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Abundance of ribosomal RNA gene copies in the genomes of schizophrenia patients

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ABSTRACT

Objective: The ribosome is a critical component of the translation machinery. The key component of ribosomes is ribosomal RNA (rRNA). Dysregulation of rRNA biogenesis has been implicated in some human diseases. One of the factors affecting rRNA biogenesis is the ribosomal RNA genes (rDNA) copy number in the genome. The aim of this study was to examine the rDNA copy number (***CN***) variation in the genomes of patients with schizophrenia (SZ) compared to healthy controls (HC).
Methods: We evaluated rDNA ***CN*** in leukocytes of 179 subjects with SZ (108 male/71 female) in comparison with 122 HC (60 male/62 female) using two techniques: qPCR and nonradioactive quantitative hybridization (NQH), which is based on the use of biotinylated rDNA probes.
Results: rDNA ***CN*** (NQH) and rDNA ***CN*** (qPCR) was higher in SZ patients than in controls (median 542 vs 384, $p = 10^{-25}$ and median 498 vs 370, $p = 10^{-12}$). NQH was experimentally proved to be less sensitive to severe DNA damage than qPCR. The more DNA damage, the higher the ratio R = ***CN*** (NQH)/***CN*** (qPCR). 15% of the SZ patients had significantly higher rDNA damage degree than the HC.
Conclusion: Genomes of some SZ patients contain more ribosomal genes than those of HC. The elevated ribosomal genes copy number in human genome can be one of the genetic factors of schizophrenia development. This hypothesis requires further experimental studies to be corroborated or disproved.

1. Introduction

The nucleolus is a nuclear organelle that coordinates rRNA transcription and ribosome subunit biogenesis. The ribosome is a complex molecular machine that serves as the site of biological protein synthesis (translation). Ribosomes consist of ribosomal RNA (rRNA) molecules and a variety of ribosomal proteins. Ribosomal RNA genes (rDNA) encode the rRNA species that form the ribosomes. The diploid human genome contains several hundred copies of a 43-kb rDNA unit tandemly arrayed in nucleolar organizer regions on five acrocentric chromosomes. Each unit contains 13.3 kb-long sequence encoding the 28S, 5.8S and 18S rRNAs and a non-coding intergenic spacer (IGS) (McStay and Grummt, 2008), Fig.1A. Together with the 5S rRNA these rRNAs form the nucleic acid backbone of the ribosome. Approx. 50% of rDNA copies are not transcribed (Hamperl et al., 2013; Lyapunova et al., 2013). The non-active copies are assumed to be important for supporting genome stability (Chubb et al., 2002; Paredes and Maggert, 2009; Ide et al., 2010).

Ribosome biogenesis dictates the capacity of the cell to grow, proliferate and successfully carry out its functions. Dysregulation of rRNA biogenesis has been implicated in some human diseases (Hannan et al., 2013). One of the factors affecting rRNA biogenesis is the ribosomal genes copy number (Larson et al., 1991; Laferte et al., 2006). There are few publications on rDNA content in the genomes of patients with mental disorders. Schizophrenia (SZ) patients were shown to have an elevated transcriptional activity of rDNA in brain and lymphocytes, which can result from an elevated rDNA content in SZ genomes compared to healthy controls (HC) (Veiko et al., 2003; Krzyżanowska et al., 2015). Previously, an increased rDNA copy number in the leukocytes of SZ patients (N = 42) compared to HC (N = 33) was revealed in a small sample using nonradioactive quantitative hybridization (NQH) (Veiko et al., 2003). In order to prove demonstratively the fact of an elevated rDNA abundance in the genomes of schizophrenia patients, it was necessary, firstly, to enlarge the sample size of both SZ patients and HC and, secondly, to apply real-time PCR (qPCR), a modern technique, which is generally recognized for genetic studies at present. The qPCR is most

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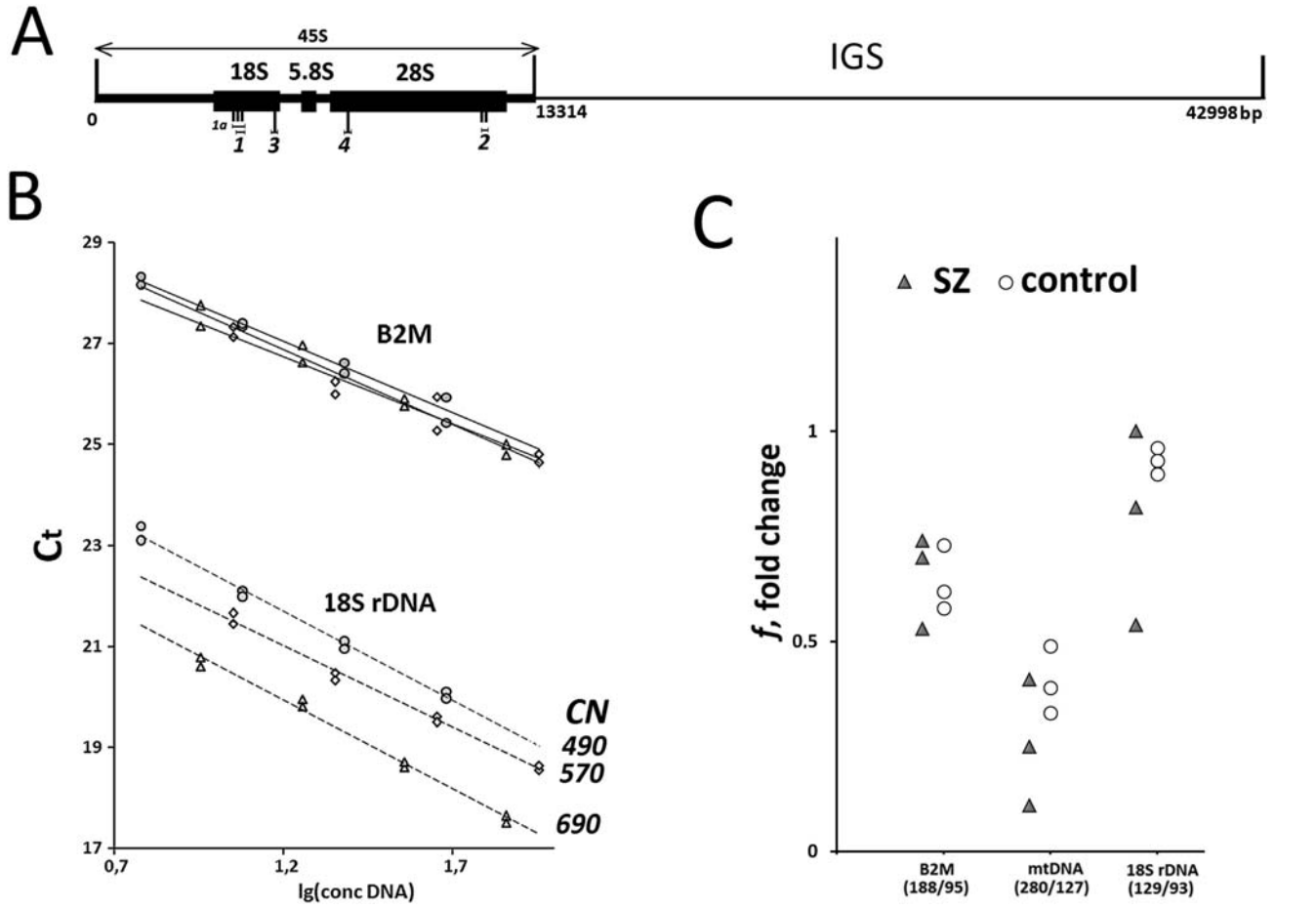

Fig. 1. Determination of rDNA copy numbers in human leukocyte DNA using qPCR. A. Scheme of the human ribosomal repeat. Segments of 18S rDNA and 28S rDNA analyzed with qPCR (1, 1a and 2) and with NQH (3 and 4) are shown. B. Dependence of Ct on the logarithm of DNA concentration in the sample. Data for three DNA samples are presented. The rDNA copy numbers (***CN***) were determined previously using blot hybridization (Veiko et al., 2003). The plot indicates that the more is ***CN***, the lower is Ct at each DNA concentration tested in the samples. C. Three DNA samples from the control group and three DNA samples from the SZ group were selected. The indices of degradation ***f*** for nDNA (gene B2M), 18S rDNA, and mtDNA were calculated by normalizing the increasing amplicons to short amplicons. The fragment length is shown on the plot for each gene.

frequently applied for the quantification of DNA by means of relative comparison of the standard and target loci.

Nonetheless, when planning the study, we decided not to reject NQH due to the following reasons. Damaged DNA is obviously a bad template for polymerase chain reaction. The ribosomal repeat is a hard region to be assayed. Even at normal conditions, a considerable number of DNA breaks are detected within rDNA (Pope et al., 2016). The transcribed region of the ribosomal repeat contains many Gn (n > 2) motifs (Kostyuk et al., 2015). Deoxyguanosine included in these motifs has a very low oxidation potential (von Sonntag, 2006). The DNA repair processes are also known to be altered in rDNA (Christians and Hanawalt, 1993; Stevnsner et al., 1993; Fritz and Smerdon, 1995; Pelloux et al., 2012). Therefore, the transcribed region of rDNA is probably affected to a higher degree than the other genomic sequences under oxidative stress conditions.

Furthermore, oxidative DNA damage is deemed a cause of the disease in recent hypotheses concerning the pathophysiology of SZ (Raza et al., 2016; Copoglu et al., 2015). We also uncovered recently a considerable enrichment of the blood lymphocyte pool with cells that had very high degree of DNA oxidation in a fraction of SZ patients (Ershova et al., 2017). Previously, we found that qPCR on damaged cell free rDNA gave underestimated results (Korzeneva et al., 2016). Interestingly, other authors showed earlier that DNA derived from the aged cells accumulated defect sites which affected the qPCR efficiency in some genome regions (Ploskonosova et al., 1999).

The DNA hybridization technique requires more amount of DNA for assay and is less sensitive than qPCR. However, NQH does not involve any usage of enzymes, therefore it is much more resistant to DNA damage (Korzeneva et al., 2016). We applied NQH because of an assumption that the SZ group might contain damaged DNA samples, for which the PCR efficiency would be substantially reduced.

The simultaneous use of qPCR and NQH allowed us to conclude that rDNA ***CN*** in the genomes of the SZ patients is higher than in healthy controls.

2. Methods

2.1. Subjects

The investigation was carried out in accordance with the latest version of the Declaration of Helsinki and approved by the Regional Ethics Committee of RCMG (approval #5). All participants signed an informed written consent to participate after the nature of the procedures had been fully explained to them. 108 male and 71 female paranoid SZ patients with acute psychotic disorders were recruited from general psychiatric units for the treatment of acute forms of mental disorders (Psychiatric Hospital of Moscow City Health Department and Mental Health Research Center, Moscow). Age of the patients is shown in Table 1. Psychopathology and functionality of patients were measured according to the Positive and Negative Syndrome Scale (PANSS). Patients were diagnosed with paranoid schizophrenia (F20.00 or F20.01) according to ICD-10 criteria using structured interviews (MINI). Diagnoses were also confirmed pursuant to DSM-IV criteria. For the treatment of the acute disorders standard antipsychotics were used: haloperidol,

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