693-1.176	0.447
rs3133269	C/T	0.217	0.202	1.097	0.840-1.433	0.497
rs4147780	C/T	0.426	0.428	0.992	0.794-1.237	0.940
rs10896289	A/C	0.122	0.143	0.834	0.598-1.162	0.282
rs11228127	A/G	0.307	0.317	0.953	0.752-1.208	0.692
NDUFV1						
rs1871042	T/C	0.169	0.172	0.979	0.732-1.311	0.889
rs4024254	C/T	0.201	0.207	0.965	0.735-1.268	0.799
rs3741165	G/A	0.143	0.134	1.078	0.787-1.477	0.639
rs7124513	T/C	0.101	0.127	0.767	0.536-1.099	0.147
rs3765088	G/A	0.347	0.326	1.095	0.869-1.379	0.441
NDUFV2						
rs4798765	T/C	0.275	0.323	0.794	0.622-1.013	0.063
rs12457810	G/T	0.101	0.107	0.933	0.649-1.341	0.707
rs12964485	T/C	0.384	0.394	0.955	0.763-1.197	0.691

Continued

SNP ID	A_{12}[a]	Frequency of minor allele		OR	95% CI	*P*-value[b]
		EOS	control			
rs8084822	T/A	0.228	0.250	0.886	0.683-1.149	0.361
rs2377961	C/T	0.386	0.345	1.197	0.955-1.501	0.118
rs2279992	G/A	0.309	0.275	1.178	0.928-1.497	0.179
rs874250	A/G	0.238	0.261	0.887	0.687-1.146	0.358
rs4798772	G/A	0.271	0.299	0.875	0.684-1.119	0.287
rs4797356	A/T	0.263	0.283	0.907	0.706-1.164	0.441

Table 2. Association results of the 46 SNPs in 189 early onset schizophrenia patients and 1042 controls. [a]A_{12}, minor allele and major allele. [b]*P*-value < 0.05 was marked in bold. OR - odds ratio; 95% CI – 95% confidence interval

PGC data analysis. To further explore if the studied SNPs are associated with schizophrenia, we extracted the genetic association data from the Psychiatric Genomics Consortium (PGC, http://www.broadinstitute.org/mpg/ricopili/)[39] and reanalyzed this data set as an independent validation sample. In brief, 35,476 schizophrenia cases and 46,839 controls were included in the PGC dataset. The genotyping of each primary GWAS study composing the PGC data was performed by Affymetrix or Illumina array and the genetic association analysis was conducted by PLINK[37] under an additive logistic regression model. More detailed information about the PGC can be found in the original publication[26].

References

1. Sullivan, P. F., Kendler, K. S. & Neale, M. C. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. *Arch Gen Psychiatry* **60,** 1187–1192 (2003).
2. Shao, L. *et al.* Mitochondrial involvement in psychiatric disorders. *Ann Med* **40,** 281–295 (2008).
3. Mink, J. W., Blumenschine, R. J. & Adams, D. B. Ratio of central nervous system to body metabolism in vertebrates: its constancy and functional basis. *Am J Physiol* **241,** R203–212 (1981).
4. Zsurka, G. & Kunz, W. S. Mitochondrial involvement in neurodegenerative diseases. *IUBMB Life* **65,** 263–272 (2013).
5. Maurer, I., Zierz, S. & Moller, H. Evidence for a mitochondrial oxidative phosphorylation defect in brains from patients with schizophrenia. *Schizophr Res* **48,** 125–136 (2001).
6. Karry, R., Klein, E. & Ben Shachar, D. Mitochondrial complex I subunits expression is altered in schizophrenia: a postmortem study. *Biol Psychiatry* **55,** 676–684 (2004).
7. Iwamoto, K., Bundo, M. & Kato, T. Altered expression of mitochondria-related genes in postmortem brains of patients with bipolar disorder or schizophrenia, as revealed by large-scale DNA microarray analysis. *Hum Mol Genet* **14,** 241–253 (2005).
8. Prabakaran, S. *et al.* Mitochondrial dysfunction in schizophrenia: evidence for compromised brain metabolism and oxidative stress. *Mol Psychiatry* **9,** (684-697), 643 (2004).
9. Rosenfeld, M., Brenner-Lavie, H., Ari, S. G., Kavushansky, A. & Ben-Shachar, D. Perturbation in mitochondrial network dynamics and in complex I dependent cellular respiration in schizophrenia. *Biol Psychiatry* **69,** 980–988 (2011).
10. Verge, B. *et al.* Mitochondrial DNA (mtDNA) and schizophrenia. *Eur Psychiatry* **26,** 45–56 (2011).
11. Zhang, W. *et al.* A matrilineal genetic legacy from the last glacial maximum confers susceptibility to schizophrenia in han chinese. *J Genet Genomics* **41,** 397–407 (2014).
12. Bandelt, H. J., Yao, Y. G. & Kivisild, T. Mitochondrial genes and schizophrenia. *Schizophr Res* **72,** 267–269 (2005).
13. Fachal, L. *et al.* No evidence of association between common European mitochondrial DNA variants in Alzheimer, Parkinson, and migraine in the Spanish population. *Am J Med Genet B Neuropsychiatr Genet* **168B,** 54–65 (2015).
14. Mosquera-Miguel, A. *et al.* No evidence that major mtDNA European haplogroups confer risk to schizophrenia. *Am J Med Genet B Neuropsychiatr Genet* **159B,** 414–421 (2012).
15. Torrell, H. *et al.* Mitochondrial DNA (mtDNA) variants in the European haplogroups HV, JT, and U do not have a major role in schizophrenia. *Am J Med Genet B Neuropsychiatr Genet* **165B,** 607–617 (2014).
16. Brandt, U. Energy converting NADH:quinone oxidoreductase (complex I). *Annu Rev Biochem* **75,** 69–92 (2006).
17. Yip, C. Y., Harbour, M. E., Jayawardena, K., Fearnley, I. M. & Sazanov, L. A. Evolution of respiratory complex I: "supernumerary" subunits are present in the alpha-proteobacterial enzyme. *J Biol Chem* **286,** 5023–5033 (2011).
18. Efremov, R. G. & Sazanov, L. A. The coupling mechanism of respiratory complex I - a structural and evolutionary perspective. *Biochim Biophys Acta* **1817,** 1785–1795 (2012).
19. Hirst, J., Carroll, J., Fearnley, I. M., Shannon, R. J. & Walker, J. E. The nuclear encoded subunits of complex I from bovine heart mitochondria. *Biochim Biophys Acta* **1604,** 135–150 (2003).
20. Dror, N. *et al.* State-dependent alterations in mitochondrial complex I activity in platelets: a potential peripheral marker for schizophrenia. *Mol Psychiatry* **7,** 995–1001 (2002).
21. Taurines, R. *et al.* Expression analyses of the mitochondrial complex I 75-kDa subunit in early onset schizophrenia and autism spectrum disorder: increased levels as a potential biomarker for early onset schizophrenia. *Eur Child Adolesc Psychiatry* **19,** 441–448 (2010).
22. Ben-Shachar, D. *et al.* Increased mitochondrial complex I activity in platelets of schizophrenic patients. *Int J Neuropsychopharmacol* **2,** 245–253 (1999).
23. Ben-Shachar, D. & Karry, R. Sp1 expression is disrupted in schizophrenia; a possible mechanism for the abnormal expression of mitochondrial complex I genes, NDUFV1 and NDUFV2. *PLoS One* **2,** e817 (2007).
24. Washizuka, S. *et al.* Association of mitochondrial complex I subunit gene NDUFV2 at 18p11 with schizophrenia in the Japanese population. *Am J Med Genet B Neuropsychiatr Genet* **141B,** 301–304 (2006).
25. Ben-Shachar, D. & Karry, R. Neuroanatomical pattern of mitochondrial complex I pathology varies between schizophrenia, bipolar disorder and major depression. *PLoS One* **3,** e3676 (2008).

26. Schizophrenia Working Group of the Psychiatric Genomics Consortium. Biological insights from 108 schizophrenia-associated genetic loci. *Nature* **511,** 421–427 (2014).
27. Ben-Shachar, D. Mitochondrial dysfunction in schizophrenia: a possible linkage to dopamine. *J Neurochem* **83,** 1241–1251 (2002).
28. Ben-Shachar, D. *et al.* Cerebral glucose utilization and platelet mitochondrial complex I activity in schizophrenia: A FDG-PET study. *Prog Neuropsychopharmacol Biol Psychiatry* **31,** 807–813 (2007).
29. Gubert, C. *et al.* Mitochondrial activity and oxidative stress markers in peripheral blood mononuclear cells of patients with bipolar disorder, schizophrenia, and healthy subjects. *J Psychiatr Res* **47,** 1396–1402 (2013).
30. Ma, L. *et al.* No association between genetic polymorphisms of the NDUFS7 gene and schizophrenia in Han Chinese. *Psychiatr Genet* **23,** 29–32 (2013).
31. Zhu, Y. *et al.* Genetic variant in NDUFS1 gene is associated with schizophrenia and negative symptoms in Han Chinese. *J Hum Genet* **60,** 11–16 (2015).
32. Ma, L. *et al.* Evaluating risk loci for schizophrenia distilled from genome-wide association studies in Han Chinese from Central China. *Mol Psychiatry* **18,** 638–639 (2013).
33. Barrett, J. C., Fry, B., Maller, J. & Daly, M. J. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* **21,** 263–265 (2005).
34. Bi, R. *et al.* No association of the LRRK2 genetic variants with Alzheimer's disease in Han Chinese individuals. *Neurobiol Aging* **35,** 444 e445–e449 (2014).
35. Li, X. *et al.* No association between genetic variants of the LRRK2 gene and schizophrenia in Han Chinese. *Neurosci Lett* **566,** 210–215 (2014).
36. Holland, M. M. & Parson, W. GeneMarker(R) HID: A reliable software tool for the analysis of forensic STR data. *J Forensic Sci* **56,** 29–35 (2011).
37. Purcell, S. *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* **81,** 559–575 (2007).
38. Gauderman, W. J. Sample size requirements for matched case-control studies of gene-environment interaction. *Stat Med* **21,** 35–50 (2002).
39. Sullivan, P. F. The psychiatric GWAS consortium: big science comes to psychiatry. *Neuron* **68,** 182–186 (2010).

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Author Contributions

W.Z. and Y.G.Y. designed the study; X.C., J.T. and L.T. collected the samples and clinical information; X.L. carried out the experimental procedures; X.L., W.Z., X.j.L. and Y.G.Y. analyzed data; X.L., W.Z. and Y.G.Y. drafted the manuscript. All authors contributed to and have approved the final manuscript.

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Do nuclear-encoded core subunits of mitochondrial complex I confer genetic susceptibility to schizophrenia in Han Chinese populations?

Xiao Li[1,3,a], Wen Zhang[1,a], Jinsong Tang[2], Liwen Tan[2], Xiong-jian Luo[1], Xiaogang Chen[2,*], Yong-Gang Yao[1,4*]

[1]Key Laboratory of Animal Models and Human Disease Mechanisms of the Chinese Academy of Sciences & Yunnan Province, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, China;
[2]Institute of Mental Health, the Second Xiangya Hospital, Central South University, Changsha 410011, China;
[3]Kunming College of Life Science, University of Chinese Academy of Sciences, Kunming 650223, China;
[4]CAS Center for Excellence in Brain Science, Chinese Academy of Sciences, Shanghai, 200031, China.
[a]These authors contributed equally to this work.

[*]Corresponding author: Dr. Yong-Gang Yao, Key Laboratory of Animal Models and Human Disease Mechanisms, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650223, China. Tel/Fax: 86-871-65180085; E-mail: yaoyg@mail.kiz.ac.cn; or Dr. Xiaogang Chen, Institute of Mental Health, the Second Xiangya Hospital, Central South University, Changsha 410011, China. E-mail: chenxghn@163.com

Supplementary Table 1 Genotype frequencies of the 46 SNPs in 918 schizophrenia patients and 1042 healthy controls from Hunan Province.

SNP ID	Genotype	Number of samples		*P*-value[a]	HWE *P*-value
		case	control		
NDUFS1					
rs4147713	GG/GT/TT	90/433/395	115/448/479	0.170	0.075/0.525
rs6435330	TT/GT/GG	69/401/448	78/428/536	0.482	0.112/0.591
rs1044120	TT/GT/GG	55/329/528	64/376/599	0.990	0.717/0.613
rs13024804	GG/AG/AA	22/246/650	35/293/714	0.328	0.902/0.452
NDUFS2					
rs10908826	TT/CT/CC	101/405/410	125/472/445	0.608	0.941/1.000
rs4656993	AA/AG/GG	9/195/713	10/189/843	0.218	0.336/1.000
rs3924264	AA/AG/GG	230/459/229	258/511/273	0.816	1.000/0.536
rs4656994	AA/AG/GG	134/438/346	169/481/392	0.582	0.336/1.000
rs1136224	CC/CT/TT	91/372/455	87/459/496	0.209	0.241/0.213
rs2070902	TT/CT/CC	172/471/274	218/512/312	0.443	0.256/0.755
rs11421	CC/CT/TT	124/428/366	154/492/396	0.597	1.000/0.948
rs4489574	TT/CT/CC	217/442/259	249/489/304	0.851	0.290/ 0.062
rs12721035	AA/AG/GG	21/235/656	29/245/767	0.442	1.000/0.082
rs5085	CC/CG/GG	77/381/460	99/438/505	0.614	0.936/0.770
rs5082	CC/CT/TT	7/172/739	8/167/867	0.285	0.470/1.000
rs4233368	AA/AC/CC	128/445/345	172/485/385	0.282	0.442/0.365
rs2307424	CC/CT/TT	208/481/229	256/511/275	0.327	0.165/0.536
NDUFS3					
rs2280231	TT/CT/CC	76/353/489	81/417/544	0.754	0.282/0.938
rs4147730	AA/AG/GG	105/383/430	111/440/491	0.856	0.174/0.392
NDUFS7					
rs2668419	AA/AG/GG	183/443/292	210/493/339	0.913	0.547/0.209
rs7256029	GG/AG/AA	166/409/343	185/476/381	0.882	0.024/0.095
rs4011457	CC/CG/GG	26/215/676	17/269/756	0.110	0.084/0.261
rs3786978	CC/CT/TT	149/433/336	148/527/367	0.254	0.630/0.069
NDUFS8					
rs581105	GG/GT/TT	159/429/330	165/483/394	0.570	0.339/0.398
rs105147	CC/CT/TT	201/457/243	235/496/308	0.348	0.6401/0.191
rs999571	AA/AG/GG	33/261/623	37/308/697	0.867	0.369/0.679
rs2075626	CC/CT/TT	55/311/552	51/361/630	0.555	0.224/1.000
rs1104739	CC/AC/AA	57/313/543	66/357/618	0.996	0.196/0.143
rs3133269	CC/CT/TT	39/297/581	46/327/666	0.903	0.919/0.500
rs4147780	CC/CT/TT	179/431/304	192/508/342	0.729	0.252/0.899
rs10896289	AA/AC/CC	23/213/682	26/245/771	0.987	0.219/0.207
rs11228127	AA/AG/GG	92/390/436	102/457/483	0.829	0.759/0.721
NDUFV1					
rs1871042	TT/CT/CC	23/246/649	33/293/716	0.513	1.000/0.664
rs4024254	CC/CT/TT	34/291/593	43/345/654	0.677	0.916/0.850

rs3741165	GG/AG/AA	15/225/677	24/231/787	0.293	0.580/0.180
rs7124513	TT/CT/CC	8/182/728	17/231/794	0.130	0.491/1.000
rs3765088	GG/AG/AA	104/402/412	117/446/479	0.886	0.710/0.398
NDUFV2					
rs4798765	TT/CT/CC	72/395/448	109/456/477	0.099	0.266/1.000
rs12457810	GG/GT/TT	12/172/734	11/201/830	0.841	0.602/0.872
rs12964485	TT/CT/CC	150/423/345	158/506/378	0.525	0.300/0.650
rs8084822	TT/AT/AA	56/319/538	60/399/581	0.293	0.359/0.457
rs2377961	CC/CT/TT	122/438/356	126/466/450	0.149	0.525/0.784
rs2279992	GG/AG/AA	81/382/452	86/391/548	0.197	1.000/ 0.182
rs874250	AA/AG/GG	57/335/526	66/411/565	0.376	0.721/0.470
rs4798772	GG/AG/AA	79/364/474	92/438/512	0.519	0.465/0.941
rs4797356	AA/AT/TT	77/342/489	79/431/531	0.230	0.132/0.542

[a]*P* value were calculated by the Chi-square test.

Supplementary Table 2 Haplotype frequencies of the 46 SNPs in the 7 genes in 918 schizophrenia patients and 1042 healthy controls from Hunan Province.

Gene	Haplotype	Freq.		*P*-value
		case	control	
NDUFS1	TGGG	0.157	0.174	0.171
	GTTA	0.241	0.239	0.895
	GTGA	0.051	0.039	0.070
	GGGA	0.043	0.047	0.553
	TGGA	0.508	0.502	0.684
NDUFS2	TGAATCCCAGTCT	0.093	0.086	0.496
	CGGGTTTTGGTAC	0.106	0.116	0.393
	TGAATCCCGCTCT	0.186	0.210	0.093
	CGGGCTTTGGTCT	0.055	0.043	0.113
	CAAGTCTCGGCCC	0.082	0.074	0.353
	TGAATCTTGGTAC	0.060	0.053	0.355
	CGGGTTCCGCTCT	0.055	0.049	0.394
	CGGGTCCCGCTCT	0.031	0.030	0.804
	CGAATCTCAGTCT	0.025	0.026	0.864
	CGGGCTTTGGTAC	0.208	0.226	0.197
	CAAGTCTTGGTCT	0.033	0.022	0.048
	CGAATCTTGGTAC	0.017	0.017	0.951
	CGGGCTTCAGTCT	0.015	0.014	0.909
	CGGGCTCCGCTCT	0.018	0.022	0.375
	TGAATCTCGGCCC	0.014	0.012	0.661
NDUFS3	CA	0.323	0.318	0.721
	TG	0.275	0.278	0.846
	CG	0.402	0.404	0.871
NDUFS7	GACC	0.114	0.120	0.573
	GGGC	0.148	0.145	0.796
	AAGC	0.127	0.122	0.623
	GAGC	0.018	0.014	0.353
	GACT	0.021	0.018	0.584
	GGGT	0.252	0.259	0.633
	AAGT	0.320	0.322	0.906
NDUFS8	GCGTATTCG	0.036	0.027	0.157
	GCACATTCG	0.023	0.023	0.976
	GCACCTCAA	0.115	0.126	0.329
	TTGTATTCG	0.516	0.516	0.973
	GCGCATTCG	0.015	0.016	0.889
	TTGTATTCA	0.009	0.013	0.350
	TCGTATTCG	0.014	0.011	0.359
	TTGTACCCA	0.015	0.023	0.082
	GCGTACCCA	0.120	0.106	0.222
	GCGTACCCG	0.037	0.042	0.494

	GCACACCCA	0.025	0.020	0.350
	TCGTCTCCG	0.063	0.065	0.810
	TCGTCTCCA	0.012	0.011	0.897
NDUFV1	TCATG	0.072	0.086	0.102
	CCATG	0.034	0.039	0.441
	TCACG	0.081	0.079	0.791
	CTACG	0.141	0.120	0.054
	CTGCA	0.136	0.133	0.765
	CTACA	0.535	0.542	0.641
NDUFV2	CTTATAGAT	0.259	0.258	0.930
	TTTATAGAT	0.092	0.106	0.161
	CTCACAGAT	0.032	0.030	0.649
	CTCACGGAT	0.261	0.241	0.169
	CTCATAGAT	0.040	0.040	0.984
	CTTACAGAT	0.044	0.037	0.290
	TGCTTAAGA	0.074	0.071	0.692
	CTCTTAAGA	0.093	0.099	0.501
	TTCTTAAGA	0.065	0.078	0.146
	TGCACGGAT	0.019	0.022	0.532
	TTCATGGGA	0.020	0.017	0.641

NDUFS1: rs4147713|rs6435330|rs1044120|rs13024804.

NDUFS2: rs10908826|rs4656993|rs3924264|rs4656994|rs1136224|rs2070902|rs11421|rs4489574|rs12721035|rs5085|rs5082|rs4233368|rs2307424.

NDUFS3: rs2280231|rs4147730.

NDUFS7: rs2668419|rs7256029|rs4011457|rs3786978.

NDUFS8: rs581105|rs105147|rs999571|rs2075626|rs1104739|rs3133269|rs4147780|rs10896289|rs11228127.

NDUFV1: rs1871042|rs4024254|rs3741165|rs7124513|rs3765088.

NDUFV2: rs4798765|rs12457810|rs12964485|rs8084822|rs2377961|rs2279992|rs874250|rs4798772|rs4797356.

Supplementary Table 3 SNP-SNP interaction in 918 schizophrenia patients and 1042 controls.

CHR1	SNP1	CHR2	SNP2	OR_INT[a]	STAT[b]	*P*-value[c]
1	rs10908826	1	rs2307424	1.292	6.402	0.011
1	rs3924264	1	rs2307424	1.255	6.189	0.013
1	rs3924264	2	rs1044120	0.758	6.621	0.010
1	rs3924264	2	rs6435330	0.7912	5	0.025
1	rs3924264	2	rs4147713	0.7822	6.308	0.012
1	rs3924264	19	rs7256029	0.8338	4.1	0.043
1	rs4656994	1	rs2307424	1.273	6.324	0.012
1	rs4656994	2	rs1044120	0.795	4.454	0.035
1	rs1136224	1	rs2307424	0.8022	4.589	0.032
1	rs1136224	2	rs1044120	1.265	3.89	0.049
1	rs1136224	2	rs6435330	1.317	5.708	0.017
1	rs1136224	2	rs4147713	1.298	5.863	0.015
1	rs1136224	18	rs2279992	1.253	4.395	0.036
1	rs2070902	1	rs2307424	0.8247	4.337	0.037
1	rs2070902	2	rs1044120	1.287	5.194	0.023
1	rs2070902	2	rs4147713	1.244	4.779	0.029
1	rs4489574	1	rs2307424	0.7807	7.53	0.006
1	rs4489574	2	rs4147713	1.226	4.58	0.032
1	rs4489574	19	rs4011457	0.7614	4.809	0.028
1	rs12721035	1	rs2307424	1.357	3.869	0.049
1	rs5082	19	rs4011457	1.559	3.842	0.050
1	rs4233368	11	rs3133269	1.273	4.336	0.037
1	rs4233368	19	rs7256029	1.256	6.289	0.012
1	rs4233368	19	rs4011457	0.7309	5.693	0.017
1	rs2307424	18	rs12457810	1.392	4.774	0.029
1	re2307424	19	rs7256029	1.199	4.028	0.045
2	rs13024804	11	rs2280231	1.549	10.6	0.001
11	rs4147730	11	rs3765088	1.494	14.95	1.102×10^{-4}
11	rs4147730	11	rs4147780	1.323	8.097	0.004
11	rs4147730	11	rs10896289	1.321	4.056	0.044
11	rs3741165	19	rs4011457	0.5953	6.781	0.009
11	rs581105	18	rs4798765	1.27	5.602	0.018
11	rs581105	18	rs2377961	0.8048	5.037	0.025
11	rs581105	18	rs4798772	1.238	4.523	0.033
11	rs581105	18	rs4797356	1.238	4.369	0.037
11	rs105147	18	rs2377961	0.7881	6.069	0.014
11	rs105147	18	rs874250	1.242	4.168	0.041
11	rs105147	18	rs4798772	1.252	4.925	0.026
11	rs999571	19	rs4011457	1.473	5.565	0.018
11	rs2075626	18	rs2377961	0.8063	3.867	0.049
18	rs4798765	19	rs3786978	0.81	4.039	0.044
18	rs12964485	19	rs2668419	1.248	5.697	0.017

18	rs12964485	19	rs3786978	0.8152	4.577	0.032
18	rs2279992	19	rs3786978	1.242	4.279	0.039

[a]OR_INT, Odds ratio for interaction.

[b]STAT, Chi-square statistic, 1df.

[c]The significant level for *P*-value should be 4.8×10^{-5} based on Bonferroni correction for multiple test (1035 tests in this analysis, only *P*-values less than 0.05 were shown).

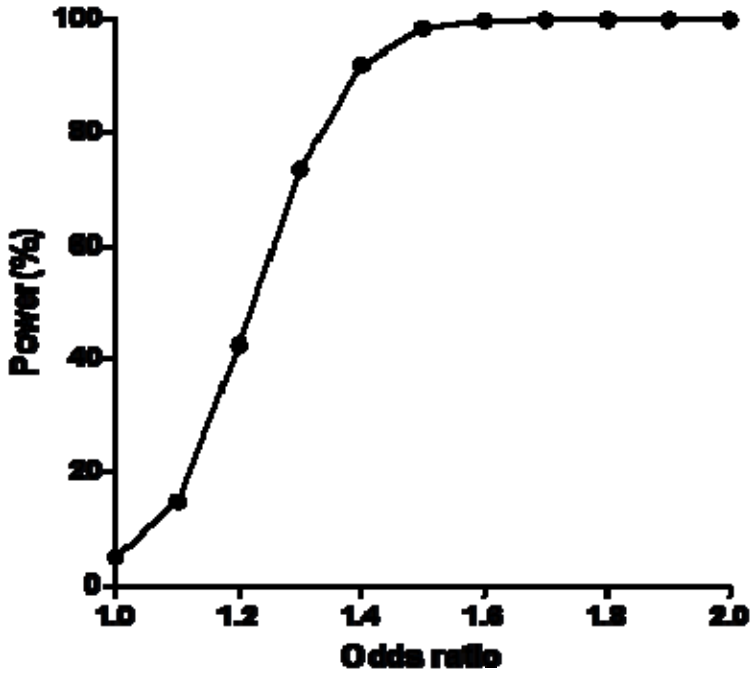
Supplementary Table 4 SNP-SNP interaction in 189 EOS patients and 1042 controls.

CHR1	SNP1	CHR2	SNP2	OR_INT[a]	STAT[b]	*P*-value[c]
1	rs1136224	1	rs5085	0.5819	4.71	0.030
1	rs1136224	1	rs6435330	1.588	4.547	0.033
1	rs2070902	18	rs4797356	1.446	4.309	0.038
1	rs4489574	1	rs5085	0.6596	4.202	0.040
1	rs4489574	19	rs4011457	0.6216	4.143	0.042
1	rs5082	2	rs13024804	2.297	6.356	0.012
1	rs5082	11	rs1104739	1.927	4.684	0.030
1	rs2307424	2	rs13024804	1.608	6.062	0.014
11	rs2280231	11	rs999571	1.593	4.367	0.037
11	rs2280231	11	rs11228127	1.497	4.732	0.030
11	rs2280231	19	rs3786978	1.526	5.874	0.015
11	rs4147730	11	rs3765088	1.71	8.882	0.003
11	rs1871042	18	rs2279992	0.5358	6.864	0.009
11	rs4024254	11	rs3133269	1.671	4.911	0.027
11	rs4024254	11	rs4147780	1.535	4.269	0.039
11	rs4024254	11	rs11228127	1.588	4.714	0.030
11	rs4024254	18	rs12964485	1.488	3.842	0.050
11	rs4024254	18	rs2279992	0.4341	12.38	4.35×10^{-4}
11	rs3741165	19	rs7256029	1.627	4.848	0.028
11	rs3765088	18	rs12964485	1.423	4.236	0.040
11	rs3765088	18	rs2279992	0.6356	6.483	0.011
11	rs581105	11	rs1104739	0.6657	4.237	0.040
11	rs999571	11	rs10896289	0.3471	6.643	0.010
11	rs999571	19	rs4011457	2.236	7.885	0.005
11	rs2075626	11	rs10896289	0.4381	4.254	0.039
18	rs4798765	19	rs4011457	0.4837	6.018	0.014
18	rs12964485	19	rs2668419	1.589	7.915	0.005
18	rs12964485	19	rs7256029	0.5992	9.866	0.002
18	rs874250	19	rs7256029	1.475	4.438	0.035
18	rs4798772	19	rs7256029	1.497	5.389	0.020
18	rs4797356	19	rs7256029	1.549	5.924	0.015
18	rs4797356	19	rs4011457	0.5294	4.047	0.044

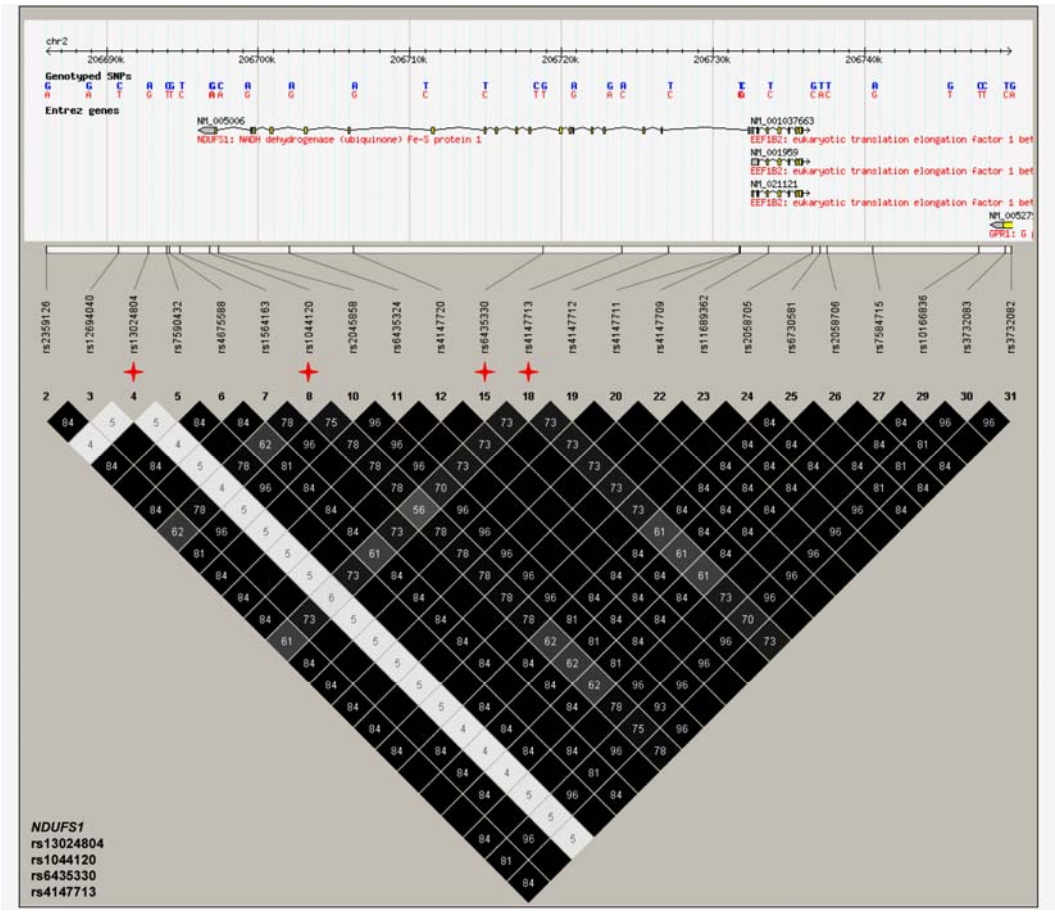
[a]OR_INT, Odds ratio for interaction.

[b]STAT, Chi-square statistic, 1df.

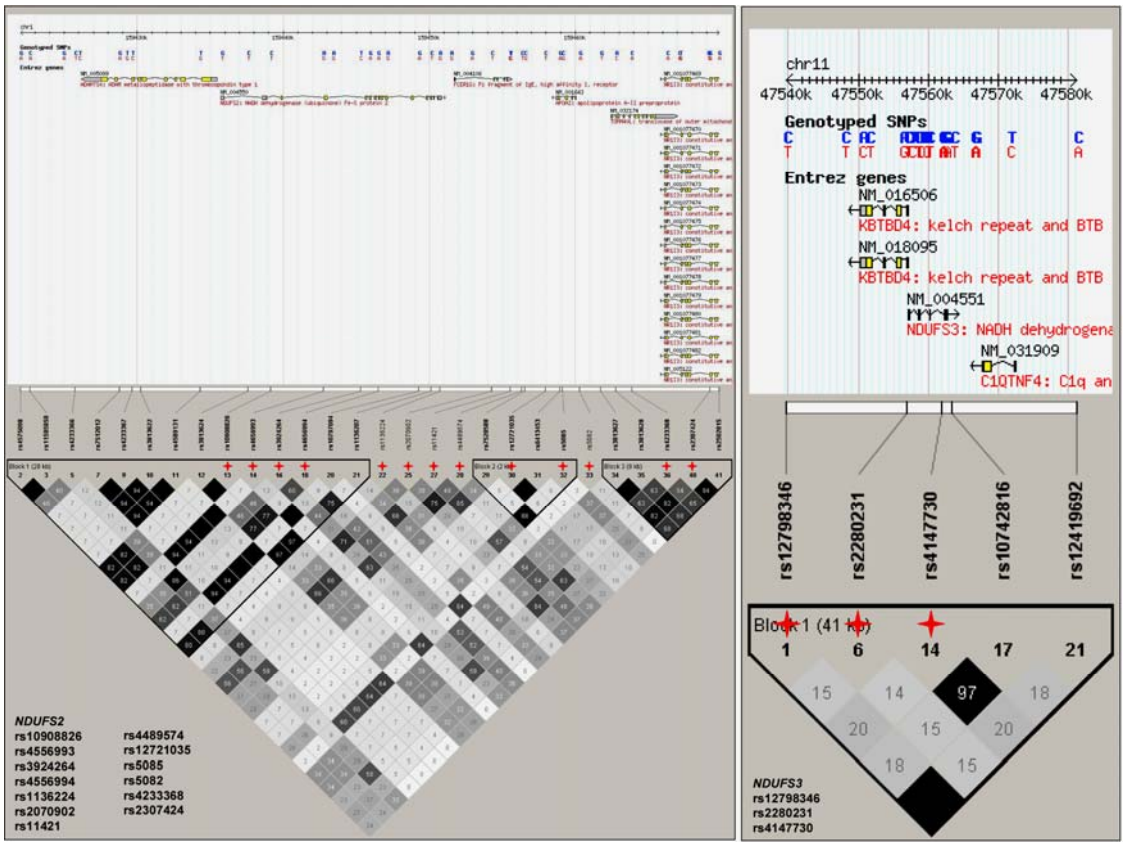
[c]The significant level for *P*-value should be 4.8×10^{-5} based on Bonferroni correction for multiple test (1035 tests in this analysis, only *P*-values less than 0.05 were shown).



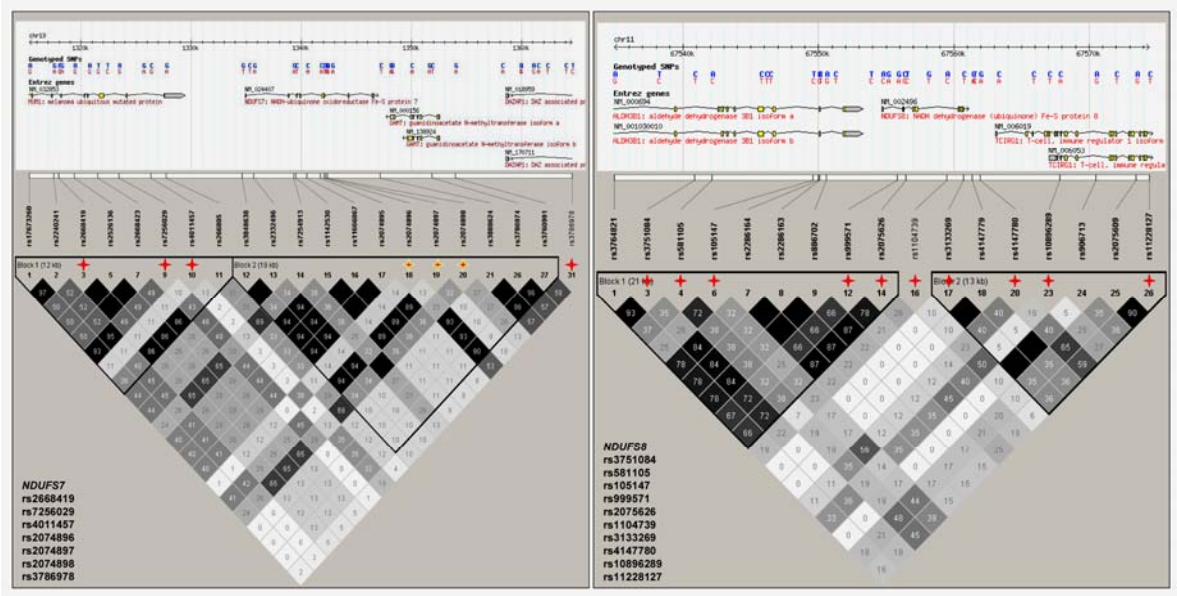
Supplementary Figure 1 Power estimates for the case-control association analysis. Statistical power was computed under the gene only hypothesis and log additive model, with the following parameters: risk allele frequency = 0.1; overall disease risk in the general population = 0.01; sample size = 918 cases vs. 1042 controls; range of OR from 1.0 to 2.0 in increments of 0.1; two-sided type I error rate = 0.05.



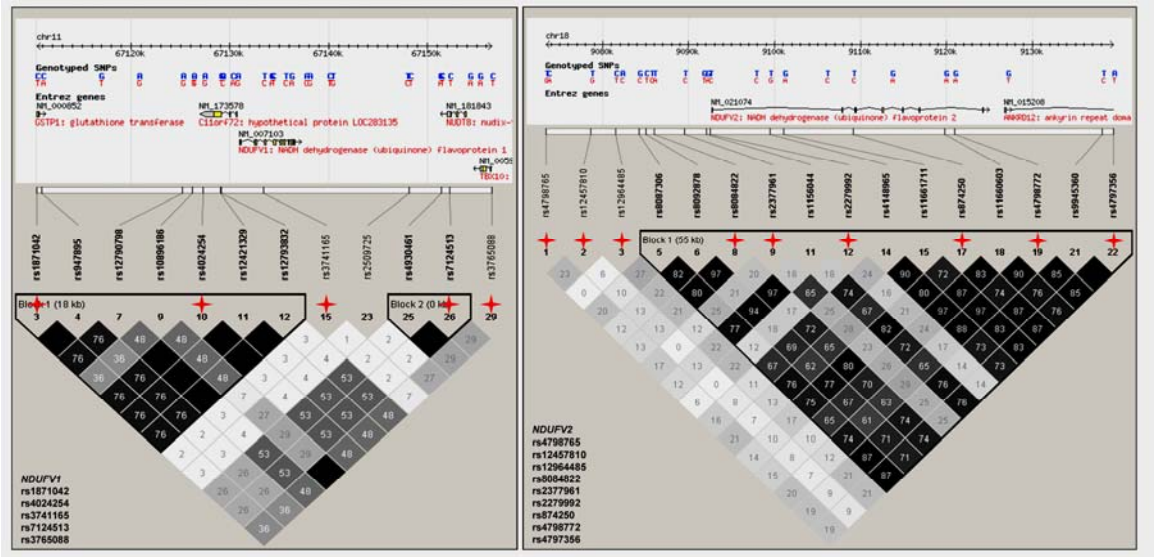
Supplementary Figure 2 Linkage disequilibrium (LD) pattern of the *NDUFS1* gene (Genomic span: Chr2: 206,696,049 to 206,732,432 according to HapMap Data Rel 28 (same as below)) in CHB population from the HapMap database. The genotyped SNPs in this study were marked by red asterisks.



Supplementary Figure 3 Linkage disequilibrium (LD) pattern of the *NDUFS2* (Genomic span: Chr1: 159,435,729 to 159,450,806) and *NDUFS3* (Genomic span: Chr11: 47,557,208 to 47,562,689) genes in CHB population from the HapMap database. The genotyped SNPs in this study were marked by red asterisks.



Supplementary Figure 4 Linkage disequilibrium (LD) pattern of the *NDUFS7* (Genomic span: Chr19: 1,334,906 to 1,346,582) and *NDUFS8* (Genomic span: Chr11: 67,554,685 to 67,560,690) genes in CHB population from the HapMap database. The genotyped SNPs in this study were marked by red asterisks. Three SNPs (rs2074896, rs2074897 and rs2074898) of the *NDUFS7* gene marked in yellow asterisks were genotyped in our previous study and were not included in the current study.



Supplementary Figure 5 Linkage disequilibrium (LD) pattern of the *NDUFV1* (Genomic span: Chr11: 67,130,983 to 67,136,581) and *NDUFV2* (Genomic span: Chr18: 9,092,725 to 9,124,336) genes in CHB population from the HapMap database. The genotyped SNPs in this study were marked by red asterisks.