

A Comprehensive Analysis of Nuclear-Encoded Mitochondrial Genes in Schizophrenia

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ABSTRACT

BACKGROUND: The genetic risk factors of schizophrenia (SCZ), a severe psychiatric disorder, are not yet fully understood. Multiple lines of evidence suggest that mitochondrial dysfunction may play a role in SCZ, but comprehensive association studies are lacking. We hypothesized that variants in nuclear-encoded mitochondrial genes influence susceptibility to SCZ.

METHODS: We conducted gene-based and gene-set analyses using summary association results from the Psychiatric Genomics Consortium Schizophrenia Phase 2 (PGC-SCZ2) genome-wide association study comprising 35,476 cases and 46,839 control subjects. We applied the MAGMA method to three sets of nuclear-encoded mitochondrial genes: oxidative phosphorylation genes, other nuclear-encoded mitochondrial genes, and genes involved in nucleus-mitochondria crosstalk. Furthermore, we conducted a replication study using the iPSYCH SCZ sample of 2290 cases and 21,621 control subjects.

RESULTS: In the PGC-SCZ2 sample, 1186 mitochondrial genes were analyzed, among which 159 had p values $< .05$ and 19 remained significant after multiple testing correction. A meta-analysis of 818 genes combining the PGC-SCZ2 and iPSYCH samples resulted in 104 nominally significant and nine significant genes, suggesting a polygenic model for the nuclear-encoded mitochondrial genes. Gene-set analysis, however, did not show significant results. In an *in silico* protein-protein interaction network analysis, 14 mitochondrial genes interacted directly with 158 SCZ risk genes identified in PGC-SCZ2 (permutation $p = .02$), and aldosterone signaling in epithelial cells and mitochondrial dysfunction pathways appeared to be overrepresented in this network of mitochondrial and SCZ risk genes.

CONCLUSIONS: This study provides evidence that specific aspects of mitochondrial function may play a role in SCZ, but we did not observe its broad involvement even using a large sample.

Keywords: Gene-gene interaction, GWAS-HD, MAGMA, Mitochondria, Oxidative phosphorylation, Schizophrenia, Stratified FDR

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Schizophrenia (SCZ) is a severe psychiatric disorder for which the underlying causes are far from fully understood. The brain is highly dependent on oxidative metabolism as the primary source of energy to maintain its functions (1), and a disturbance in brain energy metabolism has been suggested as part of the pathogenesis of SCZ (2). Studies have indicated that abnormalities in glucose oxidative metabolism may contribute to an individual's susceptibility of developing psychosis (1,3). Indeed, there is compelling evidence that patients with SCZ have impaired glucose metabolism, with decreased metabolic rates and reduced blood flow in the frontal cortex exhibited during cognitive tasks (4–7).

Impaired oxidative metabolism suggests mitochondrial dysfunction mainly because these organelles are the primary sources of aerobic energy via oxidative phosphorylation (OXPHOS) for neuronal function. Furthermore, it has been shown that mitochondria play an important role in cellular processes that are disturbed in SCZ, such as synaptic

transmission (8–10) and oxidative stress (11). Comorbidity between mitochondrial diseases and neuropsychiatric symptomatology has also been reported (12,13).

SCZ has a strong genetic basis, with estimated heritability around 80% (14). However, few studies aim specifically to discover mitochondrial loci associated with SCZ, and consequently, genetic data supporting the association of mitochondrial loci with SCZ are lacking. There is some evidence that mitochondrial genes play a role in SCZ [for a review, see Hjelm *et al.* (9)]. For example, the most recent results from the Psychiatric Genomics Consortium Schizophrenia Phase 2 (PGC-SCZ2) genome-wide association study (GWAS) (35,476 cases and 46,839 control subjects), the largest dataset to date used for genetic association studies of SCZ, reported evidence for 22 nuclear-encoded mitochondrial genes (15), but mitochondria-related pathways showed no significant association with the disease in another large study of SCZ (9379 cases and 7736 control subjects) (16). Further evidence

includes enrichment of expression quantitative trait loci (17), and an excess of large (>500 Kb) deletions, and rare copy number variants in nuclear-encoded mitochondrial genes in individuals with SCZ (18).

Here we tested the hypothesis that variants in nuclear-encoded mitochondrial genes influence SCZ risk. Because mitochondrial genes have unique features (being encoded by both the nuclear and mitochondrial genomes) and contain highly connected signaling pathways (for maintenance of mitochondria homeostasis), we performed both gene-based and gene-set/pathway analyses.

Of particular interest is the set of nuclear-encoded OXPHOS genes, the mitochondrial pathway that produces adenosine triphosphate (ATP) for neuronal functioning. This specific pathway has been more intensively investigated in SCZ, mainly due to the critical role of mitochondrial reactive oxygen species in triggering oxidative stress. SCZ patients have been reported to show increased levels of oxidative stress (2), and the SCZ-oxidative stress association has been noted in functional studies (19). Moreover, there is compelling evidence linking reactive oxygen species with immune-inflammatory pathways (20,21), which have also been implicated in SCZ (22,23).

Specific nuclear-encoded OXPHOS genes physically interact with other subunits encoded by the mitochondrial DNA. Therefore, the mitochondria and nucleus must coordinate transcription, translation, import, and function of mitochondrial protein complexes (24). Disturbances in this crosstalk may lead to deficiencies in the mitochondrial ATP production process, causing or contributing to disease.

We defined three sets of genes to test our hypothesis that variants in mitochondrial or mitochondria-related genes influence SCZ risk: 1) nuclear-encoded OXPHOS genes, 2) other nuclear-encoded mitochondrial genes (excluding the OXPHOS genes), and 3) genes involved in the crosstalk between the nucleus and mitochondria (25). Using the MAGMA tool (26) alongside a hypothesis-driven GWAS (GWAS-HD) approach (27), we aimed to 1) identify and replicate new mitochondrial susceptibility loci for SCZ, 2) evaluate the significance of the three mitochondrial gene sets in SCZ, and 3) use *in silico* tools to predict the biological processes involving mitochondrial genes identified in this study, to potentially reveal novel abnormal cellular circuits in SCZ.

METHODS AND MATERIALS

Samples

We used the summary association statistics available from the most recent PGC-SCZ2 GWAS (15) to perform our analyses in the discovery sample. The original quality control steps for 35,476 SCZ cases and 46,839 control subjects from 52 cohorts, and detailed association analyses of 9,444,230 single nucleotide polymorphisms (SNPs) are described elsewhere (15).

To replicate findings, we used the iPSYCH GWAS data (www.ipsych.au.dk) with 2290 SCZ cases and 21,621 control subjects. Subjects were genotyped using the Illumina Infinium PsychChip v1.0 (Illumina, Inc., San Diego, CA) in accordance with the manufacturer's instructions. The total number of genotyped SNPs was 588,454 (28). Preimputation quality control steps included removal of SNPs with minor allele

frequency <0.05 and Hardy-Weinberg equilibrium $p < 10^{-6}$ in control subjects, and removal of individuals with call rate <95% and (cryptic) first- and second-degree relatives using KING (v1.9) (29). The data were then phased using Shapeit3 (v2.r837) (30) and imputed using IMPUTE2 (v2.3.2) (31), with the 1000 Genomes Project Phase 3 as the reference panel. Postimputation quality control included removal of SNPs with INFO score <0.2, minor allele frequency <0.001, genotype missing rate >10%, and Hardy-Weinberg equilibrium $p < 10^{-6}$, resulting in a total of 8,842,045 SNPs for analysis.

Nuclear-Encoded Mitochondrial Genes and Mitochondria-Related Gene Sets

The OXPHOS gene set started with 95 nuclear-encoded genes selected using KEGG (32). All 95 were listed in the MitoCarta 2.0 release 2015 (33), a database of human genes with strong evidence for mitochondrial location based on computational and microscopy evidence. In total, 89 genes tagged by 6686 PGC-SCZ2 GWAS SNPs formed the OXPHOS gene set.

The mitochondrial gene set originally included 1158 nuclear-encoded genes downloaded from MitoCarta. Two genes encoded in the extended major histocompatibility complex (34) were excluded; the major histocompatibility complex region is known to be highly associated with SCZ and was thus excluded for a conservative analysis. After removal of these genes, 1038 genes tagged by 131,502 SNPs remained, and they formed the mitochondrial gene set (excluding the OXPHOS genes defined by the gene set above).

The nucleus-mitochondria crosstalk gene set included 76 genes selected based on previous literature (24,35–37). These genes were tagged by 18,781 SNPs. One gene also appeared in the OXPHOS group, and 16 genes appeared in the mitochondrial group. We decided to keep these overlapping genes in both groups because they may be part of several different cellular processes/circuits involving mitochondria.

In total, 155,843 unique SNPs were located within the genomic region of 1186 unique mitochondrial or mitochondria-related genes; only SNPs within transcribed regions were included to minimize overlapping of SNPs between multiple genes and inclusion of null SNPs (38).

MAGMA Gene-Based and Gene Set Analyses

The PGC-SCZ2 GWAS p values (after the genomic control [GC] adjustment) were the primary input data for MAGMA (v1.06) with the 1000 Genomes Project Phase 3 (Build 37/European data only) used as the reference panel. We calculated the linkage disequilibrium score intercept for the PGC-SCZ2 GWAS data and found a value of 1.052, suggesting a slight inflation even after correction for the polygenic nature of SCZ. Thus, we applied the conservative GC correction to the original SNP association results before conducting our analyses, ensuring that the remaining enrichment was genuinely due to a polygenic model.

The MAGMA gene-based results (using the SNP-wise option) were then corrected for multiple testing of the 1186 mitochondrial genes using the Bonferroni method. MAGMA gene-set competitive analysis [with default parameter values and correcting for potential confounders and linkage disequilibrium score between genes (26)] was then performed to examine if any of the three mitochondrial gene sets was

enriched in variants associated with SCZ (three hypotheses tested).

Hypothesis-Driven GWAS

Given that the MAGMA method only analyzed the defined genes and gene sets, we used the GWAS-HD and stratified false discovery rate (sFDR) (27) to complement the MAGMA analysis. GWAS-HD re-evaluates (upweight or downweight through the sFDR control) all individual SNP associations genome wide, based on the prior information contained in the biological hypothesis. SNPs annotated to the three mitochondrial gene sets were assigned to three high-priority strata, and the remaining SNPs formed a separate (low priority) stratum. We emphasize here that SNP stratification must be done a priori independent of the p values to be stratified. The sFDR method improves power if the prior information is informative; the high-priority group(s) indeed contains proportionally more truly associated SNPs as compared with no stratification (39).

Replication Study

To replicate association between individual SNPs and SCZ, a list of 657 SNPs (with sFDR q value $< .05$) were sent to the iPSYCH Mitochondria Group investigators in Denmark. The same MAGMA approach used in the discovery PGC-SCZ2 sample was applied to the iPSYCH SCZ sample to replicate the gene-based findings. A meta-analysis using Fisher's method to combine the discovery and replication gene-based p values was performed. Genes with p values less than the conservative Bonferroni correction threshold were declared significant.

Mitochondrial Gene Expression in the Brain

We used the Allen Human Brain Atlas to explore the expression of the associated genes across human brain regions to look for additional evidence of their potential role in SCZ. Microarray data for 6 subjects (4 men and 2 women 18 to 68 years of age with no known neuropsychiatric or neuropathological history) were downloaded from Allen Human Brain Atlas (<http://human.brain-map.org/static/download>) in July 2017. The Atlas used a custom design Agilent 8x60K array that included the 4x44K Agilent Whole Human Genome probe set supplemented with an additional 16,000 probes. As probes for the same gene were highly correlated, only one probe per gene was selected to avoid collinearity. This probe selection was based on expression levels, with moderate expression levels defined conservatively as >6 ; the median expression for all genes across the brain for the 6 subjects was 4.99 and the 75th quartile was 7.13.

Differential Expression of Genes in SCZ

The .bam files were obtained through the CommonMind Consortium Knowledge Portal (<https://www.synapse.org/CMC>) (the largest RNA sequencing dataset for human dorso-lateral prefrontal cortex) with permission and analyzed at University of California, Irvine (40). Data from 10 duplicate samples and 89 individuals with incomplete demographic information were excluded. The analysis included 514 samples and the following factors were used to correct for differences between brain samples: pH, age, postmortem interval, RNA integrity index, batch, institution, and the manner of death.

The linear regression model included the following terms in R package (glm) (R Project for Statistical Computing, Vienna, Austria): Gene Expression (log counts per million) = $\beta_0 + \beta_1 \times$ (diagnosis) + $\beta_2 \times$ (brain pH) + $\beta_3 \times$ (postmortem interval) + $\beta_4 \times$ (age) + $\beta_5 \times$ (sex) + $\beta_6 \times$ (RIN) + $\beta_7 \times$ (institution) + $\beta_8 \times$ (cause of death) + ϵ .

Log counts per million values were obtained from RNA sequencing read counts divided by the corresponding library size (in millions) to yields counts per million (41). Postmortem interval is in hours, RNA integrity number was obtained from Agilent BioAnalyzer, and institution was coded for each of three sites (Icahn School of Medicine at Mount Sinai, University of Pittsburgh, University of Pennsylvania).

Gene-Gene Interaction and Overrepresentation Analyses

Genes that belong to the same molecular networks and cellular pathways are often involved in the pathophysiology of the same or similar conditions (41). Therefore, we mapped functionally relevant physical interactions using an in silico protein-protein interaction (PPI) network analysis. We tested gene-gene interactions among genes that code for interacting proteins, particularly the 19 nuclear-encoded mitochondrial genes significantly associated with SCZ in the discovery sample using a gene-based approach, as well as the 327 genes in the 108 genome-wide significant loci reported in the original PGC-SCZ2 study (15).

We first built a PPI network with the 327 SCZ risk genes (of which 10 were not recognized by the program for encoding microRNAs) to visualize how these genes interact among each other in a functional network using GeneMANIA Cytoscape Plugin (Cytoscape_v3.5.1) (42). These initial 327 genes were referred as seed genes. To visualize and analyze topological parameters of the network, we used the Cytoscape platform (43) and the CentiScape plugin (44). After constructing the network, we then used GeneNets toolbox in MATLAB (R2015a; The MathWorks, Inc., Natick, MA) (45) to determine whether the connectivity among the 327 seed genes was statistically significant compared with a randomly selected network of similar size (taking into account number of genes and genes sizes).

We then used GeneNets to investigate whether this network formed by 327 seed genes (the SCZ risk genes) significantly connect with the backbone genes (i.e., mitochondrial genes considered significant in this study), using the default seed randomization option. We called the network built from this analysis the mito-SCZ network. Mitochondrial and SCZ genes physically interacting in the mito-SCZ network were selected for gene-set overrepresentation analysis in the GeNets software (Broad Institute of MIT and Harvard, Cambridge, MA).

GeNets is a web platform (<http://apps.broadinstitute.org/genets>) for analysis and interpretation of genetic variation in the context of gene-gene networks (46). GeNets allowed us to visualize genes that interact with each other as well as their communities (i.e., a group of genes relatively more connected with each other in a network), while taking into account the biological process related to the genes. Additional candidate genes could be predicted as well. This software investigates

which pathways within the gene set of interest are enriched, using a Bonferroni-adjusted hypergeometric test. In our study, only gene sets from the Molecular Signatures Database were used.

GeNets also includes Quack (<https://www.biorxiv.org/content/biorxiv/early/2017/10/04/196303.full.pdf>), a machine learning algorithm that can be trained to find nontrivial pathway relationships between genes of interest. The hypothesis is that genes in a common pathway would share pathway-specific topological properties (connectivity profiles) that systematically distinguish them from genes that are not part of the pathway under analysis. The algorithm generates a confidence score (from 0 to 1) that ranks each gene; genes with higher scores have greater probability being related to the pathway under analysis.

Last, we used Ingenuity Pathway Analysis software (v33559992, release date March 28, 2017; QIAGEN Bioinformatics, Redwood City, CA) with default parameter values to determine whether the genes in the mito-SCZ network were enriched for specific biological pathways. The p values were adjusted using the default option, i.e., the FDR-adjusted p (Benjamini-Hochberg method) (47). We used the lenient FDR control to identify as many candidate pathways as possible while still maintaining an acceptable false positive rate.

RESULTS

Gene-Based and Gene-Set Analysis

We used MAGMA to conduct gene-based analysis of the 1186 OXPHOS, mitochondrial, and nucleus-mitochondria crosstalk genes using PGC-SCZ2 as our discovery sample. In total, 159 mitochondrial genes had MAGMA gene-based

$p < .05$, 19 of which survived Bonferroni correction (Table 1). In total, 818 mitochondrial genes had data in both the PGC-SCZ2 discovery and iPSYCH replication samples, and meta-analysis resulted in 104 genes with gene-based $p < .05$ and nine with $p < .05/818$ (Bonferroni correction) (Table 2). Notably, among 1000 randomly selected sets of 818 genes from the nuclear genome, the numbers of gene-based $p < .05$ ranged from 66 to 123, with a mean of 92.15 (first quartile of 84, median of 92.5, and third quartile of 98). Among the total of 1000 replicates, there were 46 replicates with counts ≥ 104 (the value observed in the set of nuclear encoded mitochondrial genes), resulting in an empirical $p = .046$ for the enrichment analysis of truly associated genes. This finding suggests that the polygenic model known for the nuclear genome appears to hold for the set of nuclear-encoded mitochondrial genes analyzed. Furthermore, q-q plots, expressed on a $-\log_{10}(p)$ scale in Figure 1, show evidence for enrichment, with an estimated proportion of associated genes of 3%, 8%, and 13% for the discovery, replication, and combined samples, respectively. These estimates are subject to sampling variation (48). We also note that the q-q plot for a set of p values with a higher estimated proportion of truly associated variants (e.g., Figure 1B for the replication sample) could look more “flat” than Figure 1A for the discovery sample, if the corresponding statistical effect sizes (which depends on both genetic effect size of a variant and the sample size) differ.

With respect to gene-set analysis, none of the three sets of genes showed statistically significant enrichment of association based on MAGMA, with gene-set p values equal to .40, .64, and .19 for the OXPHOS, mitochondrial excluding

Table 1. Mitochondrial Genes Significantly Associated With SCZ Risk at the $p < .05$ Level After the Conservative Bonferroni Correction for 1186 Multiple Hypothesis Tests

Symbol	CHR	Start	Stop	No. of SNPs Annotated	No. of Relevant Parameters Used in the Model	n	ZSTAT	p Value	Correct _p ^a
<i>APOPT1</i>	14	104029299	104057236	59	17	82,315	5.9449	1.38E-09	.000002
<i>C12orf65</i>	12	123717844	123742651	68	11	82,315	5.8275	2.81E-09	.000003
<i>HSPE1-MOB4</i>	2	198364721	198418423	63	16	82,315	5.5313	1.59E-08	.000019
<i>C2orf69</i>	2	200775979	200792996	43	13	82,315	5.5011	1.89E-08	.000022
<i>C2orf47</i>	2	200820040	200828848	19	10	82,315	4.8352	6.65E-07	.0008
<i>PCCB</i>	3	135969167	136056737	148	43	82,315	4.8174	7.27E-07	.0009
<i>ATPAF2</i>	17	17921334	17942483	25	11	82,315	4.7175	1.19E-06	.0014
<i>HSPA9</i>	5	137890571	137911318	37	16	82,315	4.5898	2.22E-06	.0026
<i>DNAJA3</i>	16	4475806	4506776	71	29	82,315	4.3822	5.87E-06	.0070
<i>NT5DC2</i>	3	52558385	52569093	26	8	82,315	4.34	7.12E-06	.0084
<i>LETM2</i>	8	38240041	38267045	36	13	82,315	4.3314	7.41E-06	.0088
<i>NDUFA6</i>	22	42481530	42486888	9	5	82,315	4.3137	8.03E-06	.0095
<i>HSPD1</i>	2	198351308	198364998	18	7	82,315	4.2994	8.56E-06	.01
<i>DNAJC19</i>	3	180701497	180707562	4	4	82,315	4.267	9.91E-06	.01
<i>COQ10B</i>	2	198318231	198339851	20	6	82,315	4.1766	1.48E-05	.02
<i>SMDT1</i>	22	42475695	42480288	5	3	82,315	4.1677	1.54E-05	.02
<i>FOXO3</i>	6	108881026	109005972	171	32	82,315	4.1328	1.79E-05	.02
<i>HSPE1</i>	2	198364721	198368187	2	2	82,315	4.0746	2.3E-05	.03
<i>ABC9</i>	12	123405498	123451056	42	18	82,315	4.0223	2.88E-05	.03

CHR, chromosome; SCZ, schizophrenia; SNPs, single nucleotide polymorphisms; ZSTAT, Z value for the gene.

^a p values after the conservative Bonferroni correction.

Table 2. List of Nine Mitochondrial Genes Significantly Associated With SCZ Risk Through Meta-analysis at the $p < .05$ Level After the Conservative Bonferroni Correction for 818 Multiple Hypothesis Tests

Symbol	CHR	Start	Stop	Discovery			Replication			Meta-analysis	
				<i>n</i>	ZSTAT	disc_ <i>p</i>	<i>n</i>	ZSTAT	rep_ <i>p</i>	Fisher_Combined_ <i>p</i>	Correct_ <i>p</i> ^a
<i>HSPE1-MOB4</i>	2	198364721	198418423	82,315	5.5313	1.59E-08	23,911	-0.683	.7527	2.30E-07	.0002
<i>C2orf47</i>	2	200820040	200828848	82,315	4.8352	6.65E-07	23,911	0.55423	.2897	3.17E-06	.0026
<i>SMDT1</i>	22	42475695	42480288	82,315	4.1677	1.54E-05	23,911	2.0424	.0206	5.05E-06	.0041
<i>ATPAF2</i>	17	17921334	17942483	82,315	4.7175	1.19E-06	23,911	0.49159	.3115	5.88E-06	.0048
<i>PCCB</i>	3	135969167	136056737	82,315	4.8174	7.27E-07	23,911	-1.4194	.9221	1.02E-05	.0083
<i>NDUFA6</i>	22	42481530	42486888	82,315	4.3137	8.03E-06	23,911	1.2234	.1106	1.33E-05	.0108
<i>HSPA9</i>	5	137890571	137911318	82,315	4.5898	2.22E-06	23,911	-0.24631	.5973	1.93E-05	.0158
<i>DNAJA3</i>	16	4475806	4506776	82,315	4.3822	5.87E-06	23,911	0.35949	.3596	2.97E-05	.0243
<i>FOXO3</i>	6	108881026	109005972	82,315	4.1328	1.79E-05	23,911	0.69366	.2440	5.83E-05	.0477

CHR, chromosome code; disc, discovery sample; rep, replication sample; SCZ, schizophrenia; ZSTAT, Z value for the gene.
^a*p* values after the conservative Bonferroni correction.

OXPPOS, and nucleus-mitochondria crosstalk gene sets, respectively. This result could be a true negative, although genetic heterogeneity may also drive these nonsignificant findings.

GWAS-HD Analysis

In total, summary statistics of 9,444,230 PGC-SCZ2 GWAS SNPs were available, among which 155,843 unique SNPs were located within the genomic region of 1186 mitochondrial genes as defined above. GC-corrected *p* values were then used for the GWAS-HD analysis. Specifically, FDR control was simultaneously applied to the four strata of SNPs: stratum 1, containing 6686 OXPPOS SNPs; stratum 2, containing 131,502 mitochondrial SNPs; stratum 3, containing 18,781 nucleus-mitochondria crosstalk SNPs (1126 overlap with stratum 2); and stratum 4, containing the remaining 9,239,959 GWAS SNPs, excluding the major histocompatibility complex region as discussed before.

In total, 728 mitochondrial SNPs had an sFDR *q* value < .05, including 86 SNPs previously reported as genome-wide

significant [$p < 5 \times 10^{-8}$ (49)] (Supplemental Table S1). The GWAS-HD significant SNPs mapped to 54 genes, of which all had MAGMA gene-based *p* < .05, including the 19 genes declared significant in the PGC-SCZ2 discovery sample using our approach.

Of the 728 mitochondrial SNPs, 657 with rs numbers (indels variants excluded) were used for replication analysis using the independent iPSYCH SCZ sample. In total, 40 SNPs had meta-analysis *p* value < .05, 35 of which demonstrated an effect in the same direction as in the PGC-SCZ2 discovery sample (Table 3). These 35 SNPs were located within five genes (*APOPT1*, *NDUFA6*, *SMDT1*, *MSRA*, and *CAMKK2*), all of which showed at least nominally significant MAGMA gene-based association with SCZ in our discovery sample.

Mitochondrial Gene Expression in the Brain

All 19 mitochondrial genes associated with SCZ in this study were expressed at significant levels in human brain tissue (Supplemental Figure S1), and 17 showed particularly high

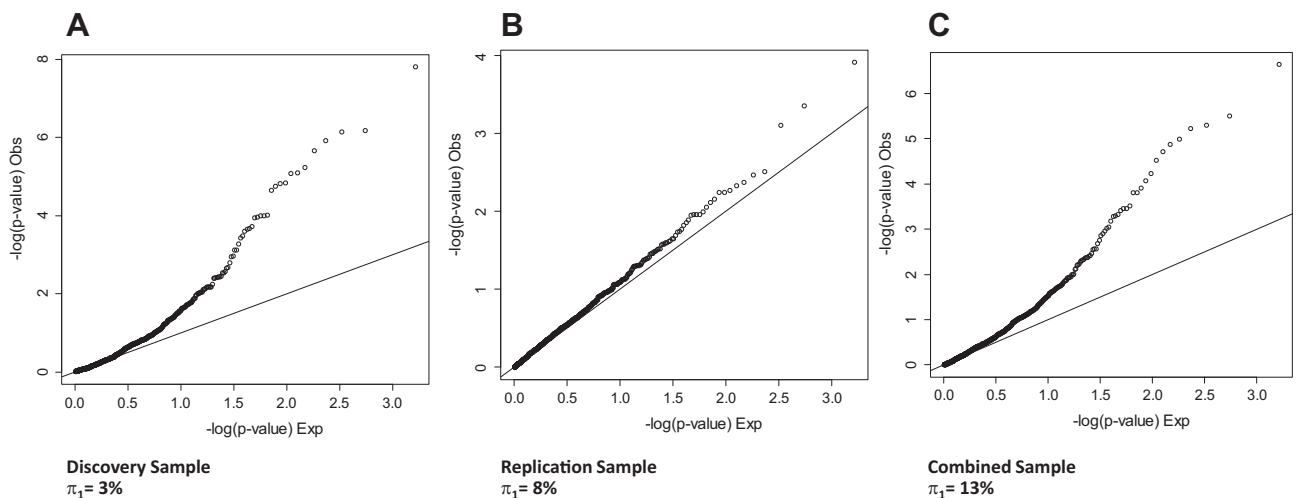


Figure 1. The q-q plot for the (A) discovery, (B) replication, (C) and combined discovery and replication samples. The estimated proportion of associated genes (π_1) is shown. Exp, expected; Obs, observed.

Table 3. List of 35 SNPs Replicated in the iPSYCH SCZ Sample

SNP	CHR	POS	A1	A2	Discovery				Replication				Meta-analysis	
					<i>n</i>	OR	SE	<i>p</i> Value	<i>n</i>	OR	SE	<i>p</i> Value	meta_P	corrected_meta_P
rs10431750	14	104040065	T	C	82,315	-	0.0115	2.11E-09	23,371	-	0.03401	.03	2.67E-10	9.35E-09
rs1063843	12	121681687	T	C	82,315	+	0.0131	4.37E-04	23,911	+	0.03833	.02	2.26E-05	7.92E-04
rs12887734	14	104046834	T	G	82,315	+	0.0117	7.03E-10	23,229	+	0.03413	.03	1.06E-10	3.70E-09
rs12889403	14	104034746	T	C	82,315	+	0.0116	7.06E-09	23,562	+	0.034	.04	1.25E-09	4.38E-08
rs12889721	14	104034863	T	C	82,315	+	0.0116	9.54E-09	23,575	+	0.03399	.05	2.06E-09	7.20E-08
rs12890820	14	104034849	T	C	82,315	-	0.0116	5.71E-09	23,584	-	0.03399	.05	1.19E-09	4.17E-08
rs12894729	14	104035292	A	G	82,315	+	0.0116	8.18E-09	23,556	+	0.03401	.04	1.47E-09	5.13E-08
rs1653591	12	121681138	A	G	82,315	+	0.0131	1.69E-04	23,840	+	0.03844	.02	9.59E-06	3.35E-04
rs2071711	22	42477650	C	G	82,315	-	0.0114	3.55E-05	23,842	-	0.03409	.02	1.92E-06	6.72E-05
rs2269524	22	42475703	T	G	82,315	+	0.0113	2.75E-05	23,845	+	0.03404	.02	1.61E-06	5.64E-05
rs2274267	14	104029449	A	G	82,315	+	0.0117	1.14E-08	23,418	+	0.03404	.03	1.35E-09	4.71E-08
rs2296482	14	104029819	T	G	82,315	-	0.0116	7.01E-09	23,708	-	0.03387	.02	6.78E-10	2.37E-08
rs2403197	14	104053764	T	C	82,315	+	0.0115	1.89E-09	23,205	+	0.03424	.05	4.27E-10	1.49E-08
rs2668247	12	121684176	C	G	82,315	-	0.0131	1.69E-04	23,819	-	0.03848	.02	1.02E-05	3.57E-04
rs2686340	12	121682051	T	C	82,315	+	0.0131	1.81E-04	23,742	+	0.03855	.02	9.45E-06	3.31E-04
rs2686342	12	121685188	A	T	82,315	-	0.0131	2.17E-04	23,785	-	0.03846	.02	1.34E-05	4.70E-04
rs2686344	12	121690548	T	C	82,315	+	0.0125	4.96E-04	22,506	+	0.03729	.02	2.95E-05	1.03E-03
rs2686345	12	121690555	T	C	82,315	+	0.0125	5.01E-04	22,506	+	0.03729	.02	2.99E-05	1.04E-03
rs34026011	14	104050883	A	G	82,315	+	0.0115	1.12E-09	23,205	+	0.03418	.03	1.90E-10	6.66E-09
rs35026580	14	104042753	A	G	82,315	-	0.0115	1.96E-09	23,316	-	0.03405	.02	1.93E-10	6.74E-09
rs35229468	14	104052057	T	C	82,315	+	0.0115	1.97E-09	23,212	+	0.03421	.04	3.45E-10	1.21E-08
rs35496194	14	104031351	T	C	82,315	+	0.0117	2.66E-09	23,651	+	0.03395	.04	5.42E-10	1.90E-08
rs4147640	22	42485292	T	C	82,315	+	0.0113	1.94E-05	23,850	+	0.03405	.02	1.23E-06	4.29E-05
rs4147641	22	42482502	C	G	82,315	+	0.0113	2.43E-05	23,846	+	0.03405	.02	1.56E-06	5.47E-05
rs4906336	14	104033265	C	G	82,315	-	0.0116	5.17E-09	23,558	-	0.034	.05	1.13E-09	3.94E-08
rs4906337	14	104040414	A	C	82,315	+	0.0115	2.12E-09	23,349	+	0.03403	.02	2.13E-10	7.47E-09
rs4906338	14	104050357	A	G	82,315	-	0.0115	1.25E-09	23,173	-	0.03419	.03	1.90E-10	6.66E-09
rs58033365	14	104030901	T	C	82,315	-	0.0116	6.50E-09	23,711	-	0.03389	.03	8.01E-10	2.80E-08
rs6002592	22	42476754	A	C	82,315	+	0.0113	3.46E-05	23,844	+	0.03404	.02	2.04E-06	7.13E-05
rs6002596	22	42484466	A	G	82,315	-	0.0114	1.36E-05	23,846	-	0.03406	.02	8.94E-07	3.13E-05
rs7140568	14	104041374	T	C	82,315	+	0.0115	2.12E-09	23,335	+	0.03404	.02	1.97E-10	6.88E-09
rs71417868	14	104046592	A	T	82,315	-	0.0115	1.32E-09	23,237	-	0.03411	.03	1.83E-10	6.40E-09
rs73191548	8	10035997	A	G	82,315	+	0.0109	6.21E-05	23,451	+	0.03213	.04	6.73E-06	2.36E-04
rs8017628	14	104047428	T	C	82,315	+	0.0115	1.44E-09	23,233	+	0.03413	.03	2.24E-10	7.85E-09
rs8017993	14	104047734	A	G	82,315	-	0.0115	1.69E-09	23,217	-	0.03417	.03	2.78E-10	9.73E-09

A1, allele 1; A2, allele 2; CHR, chromosome code; OR, odds ratio; POS, chromosome location; SCZ, schizophrenia; SE, standard error; SNP, single nucleotide polymorphism.

levels of expression in the thalamic, midbrain, and striatal areas. Importantly, these brain regions are known to be altered in SCZ (50). For 17 of these 19 genes, we observed moderate-to-high levels of log₂ expression (log₂ score >6) across the human brain (Supplemental Table S2). The two other genes had moderate to high expression levels in specific areas such as the frontal lobe for *ABCB9* or the cerebellar cortex for *LETM2*.

Differential Gene Expression of Replicated Genes in SCZ

Of the nine replicated genes shown in Table 2, three were downregulated in a nominally significant manner in SCZ compared with control subjects (Table 4).

PPI Network and Gene Set Overrepresentation Analysis

The PPI analysis of the 337 SCZ risk genes showed a connected network including 327 seed genes and 5790 interactions (edges in Supplemental Figure S2) between them. The randomization method showed that the connectivity among 327 genes was statistically significant ($p = .034$, 1000 randomizations). The greatest number of interactions between genes in the network was found for those with coexpression, (i.e., two genes showing similar expression levels in the Gene Expression Omnibus database, suggestive of coexpression) (Supplemental Figure S2).

In examining the interaction between the 19 mitochondrial genes (backbone genes) with the 327 SCZ risk genes

Table 4. Fold Change of Replicated Mitochondria Genes in the Dorsolateral Prefrontal Cortex From the CommonMind Consortium

Symbol	Average Expression (CPM)	<i>t</i>	<i>p</i> Value	Fold Change (Schizophrenia/Control Subjects)	FDR ^a
<i>NDUFA6</i>	5.91	-2.13	.03	-1.21	0.25
<i>PCCB</i>	5.78	-2.73	.01	-1.19	0.14
<i>SMDT1</i>	4.28	-2.86	.00	-1.23	0.12

Three mitochondria genes shown in Table 2 were nominally significantly decreased in the expression for schizophrenia compared with control subjects for the CommonMind Consortium dorsolateral prefrontal cortex after correction for brain pH, age, postmortem interval, RNA integrity number, batch, institution, and the manner of death.

CPM, counts per million; FDR, false discovery rate.

^aBenjamini-Hochberg-adjusted *p* value.

(seed genes), we observed that 14 mitochondrial genes were connected with 158 SCZ genes in a significant network after the randomization test ($p = .023$, 1000 randomizations) (Figure 2 and Supplemental Figure S3). The genes *DNAJC19*, *HSPA9*, *PCCB*, and *HSPE1* showed the greatest number of connections among mitochondrial genes, interacting with many genes of the SCZ network.

For the GeNets analysis of functional connectivity among the 14 mitochondrial genes and 158 SCZ genes, 70 additional candidates were included to the network, based on their connectivity with our input genes (selected by the software). Of the 242 genes examined, 138 genes were connected in a significant network ($p = .002$), of which 18 were mitochondrial. Moreover, mitochondrial genes comprised one of the largest communities in the network (Community 7; Supplemental Figure S4). For this community, two significant canonical

pathways were overrepresented: 1) respiratory electron transport and 2) ATP synthesis. Furthermore, the gene *NDUFA6* from our study was one of the genes included in this community (Supplemental Table S4). Additionally, GeNets classified the mitochondrial genes *DRG2*, *NDUFA2*, *NDUFA6*, and *NDUFA13* as the most important genes for the network based on Quack (Supplemental Table S5).

Finally, the Ingenuity Pathway Analysis of the 14 mitochondrial genes and the 158 SCZ genes (mito-SCZ network) found canonical biological pathways related to mitochondrial function as overrepresented in this network (after FDR correction). Particularly, mitochondrial dysfunction and aldosterone signaling in epithelial cells were most overrepresented (Supplemental Table S6).

DISCUSSION

Regions of the genome robustly associated with schizophrenia by the PGC-SCZ2 GWAS provide a framework for secondary analysis to identify important biological pathways and help to shed further light on disease etiology. Although there were 22 mitochondrial genes among the top PGC-SCZ2 GWAS hits, the mitochondrial system is not yet widely considered as a pathophysiologic mechanism in SCZ. To the best of our knowledge, the study presented here is the first to examine explicitly the role of mitochondria-related genes in SCZ.

Using MAGMA gene-based analysis, we identified 19 mitochondrial or mitochondria-related genes significantly associated with SCZ at gene level, all of which were corroborated in a separate GWAS-HD sFDR analysis. Of the 19 significant genes identified in the PGC-SCZ2 sample, 12 were from the mitochondrial gene set, five were from the nucleus-mitochondria-crosstalk gene set, and two were from the OXPPOS gene set. We note that the significance here is

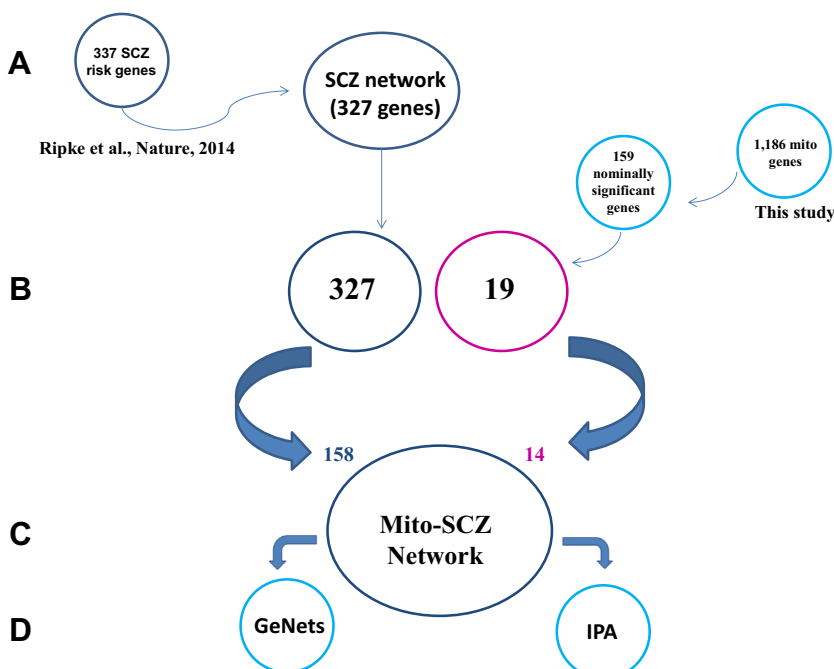


Figure 2. Protein-protein interaction analysis workflow. (A) Protein-protein interaction analysis of 337 schizophrenia (SCZ) risk genes (15) showed a significant connected network including 327 of these genes ($p = .03$). (B) These 327 genes were further investigated using GeneNets and found to be connected in a network with significant mitochondrial genes from our study ($n = 19$). (C) A total of 158 SCZ risk genes were connected with 14 mitochondrial genes in a significant network ($p = .02$) (mito-SCZ network). (D) Pathways and biological processes associated with the mito-SCZ network were investigated using GeNets and Ingenuity Pathway Analysis (IPA) software.

defined based on Bonferroni correction for the 1186 genes tested using MAGMA. However, eight genes overlapped with those declared as genome-wide significant loci by the PGC-SCZ2 GWAS; the remaining 11 novel genes only passed the less conservative threshold as defined here. We did not include regulatory regions in our gene-based analysis and it remains to be explored further in the future.

Meta-analysis of 818 genes with data in both the PGC-SCZ2 (discovery) and iPSYCH (replication) samples identified 104 genes with meta-analysis $p < .05$, and nine were significant after Bonferroni correction for multiple testing.

The q-q plots of gene-based p values in Figure 1 were suggestive of enrichment of variants associated with SCZ. This is supported by the stratified q-q plots of SNP-based p values in Supplementary Figure S5 where the estimated proportion of associated SNPs was 3% for the mitochondrial gene set (GC $\lambda = 1.04$), and 4% for the nucleus-mitochondria crosstalk gene set ($\lambda = 1.10$), as compared with 0.01% for the protein-coding control group ($\lambda = 0.99$). The SNPs in the OXPHOS gene set, however, did not show apparent enrichment ($\lambda = 0.78$, 0.00%).

However, we did not find enrichment for the three mitochondrial gene sets tested when we used the MAGMA pathway analysis tool. One possible explanation is that the mitochondrial system includes many internal pathways that are connected with several distinct cellular circuits. Thus, future studies need to be more refined in terms of defining the mitochondria-internal pathways to be tested.

We then performed a network-based analysis to explore the biological significance of our findings. Our in silico investigations suggested that 14 mitochondrial genes were functionally connected with risk genes for SCZ and identified several biological processes in which mitochondrial genes were involved in SCZ (respiratory electron transport, respiratory electron transport/ATP synthesis, and aldosterone signaling in epithelial cells).

Of interest, *HSPA9* is a chaperone involved in protein quality control, folding, and degradation, and it has been associated with Alzheimer's disease (51). The expression of *DNAJC19* has also been found to be downregulated in the motor cortex and thalamus of patients with autism (52). The involvement of respiratory electron transport and ATP synthesis pathways support impaired OXPHOS functioning in SCZ subjects, which has already been proposed as part of the etiology of the disease (11).

The aldosterone signaling pathway is involved in the regulation of mineral metabolism. Interestingly, aldosterone is thought to disinhibit *N*-methyl-D-aspartate receptors due to its role in the excretion of magnesium ions (Mg^{2+}), which may reduce intracerebral Mg^{2+} levels (53). *N*-methyl-D-aspartate receptor hypofunction might explain (at least in part) common clinical manifestations of SCZ including cognitive deficits, impaired memory and learning, positive-negative symptoms, and executive dysfunction (54).

NDUFA6 was the mitochondrial gene with the highest Quack score (0.104). This gene has been found to be downregulated in bipolar patients and has a central role in energy metabolism and mitochondrial function (55). *NDUFA6* is also downregulated in the analysis of CommonMind Consortium dorsolateral prefrontal cortex expression in SCZ compared with control subjects. Another gene, *DRG2*, is a SCZ risk gene

found to be involved in the regulation of mitochondrial morphology and function (56).

Furthermore, we examined whether the mitochondrial genes connect directly with the SCZ genes, which are considered important for disease etiology or treatment (15). We found that *DNAJC19*, *HSPA9*, and *PCCB* had high connectivity with the following SCZ genes: *ATP2A2* (calcium signaling), *CNTN4* (neuronal network formation and plasticity), and *FXR1* (neurodevelopment).

We have also identified four nucleus-mitochondria crosstalk genes listed as associated with SCZ in our gene-based study. These genes are involved with the mitochondrial unfolded protein response. This pathway is activated under mitochondrial stress and induces the expression of protective genes (chaperones and proteases) to re-establish protein homeostasis within mitochondria. It may also result in the maintenance of mitochondria with heteroplasmic deleterious mitochondrial DNA variants (57).

In conclusion, our study was able to identify novel nuclear-encoded mitochondrial and mitochondria-related (nucleus-mitochondria crosstalk) genes associated with SCZ using a gene-based approach. We did not obtain significant gene-set findings, potentially limited by low signal-to-noise ratio, but we were able to provide supporting evidence for the variants' association from a GWAS-HD analysis of individual SNPs. Our in silico analysis of the top findings suggests that mitochondrial pathways should be further explored in SCZ research. Importantly, our approach can be applied, with relative ease, to other complex diseases with a hypothesized mitochondrial component such as bipolar disorder, Alzheimer's disease, Parkinson's disease, and diabetes.

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