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Archival Report

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

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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