RIBOSOMAL GENE COPY NUMBER IN PERIPHERAL LEUKOCYTES OF WOMEN WITH NORMAL AND COMPLICATED PREGNANCY

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Pregnancy requires the cells of the woman's body to ensure increased ribosomal biogenesis in order to enhance the protein synthesis intensity. The number of ribosomes depends on the copy number of ribosomal genes (rDNA) in the genome. The study aimed to test the hypothesis about the association of the rDNA copy number in the woman's genome with the course of normal and complicated pregnancy. The sample of 488 pregnant women (25–39 weeks) included the following groups: 1) normal pregnancy (control); 2) impaired uteroplacental blood flow and fetoplacental insufficiency; 3) congenital malformations; 4) isthmic-cervical insufficiency; 5) early placental maturation; 6) dichorionic diamniotic twins; 7) polyhydramnios; 8) macrosomia. The rDNA copy number was determined by the quantitative hybridization method in the DNA extracted from peripheral leukocytes. The rDNA copy number varied between 226 and 800 (n = 488). DNA samples with the rDNA copy number below 290 were lacking in groups 3–8. Groups 5–8 included no samples with the rDNA copy number exceeding 520; these in total differed from group 1 by low rDNA copy number values (the average values were 360–381 for groups 3–8 and 452 for group 1; $p < 10–7$). The rDNA copy number range of 290–520 in the woman's genome (the adaptive norm typical for long-lived individuals) is optimal in terms of successful completion of pregnancy in the presence of pregnancy complications. The low rDNA copy number (200–290) in the genome is associated with the failure to complete embryogenesis when there are some fetal abnormalities/features. A high rDNA content (over 600 copies) indicates the presence of genetic variants in the woman's genome that can interfere with the complicated pregnancy course. Determining the rDNA copy number in the genome of married couples may be useful for planning and predicting the course of pregnancy.

Keywords: pregnancy, pregnancy pathology, ribosomal genes, rDNA

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Compliance with ethical standards: the study was approved by the Ethics Committee of the Pirogov Russian National Research Medical University (protocol No. 228 dated 17 April 2023). The informed consent was submitted by all study participants.

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КОЛИЧЕСТВО КОПИЙ РИБОСОМНЫХ ГЕНОВ В ЛЕЙКОЦИТАХ ПЕРИФЕРИЧЕСКОЙ КРОВИ У ЖЕНЩИН С ФИЗИОЛОГИЧЕСКОЙ И ОСЛОЖНЕННОЙ БЕРЕМЕННОСТЬЮ

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Беременность требует от клеток организма женщины повышенного уровня биогенеза рибосом для увеличения интенсивности синтеза белка. Количество рибосом зависит от числа копий рибосомных генов в геноме (ЧК рДНК). Целью исследования было проверить гипотезу об ассоциации ЧК рДНК в геноме женщины с протеканием нормальной и осложненной беременности. Выборка 488 беременных (25–39 недель) включала группы: 1) беременность без патологии (контроль); 2) нарушение маточно-плацентарного кровотока и фетоплацентарная недостаточность; 3) врожденные пороки развития; 4) истмико-цервикальная недостаточность; 5) преждевременное созревание плаценты; 6) дихориальная диамниотическая двойня; 7) многоводие; 8) крупный плод. ЧК рДНК определяли методом количественной гибридизации в ДНК, выделенной из лейкоцитов периферической крови. ЧК рДНК варьировало от 226 до 800 (n = 488). В группах 3–8 отсутствовали образцы ДНК с ЧК рДНК менее 290. Группы 5–8 не содержали образцов с ЧК рДНК более 520 и суммарно отличались от группы 1 низкими значениями ЧК рДНК (средние значения 360–381 для групп 3–8 и 452 копии для группы 1; $p < 10–7$). Диапазон ЧК рДНК от 290 до 520 в геноме женщины (адаптивная норма, характерная для долгожителей) является оптимальным с точки зрения успешного завершения беременности при наличии осложнений. Низкое ЧК рДНК (200–290) в геноме ассоциировано с невозможностью реализации эмбриогенеза при наличии патологии/особенности плода. Большое содержание рДНК (более 600 копий) указывает на наличие в геноме женщины генетических вариантов, которые могут препятствовать протеканию осложненной беременности. Определение ЧК рДНК в геноме супружеских пар может быть полезным для планирования и прогнозирования течения беременности.

Ключевые слова: беременность, патология беременности, рибосомные гены, рДНК

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The study of the impact of maternal genetic features on the reproductive function and embryogenesis processes are of great importance for addressing the issue of increasing birth rate. Pregnancy and delivery need the cells of the woman's body to effectively respond to stress and to be capable of significantly enhancing protein synthesis in the body. Protein synthesis is a central event of the eukaryotic cell functioning, including in response to stress of any origin. This process referred to as translation is implemented by specific molecular machinery: ribosomes. A human ribosome consists of two components: ribosomal RNA (rRNA) and 70–80 ribosomal proteins [1]. The 28S, 5,8S, and 18S rRNA (rDNA) genes in the human genome are represented by multiple copies. The rDNA copies are arranged into tandem repeats sized 43 kbp in five pairs of acrocentric chromosomes. Each single repeat comprises a transcribed region sized 13.3 kbp (47S rRNA), which contains the 28S, 5,8S, 18S rRNA genes, the transcribed spacers (5′ETS and 3′ETS), and a non-transcribed intergenic spacer (IGS). Together with the 5S rRNA (the genes are located on the first chromosome) these rRNA form ribosomes [2]. The rRNA synthesis for ribosomes is the main function of ribosomal repeats. The rDNA transcription is accomplished by RNA polymerase I in the nucleolus, a specific cell structure in the nucleus (Fig. 1A).

The human genome contains about 200–1000 copies of tandem ribosomal repeats [3–5]. The number of rDNA copies in the genomes of cells of various types within the same body is constant; it does not change with age or under exposure to stress. The rDNA copy number is also the same within the same population of cells. In other words, the rDNA copy number can be considered as a stable genetic trait that remains unchanged throughout human life [3]. In recent years, there are more and more reports suggesting the role of the rDNA copy number in the genome in the human body functioning, as well

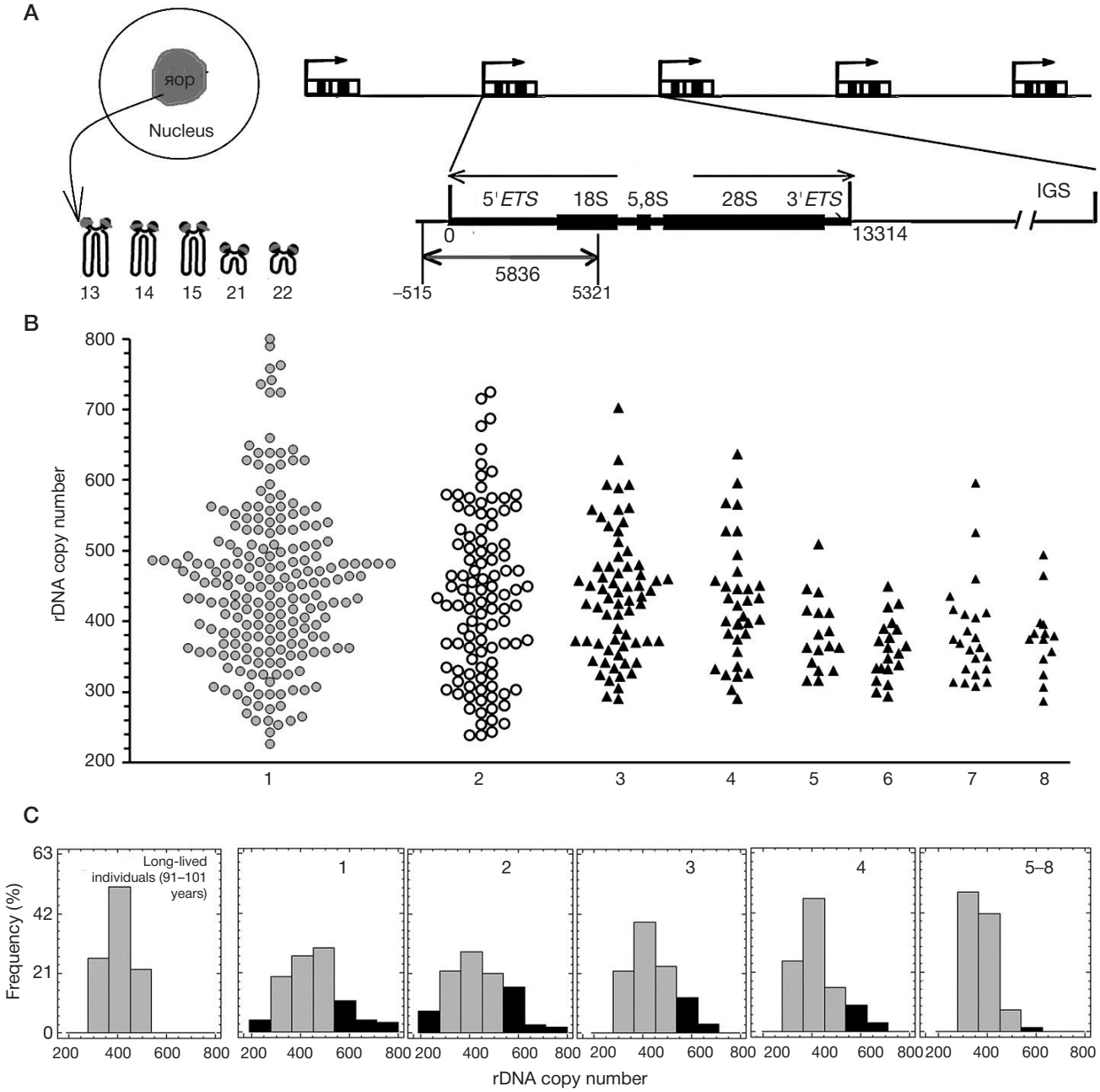

Fig. 1. Ribosomal repeat copy number variation in the genomes of pregnant women. **A**. Ribosomal repeat scheme. The repeat fragment determined with the DNA probe by hybridization is shown. **B**. Experimental data reflecting the rDNA repeat content in the studied groups. C. Distribution of DNA samples in the groups by the rDNA copy number in the genome

Table 1. Characteristics of the groups of women, for whom the rDNA copy number was determined. Descriptive statistics are provided

№	Group	N	Mean ± SD	I min–max	CI (95%)	Median	C_{var}
1	Control (normal pregnancy)	207	452 ± 113	226–800	436–467	450	0.25
2	Impaired uteroplacental blood flow and fetoplacental insufficiency	107	436 ± 115	237–724	413–457	432	0.26
3	Congenital malformations, ch. a.	66	436 ± 88	290–702	414–459	434	0.2
4	Isthmic-cervical insufficiency	34	423 ± 88	290–637	395–459	415	0.21
5	Early placental maturation	17	381 ± 53	316–509	356–416	365	0.14
6	Dichorionic diamniotic twins	20	360 ± 43	294–449	338–382	358	0.12
7	Polyhydramnios	23	379 ± 74	289–596	348–415	363	0.19
8	Macrosomia	14	375 ± 57	287–494	332–415	374	0.15
D	Long-lived individuals (91–101 years) [3]	103	404 ± 55	290–519	393–414	403	0.13

as the association of this trait with disorders and aging. It has been shown that the rDNA copy number is associated with the chronic inflammation level, kiney disease [6], body weigh [7], as well as with the fact of having a monogenic (cystic fibrosis) or polygenic (schizophrenia) disorder [3]. The low rDNA copy number is associated with cognitive impairment in the elderly [8], slower metabolism, and low human cells' resistance to stress [7, 9]. The rDNA content of blood cells in long-lived individuals varies within a narrow range: from about 290 to 530 copies. People with the lower or higher rDNA copy number values don't live to the age of 90 or older [10].

Approximately one-third of all rDNA copies, which are referred to as active copies, are transcribed in the nucleolus. These copies are not methylated, in contrast to the transcriptionally inactive copies, in which the transcribed region of rDNA is methylated. The number of active rDNA copies is proportional to the total number of copies in the genome [11]. The data are provided that are consistent with the hypothesis about the stabilizing selection occurring at the zygote and/or early embryogenesis level and aimed at maintaining the number of active rDNA copies between ~ 94 and ~ 277 copies (beyond the specified threshold values, the cell is not viable). The zygotic loss rate has been determined for this trait (about 10%) [11, 12]. The data obtained suggest that the loss of zygotes/embryos under conditions of insufficient or excess active copies of ribosomal genes in the genome can be one of the factors determining reduced fertility in some married couples [13].

The impact of the rDNA copy number in the woman's genome on the reproductive function and embryogenesis has not yet been adequately studied. The literature provides only the data of the authors, who have revealed a positive association between the IVF success and the rDNA copy number in the women's leukocytes [14]. It has been also shown that silent miscarriage is associated with severe imbalance of the rDNA content in the embryo's genome and maternal genome. In most cases, the genome of a non-developing embryo contains significantly fewer rDNA copies, than the maternal genome and genomes of other embryos, the development of which has not been spontaneously interrupted [15].

It should be noted that the quantitative PCR method widely used for analysis of genes is poorly applicable to the analysis of multicopy ribosomal repeats due to a number of reasons discussed in detail earlier [16]. The tandem nature of repeats, large number of self-complementary regions, various copy methylation levels, increased oxidative modification of multiple Gn-rich rDNA regions result in the fact that rDNA represents a very bad matrix for Taq polymerase. We observed a nonlinear relationship between the amplification reaction effectiveness and the rDNA concentration and oxidation, in contrast to other genome sequences. The non-radioactive quantitative hybridization (NQH) method, that does not depend on the DNA methylation, oxidation, and fragmentation levels since it does not involve PCR, has been developed specifically for rDNA quantification. The alkali-denatured DNA fragments immobilized on the filter are through hybridization with a long biotin-labeled DNA probe. Several calibration DNA samples with the known ribosomal repeat content are used as standards. The results obtained using NQH when studying rDNA in the sample of healthy donors were fully confirmed by later studies by the authors, who used a new long DNA fragment analysis method not involving the amplification reaction (Oxford Nanopore sequencing [17]).

The study aimed to assess the association of the ribosomal gene copy number in the maternal genome with the risk of various pregnancy complications. For that we determined the rDNA copy number by NQH in the leukocyte genomes of women with normal pregnancy and pregnancy with the complications/specific features caused by various factors.

METHODS

Blood samples of women with normal pregnancy and complicated pregnancy were obtained through collaboration with the department of obstetrics and gynecology at the Pediatric Faculty, Pirogov Russian National Research Medical University.

The venous blood samples for analysis of the rDNA copy number in leukocytes were collected from 488 pregnant women aged 18–45 years (average age 32 ± 5 years, gestational age 25–39 weeks) living in Moscow (RF) in the same social environment. Furthermore, the previously published data on the rDNA content in the genomes of long-lived individuals (n = 103, females 84%) aged 91–101 years were taken for comparison [3]. Groups 1–8 were formed (Table 1).

Inclusion criteria: group 1 (control group) — women with the normal course of pregnancy without any disorder identified, who gave birth to healthy children showing no signs of hypoxia and hypotrophy; groups 2–8 — women with the abnormal course of pregnancy diagnosed with the disorders specified in Table 1.

Exclusion criteria: patients having chronic (diabetes, autoimmune diseases, cardiovascular diseases, cancer) and hereditary disorders; acute infection at the time of blood collection; smoking, drinking alcohol, taking drugs or medications; history of unsuccessful pregnancies.

Special tests

DNA was isolated from 1 mL of blood by phenol extraction. Red blood cells were lysed (0.25% ammonium chloride), leukocytes were precipitated by centrifugation at 400 g for 10 min, the sediment was added 1 mL of the lysis buffer (1% sodium lauroyl

sarcosinate, 0.02 M EDTA, pH 7) and treated with RNAse A at a concentration of 0.075 mg/mL (Sigma; USA) for 45 min (37 °C). Then the mixture was treated with proteinase K, 0.2 mg/mL (Promega; USA) for 24 h at 37 °C. After two cycles of extraction with the saturated phenol solution, DNA was precipitated by adding two volumes of ethanol in the presence of 2 M ammonium acetate. Then the sediment was twice washed with the 75% ethanol, dried and dissolved in water.

The phase of determining DNA concentration in the sample is critical for analysis. The concentration was determined by two methods: spectrophotometry (the absorption spectrum was acquired using the Shimadzu UV-160A system) and fluorometry. The PicoGreen fluorescent dye was used (Sigma; USA). Fluorescence was recorded using the LS-55 system (Perkin Elmer; USA).

The ribosomal repeat copy number in the DNA extracted from blood was determined by the non-radioactive quantitative dot-blot hybridization with the biotinylated DNA probes that had been described in detail earlier [16]. Equal quantities (20 ng) of the DNA samples denatured with the 0.1M NaOH, the set of calibration DNA samples with the known rDNA content, and the negative control for non-specific binding were in several repetitions applied onto nitrocellulose filters and then, after thermal immobilization, incubated with the biotinylated DNA probe (Fig 1A). The p(5′ETS-18S) probe represented a rDNA fragment comprising the ribosomal repeat fragment (positions — 515 to 5321 relative to the transcription starting point) cloned into the pBR322 plasmid (HSU 13369; GenBank accession No.U13369). The fragment comprises a small fragment of the non-transcribed spacer, the external transcribed spacer (5′ETS), and a part of the 18S rRNA gene. The probe was biotinylated using the Biotin NT Labeling Kit (Jena Bioscience GmbH, Jena, Germany) for nick translation.

After completing hydridization, the signal was visualized using the streptavidin-alkaline phosphatase conjugate (Merck) and the colorimetric substrate. To quantify rDNA based on the spot signal intensity, the Imager 6 software tool was used allowing one to calculate integral intensity of the signal from each spot. Signals from all the spots corresponding to the same sample were summed up, and the mean and standard error were calculated for each sample. The abundance of ribosomal repeat copies was calculated using a calibration curve showing the signal as a function of the rDNA copy number in the control DNA samples, which were applied onto the filter in the same amount as the test DNA samples. The analysis relative error was 5 ± 3%.

Statistical processing

The descriptive statistics for quantitative variables are provided in Table 1 in the format of the mean and standard deviation (±SD), the median and range of variation (l), confidence interval values (CI 95%) and variation coefficient (Cvar: standard deviation divided by the mean). Two groups were compared using the nonparametric Mann–Whitney test (p). The Kruskal–Wallis test (H, p) was used to compare several groups. Distributions of the measured parameter in the groups were compared using the Kolmogorov–Smirnov test (D, α). The critical significance level was specified as 0.05. When applying Bonferroni correction for multiple comparisons, the differences were considered significant at $p \leq 0.0062$. The StatPlus2007 (http://www.analystsoft.com/) software was used for calculation.

RESULTS

A total of 488 DNA samples isolated from blood leukocytes of pregnant women aged 18–45 years were analyzed. The rDNA copy number was determined by the non-radioactive quantitative hybridization method that had been specially developed for the analysis of tandem ribosomal repeats in the human genome [16]. A biotinylated DNA probe that was homologous to the 5836-nucleotide ribosomal repeat fragment was used for hybridization (Fig. 1A). The rDNA quantity was presented as the repeat copy number per diploid genome.

The quantitative data for the entire sample divided into eight groups are presented in Fig. 1B. Table 1 provides characteristics of the groups and descriptive statistics. Fig. 1C presents distributions of DNA samples in the groups by the rDNA copy number in the genomes. In the studied sample of 88 DNA samples, the rDNA content varies between 226 and 800 copies per diploid genome. The rDNA content in a population of generally healthy people without any obvious genetic pathology aged up to 70 years varies within the same range [3].

By comparison, Fig. 1C presents the DNA sample distribution by the rDNA copy number in the group of long-lived individuals (the data had been published earlier [3]). The rDNA copy number in the genomes of long-lived individuals varies within the narrow range: between ~ 290 and~ 520 copies. This range represents some adaptive norm for the population. People with the higher rDNA copy number (over 550) and low copy number (below 280) do not live to the age of 90 years or more. In the younger sample (3–75 years), DNA samples with the rDNA copy number outside the range of long-lived individuals constitutes about one third [3].

Comparison of the eight studied groups of pregnant women based on the rDNA copy number in the blood leukocyte genome using the nonparametric Kruskal–Wallis test revealed significant intergroup differences ($H = 30.2$; $p < 10–4$, $n = 8$). Then we used the nonparametric Mann–Whitney test to compare group 1 (women with the normal course of pregnancy, without any abnormality detected, who gave birth to children showing no signs of hypoxia and hypotrophy) with the groups 2–8. The data of comparing distributions (Kolmogorov–Smirnov test) and rDNA quantities in the groups (Mann–Whitney test) are provided in Table 2.

Considering Bonferroni correction, group 2 (impaired uteroplacental blood flow and fetoplacental insufficiency) and group 1 (control) showed no significant differences in the rDNA copy number ($p > 0.006$) and no differences in the distribution of this parameter ($D = 0.12$, $\alpha = 0.29$). However, the analysis of distributions (Fig. 1C) showed that group 2 comprised twice more low-copy rDNA variants (8%), than group 1 (4%).

Group 3 (congenital malformations and chromosomal abnormalities) and group 4 (isthmic-cervical insufficiency) also showed no differences from group 1 in the rDNA copy content ($p > 0.006$) and the DNA sample distribution by the parameter values in the groups. However, in these groups there are no DNA samples with the low rDNA copy number (below 290), in contrast to groups 1 and 2.

The analysis using the Kruskal–Wallis test has shown that groups 1–4 do not differ from each other in the rDNA copy number ($H = 2.9$, $p = 0.41$, $n = 4$).

Groups 5 (early placental maturation), 6 (dichorionic diamniotic twins), 7 (polyhydramnios), and 8 (macrosomia) do not differ from each other in the rDNA copy number ($H = 1.27$; $p = 0.74$, $n = 4$). Each of the groups 5–8 is significantly different from the control group 1 in the rDNA copy number and/or trait distribution. The groups comprise lower quantities of rDNA copies in the DNA compared to the control (Table 2). These groups comprise no DNA samples with the low rDNA copy number values and no samples with the large (over 600 copies) rDNA quantities. In group 1 with the normal pregnancy, the number of such samples is 12 and 18%, respectively.

Table 2. Pairwise comparison of the control group 1 (normal pregnancy without any abnormalities) and the sample D (adaptive norm, long-lived individuals) with other groups of pregnant women based on the rDNA copy number in the DNA and the distribution of this parameter

Groups compared		Kolmogorov–Smirnov test		*U*-test
X1	X2	D	α	*p*
1	2	0.12	0.29	0.2
1	3	0.14	0.24	0.34
1	4	0.19	0.23	0.25
1	5	0.4	0.008	0.005*
1	6	0.5	0.0001*	0.0001 *
1	7	0.42	0.001*	0.002*
1	8	0.45	0.006*	0.008
1	∑(5–8)	0.45	3·10–9	10–8
D	1	–0.29	0.00002*	0.0002*
D	2	–0.26	0.001*	0.12
D	3	–0.23	0.02	0.02
D	4	–0.21	0.21	0.34
D	5	0.2	0.5	0.09
D	6	0.34	0.04	0.002*
D	7	0.25	0.2	0.02
D	8	0.29	0.33	0.08

Note: * — significant differences ($p \leq 0.0062$)

In terms of the the rDNA content in the genome and distribution of this parameter, groups 5–8 are most close to the adaptive norm of the rDNA copy number in the group of long-lived individuals (Fig. 1; Tables 1 and 2). The relatively low parameter values, low variation coefficients (0.12–0.19) compared to group 1 (C_{var} = 0.25) and the lack of low-copy (less than 290 copies per genome) rDNA variants are typical for these groups.

DISCUSSION

Human ribosomal repeats located in the r-regions of five pairs of acrocentric chromosomes are characterized by the pronounced quantitative polymorphism. In the sample we have studied, the range of rDNA variation is 595 copies, which confirms the earlier reported data on the ribosomal repeat variability [3, 5, 11]. In humans, the rDNA copy number is a stable genetic trait that is similar in all cells of the body and is not changed during life and under exposure to stress factors [3]. The fact of getting pregnant is also not likely to change the overall rDNA copy number in the genome of the woman's leukocytes.

We studied the rDNA copy number variation in the group of women with the normal course of pregnancy (group 1; Table 1) compared to the women, whose pregnancy was accompanied by various complications. The entire sample was divided into two parts after assessing the rDNA copy number in the genome.

Impaired uteroplacental blood flow and fetoplacental insufficiency (group 2), congenital malformations and chromosomal abnormalities (group 3), isthmic-cervical insufficiency (group 4) — these complications are not associated with alteration of the rDNA copy number in the women's genomes compared to the genomes of women with normal pregnancy without any complications (group 1). However, one nuance that had no effect on the overall analysis was reported for groups 3 and 4. In these groups, there were no DNA samples with the very low (below 290 copies) rDNA content. In groups 1 and 2, such samples accounted for 4 and 8%.

Groups 5–8, which include women with early placental maturation, dichorionic diamniotic twins, polyhydramnios and macrosomia, also comprise no low-copy rDNA variants, in contrast to the control group and group 2 (Fig. 1C). This can indicate that the maternal genome must contain more than 290 rDNA copies to ensure realization of embryogenesis complicated by the factors specified for groups 3–8. The woman's body with the lower rDNA copy number is likely to be unable to ensure acceptable ribosomal biogenesis to respond to the complicated pregnancy-induced stress.

This is confirmed by the data on the association of the rDNA copy number with the IVF procedure effectiveness. Women with the low rDNA copy number in their genomes (average number 305 ± 57) failed to get pregnant after several attempts, while women with the higher rDNA copy number (499 ± 62) successfully got pregnant on the first try [14].

We believe that the lack of low-copy rDNA variants in the genomes of women in groups 3–8 is associated with selection during early embryogenesis. The fact of an abnormality/specific feature in the embryo probably requires the maternal genome to ensure enhanced ribosomal biogenesis to realize the embryo development. Low rDNA quantity in the maternal genome can be associated with the arrest of the development of the embryo having the abnormality/specific feature at early stage. That is why in groups 3–8 at the 25–39th week of gestation no low-copy variants are found. Furthermore, low rDNA copy number in a mother can be inherited by the embryo, especially when the number of copies in the paternal genome is also low. It has been shown that embryos with the low rDNA copy number significantly more often fail to develop (silent miscarriage) compared to the embryos with the normal rDNA copy number [15]. The negative role of the low rDNA copy number in the paternal genome has been demonstrated earlier [18]. The authors have found that the total rDNA copy number in sperm is correlated to the rDNA methylation level and, therefore, to the number of transcriptionally active rDNA copies that ensure the required ribosomal biogenesis level. Sperm of males with idiopathic infertility contained the significantly lower total rDNA copy number and, therefore, the lower number of active copies, than sperm of males with normal fertility.

It is interesting to note that low rDNA copy number in associated with not only insufficient ribosomal biogenesis. The

functions of ribosomal repeats being parts of the nucleoli are not limited to production of subunits for ribosomes [19, 20]. The nucleolus is a center, where the synthesis of ribosomes, cell cycle progress, and the cells' response to various types of stress are coordinated. The research has shown that the epigenetic status of ribosomal genes and the nucleolar structure integrity can modulate cellular homeostasis [21–23]. The discovery of structural and functional links between the nucleolus and other genome of the cell made it possible to hypothesize that the nucleolus plays a key role in the nuclear architecture organization. The low rDNA copy number destabilizes heterochromatin and increases the likelihood of chromosomal rearrangements [24]. The ribosomal repeat copy number variation alters the cells' response to DNA damage. Cells with the low rDNA content are more susceptible to various stress factors [25].

The fact seems paradoxical that the average rDNA content in the group with abnormal pregnancy/specific features of pregnancy is lower compared to the control group, despite the lack of low-copy rDNA variants (Fig. 1B, C; Table 1). In these groups the lack of low-copy DNA samples is accompanied by the reduced number of high-copy samples. The variation interval and variation coefficient of these groups are considerably lower compared to group 1 (normal pregnancy). It would seem that a large number of rDNA copies in the genome should provide a greater number of ribosomes and a better response to stress associated with abnormalities. To answer this question we brought the earlier published data on the rDNA copy number variation associated with genetic abnormalities in the group of long-lived individuals [3]. According to these data, the larger number of rDNA copies in the human genome is associated with genetic abnormalities. During embryogenesis the embryo's genome requires the more intense protein synthesis to respond to the abnormality-induced stress. When the rDNA quantity is insufficient to maintain the ribosomal biogenesis level that is appropriate for genome realization, the embryogenesis failure occurs. The high rDNA copy number have been found in the genomes of patients with monogenic (cystic fibrosis) and polygenic (hereditary forms of schizophrenia) disorders [3], as well as in the genomes of people with renal failure and chronic inflammation [6]. Thus, the high rDNA copy number in the genome is a special marker of the presence of mutations/polymorphic DNA sequence variants in the genome affecting many body's processes, longevity, and probably successful reproductive function realization.

In people who have lived to the age of centenarians (over 90 years), the rDNA copy number varies within a narrow range; it is slightly lower relative to the population of people under the age of 70. The genomes of long-lived individuals comprise neither high-copy, nor low-copy rDNA variants [3]. It is interesting to note that the distribution of DNA samples by the rDNA copy number in groups 5–8 shows no differences from the distribution in the group of long-lived individuals (Table 2). It is likely that only the genome containing the rDNA quantity that is large enough for normal ribosomal biogenesis and not containing any DNA sequence variants that are harmful in terms of normal cell functioning (the abnormally high rDNA content is a marker) allows the woman to nurture the fetus, despite early placental maturation, polyhydramnios, twin pregnancy, or macrosomia.

Study limitations

It should be noted that the conclusions about small groups of women (5, 6, and 8; $n < 20$) are preliminary, these should be verified in the larger cohorts.

CONCLUSIONS

The rDNA copy number ranging from ~ 300 to 500 in the woman's genome (the adaptive norm typical for long-lived individuals) is likely to be optimal in terms of successful completion of pregnancy, even when complications occur. The low rDNA copy number in the woman's genome is associated with the impossibility of embryogenesis realization when there are fetal abnormalities/specific features. The high rDNA content suggests that the woman's genome contains genetic variants that can impede the complicated pregnancy course. Determining the rDNA copy number in the genomes of females and males can be useful for planning and predicting the course of pregnancy. This approach should be further tested for possible introduction into clinical practice.

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