



OPEN Plasma circulating free DNA in chronic schizophrenia: a case-control study

Kamila Meca^{1✉}, Łukasz Czogalik¹, Piotr Lewandowski², Marcin Rojek², Szymon Florek³, Piotr Gorczyca⁴ & Magdalena Piegza⁴

Circulating cell-free DNA (cf-DNA) reflects ongoing cell injury and inflammation, but its role in schizophrenia remains unclear. We conducted a case-control study of 33 clinically stabilized patients with chronic schizophrenia and 30 healthy volunteers. Plasma cf-DNA was quantified by fluorometry and droplet digital PCR (ddPCR) targeting nuclear (cf-nucDNA) and mitochondrial (cf-mtDNA) genomes. In unadjusted analyses, patients showed higher cf-nucDNA than controls ($p = 0.020$), while cf-mtDNA did not differ. After adjusting for age, sex, and smoking, the case-control difference was no longer significant; however, this result should be interpreted with caution due to substantial confounding between disease status, male sex, and smoking habits within our cohort. No associations were found between cf-DNA and symptom severity, despite a predominance of negative symptoms in the study group. These findings suggest that the observed variance in cf-DNA levels is heavily influenced by demographic and lifestyle factors, which complicates the isolation of a distinct disease-specific biological signal in this study group. While ddPCR is a sensitive tool for psychiatric research, current results do not support the routine clinical application of cf-DNA as a biomarker.

Keywords Chronic schizophrenia, Circulating free DNA, Biomarkers

What is cf-DNA?

In recent years, interest in circulating cell-free DNA (cf-DNA) has increased substantially due to its potential role as a biomarker in a wide range of medical conditions. Cf-DNA consists of short DNA fragments present in the bloodstream, primarily derived from cells undergoing apoptosis or necrosis. It circulates continuously and is rapidly cleared from the circulation, which makes its concentration a dynamic indicator of ongoing cellular damage and physiological stress¹.

Cf-DNA concentrations in health and disease

In healthy individuals, plasma cf-DNA levels are generally low and relatively stable, although they may fluctuate in response to physiological and environmental influences. A large population-based study demonstrated associations between cf-DNA levels and features of aging such as frailty, biological age, and overall health status, as well as with health-related behaviors including smoking. Higher concentrations were observed in men, older individuals, and subjects with elevated inflammatory markers and unfavorable metabolic profiles, including increased homocysteine levels². These findings suggest that cf-DNA may serve as a global marker of biological burden and systemic dysregulation.

Marked elevations of cf-DNA have been reported in cancer, where rapid tumor cell turnover results in massive DNA release into the circulation^{3–5}. This phenomenon forms the basis of liquid biopsy, a non-invasive approach used to monitor disease progression, assess treatment response, detect recurrence, and identify actionable mutations⁶.

Elevated cf-DNA concentrations are also observed in cardiovascular diseases, where ischemia, inflammation, and hemodynamic overload lead to cardiomyocyte injury. In this context, cf-DNA has been investigated as a

¹Student Science Club, Department of Psychiatry, Medical University of Silesia, Faculty of Medical Sciences in Zabrze, Pyskowicka 49, Tarnowskie Góry 42-612, Poland. ²Department of Histology and Cell Pathology, Medical University of Silesia, Faculty of Medical Sciences in Zabrze, Jordana 19, Zabrze 41-808, Poland. ³Department of Psychoprophylaxis, Medical University of Silesia, Faculty of Medical Sciences in Zabrze, Pyskowicka 49, Tarnowskie Góry 42-612, Poland. ⁴Department of Psychiatry, Medical University of Silesia, Faculty of Medical Sciences in Zabrze, Pyskowicka 49, Tarnowskie Góry 42-612, Poland. ✉email: kamilameca01@gmail.com

biomarker of heart failure severity and prognosis. Measurement of donor-derived cf-DNA (dd-cf-DNA) after heart transplantation allows early non-invasive detection of graft rejection episodes^{6–10}.

Similarly, increased cf-DNA levels have been described in sepsis and autoimmune diseases, reflecting systemic inflammation and tissue damage. In sepsis, cf-DNA correlates with disease severity, organ failure, and mortality¹¹. In systemic lupus erythematosus, cf-DNA levels are associated with disease activity and organ involvement. In rheumatoid arthritis, concentrations rise during periods of active inflammation and decrease with treatment, in some cases falling below healthy reference levels in long-standing stable disease^{12–15}.

In neurological disorders, cf-DNA is emerging as a potential diagnostic and prognostic biomarker. In Alzheimer's disease, analysis of neuronal cf-DNA methylation patterns enables early detection and prediction of disease progression. In multiple sclerosis, cf-DNA may reflect disease activity and treatment response. Findings in epilepsy remain inconsistent, with both increased and decreased levels reported depending on disease type and duration^{16,17}.

Across these diverse conditions, a recurring theme emerges: cf-DNA reflects integrated processes such as inflammation, oxidative stress, cellular turnover, and tissue injury. Rather than representing a disease-specific signal, cf-DNA may function as a dynamic index of cumulative biological stress.

Usefulness of cf-DNA in the diagnosis and monitoring of schizophrenia

Schizophrenia is a severe psychiatric disorder of heterogeneous etiology, characterized by profound disturbances of thought, perception, and affect, leading to significant functional impairment and social withdrawal^{18,19}. Its course is frequently chronic and marked by periods of exacerbation and remission.

Although its pathophysiology remains incompletely understood, two complementary frameworks predominate. The neurodevelopmental model attributes the onset of schizophrenia to early-life disturbances in central nervous system maturation caused by prenatal or perinatal insults. In contrast, the neurodegenerative model proposes progressive brain changes resembling accelerated aging, particularly in regions involved in cognition and emotional regulation^{20,21}. Both perspectives imply sustained alterations in cellular vulnerability and long-term biological dysregulation.

An increasing body of evidence supports a role for immune activation and chronic low-grade inflammation in schizophrenia. Elevated levels of inflammatory markers, including IL-6, IL-1 β , TNF- α , and CRP, have been consistently reported^{22,23}. Chronic inflammation may promote increased cellular turnover, apoptosis, and oxidative DNA damage, all of which can contribute to the release of cf-DNA into the circulation.

Within the neurodevelopmental framework, early-life insults may induce persistent alterations in immune reactivity and cellular resilience, predisposing to long-term increases in cf-DNA release. From a neurodegenerative perspective, progressive neuronal dysfunction and features of accelerated biological aging^{20,21} may similarly manifest as elevated circulating DNA fragments. Thus, cf-DNA may represent a peripheral marker integrating developmental vulnerability, ongoing neurobiological changes, and systemic inflammatory activity.

Recent studies have demonstrated elevated cf-DNA levels in patients with schizophrenia compared to healthy controls²⁰. Moreover, cf-DNA concentrations have been shown to decrease following antipsychotic treatment, suggesting a potential role as an indicator of disease activity and therapeutic response²⁴. However, most existing studies have examined heterogeneous patient populations without stratification according to illness duration or chronicity.

Despite extensive efforts to identify reliable biomarkers of schizophrenia, many candidates demonstrate limited specificity and inconsistent reproducibility across diverse cohorts. Given that cf-DNA reflects cumulative biological stress rather than a single molecular pathway, it may provide a more integrative index of disease-related burden. This approach may be particularly relevant in chronic schizophrenia, where long-term illness course and prolonged antipsychotic exposure may shape a distinct biological phenotype.

Currently, there is very limited literature examining cf-DNA levels specifically in patients with chronic schizophrenia receiving long-term treatment. Further investigation of cf-DNA concentrations in this subgroup may provide insight into molecular mechanisms underlying disease chronicity and contribute to the development of more precise biomarker stratification strategies.

Materials and methods

Aim, design and setting

This was a single-center case-control study conducted at the Clinical Department of Psychiatry, Faculty of Medical Sciences in Zabrze, Medical University of Silesia, Poland, between August 2023 and November 2024. The study aimed to determine plasma concentrations of circulating free DNA in patients with chronic schizophrenia and to assess potential associations with clinical features of the disorder.

All procedures involving human participants were conducted in accordance with the ethical standards of the local Bioethics Committee and the 1964 Helsinki Declaration and its later amendments, as well as relevant guidelines and regulations. The study protocol was approved by the Bioethics Committee (BNW/NWN/0052/KB1/22/23), and written informed consent was obtained from all participants.

Study population

A total of 34 patients with schizophrenia and 30 healthy controls were initially recruited. One patient experiencing a first episode of psychosis was excluded, leaving 33 patients in the final analysis. Patients met ICD-10 criteria for schizophrenia (F20) and were enrolled after clinical stabilization that enabled provision of informed consent.

Eligibility criteria included: (1) age ≥ 18 years, (2) at least a second psychiatric hospitalization due to schizophrenia, and (3) a minimum of two weeks of stable treatment with second-generation antipsychotics prior to enrollment. In this study, chronicity was defined by the 2nd and 3rd included criteria and applied to the patients with non-severe symptoms (mean SANS sum of 49.26 ± 29.13 and a mean SAPS sum of 45.55 ± 32.62).

rather than treatment resistance. Exclusion criteria included: (1) first-ever hospitalization for psychosis, (2) diagnosis of dementia or other organic mental disorders (F00–F09), substance use disorders (F10–F19), mood disorders (F30–F39), neurotic or stress-related disorders (F40–F48), developmental and intellectual disabilities (F70–F98), (3) somatic diseases potentially affecting cf-DNA levels (e.g., cancer, myocardial infarction, stroke, as well as acute infections like pneumonia or urinary tract infections, and active systemic inflammatory states). All patients were receiving stable doses of second-generation antipsychotics (e.g., olanzapine, clozapine) for at least two weeks prior to inclusion; however, detailed drug exposure was not analysed in this study.

The control group consisted of 30 healthy volunteers recruited from the general community. Based on structured interviews, they reported no history of psychiatric illness, substance use disorders, illicit drug use, alcohol abuse, or significant somatic disease.

Clinical assessment

Prior to enrollment, each participant's medical history and current clinical status were thoroughly reviewed to ensure they met the health-related inclusion criteria. This evaluation was specifically aimed at excluding individuals with major somatic comorbidities known to significantly alter cf-DNA levels, such as cancer, recent myocardial infarction, or stroke. Furthermore, on the day preceding blood collection, a structured physical screening was performed to rule out acute inflammatory processes; individuals exhibiting symptoms of fever, cough, rhinitis, localized inflammatory signs, or general malaise were excluded from the study to minimize the influence of acute inflammation on cf-DNA levels. Following this somatic clearance, each patient underwent a comprehensive psychiatric interview conducted by an experienced psychiatrist. The clinical assessment was performed to confirm the diagnosis of schizophrenia based on ICD-10 criteria and to ensure clinical stability. Negative and positive symptom severity was assessed using the Scale for the Assessment of Negative Symptoms (SANS) and the Scale for the Assessment of Positive Symptoms (SAPS), which are validated instruments widely used in schizophrenia research to quantify symptom burden. The SANS evaluates the severity of negative symptoms, such as affective blunting, avolition/apathy, anhedonia/asociality, and attentional deficits. The SAPS assesses positive symptoms, including hallucinations, delusions, bizarre behavior, and positive formal thought disorder. Each scale uses a 6-point rating system (0–5), with higher scores indicating greater symptom severity (SANS total score 0–125; SAPS total score 0–170)²⁵.

Blood Collection and Storage

Peripheral venous blood was collected into Streck Cell-Free DNA BCT[®] tubes (Streck, USA), designed to minimize contamination and preserve cf-DNA integrity. The tubes were gently inverted to mix and transported at 4 °C to the laboratory, where they were processed within 24 h. Samples were centrifuged in two stages: first at 300×g for 20 min, after which the supernatant was transferred into Eppendorf tubes and centrifuged again at 5000×g for 10 min. The resulting plasma was aliquoted into 1 mL portions and stored at –80 °C until further analysis.

cf-DNA Isolation

Cell-free DNA was extracted using the MagCore[®] Plus II instrument with the Code 105 MagCore[®] Plasma DNA Extraction Kit (1.2 mL), following the manufacturer's protocol optimized for high-purity cf-DNA extraction. This automated method ensured consistent yield and minimized manual handling errors.

Fluorometric Quantification of cf-DNA

The concentration of cf-DNA was measured using the 1× dsDNA (double-stranded DNA) High Sensitivity kit on the Invitrogen Qubit 3.0 Fluorometer (Thermo Fisher Scientific, USA). Measurements were performed using 10 µL of eluate for each measurement. The results were reported in ng/µL and converted to ng/mL of plasma.

ddPCR analysis

The exact copy number of mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) was quantified using droplet digital polymerase chain reaction (ddPCR) on the Bio-Rad QX600 system. Specific primers and probes were designed to target regions of interest in the mitochondrial and nuclear genomes. The sequences were as follows: hRPP30F: AACTTGTAAGTGGTAGTGCATAGA, hRPP30R: GTAGGAGGACATTTGAGGAGTG, hRPP30Probe: 6-FAM-TCAGGCAGACTGACACTAGAGTTCACA-BHQ-1, hND1F: CTGATCAGGGTGAGCATCAAA, hND1R: GAATGATGGCTAGGGTGACTTC, and hND1probe: HEX-TGCGAGCAGTAGCCCCAAACAATCT-BHQ-1. These sequences were adopted from a previously published study²⁶. All primers and probes were manufactured by Genomed, Poland. The ddPCR reactions were prepared using Bio-Rad's ddPCR Multiplex Supermix for Probes, and each reaction had a final volume of 20 µL. Each reaction was prepared with 10 µL of Supermix, 0.18 µL of each primer (100 µM), 0.07 µL of each probe (100 µM), 3 µL of sample, and nuclease-free water to 20 µL. Reaction conditions were in accordance with the manufacturer's instructions, and each reaction was performed in two technical replicates. The concentrations of mitochondrial and nuclear DNA were calculated using the QX Manager Software Standard Edition (version 2.3.1; Bio-Rad, Hercules, CA, USA; <https://www.bio-rad.com>). The total number of copies in the eluate was calculated and divided by the plasma volume used for isolation to express results as the number of copies per mL of plasma.

Statistical analysis

The dataset was analyzed in Python (version 3.11.10; <https://www.python.org>) using the following libraries: NumPy (<https://numpy.org>), SciPy (<https://scipy.org>), Plotly (<https://plotly.com>), and Matplotlib (<https://matplotlib.org>). A variety of statistical tests were used depending on the type of data analyzed. Spearman's rank

correlation was applied for the analysis of pairs of quantitative data, while the Mann-Whitney U test, Student's t-test, or Welch's t-test were used for the analysis of quantitative variables against categorical variables, depending on whether the criteria regarding the nature of data distribution were met. Significance was defined as $p < 0.05$.

Results
Participants characteristics

We analyzed 33 patients with chronic schizophrenia (Study) and 30 healthy volunteers (Control). Participants were recruited between August 2023 and November 2024. The Study group included 12 women (36.4%) and 21 men (63.6%), whereas the Control group comprised 21 women (70.0%) and 9 men (30.0%); the sex distribution differed significantly between groups (χ^2 , $p = 0.0156$). Mean age was 39.5 ± 12.6 years in the Study group and 31.1 ± 10.5 years in Controls (Welch's t, $p = 0.0059$). Current smoking was reported by 21/33 (63.6%) participants in the Study group and 2/30 (6.7%) in Controls (χ^2 , $p = 5.6 \times 10^{-6}$). These imbalances reflect a significant overlap between the patient group and these variables, introducing substantial confounding that was further explored in subsequent adjusted analyses (see Table 1).

Between-group differences in plasma cf-DNA (unadjusted)

Median nuclear cf-DNA (cf-nucDNA) concentrations were higher in patients than in Controls (628 [386–939] vs. 452 [250–562] copies/mL; Mann–Whitney $p = 0.020$; Welch's t on $\log_{10}$ -transformed data $p = 0.012$; Cliff's $\delta = 0.34$) (Fig. 1). Mitochondrial cf-DNA (cf-mtDNA) did not differ significantly between groups (111,270 [46,121–181,324] vs. 94,613 [44,010–145,877] copies/mL; $p = 0.413$). Total dsDNA showed a non-significant trend toward higher values in patients (2.09 [1.54–3.04] vs. 1.83 [1.48–2.23] ng/mL; $p = 0.088$; $\log_{10}$ Welch's $p = 0.069$). Given the substantial differences in sex distribution and smoking status between groups, which represent significant sources of confounding, we present adjusted analyses later in this section to investigate whether the observed case-control difference remains independent of these factors.

Adjusted analysis of cf-nucDNA (primary)

After adjustment for age, sex, and smoking, the case–control difference in $\log_{10}$ was not significant ($\beta = 0.091$, 95% CI -0.058 to 0.241 , $p = 0.237$; model $R^2 = 0.318$). Male sex remained a strong positive predictor ($\beta = 0.256$, 95% CI 0.134 to 0.377 , $p = 1.28 \times 10^{-4}$), whereas age and current smoking were not associated with cf-nucDNA (both $p > 0.22$). Full coefficients are shown in Table 2. It is important to emphasize that our study cohort is characterized by a marked imbalance in smoking and sex distributions, as the vast majority of smokers and male participants were in the patient group. This overlap introduces significant statistical collinearity, suggesting that the attenuation of significance in the adjusted model ($\beta = 0.091$, $p = 0.237$) may be a consequence of limited statistical power and group confounding rather than a true absence of a disease-related effect.

Method concordance and parameter correlations

Fluorometric total dsDNA correlated strongly with cf-nucDNA measured by ddPCR (Spearman $\rho = 0.871$; $p = 1.9 \times 10^{-20}$; $n = 63$), with no association to cf-mtDNA ($\rho = -0.127$; $p = 0.322$). In control women, cf-mtDNA increased with age ($\rho = 0.666$; $p = 0.0010$; $n = 21$) (see Supplementary Fig. S2, which illustrates the age-related distribution of mtDNA across the total sample). No correlations were observed between SANS/SAPS scores and any cf-DNA measure (all $p > 0.16$; Supplementary Fig. S3).

Raw data available as Supplementary Materials 1.

Discussion

In our study, unadjusted analyses initially suggested that patients with chronic schizophrenia had higher levels of cf-nucDNA compared to healthy controls. However, after rigorous adjustment for age, sex, and smoking status, this difference was no longer statistically significant, and male sex emerged as a robust positive predictor of cf-nucDNA. We also observed a strong method concordance between Qubit dsDNA and ddPCR cf-nucDNA, and a positive association between age and cf-mtDNA specifically within the subgroup of control women. These results demonstrate that the relationship between cf-DNA and schizophrenia is highly sensitive to demographic and lifestyle factors, which account for a significant portion of the observed variance in our sample. However, because diagnosis, sex, and smoking status are deeply confounded in this cohort, no independent disease-specific association could be conclusively demonstrated. The lack of significance in the adjusted analysis likely

Variable	Study ($n = 33$)	Control ($n = 30$)	p -value
Age, years — mean $\pm$ SD	39.5 ± 12.6	31.1 ± 10.5	0.0059
Sex — female, n (%)	12 (36.4%)	21 (70.0%)	0.0156
Sex — male, n (%)	21 (63.6%)	9 (30.0%)	—
Smoking — yes, n (%)	21 (63.6%)	2 (6.7%)	5.6×10^{-6}
Psychoactive substances — yes, n (%)*	2 (6.1%)	0 (0.0%)	0.49†

Table 1. Participant characteristics (study vs. control). Age: Welch's t-test; Sex/Smoking: χ^2 test; †Drugs: Fisher's exact. Report exact p-values; bold indicates $p < 0.05$. *Two patients reported occasional recreational psychoactive substance use with no use within > 2 weeks before blood sampling.

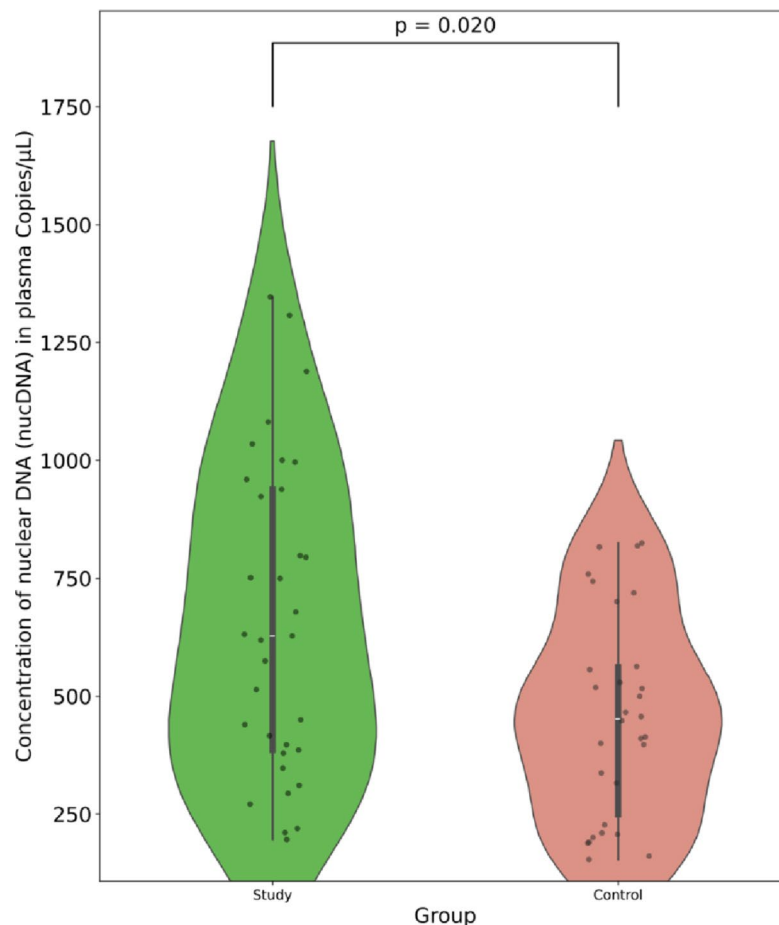


Fig. 1. Plasma nuclear cell-free DNA in patients with chronic schizophrenia (Study, $n = 33$) and healthy controls (Control, $n = 30$). Violin plots show kernel density; points are individual participants; horizontal lines denote medians with interquartile ranges. P-value from Mann–Whitney ($p = 0.020$) is shown. Units: copies/mL.

Predictor	β (log10-scale)	95% CI	p-value	Approx. fold-change (10^{β})
Case — Study = 1	0.091	−0.058 to 0.241	0.2366	1.23×
Age — years	0.003	−0.002 to 0.008	0.2520	1.01×
Male — 1 = Male	0.256	0.134 to 0.377	0.0001	1.80×
Smoker — 1 = Yes	−0.091	−0.236 to 0.054	0.2222	0.81×

Table 2. Adjusted regression for $\log_{10}$.

reflects the statistical difficulty of decoupling these variables in an imbalanced sample rather than a definitive lack of biological relevance to schizophrenia.

Our findings contrast with the meta-analysis by Melamud et al., which reported significantly elevated cf-DNA in various psychiatric disorders, including schizophrenia²⁰. This discrepancy likely stems from the clinical phase of the study populations. While many studies included in meta-analyses focus on acute phases or first-episode psychosis, our cohort consisted of clinically stabilized patients with chronic illness. We defined chronicity as having at least a second psychiatric hospitalization and receiving stable antipsychotic treatment for at least two weeks prior to enrollment. Notably, our patients did not present with “stormy” or acute psychotic symptoms - such as massive delusions or psychomotor agitation - which allowed for informed consent but also suggests a more biologically stable state. The chronic nature of our group was further evidenced by a predominance of negative symptoms, with a mean SANS sum of 49.26 ± 29.13 and a mean SAPS sum of 45.55 ± 32.62 . Unlike previous research investigating first-episode patients where changes in cf-mtDNA correlated with PANSS improvements, we observed no significant correlations between SANS/SAPS scores and any cf-DNA measure. This may imply that cf-DNA is not a direct marker of current symptom severity in stable, chronic patients, but rather reflects other pathophysiological aspects.

From a pathophysiological perspective, although not directly confirmed by our adjusted analysis, it has been hypothesized in the literature that elevated cf-DNA levels might reflect a persistent state of disturbed cellular homeostasis²⁷. Within this theoretical framework, cf-DNA has been proposed to potentially play a role in sustaining immune responses as a damage-associated molecular pattern^{22,23,28,29}. Such a mechanism may help explain persistent symptoms despite treatment and the presence of biological alterations in patients in clinical remission. It should be emphasized that the markers targeted in this study-RPP30 for nuclear DNA and ND1 for mitochondrial DNA-reflect total systemic cf-DNA concentrations rather than brain-specific or neuron-derived pathology. Therefore, our results should be interpreted as reflecting a general disturbance in cellular homeostasis rather than direct neuronal damage, and these findings should be considered speculative and hypothesis-generating in nature.

Regarding demographic factors, our findings align partially with previous reports but also highlight the significant heterogeneity of existing data. In our study, male sex emerged as a robust positive predictor of nuclear cf-DNA levels. Some studies, such as that by Zhong et al., found no sex-related differences in cf-DNA concentrations, except in women over 60 years of age, who exhibited higher plasma cf-DNA levels, suggesting that aging-related biological factors specific to women may influence cf-DNA release³⁰. Conversely, a large cohort study involving over 5,000 participants by Kananen et al.² reported significantly higher cf-DNA levels in men across all age groups, particularly in middle-aged and older men with higher frailty indices. Notably, older women (> 65 years) in that study also showed elevated cf-DNA levels, indicating that age- and frailty-related mechanisms may modulate cf-DNA dynamics in a sex-specific manner². The association between cf-DNA and age remains inconsistent across studies. Meddeb et al. reported an increase in nuclear cf-DNA - but not mitochondrial cf-DNA - with age, whereas other analyses found no uniform trend in cf-DNA concentrations. Instead, age-related alterations in cf-DNA profiles have been described, including differences in fragmentation patterns and chromatin origin, reflecting potential epigenetic or structural changes linked to aging^{31,32}. Taken together, the evidence suggests that both sex and age may significantly affect cf-DNA characteristics, although findings remain variable. These discrepancies likely stem from methodological differences, population-specific factors, and the complex biology underlying cf-DNA release. It's also crucial to acknowledge that schizophrenia is a heterogeneous syndrome, and it is unlikely that a single pathophysiological mechanism explains cf-DNA elevations across all patients. In our cohort, the substantial imbalance in sex distribution and smoking status between groups likely introduced collinearity into the model. This statistical confounding, rather than a definitive absence of a disease effect, may explain why the initial case-control difference in cf-nucDNA was attenuated after adjustment.

In the present study, no significant correlations were observed between SANS/SAPS scores and cf-DNA concentrations (Supplementary Fig. S3), which was further confirmed by visual inspection of scatter plots to rule out the influence of outliers. This suggests that in the analyzed population, cf-DNA levels do not directly reflect the severity of clinical symptoms of schizophrenia. This lack of association may imply that cf-DNA is not a direct marker of symptom burden during stable phases of the illness but rather reflects other underlying aspects of disease pathophysiology. For the purposes of this study, we defined chronicity specifically as having at least a second psychiatric hospitalization and a minimum of two weeks of stable treatment with second-generation antipsychotics prior to enrollment. Our study group was notably characterized by a relative predominance of negative symptoms, which aligned with our criteria for chronicity. This was reflected in the clinical assessment, showing a mean SANS sum of 49.26 ± 29.13 and a mean SAPS sum of 45.55 ± 32.62 . Our findings contrast with some existing literature. For instance, a 2021 study investigated the relationship between cf-mtDNA levels and PANSS scores in patients with first-episode schizophrenia undergoing antipsychotic treatment. That study found a significant correlation between changes in cf-mtDNA levels and clinical improvement, particularly in positive symptoms. However, those results were obtained in an acute population undergoing active treatment response³³, whereas our study focused on patients who did not present with “stormy” psychotic symptoms - such as massive delusions, hallucinations, or psychomotor agitation - that would have prevented them from providing informed consent. The use of SANS and SAPS scales in our study allowed for a detailed analysis of symptom domains, yet unlike previous studies using the PANSS scale, no associations were found. This suggests that the relationship between cf-DNA and symptom severity may be inconclusive or specific to the acute phase of the disorder. In future research, expanding the study population and incorporating the PANSS scale may help obtain more precise results and improve our understanding of potential associations between cf-DNA concentrations and disease progression.

Limitations

It should be noted that the present study has several important limitations that necessitate a cautious interpretation of the findings. First, the relatively small sample size may reduce the statistical power of the analyses and limit the generalizability of the results to the broader population of patients with schizophrenia. In addition, the control group was not fully matched to the study group with respect to age and sex, which required the use of adjusted statistical models to interpret the results. The cross-sectional design of the study further precludes any causal inferences regarding the relationship between cf-DNA and the disease process.

A significant methodological constraint concerned the collection of clinical history data. Our ability to provide a specific, verified number of lifetime hospitalizations was limited by the lack of an integrated electronic medical record system at our center prior to 2020. Consequently, data regarding hospitalizations before this period were collected primarily through structured clinical interviews and available current records; however, since patients may have been hospitalized in other facilities, these figures may not be fully comprehensive.

Furthermore, the results of our study must be interpreted with a high degree of caution. Crucially, the marked imbalance in smoking and sex distributions between the study and control groups represents a major methodological constraint. Since current smoking was reported almost exclusively by patients and the majority

of male participants were also in the patient group, the effects of lifestyle and sex are statistically confounded with the disease state. This confounding leads to collinearity in regression models, which, combined with the relatively small sample size, likely reduced the statistical power to detect independent disease-related signals. Therefore, the apparent dominance of lifestyle factors must be regarded as preliminary and requires validation in larger, more closely matched populations where these variables can be effectively decoupled. As such, the findings of this study do not currently allow for the application of cf-DNA measurements in routine clinical practice.

Another limitation is that this study did not assess the stability of cf-DNA levels over time. A reliable clinical or diagnostic biomarker must demonstrate high sensitivity, specificity, and temporal stability. Since our research did not include a longitudinal component, we cannot determine whether the measured parameters remain constant throughout the course of the illness. Future research should incorporate a follow-up design - for instance, re-examining the same cohort after six months - to evaluate the stability of cf-DNA as a marker.

Finally, while the SANS and SAPS scales provided a detailed assessment of symptom severity, we did not observe the correlations reported in studies using the PANSS scale. Future research involving larger patient populations should incorporate the PANSS scale to enable a more precise assessment of all three symptom domains and their potential relationship with cf-DNA concentrations.

Despite these limitations, cf-DNA remains a potential biomarker in schizophrenia research, provided that future studies utilize larger, well-matched populations and consider the degree of disease progression and clinical severity.

Conclusions and future directions

In conclusion, while nuclear cf-DNA shows modest elevations in chronic schizophrenia, the interpretation of these findings is constrained by significant confounding with demographic and lifestyle factors, particularly sex and smoking. In this cohort, it was not possible to statistically decouple these variables from the disease state with sufficient power. These findings do not currently allow for the application of cf-DNA in routine clinical practice; however, its potential relevance as a biomarker requires further investigation. Future studies should not only include larger and more clinically diverse populations but also employ longitudinal follow-up designs to determine the temporal stability of these markers. Furthermore, as suggested by Jiang et al.³⁴, focusing solely on total cf-DNA concentration may be insufficient; future research should investigate qualitative parameters, such as DNA fragment size distributions, which may offer greater sensitivity and stability for monitoring the progression of chronic schizophrenia over time.

Data availability

The raw datasets generated and analysed during the current study are provided in the Supplementary materials.

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Author contributions

KM was responsible for study design, patient recruitment and qualification, blood sample collection and laboratory analyses, as well as drafting of the manuscript. ŁC contributed to patient recruitment and qualification, and assisted with sample collection. PL contributed to study design, conducted laboratory analyses of cf-DNA, performed statistical analyses, co-drafted the manuscript, and provided methodological supervision regarding laboratory procedures and cf-DNA. MR contributed to statistical analyses. SF contributed to study design, patient recruitment and clinical assessment, provided methodological supervision in psychiatric aspects, and critically revised the manuscript for important intellectual content. PG and MP provided methodological supervision in psychiatric aspects and critically revised the manuscript for important intellectual content. All authors read and approved the final version of the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

The study was approved by the Bioethics Committee of the Medical University of Silesia, Katowice, Poland (BNW/NWN/0052/KB1/22/23). Written informed consent was obtained from all participants prior to sample collection.

Artificial intelligence

During the preparation of this work the authors used GPT-4o and GPT-5 (OpenAI, San Francisco, CA, USA; <https://openai.com>) to improve language and readability. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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Additional information

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Correspondence and requests for materials should be addressed to K.M.

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