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A CASE OF PRENATAL DETECTION OF A *DE NOVO* UNBALANCED COMPLEX CHROMOSOMAL REARRANGEMENT INVOLVING FOUR CHROMOSOMES

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Complex chromosomal rearrangements are rarely observed prenatally. Genetic counceling of CCR carriers is complicated, especially in cases of de novo origin of the rearrangement. Here we present a new case of a de novo CCR involving four chromosomes observed in amniotic fluid cells of the fetus at 17 weeks of gestation. The rearrangement was characterized as an apparently balanced four-way translocation t(1;11;7;13)(~p21;~q13.5;~q32;~q22)dn by conventional cytogenetic studies. However, array-based comparative genomic hybridization revealed 5 submicroscopic heterozygous interstitial deletions on chromosome 1, 11, 7, 13 with a total loss of 21.1 Mb of genetic material in regions close to those, designated as breakpoints by conventional cytogenetic analysis. The described case clearly illustrates that high-resolution molecular genetic analysis should be combined with conventional cytogenetic techniques to exclude subtle chromosomal abnormalities in CCR cases detected prenatally.

***Key words**: complex chromosomal rearrangement, prenatal diagnosis, unbalanced karyotype.*

Introduction. Complex chromosomal rearrangements (CCRs) are structural aberrations involving more than two chromosomal breaks with exchanges of chromosomal segments. Depending on the degree of complexity, CCRs are classified into 3 categories: 1) three-way exchanges with three chromosomal breaks and exchange of chromosomal segments; 2) exceptional translocations with more than one breakpoint per chromosome; 3) double two-way translocations corresponding to a simple co-existence of two or three simple reciprocal and Robertsonian translocations in the same carrier [1]. In terms of the transmission, CCRs can be divided into familial and *de novo* cases. The phenotypic expression of a CCR depends on its origin: most of the familial CCRs result in normal phenotype, while half of the *de novo* cases, which are apparently balanced, are associated with phenotypic abnormalities due to submicroscopic imbalances or other genetic defects [2, 3]. These abnormalities cannot be detected by conventional cytogenetic analysis due to its obvious limitations. Therefore, additional investigations by high-resolution molecular methods such as array comparative genomic hybridization (aCGH) or next generation sequencing are required for the detection of possible chromosomal imbalances and proper genetic counseling of CCR carriers, especially in those cases, when CCRs are of *de novo* origin and found prenatally. In this report we describe a case of prenatal detection of a *de novo* CCR involving chromosomes 1, 11, 7, 13 in a fetus at 17 weeks of gestation with submicroscopic imbalances in chromosomes involved in the rearrangement, located close to the breakpoints.

Materials and Methods. *Patient.* A 24 years-old pregnant female was referred to the Clinic of Reproductive Medicine «Nadiya» (Kyiv, Ukraine) for invasive prenatal diagnosis at 17 weeks of pregnancy. It was her second pregnancy achieved naturally. The first one was ectopic. Her prior family history was unremarkable with no unusual environmental exposure. Her husband had a healthy child in the previous marriage. The results of hormonal tests at 13 weeks of gestation were PAPP-A of 2.85 MoM and βHG of 4.9 MoM. The results of ultrasound examination at 13 weeks of gestation were as follows: crown-lump length – 65 mm, nuchal translucency – 3.5 mm, nasal bone could not be visualized. The calculated risk for trisomy of chromosome 21 corresponded to 1:50. Therefore, invasive prenatal diagnosis was recommended to a patient.

Cytogenetic and molecular-cytogenetic studies. 20 ml of amniotic fluid were obtained by transabdominal amniocentesis. Two separate cultures were initiated on BIOAMF-2™(«Biological Industries», Israel) and AmnioMAX™-II («Gibco», Germany) culture medium. Amniotic fluid cells were gathered after 9 days of culture and metaphase chromosomes were obtained as described previously [4]. 10 GTG-banded metaphase plates with a minimum resolution of 400–450 bands per haploid set were ana-

lyzed from each culture. Cytogenetic studies were also performed on phytohemagglutinin-stimulated lymphocytes of peripheral blood of a couple according to standard protocols.

To detect possible chromosomal imbalances aCGH was performed on cultured cells of amniotic fluid (CytoChip ISCA 44K, Illumina, Cambridge, UK). Database of Genomic Variants was used for the analysis of genes lost in the interstitial deletions [5]. For interpretation purposes, public sources like Online Mendelian Inheritance in Man were consulted.

Results and Discussion. The cytogenetic study of cultured amniotic fluid cells of a fetus at 17 weeks of gestation revealed an apparently balanced karyotype with a CCR involving chromosomes 1, 11, 7, 13 – 46,XX,t(1;11;7;13)(~p21;~q13.5;~q32;~q22) dn (Fig. 1).

Karyotypes of a couple were 46,XX and 46,XY corresponding to a *de novo* origin of a detected chromosomal abnormality in a fetus.

To search for possible cryptic imbalances at the breakpoints aCGH analysis was performed, revealing 5 submicroscopic heterozygous interstitial deletions in regions of chromosomes involved in the rearrangement: 1p32.1p31.3 (6.5 Mb), 7q21.11q21.12 (1.0 Mb), 7q31.1 (3.5 Mb), 11q22.3 (0.6 Mb) and 13q14.3q21.2 (9.5 Mb) (Fig. 2).

Therefore, the karyotype of a fetus was designated as 46,XY,t(1;11;7;13)(p21;q13.5;q32;q22) dn.arr1p32.1p31.3(59,590,232-66,044,888) x1,7q21.11q21.12(85,783,827-86,862,945) x1,7q31.1(109,002,041-112,516,472)x1,11q22.3(103,604,143-104,193,985)x1,13q14.3q21.2(50,998,797-60,541,017)x1.

Patients decided to terminate the pregnancy after genetic counseling.

CCRs are not commonly found in prenatal and postnatal cases. Approximately 70 % of CCRs are detected in phenotypically normal subjects, 20–25 % – in patients with congenital abnormalities and/or mental retardation and 5–10 % are observed in prenatal diagnosis samples [1]. A review of 269 371 prenatal cases revealed 246 apparently cytogenetically balanced chromosomal abnormalities (0.091 %); CCRs constituted only 3 % of them [6]. Familial CCRs are more prognostically favorable, than *de novo* ones. The risk of phenotypic abnormalities in carriers of *de novo* CCRs detected prenatally is high and complex cases are associated with multiple congenital anomalies

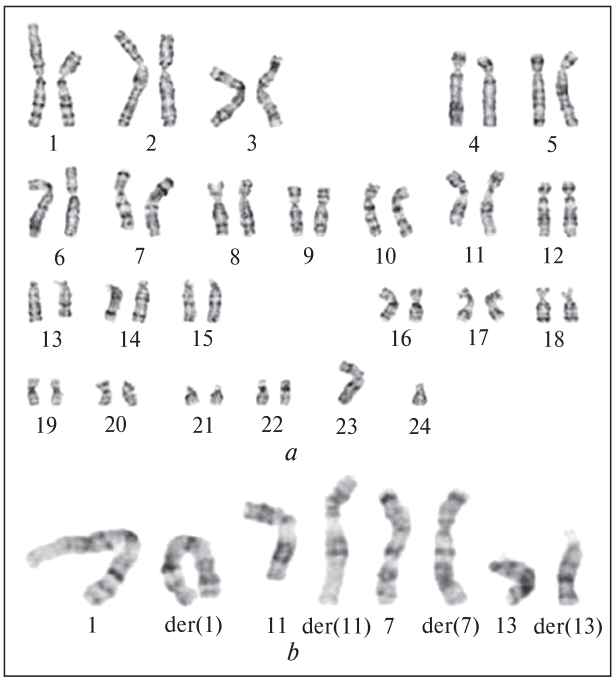

Fig. 1. Complete (*a*) and partial karyotype (*b*) of amniotic fluid cells with a CCR involving chromosomes 1, 11, 7, 13 – t(1;11;7;13)(~p21;~q13.5;~q32;~q22)

and/or mental retardation. For example, Madan et al. [7] found that 14 of 27 CCRs detected prenatally had negative outcome. Prenatal or postnatal detection of abnormal phenotype in individuals harboring CCRs is thought to be a result of chromosomal imbalances undetected by routine karyotyping, disruptions or modulation of expression of genes located at rearrangement breakpoints or position effect resulting in gene inactivation [3, 8]. Recent clinical implementation of high-resolution molecular-genetic analysis by aCGH has enabled the identification of submicroscopic abnormalities in the breakpoints or other regions of CCRs of carriers [2, 9].

In the present paper we report a case of prenatal detection of a *de novo* CCR involving chromosomes 1, 11, 7 and 13. The rearrangement was characterized as an apparently balanced four-way translocation t(1;11;7;13)(~p21;~q13.5;~q32;~q22) with four breakpoints by conventional cytogenetic analysis. The precise identification of breakpoints of CCR was complicated by a limited resolution of the method, size of the translocated segments (almost no change in the centromeric index of chromosomes 7 and 13) and high number of chromosomes involved in the

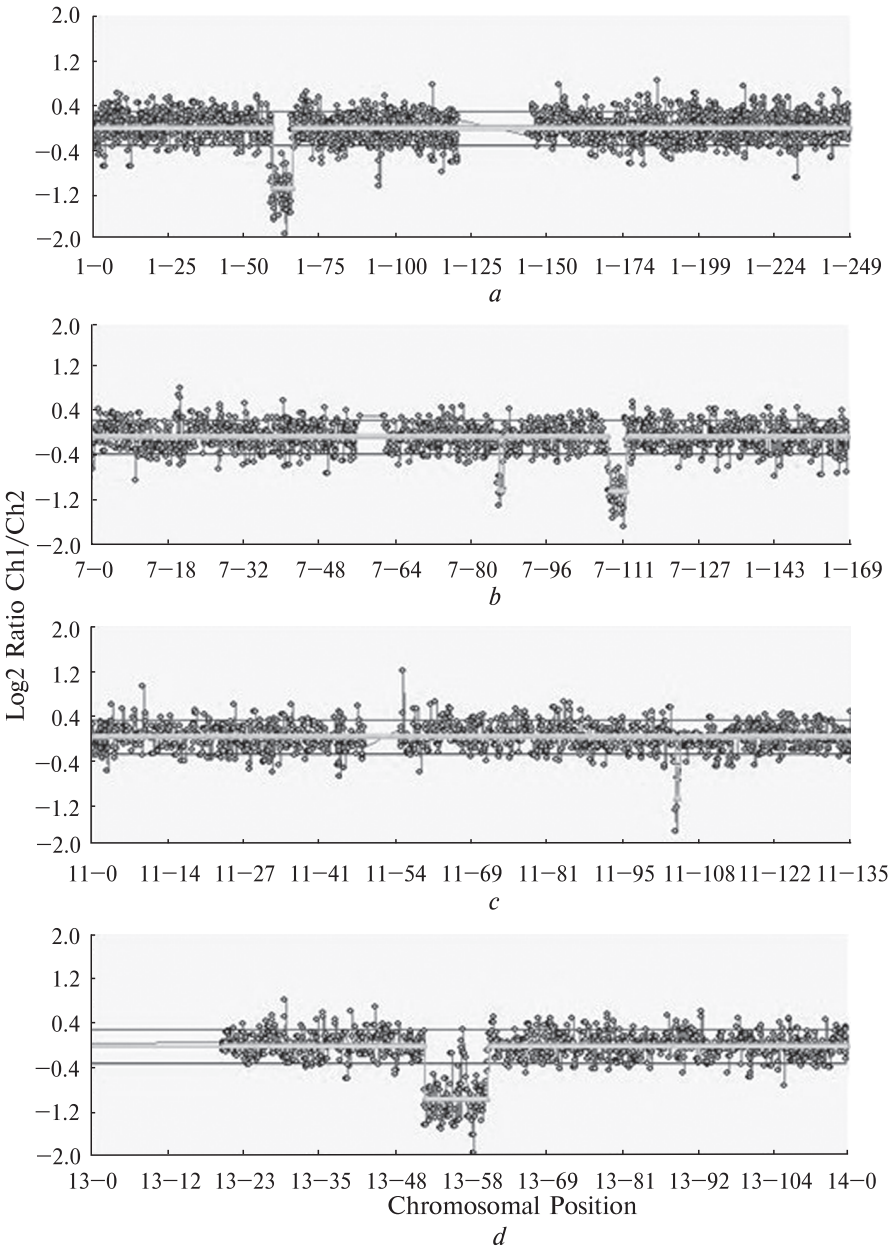

Fig. 2. Result of aCGH analysis of cultured amniotic fluid cells of a t(1;11;7;13)(~p21;~q13.5;~q32;~q22) carrier: *a*, *b*, *c*, *d* − chromosome 1, 7, 11, 13 respectively

rearrangement, as well as a limited banding quality. Since there is a growing evidence of underestimation of unbalanced karyotypes in structural chromosomal rearrangement carriers by conventional cytogenetic studies, especially in phenotypically abnormal carriers of chromosomal rearrangements [2, 3, 10], aCGH was performed to exclude submicroscopic chromosomal abnormalities in the fetus. As a result, an unbalanced karyotype was revealed with a total loss of 21.1 Mb of genetic material of chromosomes 1, 11, 7, 13 in regions close to those, designated by conventional cytogenetics as breakpoints: on 1p32.1p31.3 (6.5 Mb), 7q21.11q21.12 (1.0 Mb), 7q31.1 (3.5 Mb), 11q22.3 (0.6 Mb) and 13q14.3q21.2 (9.5 Mb). The involvement of chromosomes 1, 11, 7 and 13 in the detected rearrangement is not random,

since chromosomes 1, 3, 4, 7, 11 are reported to be preferentially implicated in CCRs [1]. This could be due to existence of «hot spots» for breakpoints in definite bands of these chromosomes. For example, regions 11q22.3 and 7q31.1 of the described rearrangement are frequently involved in chromosomal abnormalities detected in neoplasms [11] and region 7q21.1 is the most frequently observed in CCRs – 30 % of CCRs have breakpoints on chromosome 7, especially on region 7q21.1 [1]. 1.0 Mb deletion at 7q21.11q21.12 was also detected in our case.

Since the observed CCR was shown to be not a simple four-way translocation, but rather an exceptional CCR with 5 breakpoints, its origin through chromothripsis was suggested. Several cases of CCRs originating from chromotripsis with multiple breakpoints on the same chromosome are described in the literature [12–14].

Unfortunately, in our case the couple refused to take any further ulrasonographic examination after amniocentesis and decided to terminate the pregnancy after genetic counseling. Therefore, detected genetic imbalances could not be correlated to ultrosonographic markers of fetal abnormalities. However, the observed genetic imbalances involved regions associated with severe multiple abnormalities. For example, the observed 6.5 Mb deletion of chromosome 1 (chr 1: 59,590,232–66,044,888) included p32-p31 region (1p32–p31 deletion syndrome; OMIM # 613735). This abnormality has been discovered previously by aCGH in patients with macrocephaly and hypoplasia or absence of the corpus callosum, hydrocephalus or ventriculomegaly and developmental delay [15–17].

Conclusions. The described case clearly illustrates the necessity of applying high-resolution molecular genetic analysis together with G-banding cytogenetic techniques to detect subtle chromosomal abnormalities in chromosomal rearrangement carriers, especially detected prenatally in order to provide proper genetic counceling. aCGH should be advisable to all carriers of apparently balanced CCRs.

СЛУЧАЙ ПРЕНАТАЛЬНОЙ ДИАГНОСТИКИ ВОЗНИКШЕЙ *DE NOVO* НЕСБАЛАНСИРОВАННОЙ КОМПЛЕКСНОЙ ХРОМОСОМНОЙ ПЕРЕСТРОЙКИ С ВОВЛЕЧЕНИЕМ ЧЕТЫРЕХ ХРОМОСОМ

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Хромосомная перестройка выявлена при анализе GTG-окрашенных метафазных хромосом клеток амниотической жидкости плода 17 недель гестации и охарактеризирована как вероятно сбалансированная транслокация с вовлечением четырех хромосом t(1;11;7;13)(~p21;~q13.5;~q32;~q22)dn. Однако молекулярно-генетический анализ клеток амниотической жидкости методом матричной сравнительной геномной гибридизации (aCGH) позволил определить пять субмикроскопических интерстициальных делеций хромосом 1, 11, 7, 13 с суммарной потерей генетического материала размером 21,2 Mb в участках хромосом, локализованных близко к видимым точкам разрыва и соединения. Описанный случай свидетельствует о необходимости проведения не только стандартного цитогенетического исследования, но и высокоразрешающего молекулярно-генетического тестирования для исключения мелких хромосомных аномалий в случаях пренатальной диагностики комплексных хромосомных перестроек.

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