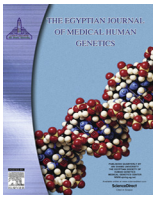
The Egyptian Journal of Medical Human Genetics xxx (2017) xxx–xxx

HOSTED BY

Contents lists available at ScienceDirect

The Egyptian Journal of Medical Human Genetics

journal homepage: www.sciencedirect.com



Original article

Alteration of rRNA gene copy number and expression in patients with intellectual disability and heteromorphic acrocentric chromosomes

Irina S. Kolesnikova [a], Alexander A. Dolskiy [a,b], Natalya A. Lemskaya [a], Yulia V. Maksimova [c,d], Asia R. Shorina [d], Alexander S. Graphodatsky [a], Ekaterina M. Galanina [b], Dmitry V. Yudkin [a,b,*]

[a] Institute of Molecular and Cellular Biology SB RAS, Lavrentieva Ave. 8/2, Novosibirsk 630090, Russia
[b] Novosibirsk State University, Pirogova Str. 2, Novosibirsk 630090, Russia
[c] Novosibirsk State Medical University, Krasnyi Ave. 52, Novosibirsk 630091, Russia
[d] Novosibirsk City Clinical Hospital No. 1, Zalesskogo Str. 6, Novosibirsk 630047, Russia

ARTICLE INFO

Article history:
Received 27 July 2017
Accepted 22 August 2017
Available online xxxx

Keywords:
Intellectual disability
Acrocentric chromosomes
Nucleolus organizer region – NOR
Ribosomal DNA – rDNA
Ribosomal RNA – rRNA

ABSTRACT

Background: Intellectual disability (ID) is an important medical and social problem that can be caused by different genetic and environmental factors. One such factor could be rDNA amplification and changes in rRNA expression and maturation.

Aim of the study: The aim of the present study was to investigate rRNA levels in patients with heteromorphism of the p-arms of acrocentric chromosomes bearing nucleolus organizer regions compared to a healthy control group.

Material and methods: Frequencies of p-arms enlargements in patients with ID and in healthy people were analyzed by G-banding screening. rRNA gene copy numbers on affected acrocentric chromosomes in peripheral blood lymphocytes were evaluated in ID patients and healthy bearers using FISH, and in immortalized lymphocytes of one patient – using FISH and real time PCR. Simultaneously, levels of 18S, 28S and 5,8S rRNA in both groups by means of qRT-PCR were investigated.

Results: No difference in acrocentric chromosome heteromorphism frequency in patients versus the healthy group were found. However, we found an amplification of rDNA, a significant elevation in 28S and 5.8S rRNA expression and changes in the 28S/18S rRNA ratio in ID patients compared to healthy controls. At the same time, FISH appeared to be not reliable enough for copy number evaluation, but RT-PCR showed rDNA copy changes in heteromorphic cells compared to normal.

Conclusion: Our findings indicate a loss of the correct regulation of rDNA activity and processing after amplification. This could disturb the ribosomal apparatus and thus lead to intellectual disability via at least two mechanisms.

1. Introduction

ID is an important medical and social problem that is commonly accompanied with comorbidities, as well as psychological issues, social difficulties and often financial pressure for patients and their relatives. Its frequency is approximately 1% of the human population, and it can be caused by different genetic and/or environmental factors. To date, more than 700 genes related to ID have been described. Many types of ID are associated with chromosome abnormalities [1]. One genetic feature that could be related to ID is enlargement of the p-arms on acrocentric chromosomes. Although acrocentric chromosomes with NOR regions with substantially enlarged p-arms were reported since the 1970s, these can be accompanied by ID or be present in healthy people [2–4] and even pass through generations [5,6]. In these cases, substantial amplification of rDNA was rarely shown because very few reports investigated rDNA in enlarged p-arms of marker chromosomes. Moreover, few cases have investigated NOR activity, and there are no reports about rRNA levels measured quantitatively in bearers of NOR-chromosomes with enlarged p-arms. In addition, it

Abbreviations: FISH, fluorescent *in situ* hybridization; ID, intellectual disability; NOR, nucleolus organizer region; PHA, phytohemagglutinin; qRT-PCR, quantitative real-time polymerase chain reaction (PCR); rDNA, ribosomal DNA; rRNA, ribosomal RNA; UID, undifferentiated intellectual disability.

Peer review under responsibility of Ain Shams University.

* Corresponding author at: Institute of Molecular and Cellular Biology SB RAS, Lavrentieva Ave. 8/2, Novosibirsk 630090, Russia.
E-mail address: dim@mcb.nsc.ru (D.V. Yudkin).

http://dx.doi.org/10.1016/j.ejmhg.2017.08.010
1110-8630/

should be noted that slight heteromorphism of p-arms in human acrocentric chromosomes is considered normal by the human chromosome atlas; moreover, these chromosomes are considered the most variable in the human karyotype [7]. It is important to note that changes and/or impairment in rRNA expression have been reported in neurodegenerative diseases, such as Alzheimer's disease [8,9] and Huntington disease [10,11], and increased NOR activity has been shown in lymphocytes and buccal epithelium of children with Down syndrome [12,13]. We propose that ID correlates with NOR activity and rRNA expression levels, and so rRNA levels could play a substantial role in the development of ID. Thus, the main aim of our present study was to examine rDNA amounts and rRNA expression levels and acrocentric chromosomes with p-arms enlargement in patients with ID and in healthy controls.

2. Ethics

The involvement of patients, their relatives and random donors in the study was strictly designed in accordance with international standards, which include the awareness of the subject and his or her informed consent to participate in the study in its entirety and guarantees of confidentiality. All studies conformed to ethical standards developed in accordance with the Helsinki Declaration of the World Medical Association, as amended in 2000. In addition, the studies were supervised by the Institutional Review Board.

3. Materials and methods

3.1. Investigated groups

A total of 88 blood samples from the IMCB SB RAS repository were studied. Fifty-six samples were from ID individuals and the remaining 32 were from healthy controls.

For the molecular investigation, 12 patients with UID and with heteromorphism of acrocentric chromosomes as determined by G-banding and without other genetic abnormalities were selected. Six of the 12 patients had diagnoses of mild intellectual disability, and the remaining patients had other diagnoses related to ID (hyperkinetic conduct disorder, infantile autism etc.). The average age of this group was 7.1 ± 1.8 years. The control group included 13 healthy persons without any known genetic diseases. The average age of this group was 27.4 ± 1.6 years. The differences in age between groups arised, because patients involved in the present study were children addressed to diagnostics due to ID, and healthy donors were their parents/sibs or randomly selected people. Healthy children had no need to be diagnosed and thus were not present in the study.

3.2. Metaphase preparation and staining

Blood samples were cultivated in RPMI-1640 medium (Gibco, USA) with 20% Fetal bovine serum (Gibco) and 1–3% PHA-M (Gibco) for 72 h. Incubation with 0.03 μg/ml of KaryoMax colcemid solution (Gibco) and 2.5 μg/ml ethidium bromide for 3 h was followed by hypotonic treatment for 25 min and fixation in Carnoy fixative (methanol/acetic acid – 3/1). Immortalized cell lines GM06895 (Coriell Institute Cell Repository, USA) and CPG148 (IMCB SB RAS cell repository) were cultivated in RPMI-1640 medium (Gibco) with 15% fetal bovine serum (Gibco) and 4 mM L-glutamine (Gibco). Metaphases were prepared by dropping the cell suspension. For G-banding [14], metaphases were treated with 0.25% trypsin solution for 1 min and staining with Giemsa stain for 2 min. Slides were analyzed on an Olympus BX-53 microscope with ×1000 total magnification. For image capture and analysis, VideoTest Karyo 3.1 (iMicroTec, Russia) software was used. For each case, no less than 12 metaphase spreads were analyzed.

3.3. Fluorescent in situ hybridization (FISH)

Plasmid pHr13 containing genes for 18S, 28S and 5.8S rRNA [15] was labeled with a BioNick DNA labeling system (ThermoFisher Scientific, USA). Before FISH, slides were pretreated with 100 μg/ml RNAse for 1 h at 40 °C and with 0.005% pepsin solution in 10 mM HCl for 10 min at 37 °C (if not G-banded before). FISH was performed with 0.06 ng of the labeled probe in 50% formamide at 40 °C overnight. Detection was performed with Alexa-555-streptavidin conjugate (ThermoFisher Scientific) or using FITC-conjugated avidin and anti-avidin antibody (both from New England Biolabs, USA). Chromosomes were counterstained with DAPI (0.08 μg/ml). Slides were analyzed on an Olympus BX-53 microscope with ×1000 total magnification. For image capture and analysis, VideoTest FISH 2.0 (iMicroTec, Russia) software was used.

3.4. RNA isolation, cDNA producing and qRT-PCR

Total RNA extraction from whole blood or blood plasma was performed using an Aurum Total RNA Mini Kit (BioRad, USA) or ExtractRNA reagent (Eurogen, Russia) followed by reverse transcription using an iScript Select cDNA Synthesis Kit (BioRad). All procedures were performed according to the manufacturers' protocols. Real-Time PCR was performed using SsoAdvanced Universal SYBR Green Supermix (BioRad) in a C1000 Touch Thermal Cycler with a CFX 96 RealTime System (BioRad).

Primers for real-time PCR (Table 1) were designed using ref. seq NR_003286.2, NR_003287.2 and NR_003285.2 for 18S, 28S and 5.8S rRNA, respectively. Beta-actin (ref.seq. NM_001101.3) was used as a reference gene. A healthy male donor (CPG57 according to IMCB SB RAS nomenclature) was used as a control for the rRNA level evaluation in all cases. Each sample was measured in 3 analyses [16].

3.5. DNA isolation and RT-PCR

Genomic DNA from immortalized cell lines GM06895 and CPG148 was extracted using a Wizard Genomic DNA purification kit (Promega, USA) according to the manufacturer's protocol. Real-time PCR was performed using the same primers as for 18S, 28S and 5.8S rRNA expression level estimation (see section above), but GAPDH was used as a single copy reference (ref.seq. NG_007073.2; for primer sequence see Table 1). Calibration curves

Table 1
Primers used for evaluation of the rRNA levels and rDNA copies.

Target	Forward primer	Reverse primer
18S rRNA	5′-GAGAAACGGCTACCACATCCAA -3′	5′-CCAATTACAGGGCCTCGAAAGA -3′
28S rRNA	5′-GGGTGGTAAACTCCATCTAAGG -3′	5′-GCCCTCTTGAACTCTCTCTTC -3′
5,8S rRNA	5′-GGTGGATCACTCGGCTCGT -3′	5′-CCGCAAGTGCGTTCGAAGTG -3′
Beta-actin	5′-CACGGCATCGTCACCAACTG -3′	5′-GCAACGTACATGGCTGGGG -3′
GAPDH	5′-CTGCAGGGCCTCACTCCTTTTGCAG-3′	5′-GGCAGGTTTTTCTAGACGGCAGGTCAG-3′