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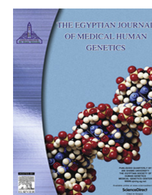

Case Report

Robertsonian translocation 13/14 associated with rRNA genes overexpression and intellectual disability

Alexander A. Dolskiy [a,b], Natalya A. Lemskaya [a], Yulia V. Maksimova [c,d], Asia R. Shorina [d], Irina S. Kolesnikova [a], 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

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ABSTRACT

Background: The Robertsonian translocations inherited from parents with a normal phenotype are often discovered through children with pathogenesis. The exact causes of pathologies in children with clinical manifestations are often unknown and vary greatly in the reported cases: uniparental disomy, de novo rearrangements, changes in methylation patterns and gene expression, including ribosomal genes.
Aim of the study: Molecular-cytogenetic investigation of a clinical case of intellectual disability.
Material and methods: GTG-banding, Ag-NOR staining, fluorescent *in situ* hybridization, PCR, real-time PCR.
Results: We describe a family case of a translocation rob (13; 14) and elevated rRNA expression in the proband with developmental delay and in his phenotypically normal mother. We show the loss of the p-arms of original chromosomes and the absence of NORs on the derived chromosome. The whole-chromosome uniparental disomy is excluded.
Conclusion: The translocated chromosome in the proband was most likely inherited from the mother and did not come about de novo with normal chromosomes 13 and 14 being obtained from the father. The cause of the pathogenesis in the proband still remains unknown. We hypothesize that it could be caused by impaired imprinting manifesting in altered methylation levels of loci on the derivative chromosome.
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1. Introduction

Translocations are chromosomal abnormalities arising due to chromosomal breakages and the incorrect reunion of fragments. Translocations are divided into two groups: unbalanced and balanced. In imbalanced translocation, an unequal exchange occurs and leads to changes in the genes copy number. When fragments exchange between two chromosomes, a new derivative chromosome is formed [1]. The frequency of Robertsonian translocations in human populations is estimated to be approx. 0.1%, but this number is higher in women with a premature termination of pregnancy (1.1%) and in infertile men (3%) [2]. Reciprocal translocation is an exchange of segments between non-homologous chromosomes. Herewith, if no amplification or loss of genetic information occurs, patients can have no visible phenotypic features. The most sensitive regions for such rearrangements are 11q23, 17q11, and 22q11, and lead to t(11;22) and t(17;22) translocations [3]. Robertsonian translocations come about due to the fusion of acrocentric chromosomes (13, 14, 15, 21 and 22) in centromeric areas [4]. This type of rearrangements can occur both between homologous and, more often, between non-homologous chromosomes [5]. Therefore, derivative chromosomes comprising long arms of original chromosomes are formed. Short p-arms also fuse, but such derivative chromosomes are mostly lost after the first cell divisions. Robertsonian translocations between chromosomes 13 and 14 (der(13;14)(q10;q10)) comprise 75% of all cases of this type of rearrangements [6].

Abbreviations: FISH, fluorescent *in situ* hybridization; NOR, nucleolus organizer region; qRT-PCR, quantitative real-time polymerase chain reaction (PCR); rRNA, ribosomal RNA.

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* 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).

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In the present paper, we describe a special family case of Robertsonian translocation rob (13;14) with a decreased diploid chromosome number 2n = 45 in two generations: in the proband and in his phenotypically healthy mother. Herewith, in both of them mosaicism and uniparental disomy (the origin of both homologous chromosomes from one parent) were excluded and elevated rRNA expression levels were shown, with a loss of two nucleoli organizer regions of chromosomes.

2. Ethics

The involvement of the patients, their relatives, and random donors in the study was strictly designed in accordance with the international standards, which include an awareness of the subject and his or her informed consent to participate in the study in its entirety and guarantee of confidentiality. All of the studies conformed to the ethical standards and were 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. Clinical study

Clinical studies included consultations with medical specialists, particularly of a clinical psychologist, neurologist, speech therapist as well as the computer tomography screening of brain.

3.2. Cell cultures and chromosomes preparation

Short-term cultures of B-lymphocytes were established from samples of peripheral venous blood of investigated donors. Cells were cultivated in a CO2-incubator at 37 °C and 5% CO2 in an RPMI-1640 medium (Gibco, USA) supplemented with 15% inactivated fetal bovine serum (Gibco) and 50 μg/ml gentamicin sulfate (KRKA, Slovenia). Cell fixation and preparation of metaphase spreads were performed according to previously described protocol [7].

3.3. Fluorescent in situ hybridization

Fluorescent *in situ* hybridization was carried out using a standard protocol [7]. Human chromosome painting probes were labeled in DOP-PCR with biotin or digoxygenin as described previously [8]. Probes for the localization of rRNA genes were obtained using plasmid pHr13, which contains DNA sequences corresponding to 18S, 5.8S, and 28S rRNA [9]. Amplification and labeling with digoxygenin was performed using GenomePlex® Whole Genome Amplification (WGA) Kit (Sigma, USA).

3.4. RNA extraction and Real-Time PCR

RNA extraction from venous blood samples was performed using Aurum Total RNA Mini Kit (BioRad, USA). For reverse transcription, iScript Select cDNA Synthesis Kit (BioRad) was used. Real-Time PCR was performed in C1000 Touch Thermal Cycler with CFX96 Touch™ Real-Time PCR detection system (BioRad) using SsoAdvanced Universal SYBR Green Supermix (BioRad). Primers were designed using ref. seq. NR_003286.2, NR_003287.2 and NR_003285.2 for 18S, 28S and 5,8S rRNA, respectively, and ref. seq. NM_001101.3 for β-actin (for normalization of results). The following primer pairs were used for amplification: 18S rRNA: F 5′-GAGAAACGGCTACCACATCCAA-3′, R 5′-CCAATTACAGGGCCTCGAAAGA-3′; 28S rRNA: F 5′-GGGTGGTAAACTCCATCTAAGG-3′, R 5′-GCCCTCTTGAACTCTCTCTTC-3′; 5,8S rRNA: F 5′-GGTGGATCACTCGGCTCGT-3′, R 5′-CCGCAAGTGCGTTCGAAGTG-3′; β-actin: F 5′-CACGGCATCGTCACCAACTG-3′, R 5′-GCAACGTACATGGCTGGGG-3′. Blood samples of healthy donors with normal karyotypes were used as a control. Each reaction was replicated three times. For a statistical evaluation and comparison of Real-Time PCR data, the $2^{-\Delta\Delta Ct}$ method and GraphPad software were used.

4. Results

4.1. Clinical investigation results

The clinical evaluation of the proband showed intellectual disability and speech impairment, ataxia, scoliosis, and hydrocephaly. The results of selective urinalysis did not reveal any specific aminoaciduria. Motion was characterized by diffuse moderately decreased tone, impaired fine motor skills, and a shaky walk. The swaying in Romberg's test and intention tremor in the finger to nose test were observed. The patient has aphasia, with defective sound pronunciation. Computer tomography of the brain did not reveal any focal lesions. Abdominal ultrasound examination revealed volvulus in the neck of the gallbladder and fundus of the gallbladder as well as the biliary dyskinesia of the hypokinetic type. Electrocardiography showed sinusoidal rhythm. The neurological diagnosis for the patient is the atonic form of infantile cerebral palsy.

4.2. Karyotyping and localization of painting probes

Karyotype analysis showed karyotype 45, XY in all lymphocytes of the proband and 45, XX – in all cells of his mother. Analyses of GTG-banded chromosomes revealed the translocation rob (13; 14). The translocation was present both in the patient and in his phenotypically healthy mother. To confirm this chromosome rearrangement, we performed the localization of human painting probes for chromosomes 13 and 14 on the metaphase spreads of both investigated individuals (Fig. 1A). Chromosome painting also showed the presence of the translocation in all cells. Therefore, there was no mosaicism for this chromosomal rearrangement in the patient or his mother.

4.3. Localization of rRNA genes

The described translocation involves chromosomes that have nucleolus organizer regions (NORs). The G-banding analyses of the derived chromosome suggested that p-arms of original chromosomes were not present. The localization of painting probes on human chromosomes marks both short and long arms and, therefore, does not allow to determine if p-arms are present on the derivative chromosome. We carried out the localization of rRNA genes, which are normally located in the short arms of acrocentric autosomes. As a result, eight NORs on all acrocentric autosomes (13, 14, 15, 21, 22) and the absence of signals on the derivative chromosome were revealed both in the proband and in his mother (Fig. 1B). Therefore, it was shown that the derivative chromosome was formed involving only the q-arms of original chromosomes.

4.4. NOR activity investigation

Considering that the number of NORs in both investigated individuals is decreased compared to the norm, we investigated the NORs' activity in order to search for the possible mechanism of pathogenesis in the patient. To evaluate the number of active NORs, we performed NOR staining using $AgNO_3$ according to the

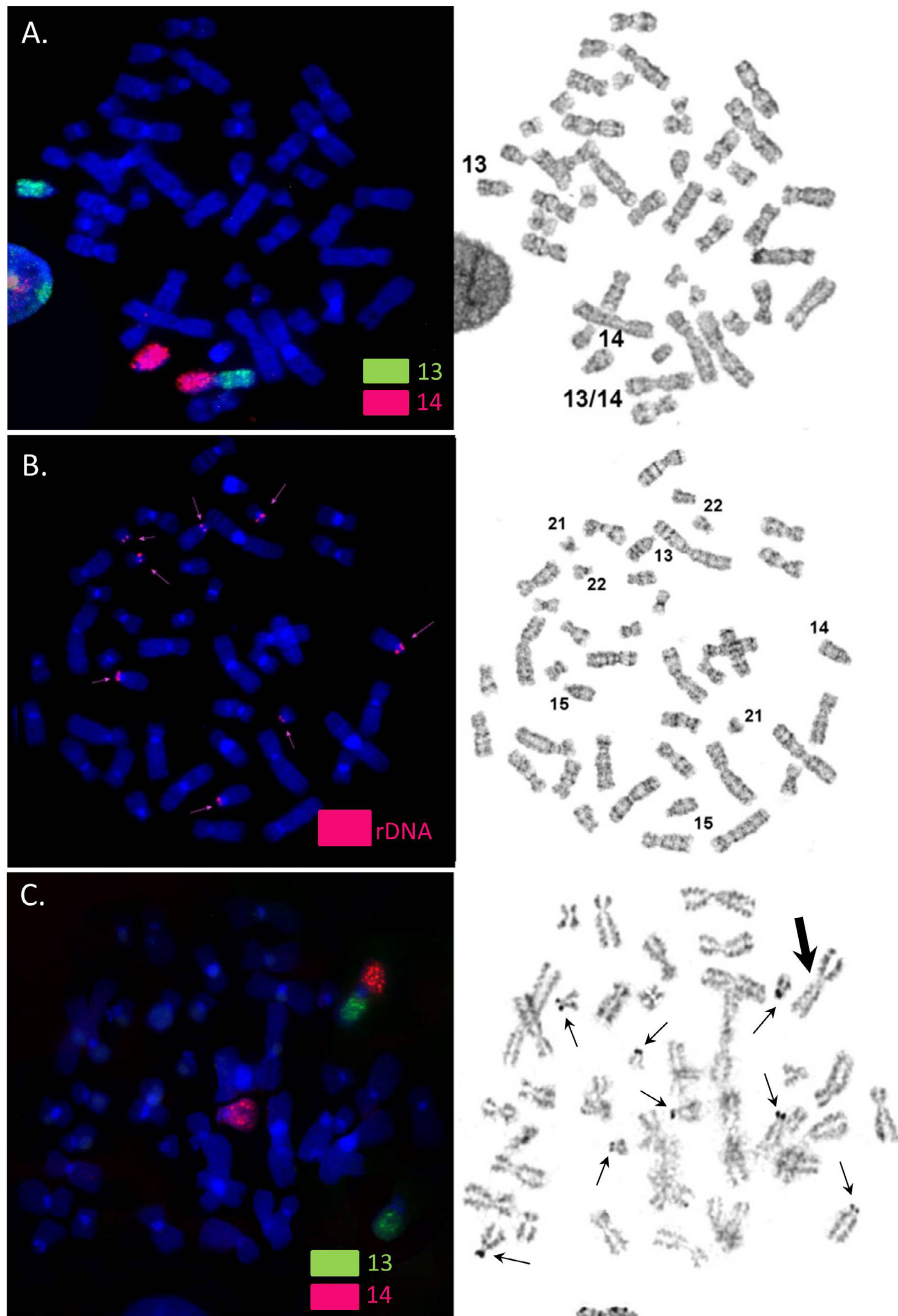

Fig. 1. A. An example of the localization of the painting probes of chromosomes 13 and 14 on a metaphase spread of the proband (left – FISH results, right – G-banded chromosomes). B. An example of localization of rRNA genes on a metaphase spread of the proband (left – FISH results, right – G-banded chromosomes). The chromosome numbers are marked. C. NOR activity study on proband's chromosomes (left – localization of the painting probes on chromosomes 13 and 14, right – $AgNO_3$ staining). The arrows indicate NORs and the thick arrow indicates the derivative chromosome.

method described previously [10]. Eight signals were revealed on acrocentric chromosomes without any visible differences between the proband and his mother (Fig. 1C) indicating that all NORs are active.

4.5. Analysis of rRNA expression levels

Two out of 10 NORs are lost in the proband and his mother. To reveal a possible mechanism of pathogenesis of the patient's clinical state, we performed an analysis of rRNA expression. As a result, both the proband and his mother have from sevenfold to tenfold elevated levels of 5.8S, 18S, and 28S rRNA expression compared to healthy controls. We did not reveal any statistically significant difference in these genes' expression between the patient and his mother.

4.6. Analysis of the presence of uniparental disomy

Robertsonian translocations are often accompanied by uniparental disomy. We investigated its presence analyzing the methylation level of DLK1/MEG3 locus in 14q32 using the previously described PCR-based method [11]. The expression of the genes DLK1 (delta, drosophila, homolog-like 1; MIM# 176290) and MEG3 (maternally expressed gene 3; MIM# 605636) comes about from maternal and paternal chromosomes, respectively. The results of methylation analysis excluded uniparental disomy in this clinical case.

5. Discussion

Robertsonian translocation rob (13, 14) is the most frequent among balanced translocation in humans, and its carriers usually have a normal phenotype [12]. The mother is a healthy bearer of this chromosomal rearrangement. Her son has clinical problems with intellectual disability as the most important. It was shown previously that such rearrangements are often accompanied by uniparental disomy [9]. Therefore, in this case, uniparental disomy could be one of possible causes of pathogenesis. However, we excluded the whole chromosome uniparental disomy by means of methylation analysis, but the partial disomy still could be present. Chromosomal rearrangement rob (13, 14) involves NOR-bearing chromosomes and led to a loss of two NORs in this clinical case. The altered NOR amount is reflected in an up to tenfold elevation of rRNA expression. The elevated rRNA expression could be connected with balancing rRNA and ribosomal proteins ratio in cells when two NORs are lost. We previously showed the correlation between elevated rRNA expression and the development of intellectual disability [13]. Disruptions of rRNA expression in some neurodegenerative diseases like Alzheimer's disease or Down syndrome are known [14,15]. However, in the clinical case that we describe here, altered rRNA expression is shown both in the patient with intellectual disability and in his healthy mother. We cannot determine the exact cause of the pathology. In general, the probability to obtain this chromosomal rearrangement namely from the mother is mostly high, despite risks of infertility and spontaneous abortion. The probability of de novo translocation in this familial case is extremely low. Most likely it was inherited from the mother [16–18].

6. Conclusion

We have shown a translocation rob (13; 14) in the proband with intellectual disability. The translocation most likely was inherited from the mother and did not come about de novo with normal chromosomes 13 and 14 being obtained from the father. Uniparental disomy is excluded, but the loss of two rDNA clusters and elevated rRNA expression are shown both in the proband and in his phenotypically normal mother. The pathological development of the proband is probably connected with altered imprinting that was caused by variation in the methylation levels of loci on the derivative chromosome. The investigation of the number of rRNA clusters and activity in cases of balanced Robertsonian translocations is important for accumulation knowledge in this poorly studied area and to the further investigate the possibility of a link of the increased or altered rRNA in clinical cases of the developmental delay of unknown genesis.

Conflict of interests

The authors declare that they have no conflicts of interest.

Authors' contribution

AAD – FISH analysis, primer design and real-time PCR, analysis of uniparental disomy, article preparation.

NAL – cell culturing and chromosome fixation, karyotyping.

YVM and ARS – clinical diagnostics.

ISK – article preparation.

DVY – study concept, financial support, article preparation.

All of the authors read and approved the final manuscript.

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