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ORIGINAL RESEARCH

Circulating Cell-Free Mitochondrial DNA in Plasma of Individuals with Schizophrenia and Cognitive Deficit in Mexican Population

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Purpose: Cognitive domains are affected in patients with schizophrenia. Mitochondrial dysfunction has been proposed as a possible origin of these symptoms. Cell-free mitochondrial DNA (cf-mtDNA) is an indicator of cellular stress, and it can be identified in individuals with age-associated disorders, this study aimed to explore the presence of cf-mtDNA in plasma of schizophrenia patients and its association with cognitive deficit.

Patients and Methods: Ninety-nine subjects were clinically evaluated; the case group included 60 patients diagnosed with schizophrenia and 39 randomly-individuals without psychiatric disorders were included in the comparison group. Cognitive status (MoCA scale) and cell-free mtDNA in blood plasma were assessed and quantified in both groups.

Results: From the original sample, cf-mtDNA was identified in 43 subjects, 40 patients with schizophrenia and 3 controls ($X^2 = 31.10$, p-value < 0.0001). Thirty-nine out of forty patients with schizophrenia had a cognitive deficit.

Conclusion: According to our findings, cognitive impairment and presence of cf-mtDNA were related in subjects with schizophrenia. Thus, while the cognitive deficit might reflect an accelerated aging process, the cf-mtDNA plays a role as a potential biomarker in this mechanism.

Keywords: schizophrenia, cell free mitochondrial DNA, cognitive impairment, mitochondrial dysfunction

Introduction

Mitochondria are double-membrane organelles, which represent the main source of cellular adenosine triphosphate (ATP) produced by the oxidative phosphorylation system (OXPHOS).[1] Besides the production of high-energy compounds, the mitochondria could regulate some cellular mechanisms like autophagy, apoptosis, and reactive oxidative species (ROS) regulation.[2–4] Mitochondrial dysfunction's main characteristics are: ATP reduction and OXPHOS dysfunctions, which mainly occur in mitochondrial disorders,[5] aging, neurodegenerative, and chronic diseases.[6,7] Mitochondrial dysfunction has been associated with neurodegenerative (Alzheimer's disease, Parkinson's disease) and neuropsychiatric disorders like depression, anxiety, and schizophrenia.[8,9]

Schizophrenia is a psychiatric disorder with a highly deleterious brain dysfunction with a prevalence of 1% world-wide. It is characterized by delusions, hallucinations, negative symptoms, as well as behavioral and cognitive dysfunction.[10–12] Multiple pathological processes (oxidative stress and mitochondrial dysfunction) have been proposed to explain the cognitive dysfunction in schizophrenia.[13,14] According to this, several indicators of oxidative status have been measured in patients with SZ. Folate deficiency,[15] as well as increased differences in thiol/disulphide balance in patients with acute psychosis compared to those with mild or stable symptoms of SZ.[16] Also, there is evidence that severe cognitive impairment and working memory impairment are related to lower antioxidant potential.[17]

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Additionally, post-mortem studies have reported a significant decrease in mitochondria numbers located in the anterior cingulate cortex, putamen, and caudate nucleus in individuals with SZ,[18] possibly pointing to a brain mitochondrial dysfunction. Also, ultrastructural studies in the autopsied anterior limbic cortex, caudate nucleus, and the prefrontal cortex from schizophrenic patients showed deformation and reduction in volume density of mitochondria on oligodendrocytes.[19] The previous findings were centered on brain mitochondrial; nevertheless, some studies have focused on mitochondrial dysfunction and biomarkers searching in peripheral tissues, such as the circulating cell-free mitochondrial DNA (ccf-mtDNA),[20] when it is detected in any biofluid, as well as cell-free mtDNA detected in plasma or serum. Besides, new evidence supports the idea that cf-mtDNA could be encapsulated and released (exosomes, microvesicles) as a physiological response, not necessarily in pathological conditions.[21] Although cf-mtDNA was identified in cancer studies, it has been proposed to be released under stress conditions as a final product in cell death.[22–25] Some studies that evaluated the cf-mtDNA influence on schizophrenia with contradictory results. Some authors have reported that individuals with SZ exhibit lower cf-mtDNA levels in whole blood samples in comparison to control subjects, and its levels were reduced with increasing psychosis severity.[26] In contrast, cf-mtDNA higher levels were observed in SZ patients with acute psychosis.[27] Nevertheless, these studies were designed to evaluate the positive and negative symptoms of the disorder, and cf-mtDNA effects on cognitive dysfunction were not considered.

Therefore, the present study aimed to evaluate the presence of cf-mtDNA in the plasma of patients with schizophrenia and to correlate with symptoms intensity and cognitive deficit, as an oxidative stress response.

Materials and Methods

Sample Population

The total sample comprised 99 individuals recruited during the period from June to September 2018. Sixty-eight individuals were males (68.6%), and 31 were females (31.3%) with a median age of 35 years.

Case Group Conformation (Case Group)

The case group was integrated by 60 patients with schizophrenia who attended appointments at the outpatient clinic of the "Hospital Regional de Alta Especialidad de Salud Mental" in Villahermosa City. All individuals included were previously diagnosed with schizophrenia by experienced psychiatrists following the DMS-5 criteria (Diagnostic and Statistical Manual of Mental Disorders Fifth Edition). The exclusion criteria were as follows: individuals under 18 years of age, patients with acute psychosis episodes, dementia, a psychotic disorder of toxic origin, a history of brain injury, and individuals with intellectual disability. The median age within the case group was 45 years, 37 individuals were males (61.6%), and 23 were females (38.3%). Also, we determined the clinical characteristics of this group through clinical interviews and they were corroborated with medical records. We found that 39.2% were diagnosed with schizophrenia before age 18. Also, the median duration of schizophrenia and the untreated psychosis duration were 16 years and 44 weeks, respectively. Moreover, 76.7% reported at least 1 psychiatric hospitalization ranging from 1 to 7 months in duration.

Comparison Group Conformation (Control Group)

Thirty-nine healthy individuals without any psychiatric illness were randomly included in the comparison group, and they were recruited from the Blood Donor Center of the Hospital Regional de Alta Especialidad: "Dr. Gustavo A. Rovirosa Pérez". Inclusion criteria were as follows: subjects >18 years of age, individuals without neurological or clinical disease background; this information was gathered through a brief interview performed by a medical physician. In this group, the median age was 25 years; 31 individuals (79.5%) were males, whereas 8 (20.5%) were females.

Clinical Assessments

Cognitive Status

The Montreal Cognitive Assessment scale (MoCA-Spanish version 7.2) was applied to the total sample. This scale detects mild cognitive deficits across 8 domains: visuospatial and executive functioning, identification, attention, language, abstraction, delayed recall, and orientation. The punctuation scale varies between 1 and 30 points; if the total score is equal to or higher than 26 points, it is considered cognitively normal. According to some authors, if the educational level is less than 12 years, another point should be added to the total score, and we did so.[28]

Schizophrenia Symptoms Intensity

To evaluate the predominance and intensity of SZ symptoms in the case group, we used the Positive and Negative Syndrome Scale (PANSS). This scale comprises 30 items, each one with 1–7 points, and the total scores are 30 as minimum and 210 as maximum.[29]

Quantification of Cell-Free mtDNA (Cf-mtDNA) in Blood Plasma

Blood samples were collected in EDTA tubes; the plasma was separated by centrifugation for 5 min at 800g. Next, we transferred it to new tubes and it was centrifuged for 15 min at 1000g. The cf-mtDNA was extracted from 200 uL of twice centrifuged plasma samples in all the individuals included in the study (cases and controls) using the QIAamp DNA Mini Kit (Qiagen, USA) protocol. The former kit allows to purify cell-free DNA from biological samples, like plasma, serum, urine, etc. The cf-mtDNA quality and quantity were evaluated by electrophoresis and spectrophotometer (NanoDrop 2000, Thermofisher, USA) at 260/280 nm. The presence or absence of cf-mtDNA was determined by RT-PCR. The primers used to amplify the MT-ND2 gene were: forward CACACTCATCACAGCGCTAA, reverse: GGATTATGGATGCGGTTGCT.[30] The amplification was performed with RT^2 SYBR Green MixEach (Qiagen, USA), with ROX as background dye. Every reaction was run on triplicate in a Quanto Studio 7 (Thermo fisher, USA). We considered the presence of cf-mtDNA if the delta of Rn (delta of fluorescence) was higher than 1 after the 30 cycle.[31]

Statistical Analysis

Statistical calculations were performed using the Statistical Package for the Social Sciences (SPSS, IBM, Armonk, NY, USA), version 24. Normal distribution was explored in all variables with Shapiro–Wilk test. Socio-demographic comparisons between cases and controls were explored by chi-squared test (X^2) or Mann–Whitney *U*-test. Data were represented as the median and interquartile range for variables without normal distribution. Mann–Whitney *U*-test was performed to compare continuous variables and chi-square test for categorical variables. We performed three statistical contrasts:

a. Case group (schizophrenia diagnosed) compared to controls (without schizophrenia).
b. Individuals with cf-mtDNA presence compared to individuals with lack of cf-mtDNA.
c. Cases with cf-mtDNA compared to cases without cf-mtDNA.

All tests were two-tailed, and a p-value <0.05 was considered significant.

Ethical Approval

This study was performed following the Helsinki Declaration and was approved by the Bioethical Committee of the Mental Health High Specialty Regional Hospital (Hospital Regional de Alta Especialidad de Salud Mental, in Spanish) No. HRAESM/DG/RP/1128/2018. All the individuals recruited in this study accepted to participate and signed a written informed consent after a full explanation of the study.

Results

Sample Description

Males were predominant in the overall sample (68.69%), and the healthy subjects (controls) were younger than the individuals with SZ (cases) (p-value < 0.0001). The cases had fewer years of education than healthy subjects (p-value = 0.0260), and 48.33 were unemployed. Cognitive deficit was detected in 96.42% (n = 54) and 48.71% (n = 19) of the case and control group (p-value < 0.001), respectively. Furthermore, we found a significant difference in the total scores of MoCA scale between cases (median = 15.5, IQR = 11.00–20.75) and controls (median = 26, IQR = 22.00–28.00); controls achieved higher scores (p-value < 0.0001). Regarding the clinical data on the case group (PANSS scale, treatment, etc.), we found that 26.7% were prescribed more than one antipsychotic in their treatment scheme. (Table 1)

Table 1 Comparisons of Sociodemographic and Clinical Characteristics of the Case and Control Groups (N = 99)

	Cases n = 60	**Controls n = 39**	**Overall sample N = 99**	**Statistics**
Sociodemographic	Median (IQR)	Median (IQR)	Median (IQR)	U (*p*-value)
Age (years)	45.00 (29.75–54.25)	25.00 (19.00–34.00)	35.00 (25.00–49.50)	375.50 (< 0.0001)
Education level (years)	9.00 (6.00–12.00)	12.00 (9.00–13.00)	9.00 (8.50–13.00)	802.00 (0.0260)
Body Mass Index (BMI kg/m^2)	27.92 (22.81–32.00)	26.04 (24.00–30.29)	27.34 (23.62–30.93)	0.1310 (0.8960)
Marital status	f (%)	f (%)	f (%)	X^2 (*p*-value)
Married	7 (1.17)	15 (38.5)	22 (22.22)	18.55 (< 0.0001)
Single	48 (80.00)	24 (61.5)	72 (72.73)	
Divorced	5 (8.33)	0 (0.00)	5 (5.01)	
Gender				
Male	37 (61.67)	31 (79.5)	68 (68.69)	3.13 (0.2090)
Female	23 (38.33)	8 (20.5)	31 (31.31)	
Socioeconomic level				
High	2 (3.33)	0	2 (2.02)	18.76 (< 0.0001)
Middle	19 (31.67)	30 (76.9)	49 (49.50)	
Low	39 (65.00)	9 (23.1)	48 (48.48)	
Occupation				
Unemployed	29 (48.33)	1 (2.6)	30 (30.30)	53.73 (< 0.0001)
Housewife	14 (28.33)	1 (2.6)	15 (15.15)	
Student	1 (1.67)	16 (41)	17 (17.17)	
Part time employment	12 (20.00)	6 (15.4)	18 (18.18)	
Full-time employment	4 (6.67)	15 (38.5)	19 (19.19)	
Clinical	Median (IQR)	Median (IQR)	Median (IQR)	U (*p*-value)
MoCA scale				
Total	15.5 (11–20.75)	26 (22–28)	20 (0 −29)	226.00 (< 0.0001)
Cognitive deficit	58 (96.42)	19 (48.72)	73 (76.84)	29.41 (< 0.0001)
PANSS scale	56 (45–69.5)			
Positive	13 (10–16)			
Negative	16.5 (11–24)			
Age of psychosis onset (years)	20.00 (16.7–25.0)			
Number of Antipsychotics	f (%)			
None	1 (1.8)			
Only 1	40 (71.4)			
Zuclopenthixol	7 (12.5)			
Risperidone	13 (23.2)			
Olanzapine	16 (28.6)			
Higher than 1	15 (26.7)			
Typical	9 (15.00)			
Atypical	34 (56.00)			
Both	16 (26.67)			

Note: Data are presented in median and interquartile range (IQR) for continuous variables, and frequencies and percentages for qualitative variables.
Abbreviations: MoCA scale, Montreal Cognitive Assessment Scale; PANSS scale, Positive and Negative Syndrome Scale.

Cell-Free Mitochondrial DNA (Cf-mtDNA)

Differences Between Carriers and Non-Carriers of Cf-mtDNA

Cf-mtDNA was identified in the plasma of 40 cases (66.7% of the 60 cases) and 3 controls (7.69% of the 39 controls) ($X^2 = 31.10$, p-value < 0.0001). Additionally, 93.02% of the individuals with cf-mtDNA had a cognitive deficit and 66.07% of those without

cf-mtDNA had a cognitive deficit (p-value = 0.0031). Also, a higher prevalence of cognitive deficit in addition to schizophrenia was observed in individuals with cf-mtDNA presence (90.70%), compared to 33.93% of the individuals without cf-mtDNA (p-value < 0.0001) (Table 2).

Forty (93.02%) out of 43 individuals with cf-mtDNA had a schizophrenia diagnosis (p < 0.0001), but 39 cases (90.70%) had schizophrenia and cognitive deficit. In contrast, only 19 individuals (33.93%) from the individuals without cf-mtDNA had schizophrenia and cognitive deficit (p <0.0001). No differences were found between cf-mtDNA, sex and body mass index values. The individuals with cf-mtDNA were older (median = 44, IQR = 27.50–55.00), compared to those without cf-mtDNA (median = 31.00, IQR = 21.75–40.75) (p-value = 0.0049) (Table 2).

The group with cf-mtDNA presence was further analyzed with deficit cognitive, age, sex and education level in a multivariate logistic regression. However, there was no relation between the cognitive deficit and age of this group (Table 3).

Table 2 Comparison Between Carriers and Non-Carriers of Cell-Free Mitochondrial DNA

	Presence n = 43	**Absence n = 56**	**Statistics**
Schizophrenia diagnosis	f (%)	f (%)	X^2 (*p*-value)
Case	40 (93.02)	20 (35.71)	31.10 (< 0.0001)
Control	3 (6.98)	36 (64.29)	
Cognitive deficit	40 (93.02)	37 (66.07)	8.73 (0.0031)
Cognitive deficit + schizophrenia (cases)	39 (90.70)	19 (33.93)	30.01 (< 0.0001)
Gender			
Male	29 (67.44)	39 (69.64)	0.0002 (0.9877)
Female	14 (32.59)	17 (30.36)	
	Median (IQR)	Median (IQR)	U (*p*- value)
Education level (years)	9.00 (6.00–12.00)	10.00 (9.00–13.00)	1465.50 (0.0621)
Body Mass Index (BMI kg/m^2)	27.28 (22.41–32.31)	27.37 (24.72–30.58)	1294 (0.5275)
MoCA scale domains			
Visuospatial	2 (1–4)	2 (0.5–4)	330 (0.9775)
Identification	3 (2–3)	3 (2–3)	324.5 (0.9062)
Attention	3 (0–4)	3 (2–4)	319.5 (0.5892)
Language	1 (0–2)	1 (0–3)	281.5 (0.2237)
Abstraction	2 (0–2)	2 (0–2)	318 (0.5495)
Delayed recall	0	0	268 (0.0537)
Orientation	5 (3–6)	5 (3–6)	316 (0.5286)
MoCA scale total score	15 (9–21)	17.5 (13.5–20.2)	312.5 (0.5140)

Abbreviation: MoCA scale, Montreal Cognitive Assessment Scale.

Table 3 Logistic Regression Model for Carriers of Cf-mtDNA

	β	**Odds ratio**	**95% CI**	***p* value**
Initial model				
Age	0.007	0.087	0.1–1.7	0.786
Education level	0.20	0.070	1.0–5.0	0.791
Cognitive deficit- Yes	0.59	1.369	0.5–16	0.242
Final model				
Education level	−0.18	0.047	1.0–1.7	0.242
Cognitive deficit- Yes	0.59	1.369	1.5–12.3	0.207

Discussion

This study aimed to investigate the association between cognitive deficit and the presence of circulating cell-free mitochondrial DNA in the plasma of individuals with schizophrenia. Cognitive symptoms are continuously studied due to their high prevalence and their negative effects on these individuals´ functionality and prognostic. It is important to mention that this is the first study focused on the cf-mtDNA possible link with cognitive deficit in a Mexican community sample. First, we observed a high prevalence of cognitive deficit in schizophrenia patients, similar to what has been reported in other populations such as the Canadian and Asian populations.[28,32] Likewise, our results suggest a high frequency of cognitive deficit in individuals without schizophrenia, principally by a reduction in scholarship, as previously reported.[33]

Second, our study measured the presence of cf-mtDNA in the plasma of individuals diagnosed with schizophrenia, compared to the control group. At this point, it is important to differentiate cf-mtDNA from mitochondrial DNA copy number (mtDNA-cn). MtDNA-cn, represents the number of genomes per cell and could be an indicator of energetic function and biogenesis.[34] Meanwhile, when mtDNA is released from cells under stress conditions it is known as cf-mtDNA.[35] The majority of the studies had centered on mtDNA-cn, with contradictory results. Chestkov et al (2018) reported that individuals diagnosed with schizophrenia and acute psychosis showed a higher number of mtDNA-cn than healthy controls in a Russian population.[27] In contrast, some studies have reported that individuals diagnosed with schizophrenia exhibit lower mtDNA-cn levels than control subjects,[26] and those levels decrease when the psychosis intensity increases.[36] Nevertheless, the circulating cf-mtDNA in schizophrenia remains unexplored. A recent meta-analysis indicates that the levels of cf-mtDNA in patients with bipolar disorder or depression are similar to healthy subjects. However, the data on SZ are scarce.[37] We found cf-mtDNA in almost 70% of the patients with schizophrenia. Thus, we could suggest that a high percentage of these patients are under higher cellular stress compared to healthy subjects.

Third, we observe that 90% of individuals with cf-mtDNA had a cognitive deficit, and more than 50% of them had schizophrenia with cognitive deficit even with a high educational level (higher than the sample median, >9 years). However, we cannot assume these findings as an indicator of neurodegeneration,[38] principally because we still do not know the source of the cf-mtDNA in neuropsychiatric disorders (brain or peripheral).[30] Some studies have proposed that plasma cf-mtDNA could be an indicator of a systemic inflammatory process (including the brain), where the blood-brain barrier permeability increases, potentiating a positive loop of cf-mtDNA release.[39] Moreover, evidence of cf-mtDNA high levels in plasma of patients with major depressive disorder, suicide attempters, and Alzheimer's disease have been correlated with immunologic responses and inflammation as well as the progression of the disease.[30,40,41] Additionally, cf-mtDNA could activate the innate immune system by toll-like receptor 9 interaction, which induces the NF-κB signaling pathway, present in all immune cells, including microglia.[42] This process culminates with the production of pro-inflammatory cytokines, generating a pro-inflammatory state and positive feedback.[43] Similarly, Chestkov et al (2018) found a positive correlation between the mtDNA content and the levels of marker 8-oxodG oxidation in patients with schizophrenia.[27] Then, increased levels of cf-mtDNA may indicate a neuro-inflammatory state in these individuals. Furthermore, schizophrenia has been linked with neuroinflammation as a potential mechanism for brain atrophy, reflected in a higher frequency of cognitive deficit in schizophrenia-diagnosed individuals.[44,45] Other authors argue that plasmatic cf-mtDNA could be an indicator of an accelerated aging process, associated with frailty.[46,47] Even though there is not a unique definition of frailty, in general, the term refers to a range of conditions in older people, including general debility and cognitive impairment, that lead to disability and increased mortality rates.[48–50] Individuals with marked frailty have higher cellular stress and remain with a pro-inflammatory status with high levels of laboratory pathological markers, and as a consequence, the cf-mtDNA levels increase.[47,51] Similarly, individuals with schizophrenia have higher frailty scores and accelerated aging.[52–56] Probably, the high frequency of cognitive deficit and the presence of cf-mtDNA in the case group could indicate a greater frailty condition and accelerated aging in the SZ patients.

Finally, some limitations of this study should be considered. First, the transversal design and the relatively small sample size; therefore, longitudinal studies with larger samples are needed to explore more clinical variables. Second, we only use one test (MoCA) for the assessment of cognitive deficit, this could be improved in future studies with different cognitive tests. Third, we did not contemplate the treatment adherence of these patients. Perhaps, retrospective data about

treatment history of individuals with SZ could confirm our speculation that these drugs cause a decrease in cf-mtDNA levels. Finally, we did not evaluate pro-inflammatory cytokines to confirm the inflammatory state.

Conclusions

The findings in this study demonstrate a high frequency of cognitive symptoms in a relatively small sample of Mexican individuals with SZ. To our knowledge, this is the first study to report differences in cf-mtDNA levels between SZ patients and healthy subjects, taking into consideration the cognitive alterations. Furthermore, it seems that cf-mtDNA is closely related to cognitive symptoms when these are part of SZ symptoms. Thus, cognitive deficit may be the clinical reflection of an accelerated cell death process where cf-mtDNA is a potential biomarker. However, further research is required to corroborate this conclusion.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Khan NA, Govindaraj P, Meena AK, Thangaraj K. Mitochondrial disorders: challenges in diagnosis & treatment. *Indian J Med Res*. 2015;141(1):13–26. doi:10.4103/0971-5916.154489
2. Chang CC, Jou SH, Lin TT, Liu CS. Mitochondrial DNA variation and increased oxidative damage in euthymic patients with bipolar disorder. *Psychiatry Clin Neurosci*. 2014;68(7):551–557. doi:10.1111/pcn.12163
3. Rezin GT, Amboni G, Zugno AI, Quevedo J, Streck EL. Mitochondrial dysfunction and psychiatric disorders. *Neurochem Res*. 2009;34(6):1021–1029. doi:10.1007/s11064-008-9865-8
4. Wu Y, Chen M, Jiang J. Mitochondrial dysfunction in neurodegenerative diseases and drug targets via apoptotic signaling. *Mitochondrion*. 2019;49:35–45. doi:10.1016/j.mito.2019.07.003
5. Schlieben LD, Prokisch H. The dimensions of primary mitochondrial disorders. *Front Cell Dev Biol*. 2020;8:600079. doi:10.3389/fcell.2020.600079
6. Swerdlow RH. Brain aging, Alzheimer's disease, and mitochondria. *Biochim Biophys Acta*. 2011;1812(12):1630–1639. doi:10.1016/j.bbadis.2011.08.012
7. Reddy PH, Reddy TP. Mitochondria as a therapeutic target for aging and neurodegenerative diseases. *Curr Alzheimer Res*. 2011;8(4):393–409. doi:10.2174/156720511795745401
8. Lezi E, Swerdlow RH. Mitochondria in neurodegeneration. *Adv Exp Med Biol*. 2012;942:269–286.
9. Dagda RK. Role of mitochondrial dysfunction in degenerative brain diseases, an overview. *Brain Sci*. 2018;8(10):178. doi:10.3390/brainsci8100178
10. Genis-Mendoza A, Gallegos-Silva I, Tovilla-Zarate CA, et al. Comparative analysis of gene expression profiles involved in calcium signaling pathways using the NLVH animal model of schizophrenia. *J Mol Neurosci*. 2018;64(1):111–116. doi:10.1007/s12031-017-1013-y
11. Tripathi A, Kar SK, Shukla R. Cognitive deficits in schizophrenia: understanding the biological correlates and remediation strategies. *Clin Psychopharmacol Neurosci*. 2018;16(1):7–17. doi:10.9758/cpn.2018.16.1.7
12. Stefanatou P, Karatosidi CS, Tsompanaki E, Kattoulas E, Stefanis NC, Smyrnis N. Premorbid adjustment predictors of cognitive dysfunction in schizophrenia. *Psychiatry Res*. 2018;267:249–255. doi:10.1016/j.psychres.2018.06.029
13. Valenti D, de Bari L, De Filippis B, Henrion-Caude A, Vacca RA. Mitochondrial dysfunction as a central actor in intellectual disability-related diseases: an overview of down syndrome, autism, fragile X and Rett syndrome. *Neurosci Biobehav Rev*. 2014;46(Pt 2):202–217. doi:10.1016/j.neubiorev.2014.01.012
14. Akarsu S, Torun D, Bolu A, et al. Mitochondrial complex I and III gene mRNA levels in schizophrenia, and their relationship with clinical features. *J Mol Psychiatry*. 2014;2(1):6. doi:10.1186/s40303-014-0006-9
15. Zhilyaeva TV, Kasyanov ED, Rukavishnikov GV, et al. Pterin metabolism, inflammation and oxidative stress biochemical markers in schizophrenia: factor analysis and assessment of clinical symptoms associations. *Prog Neuropsychopharmacol Biol Psychiatry*. 2023;127:110823. doi:10.1016/j.pnpbp.2023.110823
16. Korkmaz SA, Kaymak SU, Neselioglu S, Erel O. Thiol-disulphide homeostasis in patients with schizophrenia: the potential biomarkers of oxidative stress in acute exacerbation of schizophrenia. *Clin Psychopharmacol Neurosci*. 2024;22(1):139–150. doi:10.9758/cpn.23.1084
17. Wiedlocha M, Zborowska N, Marcinowicz P, et al. Oxidative stress biomarkers among Schizophrenia inpatients. *Brain Sci*. 2023;13(3):490. doi:10.3390/brainsci13030490
18. Roberts RC. Postmortem studies on mitochondria in schizophrenia. *Schizophr Res*. 2017;187:17–25. doi:10.1016/j.schres.2017.01.056
19. Uranova N, Orlovskaya D, Vikhreva O, et al. Electron microscopy of oligodendroglia in severe mental illness. *Brain Res. Bull.* 2001;55(5):597–610. doi:10.1016/S0361-9230(01)00528-7
20. Lowes H, Pyle A, Santibanez-Koref M, Hudson G. Circulating cell-free mitochondrial DNA levels in Parkinson's disease are influenced by treatment. *Mol Neurodegener*. 2020;15(1):10. doi:10.1186/s13024-020-00362-y

21. Trumpff C, Michelson J, Lagranha CJ, et al. Stress and circulating cell-free mitochondrial DNA: a systematic review of human studies, physiological considerations, and technical recommendations. *Mitochondrion*. 2021;59:225–245. doi:10.1016/j.mito.2021.04.002
22. Mahmoud EH, Fawzy A, Ahmad OK, Ali AM. Plasma circulating cell-free nuclear and mitochondrial DNA as potential biomarkers in the peripheral blood of breast cancer patients. *Asian Pac J Cancer Prev*. 2015;16(18):8299–8305. doi:10.7314/APJCP.2015.16.18.8299
23. Kohler C, Radpour R, Barekati Z, et al. Levels of plasma circulating cell free nuclear and mitochondrial DNA as potential biomarkers for breast tumors. *Mol Cancer*. 2009;8(1):105. doi:10.1186/1476-4598-8-105
24. Bisharyan Y, Clark TG. Calcium-dependent mitochondrial extrusion in ciliated protozoa. *Mitochondrion*. 2011;11(6):909–918. doi:10.1016/j.mito.2011.08.001
25. Zhang Q, Itagaki K, Hauser CJ. Mitochondrial DNA is released by shock and activates neutrophils via p38 map kinase. *Shock*. 2010;34(1):55–59. doi:10.1097/SHK.0b013e3181cd8c08
26. Li Z, Hu M, Zong X, et al. Association of telomere length and mitochondrial DNA copy number with risperidone treatment response in first-episode antipsychotic-naive schizophrenia. *Sci Rep*. 2015;5(1):18553. doi:10.1038/srep18553
27. Chestkov IV, Jestkova EM, Ershova ES, et al. ROS-induced DNA damage associates with abundance of mitochondrial DNA in white blood cells of the untreated schizophrenic patients. *Oxid Med Cell Longev*. 2018;2018(1):8587475. doi:10.1155/2018/8587475
28. Yang Z, Abdul Rashid NA, Quek YF, et al. Montreal cognitive assessment as a screening instrument for cognitive impairments in schizophrenia. *Schizophr Res*. 2018;199:58–63. doi:10.1016/j.schres.2018.03.008
29. Emsley R, Rabinowitz J, Torreman M, R-i-epgw G. The factor structure for the Positive and Negative Syndrome Scale (PANSS) in recent-onset psychosis. *Schizophr Res*. 2003;61(1):47–57. doi:10.1016/S0920-9964(02)00302-X
30. Lindqvist D, Wolkowitz OM, Picard M, et al. Circulating cell-free mitochondrial DNA, but not leukocyte mitochondrial DNA copy number, is elevated in major depressive disorder. *Neuropsychopharmacology*. 2018;43(7):1557–1564. doi:10.1038/s41386-017-0001-9
31. Garcia-de la Cruz DD, Juarez-Rojop IE, Tovilla-Zarate CA, et al. Association between mitochondrial DNA and cognitive impairment in schizophrenia: study protocol for a Mexican population. *Neuropsychiatr Dis Treat*. 2019;15:1717–1722. doi:10.2147/NDT.S208587
32. Wu C, Dagg P, Molgat C. A pilot study to measure cognitive impairment in patients with severe schizophrenia with the Montreal Cognitive Assessment (MoCA). *Schizophr Res*. 2014;158(1–3):151–155. doi:10.1016/j.schres.2014.07.006
33. Calderon-Garciduenas L, Mukherjee PS, Kulesza RJ, et al. Mild cognitive impairment and dementia involving multiple cognitive domains in Mexican urbanites. *J Alzheimer's Dis*. 2019;68(3):1113–1123. doi:10.3233/JAD-181208
34. Clay Montier LL, Deng JJ, Bai Y. Number matters: control of mammalian mitochondrial DNA copy number. *J Genet Genomics*. 2009;36(3):125–131. doi:10.1016/S1673-8527(08)60099-5
35. Yu M. Circulating cell-free mitochondrial DNA as a novel cancer biomarker: opportunities and challenges. *Mitochondrial DNA*. 2012;23(5):329–332. doi:10.3109/19401736.2012.696625
36. Kumar P, Efstathopoulos P, Millischer V, et al. Mitochondrial DNA copy number is associated with psychosis severity and anti-psychotic treatment. *Sci Rep*. 2018;8(1):12743. doi:10.1038/s41598-018-31122-0
37. Melamud MM, Buneva VN, Ermakov EA. Circulating cell-free DNA levels in psychiatric diseases: a systematic review and meta-analysis. *Int J Mol Sci*. 2023;24(4):3402. doi:10.3390/ijms24043402
38. Zipursky RB, Reilly TJ, Murray RM. The myth of schizophrenia as a progressive brain disease. *Schizophr Bull*. 2013;39(6):1363–1372. doi:10.1093/schbul/sbs135
39. Najjar S, Pahlajani S, De Sanctis V, Stern JNH, Najjar A, Chong D. Neurovascular unit dysfunction and blood-brain barrier hyperpermeability contribute to schizophrenia neurobiology: a theoretical integration of clinical and experimental evidence. *Frontiers in Psychiatry*. 2017;8:83. doi:10.3389/fpsyt.2017.00083
40. Hoekstra JG, Hipp MJ, Montine TJ, Kennedy SR. Mitochondrial DNA mutations increase in early stage Alzheimer disease and are inconsistent with oxidative damage. *Ann Neurol*. 2016;80(2):301–306. doi:10.1002/ana.24709
41. Lindqvist D, Fernstrom J, Grudet C, et al. Increased plasma levels of circulating cell-free mitochondrial DNA in suicide attempters: associations with HPA-axis hyperactivity. *Transl Psychiatry*. 2016;6(12):e971. doi:10.1038/tp.2016.236
42. Angajala A, Lim S, Phillips JB, et al. Diverse roles of mitochondria in immune responses: novel insights into immuno-metabolism. *Front Immunol*. 2018;9:1605. doi:10.3389/fimmu.2018.01605
43. Aucamp J, Bronkhorst AJ, Badenhorst CPS, Pretorius PJ. The diverse origins of circulating cell-free DNA in the human body: a critical re-evaluation of the literature. *Biol Rev Camb Philos Soc*. 2018;93(3):1649–1683. doi:10.1111/brv.12413
44. Rose J, Brian C, Woods J, et al. Mitochondrial dysfunction in glial cells: implications for neuronal homeostasis and survival. *Toxicology*. 2017;391:109–115. doi:10.1016/j.tox.2017.06.011
45. Na KS, Jung HY, Kim YK. The role of pro-inflammatory cytokines in the neuroinflammation and neurogenesis of schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry*. 2014;48:277–286. doi:10.1016/j.pnpbp.2012.10.022
46. Padilla-Sanchez SD, Navarrete D, Caicedo A, Teran E. Circulating cell-free mitochondrial DNA levels correlate with body mass index and age. *Biochim Biophys Acta Mol Basis Dis*. 2020;1866(12):165963. doi:10.1016/j.bbadis.2020.165963
47. Jylhava J, Nevalainen T, Marttila S, Jylha M, Hervonen A, Hurme M. Characterization of the role of distinct plasma cell-free DNA species in age-associated inflammation and frailty. *Aging Cell*. 2013;12(3):388–397. doi:10.1111/acel.12058
48. Lally F, Crome P. Understanding frailty. *Postgrad Med J*. 2007;83(975):16–20. doi:10.1136/pgmj.2006.048587
49. Ruan Q, Yu Z, Chen M, Bao Z, Li J, He W. Cognitive frailty, a novel target for the prevention of elderly dependency. *Ageing Res Rev*. 2015;20:1–10. doi:10.1016/j.arr.2014.12.004
50. Rolfson D. Successful aging and frailty: a systematic review. *Geriatrics (Basel)*. 2018;3(4). doi:10.3390/geriatrics3040079
51. Topinkova E. Aging, disability and frailty. *Ann Nutr Metab*. 2008;52(Suppl 1):6–11. doi:10.1159/000115340
52. Tsai MT, Lee SM, Chen HK, Wu BJ. Association between frailty and its individual components with the risk of falls in patients with schizophrenia spectrum disorders. *Schizophr Res*. 2018;197:138–143. doi:10.1016/j.schres.2018.01.023
53. Svensson E, Rogvin M, Hultman CM, Reichborn-Kjennerud T, Sandin S, Moger TA. Schizophrenia susceptibility and age of diagnosis--a frailty approach. *Schizophr Res*. 2013;147(1):140–146. doi:10.1016/j.schres.2013.03.004
54. Nguyen TT, Eyler LT, Jeste DV. Systemic biomarkers of accelerated aging in schizophrenia: a critical review and future directions. *Schizophr Bull*. 2018;44(2):398–408. doi:10.1093/schbul/sbx069

55. Kirkpatrick B, Kennedy BK. Accelerated aging in schizophrenia and related disorders: future research. *Schizophr Res*. 2018;196:4–8. doi:10.1016/j.schres.2017.06.034
56. Lomholt LH, Andersen DV, Sejrsgaard-Jacobsen C, et al. Mortality rate trends in patients diagnosed with schizophrenia or bipolar disorder: a nationwide study with 20 years of follow-up. *Int J Bipolar Disord*. 2019;7(1):6. doi:10.1186/s40345-018-0140-x