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A Hypothesis-Driven Association Study of 28 Nuclear-Encoded Mitochondrial Genes with Antipsychotic-Induced Weight Gain in Schizophrenia

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Mitochondria are the main source of energy for neurons and have a role in many vital neuronal functions. Mitochondrial dysfunction has been described in schizophrenia, and antipsychotics such as clozapine and olanzapine have been associated with differences in gene expression in mitochondria. We investigated the hypothesis that nuclear-encoded mitochondrial genes, particularly those involved in oxidative phosphorylation or involved in oxidative stress, mitochondrial biogenesis, inflammation, and apoptosis, would be associated with antipsychotic-induced weight gain (AIWG). In total, we selected 28 genes and analyzed 60 SNPs (50 are functional), in 283 schizophrenia subjects, treated with atypical medications for up to 14 weeks. Association between AIWG (as measured by the % of weight gain from baseline) and SNP genotypes were tested using linear regression with treatment duration, baseline body weight, and medication type as covariates. We observed a significant association between rs6435326 in the *NDUFS1* gene and AIWG in the subset of European patients ($N = 150$, $P_{corrected} = 0.02$). The haplotype carrying the risk alleles of rs6435326 and two other SNPs (rs1053517 and rs1801318) in *NDUFS1* was also nominally associated with percentage of weight gain (T-C-G *vs* A-T-A, $P = 0.005$). In addition, stepwise linear regression was performed to select important variables predictive of the outcome, and a gene–gene interaction analysis was carried out. We observed a significant interaction between the TT risk genotype of rs6435326 in *NDUFS1* and AG genotype of rs3762883 in *COX18* ($P_{corrected} = 0.001$). A permutation-based test of all 60 SNPs jointly showed significant association with weight gain ($P = 0.02$). Finally, our replication study of rs6435326, rs1053517 and rs1801318 in *NDUFS1* using samples from the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) showed that rs1801318 was significantly associated with AIWG ($N = 200$, $P_{corrected} = 0.04$), and the three SNPs were collectively associated with AIWG ($P = 0.04$). In conclusion, our findings suggest an association between *NDUFS1* and AIWG in schizophrenia subjects. To the best of our knowledge, this is the first study to explore genetic variation in the mitochondrial genes in the context of AIWG.

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INTRODUCTION

Antipsychotics are the main treatment for ameliorating the symptoms of schizophrenia, although there is substantial inter-individual variability regarding dose, response and side effects. Antipsychotic-induced weight gain (AIWG) is a common side-effect of many antipsychotics and is particularly pronounced with clozapine and olanzapine contributing to a main reason for non-compliance (Lieberman *et al*, 2005). Twin and family studies have suggested that the heritability of AIWG is between 60% and 80% (Gebhardt *et al*, 2010), indicating that genetic factors have a significant role in its development. Indeed, several genetic and biological systems appear to influence AIWG (reviewed by Muller and Kennedy (2006) and Lett *et al* (2012)). However, the mitochondrial system has been underexplored despite its importance for body energy homeostasis and neuronal functions. Mitochondria are the main source of energy, and

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neurons, with their high level of differentiation and elevated metabolic rate, are very dependent on mitochondrial oxidative phosphorylation (OXPHOS) (Kann and Kovacs, 2007). This process of energy production occurs in the mitochondrial inner membrane through the respiratory chain, which contains five protein complexes. Besides ATP, reactive oxygen species (ROS) are also products of OXPHOS and are involved in intracellular signaling cascades, synaptic transmission and communication between neurons and glia (Kann and Kovacs, 2007).

Antipsychotic medications have been associated with differences in gene expression in mitochondria, although the molecular mechanisms (including a possible genetic predisposition) by which each drug alters mitochondrial function are poorly understood. Studies have suggested that antipsychotics such as haloperidol (first-generation antipsychotic FGA) may inhibit complex I activity of OXPHOS (Balijepalli *et al*, 2001). SGAs, such as clozapine and risperidone, also inhibit complex I activity but at a lower level than haloperidol (Balijepalli *et al*, 2001). Studies involving clozapine suggest that the mechanism by which the drug alters mitochondrial activity appears to involve oxidation of proteins and induction of oxidative stress in both human neuroblastoma cells and lymphoblastoid cell lines from schizophrenia subjects (Walss-Bass *et al*, 2008); (Baig *et al*, 2010). These studies suggest that oxidative stress may be one of the mechanisms by which clozapine increases risk for metabolic syndrome and diabetes (Baig *et al*, 2010). Olanzapine is not known to induce oxidative stress (Reinke *et al*, 2004) but has been associated with decreased fatty acid oxidation, which may predispose subjects to weight gain (Graham *et al*, 2005). Olanzapine has also been associated with increased levels of citrate synthase in pre-frontal cortex, hippocampus, and striatum in rats (Agostinho *et al*, 2011). This enzyme is critical for the Krebs cycle, as it catalyzes the first reaction of the pathway, condensing acyl groups from acetyl-CoA with oxalacetate to yield citrate. Finally, Choi *et al* (2009) compared the gene expression profiles of postmortem liver tissue between FGA and SGA groups of schizophrenia patients. The authors reported that 14 mitochondrial genes were differentially expressed between the two groups with 11 genes downregulated and 3 upregulated.

Given the above, we hypothesized that polymorphisms in genes involved in mitochondrial function may predispose individuals for weight gain after exposure to SGAs. Specifically, we selected 12 nuclear-encoded mitochondrial genes according to their involvement with OXPHOS. Furthermore, we also included 16 genes involved in oxidative stress, mitochondrial biogenesis, inflammation, and apoptosis that we propose may also be part of the mechanism by which SGAs lead to weight gain (Prabakaran *et al*, 2004; Konradi *et al*, 2004; Sun *et al*, 2006; Ben-Shachar and Karry, 2008; Gigante *et al*, 2011; Da Pozzo *et al*, 2012; Scola *et al*, 2013). Compared with the traditional genome-wide association study (GWAS) design that requires very large sample sizes to achieve sufficient power, this hypothesis-driven (HD) approach allowed us to perform an association study with increased statistical power for prioritized genes (Sun *et al*, 2012). Nevertheless, power for any variants at the genome-wide level is limited by the small sample size even with the prioritization. Therefore, instead of using the original GWAS-HD approach proposed by Sun *et al* (2012), we used HD as a general principle focusing on the set of HD-selected 28 genes only.

MATERIALS AND METHODS

Subjects

Patients 18–60 years old with schizophrenia or schizoaffective disorder were diagnosed according to DSM-III-R or DSM-IV criteria and recruited from four different investigators (total sample $N=283$): DJM-1 (Berlin, Germany, $N=87$); HYM (Cleveland, USA, $N=61$); JAL (New York and North Caroline, USA, $N=74$); DJM-2 (Toronto, Canada, $N=61$). A complete description of the study was provided to the participants and written informed consent was obtained in line with each institution's ethics review board guidelines. In the case of DJM-1, patients were prescribed SGAs and assessed for up to 6 weeks. For samples HYM and JAL, patients did not have any prior exposure to SGAs and were treated for 6 weeks, or up to 14 weeks, respectively. For sample DJM-2, patients were prescribed antipsychotic medication and followed up for a minimum of 6 weeks. Demographic and clinical characteristics of the sample stratified by the four sub-samples are provided in Table 1.

Genotyping

Single-nucleotide polymorphism selection. Genomic DNA was extracted from blood samples using the high-salt method (Lahiri and Nurnberger, 1991). For this study, we genotyped only individuals with European ancestry ($N=183$). For the 28 nuclear-encoded mitochondrial genes selected based on our hypothesis, gene boundaries included 5 kb in 5′and 2 kb in 3′UTR regions. For each gene, tagging SNPs were selected using HapMap database, phase2 + phase3, release #28, CEU population, Build36 (www.hapmap.org) and Tagger in Haploview (Barrett *et al*, 2005). The threshold for the minor allele frequency (MAF) was set at 0.05. In total, 64 SNPs were selected according to MAF >0.05 and for tag SNPs, linkage disequilibrium (LD, $r^2 \geqslant 0.8$).

Most of the SNPs selected (50 out of 60 SNPs) in this study were classified as functional according to Haploreg (Ward and Kellis, 2012) and SNPinfo (Xu and Taylor, 2009). Each of the 28 genes has at least one functional SNP. For 8 out of these 28 genes, we also included non-functional SNPs due to two main reasons: 1) The gene had only one functional SNP tagged by our approach and, thus, we decided to include non-functional SNPs for better coverage of these genes. 2) The gene has shown differential gene expression in schizophrenia patients and we decided to take all tag SNPs for them (including both functional and non-functional). Details about the SNP selected are provided in Supplementary Table 1.

Genotyping and quality control (QC). Genotyping was performed on the TaqMan Openarray AccuFill System (Life Technologies, NY, USA) using 64 SNP per array. Briefly, 2.5 μl of DNA (50 ng/μl) was mixed with 2.5 μl of the TaqMan OpenArray Master Mix (40 ×) onto the OpenArrays 384-well sample plate. The OpenArray plate was

Table 1 Demographic and Clinical Characteristics of the Samples of the Study

	DJM-1	HYM	JAL	DJM-2	Total sample	*P*-value
Number of individuals	87	61	74	61	283	
Weight gain (%)	4.0 ± 4.6	4.8 ± 6.3	5.9 ± 7.9	4.8 ± 10.6	4.84 ± 7.22	0.50[a]
Baseline weight (kg)	81.1 ± 15.8	76.5 ± 13.9	82.4 ± 17.5	82.8 ± 18.8	80.5 ± 16.72	0.28[a]
Age (years)	35.2 ± 12.5	33.4 ± 8.9	39.31 ± 8.1	36.9 ± 12.2	36.1 ± 10.7	<0.008
Study duration (weeks)	5.1 ± 1.59	6.00	10.4 ± 4.2	3.7 ± 1.7	6.4 ± 3.45	<0.001[a]
Sex						<0.001
Male	55 (63.2%)	42 (68.9%)	56 (75.7%)	42 (68.9%)	195 (68.9%)	
Female	32 (36.8%)	19 (31.1%)	18 (24.3%)	19 (31.1%)	88 (31.1%)	
Race						
European	85 (98%)	42 (68.9%)	26 (35.1%)	30 (49.2%)	183 (64.7%)	<0.001
Others	2 (2%)	19 (31.1%)	48 (64.9%)	31 (50.8%)	100 (35.3%)	
Medication						
Clozapine	10 (11.5%)	61 (100.0%)	27 (36.5%)	41 (67.2%)	139 (49.1%)	
Olanzapine	15 (17.2%)		22 (29.7%)	8 (13.1%)	45 (15.9%)	
Others	62 (71.3%)		25 (33.8%)	12 (19.7%)	99 (35.0%)	
Individuals genotyped[b]	80	38	17	29	164 (89.6%)	

[a]Kruskal–Wallis test.
[b]Only Europeans were included in the study.

loaded into OpenArray case filled with immersion fluid, sealed with glue and, submitted to PCR. The cycling conditions were: initial denaturing at 93 °C 10 min, following by 50 cycles at 95 °C for 45 s, 94 °C for 13 s and 53 °C for 2 min 14 s, and a final extension at 25 °C for 2 min. After PCR, the OpenArray plate was submitted for image at OpenArray Real-Time PCR Instrument (Biotrove).

Data quality control (QC) excluded four SNPs with poor clustering in allelic discrimination plots. Using PLINK (Purcell *et al*, 2007), none of the remaining SNPs had call rates < 95% or violated Hardy–Weinberg equilibrium ($P < 0.01$). Duplicate samples (15%) were used to check genotyping accuracy, and 100% concordance was observed. QC for individuals excluded 19 samples with > 5% missing genotype rate or high/lower heterozygosity (± 3 SD from the sample mean). In total, 60 SNPs and 164 individuals passed the QC criteria and were kept for further analyses. The exact number of individuals used in each of the statistical analyses varied depending on the missing data rate of the covariates involved in that analysis.

Statistical Analyses

For detecting heterogeneity between study samples, categorical variables were compared using Pearson χ^2 test, and continuous variables were analyzed using Student *t*-test or analysis of variance (SPSS 15.0, SPSS, Chicago, IL, USA). Linear regression was used to test the association, where percentage of weight gain from baseline was the dependent variable, and genotypes, baseline weight, study duration and medication type were predictors. The genotypes were coded additively as 0, 1 and 2, representing the number of copies of the minor allele. The 'medication type' variable included clozapine and olanzapine as highest risk for weight gain (1) and the other drugs as low risk (0, the reference category) (Tiwari *et al*, 2013). The outliers for weight gain were winsorized at the 99% level for better normal approximation of the data (Supplementary Figure 1). UNPHASED version 3.1.5 (Dudbridge, 2003) was used for haplotype comparisons, and all haplotypes with frequency ⩾5% were included in the analyses. Haploview was used to display pair-wise LD between SNPs. Corrections for multiple tests were performed using Single-Nucleotide Polymorphism Spectral Decomposition (SNPSpD) (Nyholt, 2004). Power calculation was performed using Quanto 1.2.4 (Gauderman and Morrison, 2006). Stepwise linear regression was carried out using SPSS to identify predictive variables that influence the outcome (percentage of weight gain from baseline). Gene–gene interaction between rs6435326 and SNPs identified from stepwise linear regression was performed using the *mbmdr* software and significance was calculated using permutation test with 10 000 simulated replicates (Calle *et al*, 2010).

Statistical evaluation of our biological hypothesis. Our hypothesis was that nuclear-encoded mitochondrial genes, particularly those involved in OXPHOS, oxidative stress, mitochondrial biogenesis, inflammation and apoptosis, are more likely to be associated with AIWG in schizophrenia subjects. To evaluate the statistical significance of this hypothesis (ie, does the association evidence of this set of prioritized genes, collectively, exceed what is expected under the null of no association), we performed a permutation-based test as in Sun *et al* (2012). Briefly, the weight gain (%) phenotype and all covariates but the genotype were permuted jointly within the site and independently 1000 times (R; Team RC (2012)). For each permuted sample, corresponding association analysis was

performed and a sum of the Wald association statistic (QUOTE distributed) of the 60 SNPs was obtained. The empirical *P*-value was calculated as the proportion of the permutation samples whose sum of statistic was larger than that in the observed sample.

Replication of the findings. To replicate our findings (ie, rs6435326, rs1053517, and rs1801318 in *NDUFS1*), we used samples from the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) study (Lieberman *et al*, 2005). This sample consisted of 741 chronic schizophrenia patients (18–65 years of age) diagnosed with schizophrenia according to Structured Clinical Interview for DSM-IV (SCID). For our replication study, we selected 200 individuals who satisfied the following criterion: subjects of European ancestry (based on population stratification analysis); subjects with BMI <40 at baseline; subjects not previously treated with olanzapine or clozapine for more than 30 days before the CATIE study trial.

The three SNPs of interest (rs6435326, rs1053517, and rs1801318) were not genotyped in the CATIE GWAS sample. Thus, we imputed the genotypes using IMPUTE v2.2 (Howie *et al*, 2012) with the 1000 Genomes Project Phase 1 (March 2012) as the reference panel. While rs1053517 and rs1801318 had good imputation accuracy (IMPUTE INFO >0.9), rs6435326 had a call-rate $<95\%$, and we used TaqMan SNP Genotyping assays (Applied Biosystems, Foster City, CA) to obtain reliable genotype data for this SNP.

The association analysis was then conducted using a similar method as in the discovery study. Briefly, the phenotype was defined as the percentage of change in BMI from baseline, genotype was defined as number of copies of the minor allele weighted by the genotype imputation posterior probabilities (ie, genotype 'dosage'), and covariates consisted of treatment duration (in days) and medication type (olanzapine as higher risk, and risperidone and quetiapine as lower risk). The multi-SNP joint analysis of all three SNPs was performed using the exact permutation method as described above.

RESULTS

Comparison among the four samples showed statistical differences in several demographic variables, however, we did not observe significant differences in the amount of weight gain across the samples, even after adjustment for duration (DJM-1 4.0 ± 4.6 SD, HYM 4.8 ± 6.3 SD, JAL 5.9 ± 7.9 SD, DJM-2 4.8 ± 10.6 SD, $P=0.20$). Thus, we decided to pool the samples for the analysis. In terms of power, assuming MAF $=0.15$ and sample size of $N=150$ (only individuals with European ancestry and with complete covariates information), we had more than 80% power to detect a mean difference of 2.6% weight gain between carriers and non-carriers of the risk genotype in the additive model at the type 1 error rate of 0.05.

We observed a significant association between weight gain (%) and rs6435326 ($N=150$, $\beta=-2.19$, $P=0.0003$). TT homozygotes gained significantly less weight ($1.1 \pm 4.5\%$ SD; $N=36$) than heterozygotes ($3.1 \pm 4.5\%$ SD; $N=63$) and AA homozygotes ($5.1 \pm 5.1\%$ SD; $N=51$) (Figure 1). This SNP is located in an intronic region of the *NDUSF1* gene

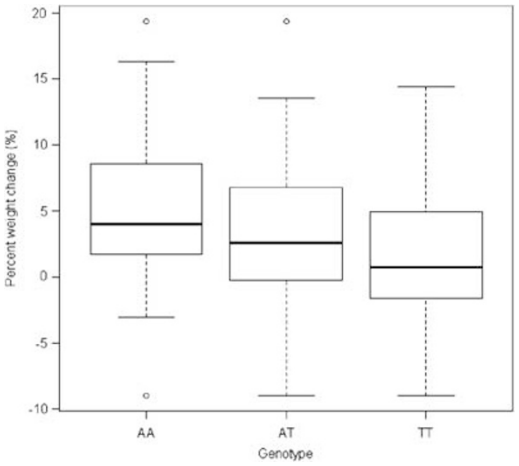

Figure 1 Box-plot graph showing the percentage of weight gain for genotype groups of the SNP rs6435326 in the *NDUFS1* gene. The boxes drawn are the median values for percentage of weight change, and the whiskers drawn correspond to minimum and maximum values observed. The outliers were defined as points more than 1.5 times of upper or lower quartiles.

(NADH dehydrogenase (ubiquinone) Fe-S protein 1, 75 KDa) which is part of the complex I of *OXPHOS*. To correct for multiple comparisons, we calculated the effective number of independent tests using SNPSpD (Nyholt test; Nyholt (2004)). The number of independent tests was 53 and association between rs6435326 and weight gain (%) remained significant after correction ($P_{\text{corrected}}=0.02$), consistent with the permutation-based adjusted *P*-value of 0.02. To dissect the association evidence observed for rs6435326, sample-specific association analysis was performed, separately, for samples DJM-1, HYM, and DJM-2 (Supplementary Table 2). The size of sample JAL was too small for meaningful statistical analysis. The frequencies of minor allele (T) were similar across all three samples and the protective effect of rs6435326 was observed in all three as well (Supplementary Table 2 and Supplementary Figure 2).

Two SNPs, rs1053517 and rs1801318, were moderately correlated with rs6435326 ($r^2=0.5$, $D'=0.95$ and $r^2=0.3$, $D'=1$, respectively) (Figure 2). Haplotype analyses revealed that the block carrying the risk alleles of rs6435326, rs1053517, and rs1801318 was also significantly protective against weight gain (%) (T-C-G vs A-T-A, $P=0.005$). The stepwise linear regression analysis selected rs6435326 in *NDUFS1* and rs3762883 in *COX18*. The gene–gene interaction analysis identified a significant interaction between rs6435326 and rs3762883 (10 000 permutations, $P=0.001$). TT homozygotes at rs6435326 and heterozygotes at rs3762883 were classified as low risk (least weight gain) ($\beta=-5.39$, $P=0.0006$).

Given the small sample size and anticipated modest effect of individual genetic variants associated with this complex trait, it was not surprising that no other SNP, beyond rs6435326 in *NDUSF1*, was significantly associated with weight gain (%). However, alternative analysis that evaluates the cumulative evidence of multiple SNPs can reveal association that might not be detectable at the individual-SNP level. Indeed, our permutation-based test of all 60 SNPs

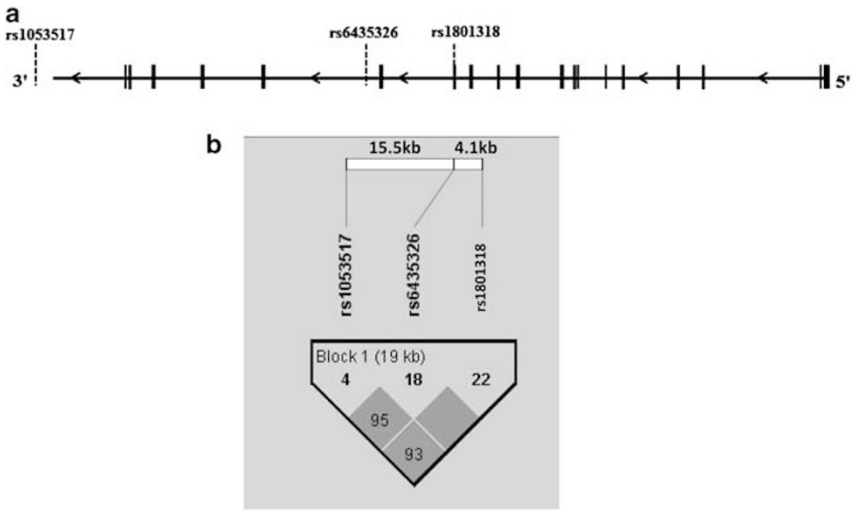

Figure 2 *NDUFS1* gene structure location of polymorphisms (a) and LD graph (b) for the SNPs genotyped. In panel a, the black boxes represent the exons and the width correspond to their sizes. In panel B, the values in the LD plot are D′ values.

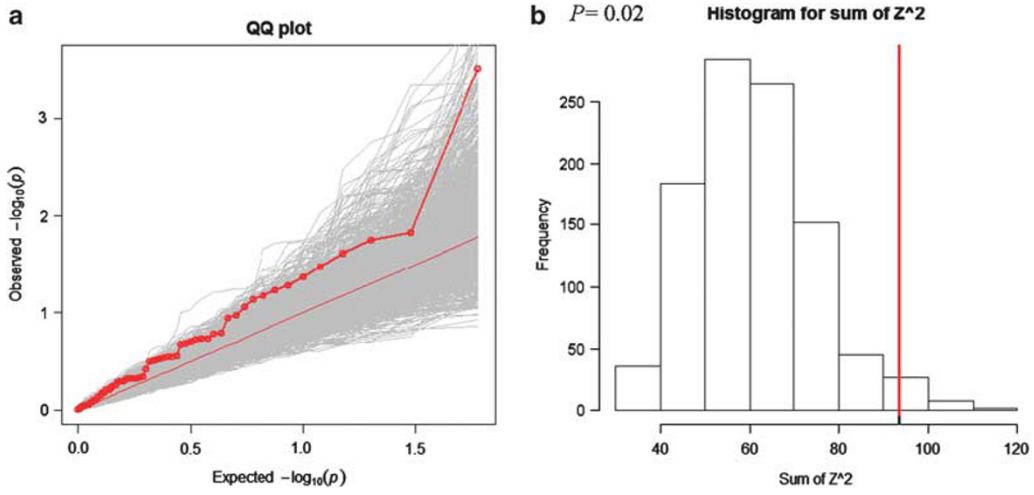

Figure 3 Mitochondria hypothesis testing. (a) QQ plot of the mitochondrial SNPs association evidence in the sample. Red curve correspond to the observed *P*-values. Gray curves correspond to the *P*-values obtained from the 1000 permutation replicates as described in the text. (b) Statistical significance of the mitochondria hypothesis. The statistical significance (permutation $P = 0.02$) was calculated by comparing the observed sum association statistic (red vertical line) aggregated over all 60 SNPs with the sum statistics calculated from the 1000 permutation replicates (shown as the histogram).

Table 2 Replication of the Top Three SNPs

Marker	Gene	MAF	LD[a]	Discovery sample ($n = 164$)		Replication sample ($n = 200$)		
				β	*P*	β	*P*	INFO[b]
rs6435326	*NDUFS1*	0.47	—	−2.19	0.0003	−1.34	0.18	NA
rs1053517	*NDUFS1*	0.43	0.8	−1.45	0.018	−1.98	0.04	0.94
rs1801318	*NDUFS1*	0.30	0.4	−1.47	0.033	−2.29	0.02	0.94

[a]Measured by r^2 between the SNP and rs6435326.

[b]Genotype of rs6435326 was obtained using the TaqMan SNP genotyping assays, and INFO was imputation accuracy measure provided by IMPUTE v2.2 (Howie *et al*, 2012).

jointly showed that the 28 nuclear-encoded mitochondrial genes selected based on our hypothesis, collectively, were associated with weight gain ($P = 0.02$, Figure 3).

In the replication sample, the directions of effect for all top three SNPs in *NDUFS1* (rs6435326, rs1053517, and rs1801318), were consistent with the discovery sample

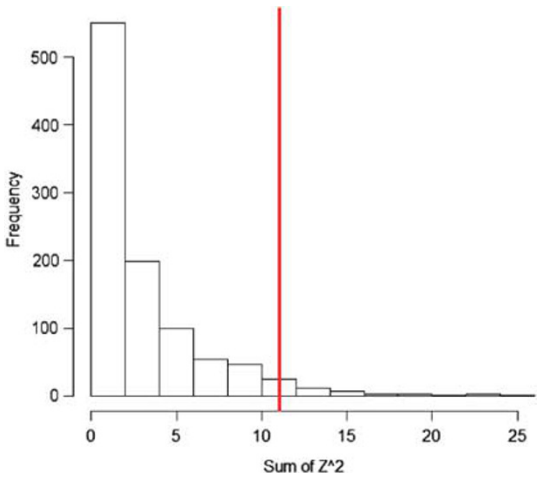

Figure 4 Replication of the association between NDUFS1 and AIWG. The statistical significance ($P_{permutation} = 0.04$) was calculated by comparing the observed sum association statistic (red vertical line) aggregated over the three SNPs within NDUFS1 with the sum statistics calculated from the 1000 phenotype permutation replicates (shown as the histogram).

(Table 2). Two of the three SNPs had replication *P*-values less than 0.05, rs1053517 ($P = 0.04$) and rs1801318 ($P = 0.02$), but only one survived adjustment of multiple hypothesis testing ($< 0.05/2 = 0.025$, where 2 is the number of independent tests among the 3 tests performed). However, the multi-SNP analysis of all three SNPs simultaneously in the CATIE replication sample provided convincing evidence that *NDUFS1* is associated with AIWG ($P_{permutation} = 0.04$, Figure 4).

DISCUSSION

In this study, we analyzed 60 SNPs in 28 nuclear genes involved in mitochondrial function, for association with AIWG, using the HD approach described by Sun *et al* (2012). Our HD analysis is technically different from what was originally proposed in Sun *et al* (2012), but the principle is the same. Here, instead of performing a genome-wide study, we decided to use the HD principle to select a small set of variants for a more focused study, providing sufficient power for these HD-selected variants given the small sample size.

Our hypothesis was that nuclear-encoded mitochondrial genes, particularly those from the *OXPHOS* pathway or those involved in oxidative stress, mitochondrial biogenesis, inflammation and apoptosis, are more likely to be associated with AIWG in schizophrenia subjects. To the best of our knowledge, this is the first study to explore genetic variation in the mitochondrial genes in the context of AIWG. In the single-SNP analysis, we observed significant association between rs6435326 and weight gain (%), even after correction for multiple testing. The haplotype containing the T allele of rs6435326, C allele of rs1053517, and G allele of rs1801318 was also significantly protective against weight gain. These SNPs are located in *NDUFS1*, which is part of the complex I of *OXPHOS*. *NDUFS1* encodes the largest and one of the core subunits of this complex and the protein is located in the iron-sulfur fragment of the enzyme (Smeitink *et al*, 1998). *NDUFS1* is part of the hydrophilic arm of the complex, which is responsible for the transfer of electrons (Finel, 1998; Scola *et al*, 2013). Mutations in this gene have been associated with isolated complex I deficiency (Hoefs *et al*, 2010); and it was proposed that dysfunction in the cellular oxidative metabolism leads to increased mitochondrial ROS (mROS) production (Iuso *et al*, 2006).

The effect of variants on mROS production may be of special importance, as it may influence the energy homeostasis in the hypothalamus. Recently, compelling evidence has shown the role of mROS for 'fuel sensing' in the melanocortin system in the hypothalamus (Leloup *et al*, 2006). Briefly, it was proposed that mROS is an important signaling pathway for stimulation of both the main types of neurons of the arcuate nucleus: 1) those that express anorectic pro-opiomelanocortin (POMC), and 2) orexigenic neurons that contain neuropeptide Y (NPY) and the agouti-gene-related transcript (AgRP). In POMC neurons, leptin starts the process of depolarizing the neuronal membrane via opening of non-specific cation channels and activating the STAT3/JAK2 signaling pathway that leads to increased *POMC* gene expression. Also, during elevated blood glucose levels, glycolysis is active and OXPHOS produces mROS and ATP. It is proposed that the increase in mROS levels is sensitive to the closure of K_{ATP} channels, a crucial step in keeping POMC neurons active. The cleavage of POMC triggers a chain of downstream events that will decrease food intake and increase energy expenditure in humans (reviewed by Horvath *et al* (2009) and Jordan *et al* (2010)). In NPY/AgRP neurons, ghrelin activates the AMPK signaling pathway, leading to inhibition of the acetyl-CoA carboxylase enzyme (ACC). As a consequence, the levels of malonyl-CoA decrease and activate the carnitine_palmitoyl_transferase I (CPT1) enzyme, which is responsible for transport of long-chain fatty acids into the mitochondrial matrix. Lower levels of glucose and high levels of fatty acid in the blood stream stimulate β-oxidation in mitochondria, increasing mROS production that leads to the expression of uncoupling protein 2 (UCP2). This protein acts by buffering mROS to keep gene expression active, allowing NPY to stimulate food intake and reduce energy expenditure. The AgRP acts as an antagonist of melanocortin 3 receptor and melanocortin 4 receptor blocking their anorectic effects (reviewed by Horvath *et al* (2009)).

Our current study also reports the significant interaction between the TT genotype of rs6435326 (NDUFS1) and AG genotype of rs3762883 (COX18). The variant, rs3762883, is predicted to be functional (DNAse hypersensitive, Haploreg v2) and *COX18* is involved in the mitochondrial biogenesis and in the assembly of the complex IV of *OXPHOS* in the inner membrane (Gaisne and Bonnefoy, 2006). Complex IV is responsible for the final transfer of electrons to oxygen. Reduction in activity for both complex I and IV was identified in postmortem brain of schizophrenia subjects (Maurer *et al*, 2001). Thus, it is possible that variants in genes that form complex I and IV may predispose individuals for the side-effect of weight gain after SGA exposure.

In support to our hypothesis, we observed significant association evidence from a multi-SNP model that jointly analyzed all 60 variants in these 28 mitochondrial genes (permutation

$P = 0.02$). The result suggests the involvement of multiple associated variants, each with small effect. A similar situation may exist regarding the polygenetic inheritance reported for schizophrenia (Gottesman and Shields, 1967).

Our study had greater than 80% power to detect the mean differences observed for rs6435326 at the type 1 error level of 0.05. Among the three top SNPs (rs6435326, rs1053517, and rs1801318 in *NDUFS1*), rs6435326 was not statistically significant ($P = 0.18$) in the CATIE replication sample. However, direction of effect is consistent with the discovery sample. Furthermore, the gene-based replication study (joint analysis of all three SNPs) replicated ($P = 0.04$) the finding that *NDUFS1* is associated with AIWG.

In conclusion, our finding suggests the role of *NDUFS1* on AIWG in schizophrenia subjects. Moreover, this study provides evidence implicating the mitochondria as a system involved in the regulation of energy homeostasis and body weight in schizophrenia subjects under SGA treatment.

FUNDING AND DISCLOSURE

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