Jiang et al. *Translational Psychiatry* (2018)8:104
DOI 10.1038/s41398-018-0153-3

Translational Psychiatry

ARTICLE **Open Access**

Analysis of the concentrations and si e distributions of cell-free DNA in schi ophrenia using fluorescence correlation spectroscopy

Jie Jiang[1], Xueli Chen[2], Liya Sun[1], Ying Qing[1], Xuhan Yang[1], Xiaowen Hu[1], Chao Yang[1], Tianle Xu[3], Jijun Wang[1,4], Peng Wang[5], Lin He[1], Chaoqing Dong[6] and Chunling Wan[1,4]

Abstract

Cell-free DNA (cfDNA), which is primarily released following cell death, has been described and developed to serve as an effective biomarker in autoimmune diseases which may share the pathogenesis with schizophrenia. In this study, we hypothesized and explored whether the concentrations and size distributions of cfDNA are abnormal in schizophrenia. A total of 65 patients with schizophrenia (SZ), 29 patients with mood disorders (MD) and 62 matched healthy controls (HC) were included in the study. Fluorescence correlation spectroscopy was used to assay the molar concentrations and size distributions of cfDNA. Fluorometric quantification and quantitative real-time PCR (qPCR) were performed to verify the results. The cfDNA levels were approximately two-fold higher in the SZ group ((29 ± 15) nM) than in the healthy controls ((15 ± 9) nM; -value = 0.00062), but the levels in patients with MD were not significantly different from those in the healthy controls ((17 ± 10) nM; -value = 0.343). According to the size distribution analysis, cfDNA in schizophrenia patients was composed of shorter DNA molecules and showed an apoptosis-like distribution pattern. Our study shows the elevated levels and short sizes of cfDNA in schizophrenia patients, which provide direct evidences supporting increased apoptotic activity in the disease. cfDNA may be developed to serve as an auxiliary diagnostic marker for the disease in the future.

Introduction

S a a a a a a a 1% ' a [1]. A a a a b a, - a ab a , a - a a ba [2], a

a a a . D a a a b a , a a a a a a a a a a a a . A , a a a , a a a a b b a a - a. F a , a a a [3] a a b b ab a a. A , a , a ab a - a b a a a a ' a [4]. Ea a a a a

Correspondence: Chaoqing Dong (cqdong@sjtu.edu.cn) or Chunling Wan (clwan@sjtu.edu.cn)
[1]Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education), Shanghai Key Laboratory of Psychotic Disorders, Shanghai Mental Health Center, Shanghai Jiao Tong University, Shanghai 200030, China
[2]College of life sciences, Anhui Normal University, Wuhu 241000, China
Full list of author information is available at the end of the article

20–80% a a
(CNS) b a [5]. S a a a a
a a b a -
a a , a a - -
, a a a a -3 a b
b a a a b [4,6–9].
DNA a fi b b Ma a M a
a a 1948[10] a a
a a. A a , DNA a
b b a a
a a a a ,
[11,12], a a, , a a a , a
, a a a a
a [13–17]. I a b b ,
, a a - a
ab a DNA a b a

Q b VR 2.0 F (I , CA) a a ' . T a PCR a ALU- a : a 115 b ALU a (ALU115) a b a DNA a , a a 247 b ALU a (ALU247) a DNA a . T a Tab S2. A 4.8 L DNA a a 1 SYBR G a (R , S a) PCR, a b a V A™ 7 R a -T PCR S (L T , USA) a 95 C 10 , 35 a- a a 95 C 30 , a a a 64 C 30 , a a 72 C 30 [13]. A PCR a a - a b a , a a a a a a a .

Statistical analysis

L a a a a a a a , , BMI, a a a , , a ab . P a a a a a . P a a a a a a fi a b a . A *P* a <0.05 a a a fi a , a a bab a . A a a a a a a - a R a a .

Results

D a a a a a a a a A a 65 a a, 29 a (18 a a a 11 b a) a 62 a a . S b ab , a a , , fla a , a a . T a a a a a a b a a Tab S1. T a fi a - b a , , b a (BMI), a ab (Tab S1).

Increased cfDNA levels in schizophrenia patients

T a a a b FCS DNA a a a a a a FCS. T DNA SZ a a - a HC , a a fi a b DNA MD a HC (SZ (29 15) M) . HC

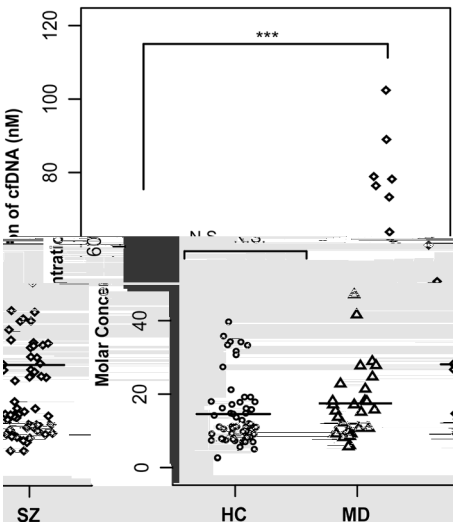

Fig. 1 Molar concentrations of cfDNA in the three groups. Statistical comparisons were performed using logistic regression analysis. SZ schizophrenia, MD mood disorders, HC healthy controls, N. S. no significant difference, *** < 0.001

((15 9) M, *P*- a = 0.00062); MD ((17 10) M) . HC ((15 9) M, *P*- a = 0.343; F . 1).

Size distribution analysis of cfDNA

A a a b a , b a DNA b a a 96 ; a 0.1 100 a a (F S3) a a . T a - a . T a DNA a DNA ba a 180 b , 360 b , a 540 b , , , a - a , . T ba a a 1.03,1.59, a 2.13 , (F . 2a). A 1000 b DNA ba a ( ) a F . 2a. T a a a a a a a - a b SZ a HC . F 2b a a DNA a 1 4 , a 180 b 1 b. T fi a a b SZ DNA a a a HC DNA. F 2 a DNA a SZ a a a HC a 0.6 1.5 , DNA a 360 b , a a a DNA SZ .

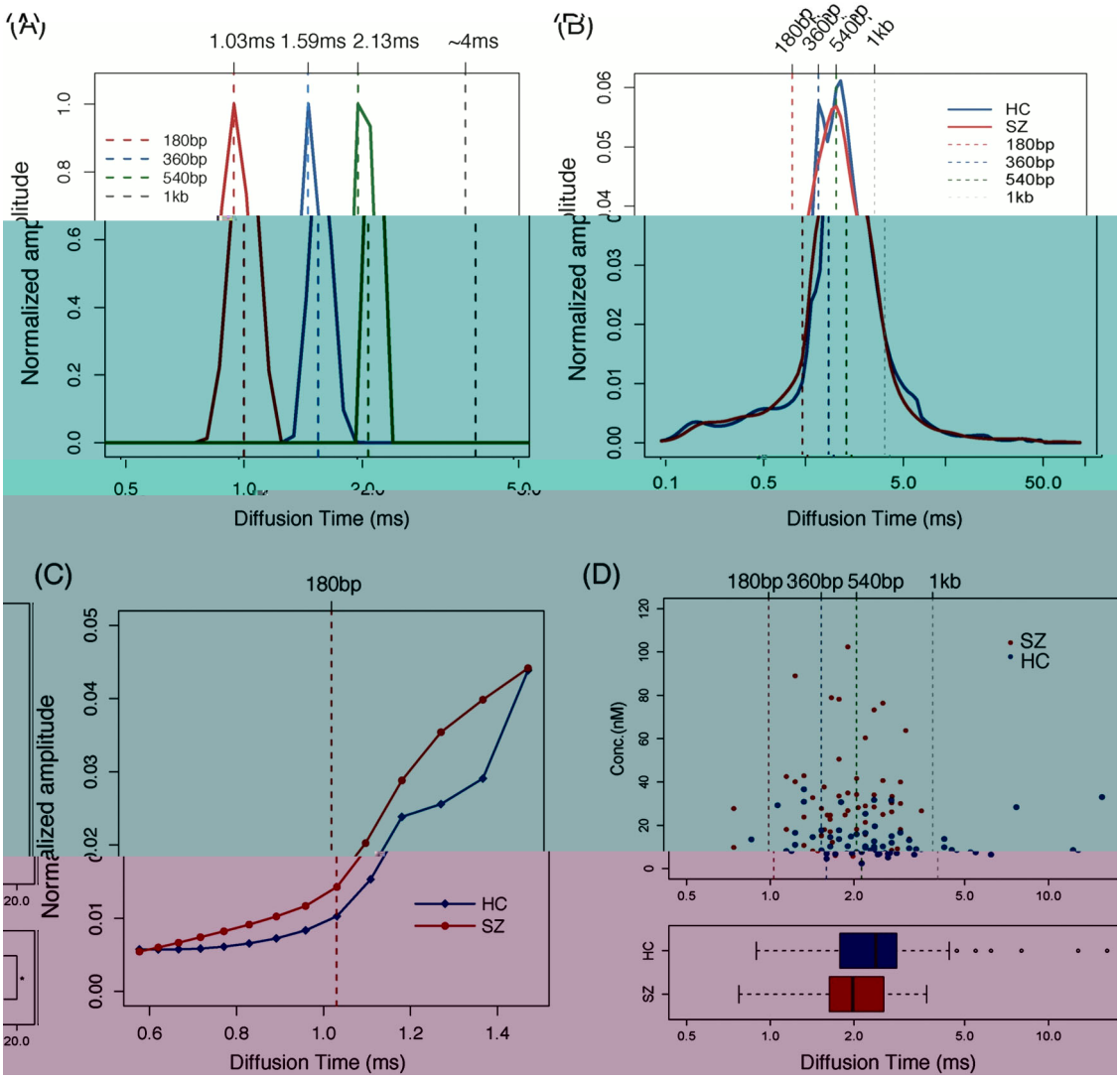

Fig. 2 Characteristics of the size distributions of cfDNA in SZ and HC groups. a The solid lines are the distribution curves and the dotted lines indicate the diffusion times of the peak points of the markers. **b** The figure shows the average amplitude of each diffusion component in the schizophrenia group and the healthy control group. **c** The enlargement part of the diffusion components from 0.6 to 1.4 ms, which represents the scope with major differences in size. The dotted lines are the positions of the markers. **d** Average diffusion time of samples. Every dot represents the average diffusion time of each sample in the upper figure. The figure below is the boxplot of the average diffusion time of each sample in the SZ and HC group. SZ schizophrenia, HC healthy controls; * < 0.05

W a a a a a a - a a DNA SZ a a a a DNA a a a DNA a . T a a a b a a . A a a a a DNA a 1 b a a HC , a a a a 540 b a a a a SZ a HC (F . 2). I a , a a SZ a fi a a - a a HC (T b F . 2 , *P*- a = 0.028). T a

Validation of results using fluorometry and qPCR

T b a b FCS, DNA a Q b VR 2.0 F a PCR. C , a a DNA a fi a a SZ a HC (SZ : (21.1 12.0) / L; HC : (12.7 5.5) / L; *P*- a = 0.000081), a

a a DNA MD a fi a a HC (MD : (12.2 5.4) / L; HC : (12.7 5.5) / L; *P*- a = 0.443). T a a b a PCR 115 b a (SZ (6.7 -08 6.8 -08) . HC (3.9 -08 2.3 -08) *P*- a = 0.0089; MD (5.9 -08 5.1 -08) . HC (3.9 -08 2.3 -08) *P*- a = 0.056, F . 3). M , a b SZ a HC - a DNA a fi b 247 b ALU a (SZ: 1.0 -08 1.2 -08; HC: 0.9 -08 0.9 -08; *P*- a = 0.506), a DNA - a a a SZ (F . 3).

W a a a a fi a- FCS PCR a fl P a ' a fifi

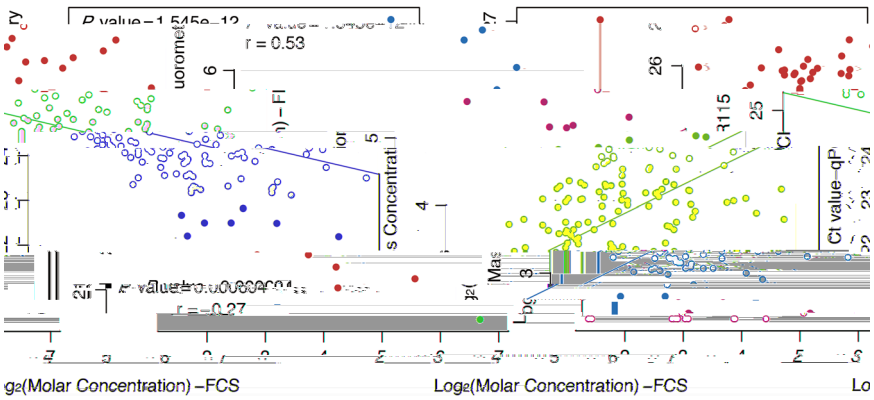

Fig. 4 Correlations between cfDNA concentrations measured by the three methods. Scatterplot comparing the correlations between molar concentrations measured by FCS and mass concentration measured by fluorometry (red) and Ct values by qPCR (blue). The correlation coefficient and the value was calculated using Pearson's correlation analysis

, a , a . FCS a a a , a a fi a a a DNA a , a a a - a 2.5 DNA a 240 a a a . T a[25]. H , a a a a DNA a >1000 b a DNA a a a a a b a a , b DNA a a b b PCR a a a a . D a a - a a a a DNA a b a a a. a a- Ba ab , , a a a a a fi DNA a a b a a a a a a . R , a a - a . a b b A a DNA a a a a " " -DNA a a a DNA a a . T a DNA a - a . F a 180 b (180 b) a a a a a , a a - a a , a b a a 10,000 b a a a - [12,18]. T a DNA b a [26]. Ma a DNA a— a a a a , a fi a a a a a fla a , a ab a a - a a a a a a . a a a — a a I a a , DNA a a [27]. T - a (<1000 b) a a a a - a a a ROS a - fi a (a a a - a DNA a) a a . F a DNA a a (F . 1). T a b a b a fl a a a a a a a a fl b b –b a ba a a a a a a a - (F . 5). E a a a a . Ea a b a , a , a - a a a a a b a a a ab a a - a a DNA , a a a a a a - a . T , a a [4,24]. A a a a a a DNA a a a - B -2 , a Ba /B -2 a a a a b . I a b Ca a -3, a a a ab a b a a - -a a a a a a a a a, b a. I (F . 5). I , a

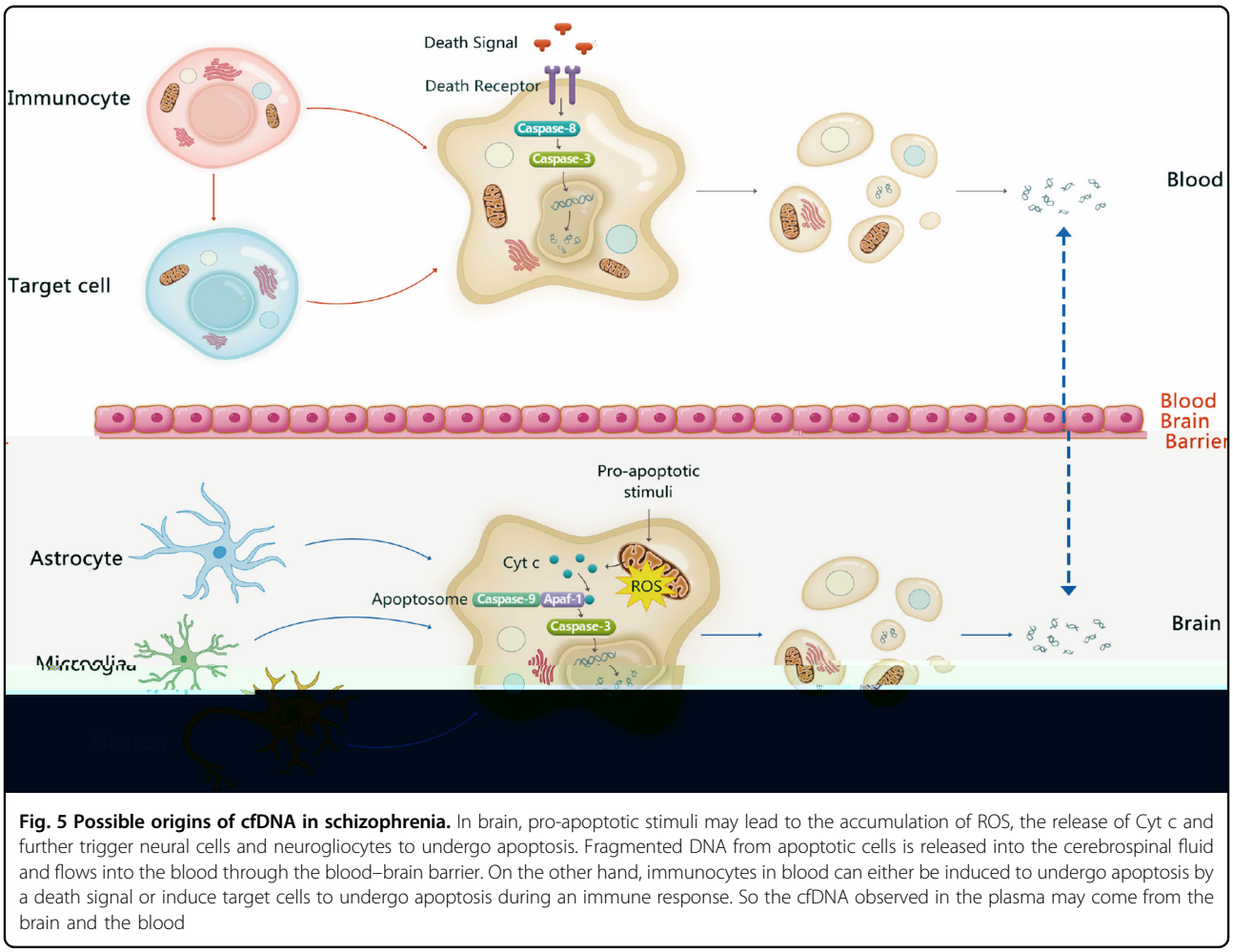

Fig. 5 Possible origins of cfDNA in schizophrenia. In brain, pro-apoptotic stimuli may lead to the accumulation of ROS, the release of Cyt c and further trigger neural cells and neurogliocytes to undergo apoptosis. Fragmented DNA from apoptotic cells is released into the cerebrospinal fluid and flows into the blood through the blood–brain barrier. On the other hand, immunocytes in blood can either be induced to undergo apoptosis by a death signal or induce target cells to undergo apoptosis during an immune response. So the cfDNA observed in the plasma may come from the brain and the blood

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Acknowledgements
This work was supported by the National Key R&D Program of China (2016YFC1306802, 2016YFC1306900), the National Nature Science Foundation of China (81271486, 21475087, 81421061), the Program for NSFC International (Regional) Cooperation and Exchange (81361120389) and Shanghai Key Laboratory of Psychotic Disorders (13dz2260500)

Author details
[1]Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education), Shanghai Key Laboratory of Psychotic Disorders, Shanghai Mental Health Center, Shanghai Jiao Tong University, Shanghai 200030, China. [2]College of life sciences, Anhui Normal University, Wuhu 241000, China. [3]Discipline of Neuroscience, Department of Anatomy, Histology and Embryology, Collaborative Innovation Center for Brain

Science, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China. [4]Collaborative Innovation Center of Genetics and Development, Shanghai 200025, China. [5]The Fourth People's Hospital of Wuhu, Wuhu 241002, China. [6]School of Chemistry and Chemical Engineering, Shanghai Jiao Tong University, Shanghai 200240, China

Conflict of interest

The authors declare that they have no conflict of interest.

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Supplementary Information accompanies this paper at https://doi.org/10.1038/s41398-018-0153-3.

Received: 3 July 2017 Revised: 3 April 2018 Accepted: 11 April 2018
Published online: 22 May 2018

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