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The somatic common deletion in mitochondrial DNA is decreased in schizophrenia

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ABSTRACT

Large deletions in mitochondrial DNA (mtDNA) can occur during or result from oxidative stress leading to a vicious cycle that increases reactive oxygen species (ROS) damage and decreases mitochondrial function, thereby causing further oxidative stress. The objective of this study was to determine if disease specific brain differences of the somatic mtDNA common deletion (4977 bp) could be observed in major depressive disorder (MDD), bipolar disorder (BD), and schizophrenia (SZ) compared to a control group.

The accumulation of the mtDNA common deletion was measured using a quantitative assay across 10 brain regions (anterior cingulate cortex, amygdala, caudate nucleus, dorsolateral prefrontal cortex, hippocampus, nucleus accumbens, orbitofrontal cortex, putamen, substantia nigra, and thalamus).

The correlation with age of the mtDNA deletion was highly significant across brain regions as previously shown. A significant decrease in the global accumulation of common deletion in subjects with SZ compared to MDD, BD, and controls was observed after correcting for age, pH, PMI, and gender. The decreases in SZ were largest in dopaminergic regions. One potential side effect of antipsychotic drugs on mitochondria is the impairment of mitochondria function, which might explain these findings.

The decreased global brain mtDNA common deletion levels suggests that mitochondrial function is impaired and might be part of an overall mitochondria dysfunction signature in subjects with schizophrenia.

1. Introduction

Mitochondrial functions include the production of adenosine triphosphate (ATP), maintenance of cytosolic calcium homeostasis, production and containment of reactive oxygen species (ROS), and modulation of apoptosis. These diverse mitochondrial functions are essential for neurotransmission, short- and long-term neuronal plasticity, apoptosis, aging, and behavioral adaptation. Thus, dysfunctions in these metabolic processes can play a role in a wide variety of brain diseases including mood disorders and SZ.

Deletions in mtDNA appear more frequently in tissues with high metabolic rates, such as brain and muscle (Piko et al., 1988; Corral-Debrinski et al., 1992; Melov et al., 1999; Pak et al., 2003; Fuke et al., 2008). MtDNA deletions can vary in size leading to decreased energy production, and they accumulate with age (Piko et al., 1988; Shoffner et al., 1989; Cortopassi and Arnheim, 1990; Corral-Debrinski et al., 1991; Ballinger et al., 1992; Corral-Debrinski et al., 1992; Cortopassi et al., 1992; Kato and Takahashi, 1996; Kato et al., 1997; Meissner et al., 1997; Meissner et al., 1999; Melov et al., 1999; Fuke et al., 2008). The mitochondrial common deletion is a 4977 bp deletion, spanning mtDNA 8470–13,447, and flanked by two 13 bp perfect repeats (Samuels et al., 2004). This common deletion (CDel) encompasses several tRNAs as well as genes encoding subunits of cytochrome oxidase, NADH dehydrogenase and ATP synthase. We and others have shown that levels of the CDel are increased in BD compared to controls, but not in SZ (Shao et al., 2008; Sequeira et al., 2012). A recent study (Torrell et al., 2013) observed higher levels of CDel in occipital cortex of MDDs and SZ compared to BD and controls, however, their results were not significant and they did not control for age, which is highly correlated with levels of the CDel in many prior studies of brain and other metabolically active tissues.

Multiple lines of evidence (transcriptomic, proteomic, neuroimaging, in vitro, peripheral tissue, genetic, and animal studies) implicates mitochondrial dysfunction in mood disorders (Kato and Kato, 2000; Bezchlibnyk et al., 2001; Konradi et al., 2004; Choudary et al., 2005; Iwamoto et al., 2005; Washizuka et al., 2005; Ryan et al., 2006; Sun et al., 2006; Vawter et al., 2006; Kato et al., 2007; Shao et al., 2008; Rao et al., 2010). Recent studies have provided evidence that mtDNA sequence variants, and subsequent mitochondrial dysfunction, are associated with an increased incidence of depression (Gardner et al., 2003; Burnett et al., 2005; Fattal et al., 2006; Vawter et al., 2006; Gardner and Boles, 2008, 2011; Shao et al., 2008; Rollins et al., 2009; Anglin et al., 2012; Inczedy-Farkas et al., 2012), suggesting that

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mitochondrial alterations might play a role in MDD. Our own mtDNA studies have consistently showed transcriptomic alterations in postmortem brain of subjects with mood disorders and SZ (Vawter et al., 2006; Shao et al., 2008; Rollins et al., 2009; Sequeira et al., 2012). Additionally, studies of isolated mitochondria from patients with mood disorders, using peripheral tissues, have reported functional differences in calcium dynamics and energy production (Emamghoreishi et al., 1997; Emamghoreishi et al., 2000; Gardner et al., 2003; Kakiuchi et al., 2003; Kato et al., 2003; Washizuka et al., 2003; Iwamoto et al., 2004a; Iwamoto et al., 2004b; Wasserman et al., 2004; Washizuka et al., 2005; Naydenov et al., 2007; Gardner and Boles, 2008; Xu et al., 2008).

In this study we compared the mtDNA common deletion levels in SZ, BD, and MDD to normal controls, taking into account age, gender, pH and PMI, to determine disease specific changes in the common deletion levels across 10 brain regions involved in neuropsychiatric disorders. We hypothesized that mood disorder subjects would show increased CDel and that SZ would show no change based upon our prior work (Shao et al., 2008; Rollins et al., 2009; Sequeira et al., 2012).

2. Methods

2.1. Post-mortem human brains

Our sample consisted of 40 post-mortem human brains: MDD (N = 10), BD (N = 10), SZ (N = 10), and psychiatrically normal controls (N = 10) (Table 1, Supplemental Table 1). Brains were obtained from the University of California, Irvine Brain Bank (UCIBB). These brains have been characterized using the UCIBB psychological autopsy protocol which is based largely on procedures validated by Kelly and Mann (1996). A partial list of medications that were prescribed to subjects with psychiatric disorders (Supplemental Table 1) showed little consistency in medications and dosages.

For each subject, 10 brain regions were dissected: anterior cingulate cortex (ACC), amygdala (AMY), caudate nucleus (CAUN), dorsolateral prefrontal cortex (DLPFC), hippocampus (HIPP), nucleus accumbens (NACC), orbitofrontal cortex (OFC), putamen (PUT), substantia nigra (SN), and thalamus (THAL). These regions were chosen due to their involvement in neuropsychiatric disorders.

2.2. DNA extraction

DNA was extracted from 30 mg of post-mortem brain tissue using the DNEasy Blood and Tissue DNA extraction kit (Qiagen). DNA concentration was measured using a spectrophotometer and standardized working solutions of 10 ng/μl were aliquoted in 96-well plates for ease of use during PCR.

2.3. Common deletion assay

Quantitative real time PCR (qPCR) was performed on the 7900HT real time instrument (Applied Biosystems) and two sets of primers designed around the large 4977 bp common deletion region (between 8224 and 13,501) to amplify and quantify by SYBR green both the wild type and the deleted mtDNA. The deleted mtDNA sequence was amplified using DelFwd: 5′-AATTCCCCTAAAAATCTTTGAAAT-3′ and WTDelRev: 5′-AACCTGTGAGGAAAGGTATTCCTGC-3′. The wild-type mtDNA sequence was amplified using WTFwd: 5′-AGGCGCTATCACCA CTCTGTTCG-3′ and WTDelRev. A common deletion copy number standard curve constructed using cloned standards (outlined in Sequeira et al., 2012; Shao et al., 2008) ranging from 1,000,000 copies/μl to 100 copies/μl for wild type mtDNA, CDel, and single copy gene (albumin). Furthermore, detection of the common deletion was confirmed using both gel electrophoresis and Sanger sequencing.

2.4. Common deletion analysis

The percent common deletion (%CDel) was calculated (outlined in Sequeira et al., 2012; Shao et al., 2008) (Fig. 1) using the following formula: %CDel = (deletion copy number) / (deletion copy number + wild type copy number) × 100. Differences in the distribution of the levels of the mitochondrial %CDel across brain regions and between diagnostic groups were carried out using Partek Genomics Suite. We conducted and analysis of covariance (ANCOVA) using PMI, pH, and age as a covariates, and diagnosis and brain region as main effects.

3. Results

3.1. Mitochondrial DNA common deletion

In total we assayed 399 of the 400 samples taken from the 40 brains included in the study. One caudate sample was eliminated due to it being an outlier. When comparing demographic variables between groups, a small difference was found in pH between BD and controls ($p = 0.03$), no other significant difference was observed for the other demographic variables. There were no significant differences between diagnoses for post-mortem interval (PMI) or gender (Table 1). Among the covariates investigated, age was highly correlated to levels of %CDel in each brain region (Table 2), while pH and PMI showed no significant correlations with %CDel in any brain region. Because we observed a significant difference in brain pH between BD and controls, while %CDel is significantly correlated with age, knowing that there is a significant correlation of %CDel with gender (Sabunciyan et al., 2007; Fuke et al., 2008), and since PMI can be used as a proxy for autolytic degradation, all these variables (age, gender, PMI, and pH) were included in the ANCOVA model. Thus, when controlling for potential confounders we found a significant effect of Diagnosis on %CDel ($p = 0.0086$) and of Region ($p = 3.5 \cdot 10^{-036}$) (Table 3). The highest average levels of %CDel are found in the caudate, putamen, nucleus accumbens and the substantia nigra, three regions containing dopaminergic cell bodies or projection areas of dopaminergic axons, in line with the association between dopamine neurotransmission and accumulation of mtDNA deletions previously described (Bender et al., 2008) (Fig. 2).

To determine potential diagnostic specificity, post-hoc comparative analyses were conducted. The SZ group displayed lower average global levels of %CDel when compared to each group (MDD $p < 0.001$), BD ($p < 0.02$), and controls ($p < 0.01$) (Fig. 3). Since the Diagnosis × Region interaction was not significant, we did not conduct a post-hoc testing of each region by diagnosis which would have increased the correction for the number of comparisons. The largest decreases between SZ and controls were observed in CAUN, SN, and PUT, followed by AMY, ACC, and DLPFC. There was a significant correlation between age

Table 1
Demographic details of the post-mortem subjects. When comparing the demographic variables between groups, pH was found to be significantly different between BD and C ($p = 0.03$).

Diagnosis (N)	Females (n)	Males (n)	Mean age (standard deviation)	Mean pH (standard deviation)	Mean PMI (standard deviation)
BD (10)	5	5	52.4 (13.2)	6.52 (0.42)	22.98 (7.30)
C (10)	3	7	48 (13.0)	6.15 (0.19)	20.52 (8.25)
MDD (10)	7	3	47.3 (11.5)	6.40 (0.39)	24.76 (7.34)
SZ (10)	5	5	45.6 (9.0)	6.44 (0.43)	22.13 (6.38)

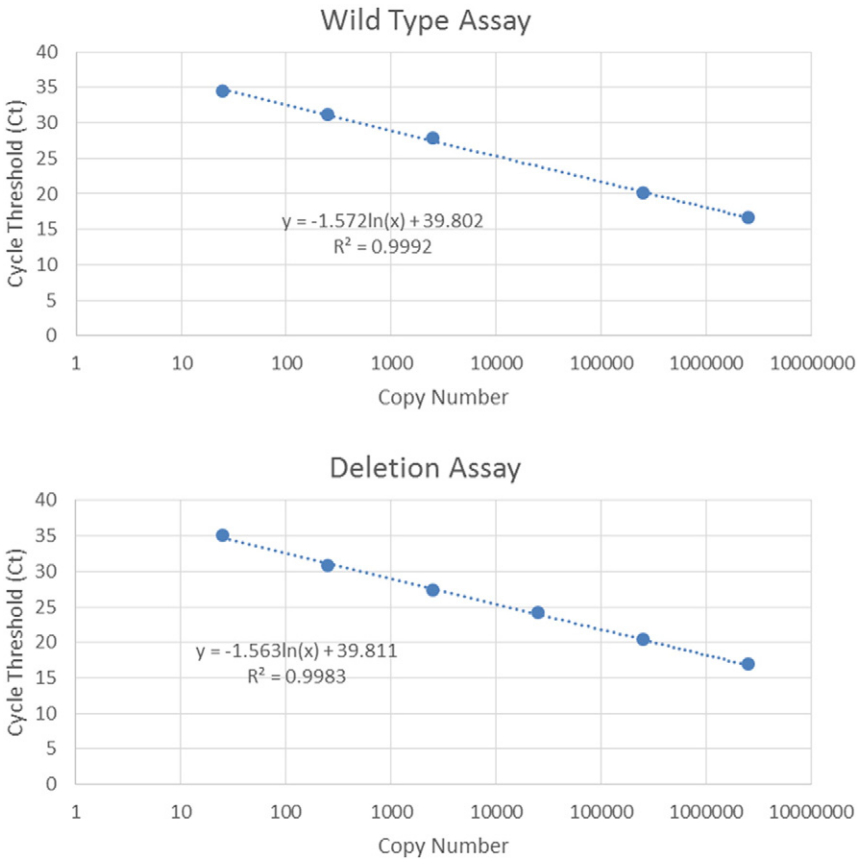

Fig. 1. Graph of cycle thresholds versus mitochondrial copy number for the wild type and deletion assays employed in the study to assess percent common deletion.

and common deletion in controls ($r = 0.8$), but not in subjects with SZ ($r = 0.2$), and the difference between the correlations was significant ($p = 0.01$). This lack of an age-related increase in the common deletion in brain replicates an earlier study of individuals with SZ (Cavelier et al., 1995).

Variability in the amount of initial mitochondrial DNA across diagnoses was taken into account by direct determination of genomic DNA, using qPCR and primers directed towards the single copy gene, human albumin. A clone of the single gene albumin was used to construct a standard curve for quantitative analyses. We analyzed the possible main effects on the ratio of copy number of mtDNA/copy number genomic DNA and found no effects on this ratio with the Diagnosis, or Diagnosis × Region factors. For the ratio of copy number of mtDNA/copy number genomic DNA there was a significant effect of Region observed ($p < 0.0001$). Thus, the decreased amount of %CDel was not due to alterations in the starting amounts of mtDNA copy number normalized to genomic DNA copy number.

Table 2
Correlations between age, pH, and PMI with percent common deletion across brain regions. Only age was significantly correlated in each brain region with the common deletion; the p-values in bold are significant at $p < 0.05$. The PMI and pH were not significantly correlated with %CDel in any of 10 brain regions tested.

Brain region	Age		pH		PMI	
	r	p-Value	r	p-Value	r	p-Value
ACC	0.33	**0.02**	0.05	0.73	−0.08	0.63
AMY	0.52	**0.0002**	−0.05	0.72	−0.11	0.46
CAUN	0.60	**0.00001**	−0.03	0.87	0.01	0.94
DLPFC	0.64	**0.0000019**	−0.04	0.81	−0.10	0.51
HIPP	0.47	**0.000895**	−0.21	0.17	−0.20	0.20
NACC	0.50	**0.0004**	0.22	0.14	−0.07	0.65
OFC	0.64	**0.000001**	−0.09	0.57	−0.01	0.94
PUT	0.46	**0.001**	0.12	0.45	−0.03	0.87
SN	0.63	**0.000002**	0.13	0.38	−0.02	0.90
THAL	0.51	**0.0003**	−0.11	0.47	−0.02	0.90

4. Discussion

Overall, the global levels of the common deletion in SZ were significantly decreased, compared to two other psychiatric groups and controls. There was no significant Diagnosis × Region interaction on common deletion levels; however the largest decreases in common deletion found in SZ compared to controls appeared most prominently in the caudate nucleus, putamen, and substantia nigra. These brain regions displaying the largest absolute decreases in the common deletion in SZ are part of the striato-nigral dopamine pathway, thus raising the question of the existence of alterations in dopamine signaling and the association between dopamine targeted antipsychotic medication and impairment of mitochondrial function.

The common deletion has been extensively studied in brain with regard to aging (Cortopassi et al., 1992; Melov et al., 1999), Alzheimer's disease (Lezza et al., 1999; Bender et al., 2008), Parkinson's disease (Bender et al., 2006) and BD and SZ (Kato et al., 1997; Kakiuchi et al.,

Table 3
Main effects included in the ANCOVA model. The effect of Diagnosis was significant, while interaction of brain region × Diagnosis was not significant.

ANCOVA interactions and main effects	p-Value
Diagnosis	0.009
Age	4.83×10^{-12}
Gender	0.496
pH	0.003
Post mortem interval	0.328
Brain region	3.59×10^{-36}
Brain region × Diagnosis	0.414

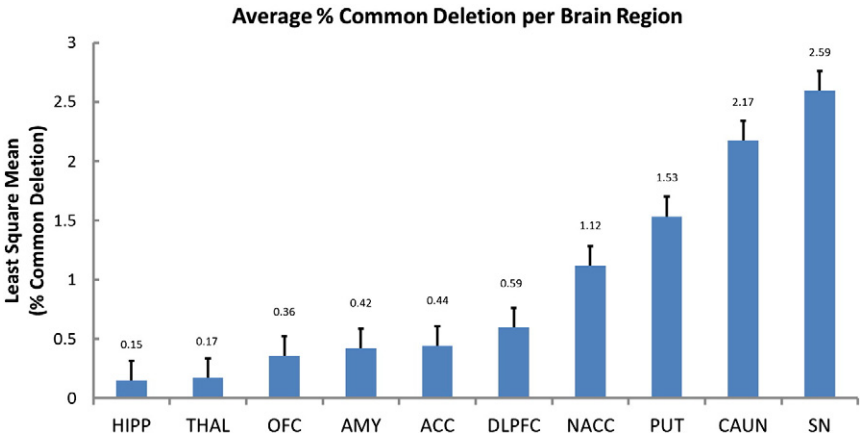

Fig. 2. The ten brain regions analyzed in this study showed an approximate 15 fold range of variation. The percentages are derived from copy number clones for wild type mtDNA and deleted copies.

2005; Sabunciyan et al., 2007; Fuke et al., 2008). Kato and colleagues first reported a significant association between increased common deletion levels and BD (Kato et al., 1997). Our results are consistent with a strong effect of age related increase of the common deletion in controls in brain. Data from our laboratory and others have shown only minimal increases in the level of the common deletion in blood with aging. The observation that the brain accumulates the common deletion with age, suggests that the brain carries larger mitochondrial deficits across the life-span (Corral-Debrinski et al., 1992; von Wurmb et al., 1998; Mohamed et al., 2004; Meissner et al., 2008; Shao et al., 2008; von Wurmb-Schwark et al., 2010). This was well characterized by Corral-Debrinski et al. (1992) when they examined CDel levels across cortical regions, the putamen and, cerebellum of control subjects with ages ranging from 24 to 94. They found greater levels or CDel in the putamen compared to the cortex, while the cerebellum demonstrated the lowest levels, as well as a strong correlation of CDel accumulation with age. Studies in psychiatric disorders have shown mixed results with regard to an age-related accumulation of CDel with several groups providing a lack of age-related accumulation in SZ (Cavelier et al., 1995; Kakiuchi et al., 2005; Sabunciyan et al., 2007; Fuke et al., 2008). In a third independent cohort reported in this paper, we have shown that considering all 40 subjects in one analysis, the human mitochondrial DNA genome accumulates the common deletion with age in brain tissue, at perhaps rates that are regionally dependent. However, when we compare the difference in correlation coefficients of common deletion and aging between schizophrenia and the control group, the control group correlation is significantly higher. This lack of an age-related accumulation of the common deletion in SZ, and the observed significant global decrease in SZ across multiple regions might be part of the signature of mitochondrial dysfunction in SZ, consistent with multiple other reports.

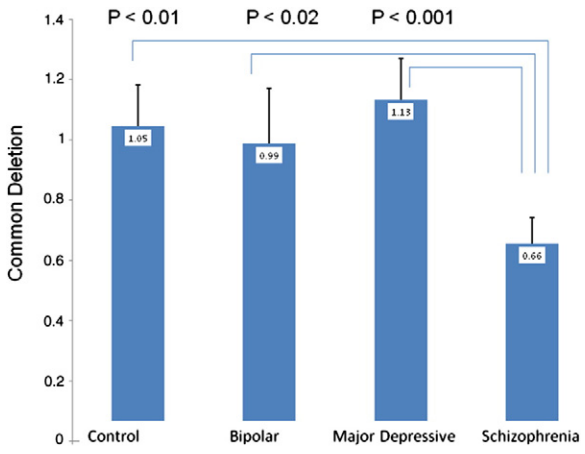

Fig. 3. Bar graph of mean %CDel across diagnoses. Each bar is the average %CDel for each diagnosis. Significant differences between SZ and each diagnostic group, and the p-value, are displayed above each bar.

Previous studies investigating the mitochondrial CDel performed thus far in SZ have been performed in few brain regions, with the frontal cortex being the predominant region. A study by Cavelier et al. (1995) investigated the levels of common deletion in the frontal cortex and caudate nucleus of subjects with Alzheimer's disease, SZ and controls. In SZ they observed differences in the amount of mitochondrial CDel across the two brain regions, with there being higher levels in the caudate nucleus compared to the frontal cortex, in concordance with the results of our independent cohort. Fuke et al. (2008) utilized a similar methodology as in this manuscript to investigate levels of CDel in the frontal cortex of subjects with BD, SZ, and controls. The authors found no significant association of CDel accumulation with either diagnosis, however, females presented with greater accumulation of the deletion compared to males. Similar to these results by Fuke and colleagues, Sabunciyan et al. (2007) found no significant difference in the amount of CDel present in the frontal cortex of BD, SZ and controls; however, no effect of gender was seen.

An important factor to consider when quantifying levels of the mitochondrial CDel is the amount of total mtDNA present and how variability in this quantity could bias the observed levels of CDel. Studies have demonstrated the existence of variability in amounts of mtDNA between brain regions, however, this does not translate into differences between psychiatric groups and controls (Cavelier et al., 1995; Sabunciyan et al., 2007; Fuke et al., 2008). A similar trend is found in this study where variability in CDel amount is not associated with mtDNA copy number. Thus, it can be postulated that CDel accumulation is occurring regardless of total mtDNA levels.

Limitations of post-mortem human brain studies apply to this paper, such as antipsychotic and antidepressant medications that are not quantified. It is not possible to rule out a possible impact of antidepressant or antipsychotic medication on the levels of common deletion; an animal model study would be necessary to quantify these impacts. The effects of PMI and pH, which are indicators of degradation or tissue quality, were controlled for in the main analysis, and neither the PMI nor pH correlated with the common deletion. We do not believe that the differences are an artifact of autolysis and DNA degradation, after careful analysis of potential confounds of PMI and pH. Because the common deletion assay specifically detects a large deleted site (primers amplify a product across the 4977 bp breakpoint region), the results cannot be explained by differential degradation of mtDNA across diagnoses. However, by using gross homogenates from brain regions it is not possible to determine the cellular localization of the observed levels

of the common deletion. The total amount of nuclear DNA extracted was not different among diagnostic groups ruling out extraction yields and amounts of starting materials in the common deletion assay.

In conclusion, we observed significantly lower global levels of a large somatic deletion in the striato-nigral circuit of subjects with SZ. Alterations within this neural circuit have been associated with key aspects of SZ such as anhedonia, emotional valence, medication response, and movement disorders (Der-Avakian and Markou, 2012). The likely decreased energy output of mitochondria in these brain regions could perhaps be a consequence of the medications targeting this dopaminergic neurocircuit. The presence of decreased levels of the mitochondrial common deletion in SZ subjects suggests an overall impairment of mitochondrial function; the cause or consequence of which is not clear. The decreased metabolic rate that seems to be caused by haloperidol treatment in turn results in reduced cellular energy leading to impairment of affected neurons. Further studies need to be carried out to assess the functional correlation of reduced deletion levels, mitochondrial respiration, antipsychotic medication, and its subsequent impact on brain and behavior.

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Contributors

MPV conceived of the study; FM, PAS,MPV, and BR performed analysis, wrote and edited the manuscript. PAS, BR, LM dissected brain tissue. BR conducted mtDNA deletion assays, and all authors reviewed and approved the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.schres.2014.08.026.

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