

## A Rare Complex Structural Chromosomal Anomaly in Mosaic Due to the Instability of a Derivative Chromosome 18 in a Female Infertile Patient

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### Abstract

Mosaicism in association with derivative chromosomes is a well-known fact. Derivative chromosomes often tend to rearrange and/or be reduced in size during karyotype evolution, because of their instability. This can determine the disappearance of a derivative chromosome at least in the most frequently studied tissue, the peripheral blood.

We report on a case of a rare complex structural chromosomal abnormality in mosaic, in a female patient who was referred to us for infertility. Patient's karyotype was performed twice: The first time two cell lines were detected (a 45 chromosomes and a 46 chromosomes cell line respectively) both with a derivative chromosome 18, resulting from a translocation between chromosome 18 and 21. Six months later, a second karyotype showed only the 45 chromosome cell line. FISH analysis and array-CGH, subsequently performed to better characterize this complex rearrangement, showed a partial deletion of the short and long arm of chromosome 21.

**Keywords:** Derivative chromosome; Translocation; Mosaicism; Infertility

### Introduction

Derivative chromosomes are the result of a reciprocal translocation. They are defined by the origin of the centromere.

Mosaicism in association with derivative chromosomes is a well-known fact. Cellular mosaicism is characterized by the simultaneous presence of two or more cell lines, which can be present in different percentage in different tissues. Constitutional chromosomal mosaicism with two cell lines carrying two different rearranged sets of chromosomes is an extremely rare condition that is generally the result of a post-fertilisation mitotic error [1,2]. Additionally, one has to expect that derivative chromosomes may tend to rearrange and/or be reduced in size during karyotype evolution, because of their instability. This can determine the disappearance of a derivative chromosome at least in the most frequently studied tissue, the peripheral blood [3].

We report on a case of a rare complex structural chromosomal abnormality in mosaic, in a female patient who was referred to us for infertility. Patient's karyotype was performed twice: the first time two cell lines were detected (a 45 chromosomes and a 46 chromosomes cell line respectively) both with a derivative chromosome 18, resulting from a translocation between chromosome 18 and 21. Six months later, a second karyotype showed only the 45 chromosome cell line. In order to better characterize this complex rearrangement we also performed FISH analysis and array-CGH, the latter showing a partial deletion of the short and long arm of chromosome 21, disagreeing with the normal phenotype of the proband.

### Materials and Methods

#### Clinical report

A 35 year old healthy woman was referred to us for infertility. She has a positive family history of myasthenia. There is no consanguinity with her partner. When the patient came to our attention, a karyotype was already performed, six months before, in another laboratory.

Karyotype was:

45,XX,der(18)t(18;21)(p11.31;q21),-21[86]/46,XX,t(18;21)(p11.31;q21) [14].

We performed a second karyotype, FISH analysis and array-CGH.

#### Cytogenetic study

The karyotype of the patient was carried out on PHA-stimulated peripheral blood lymphocytes cultured for 72 h at 37°C, using the "Rapid Method" following standard procedures established by the SIGU (Italian Society of Human Genetics) guidelines. A hundred metaphases were analyzed with G-banding and C-banding at resolution of 400 bands. The karyotype is described according to the International System for Human Cytogenetic Nomenclature.

#### FISH Analysis

Fluorescent in situ hybridisation (FISH) analysis was performed using a whole chromosome painting (WCP) probe of chromosome 18 (Cambio, Cambridge), according to manufacturers' instructions and an alphoid DNA probe specific for alphoid regions of chromosome 13 (D13Z1) and 21 (D21Z1) from Poseidon (Kreatech Diagnostics, Leica Microsystems Inc., USA).

Slides were counterstained with 4',6-diamidino-2-phenylindole (DAPI) (200 ng/ml) and analysed by fluorescence microscope Olympus BX70 equipped with a cooled CCD Video Camera Image Point, Photometrics. Image analysis was carried out with PSI MacProbe software.

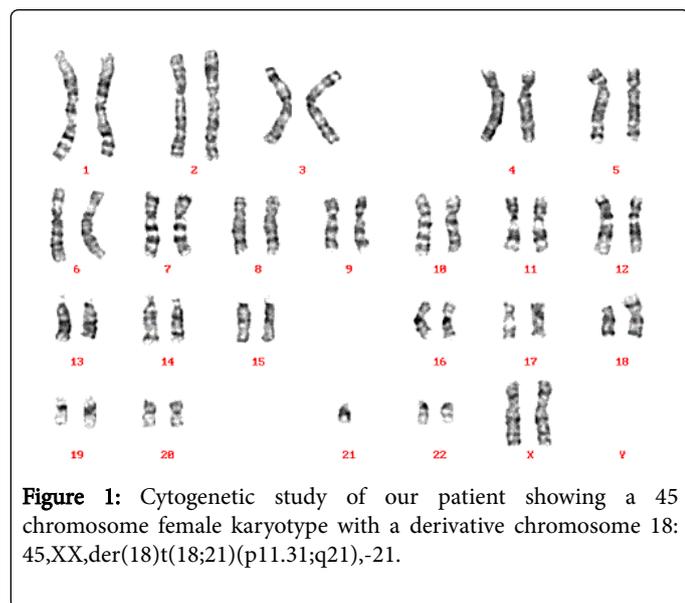
### Array-CGH analysis

Genomic DNA of the proband was extracted from peripheral blood lymphocytes using KingFisher Blood DNA Kit (Thermo Scientific, Vantaa, FI) according to manufacturers' instructions. Proband and reference DNA (Promega Corporation, Madison, WI, USA) were labeled with Cy5-dUTP and Cy3-dUTP respectively. Whole genome array-CGH was performed using Human Genome CGH Microarray Kit 8x60K (Agilent Technologies, Santa Clara, CA, USA) with an average resolution of 100 kb (Build37: Feb 2009-hg19) according to manufacturers' instructions. Images of the array were acquired with Agilent scanner G2505B and analyzed with Feature Extraction software v9.5.1 (Agilent Technologies, Santa Clara, CA, USA). Graphical overviews of results were obtained with Genomic Workbench Standard Edition software v5.0.14 (Agilent Technologies, Santa Clara, CA, USA).

### Results

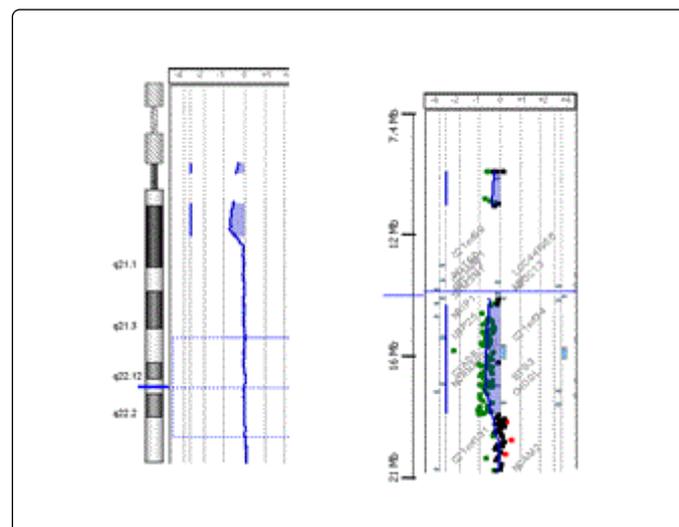
The patient had already performed a karyotype on peripheral blood lymphocytes in another laboratory, six months before coming to our attention. This previous study showed a female karyotype in mosaic, with two cell lines: A 46 chromosomes cell line with a reciprocal translocation between the short arm of a chromosome 18 and the long arm of a chromosome 21, involving bands 18p11.31 and 21q21, present in 14% of the analyzed metaphases; and a 45 chromosomes cell line characterized by the presence of only the derivative chromosome 18 involved in the translocation, present in 86% of metaphases.

The patient cytogenetic study, performed in our laboratory, showed a 45 chromosome female karyotype with the derivative chromosome 18. The patient's karyotype was so defined: 45,XX,der(18)t(18;21)(p11.31;q21),-21 (Figure 1).



FISH analysis confirmed the presence of the derivative chromosome 18 and positioned the breakpoints respectively in 18p11.31 e 21q21.

The result of Genome-wide array CGH analysis of the proband was: arr 21p11.2q21.1(9,832,448-18,983,265x1,19,035,976x2), 9 Mb deletion of the short and long arm of chromosome 21 ranging from 9,832 Mb to 18,983 Mb (Build37: Feb 2009-hg19) (Figure 2). No deletion was detected on the short arm of chromosome 18 (first oligo probe in used platform at 14,316 bp from telomere).



**Figure 2:** Genome-wide array CGH analysis: arr 21p11.2q21.1(9,832,448-18,983,265x1,19,035,976x2), 9 Mb deletion of the short and long arm of chromosome 21 ranging from 9,832 Mb to 18,983 Mb.

### Discussion

Karyotyping in our patient revealed a complex structural chromosomal abnormality in mosaic. Cellular mosaicism is characterized by the simultaneous presence of two or more cell lines, which can be present in different percentage in different tissues, thus resulting in a variable clinical expression.

In our case, the first karyotype revealed the presence of a 45 chromosomes cell line in 86% of the metaphases. This cell line, found in 100% of the metaphases in the second karyotype performed six months later, is characterized by the presence of a derivative chromosome 18, originated from the translocation involving the short arm of chromosome 18 and the long arm of chromosome 21 and loss of derivative (18;21)(p11.31;q21). Breakpoints, defined by FISH, are located in 18p11.31 and 21q21 respectively. Studying by array-CGH showed the presence of a partial deletion of the short and long arms of chromosome 21 of about 9 Mb. The chromosome 18 appears to be involved from the end part of p11.32, that considering the fact that the mosaic cell percentage is lower than that detectable by array-CGH.

The first karyotype revealed also a 46 chromosomes cell line in 14% of examined metaphases, with a reciprocal translocation between the short arm of a chromosome 18 and the long arm of a chromosome 21. As noted, when we repeated the cytogenetic study six months later, we found out only a 45 chromosome cell line with absence of derivative (18;21)(p11.31;q21) in 100% of metaphases, so we did not reveal the mosaicism. We can explain this finding because of the instability of

derivative chromosomes. Indeed, derivative chromosomes are not stable structures and they may be lost over time. Thus the percentage of mosaicism can be variable in time. However, we cannot exclude that the two cell lines are present in different percentages in other tissues, since our study limited only to peripheral lymphocytes. Moreover, considering the complexity of the rearrangement in combination with instability, mFISH (multicolor fluorescence *in situ* hybridization) analysis would probably have given additional information on the genomic composition in the sample.

The partial deletion of chromosome 21, revealed by array-CGH, involves many genes. Chromosome 21 represents around 1-1.5% of the human genome and it was sequenced and extensively studied [4]. The deletion detected in our patient includes genes such as TPTE, AJ239318.1, POTES, LIPI, RBM11, HSPA13, SAMS1, AF165138.7, NRIP1, USP25, CXADR, BTG3, C21orf91, CHODL and TMPRSS15. Among these, TPTE is the only one located on the short arm of chromosome 21 and encodes a putative tyrosine phosphatase.

LIPI gene (lipase, member I) has been associated with hypertriglyceridemia and is considered a susceptibility factor of hyperlipoproteinemia type 4.

HSPA13 encodes a heat shock protein, member of the stress-70 chaperone family, playing a role in the processing of cytosolic and secretory proteins.

NRIP1, also known as RIP140, is nuclear receptor. Sugawara et al. [5] showed that RIP140 interacted with SF1 and DAX1, which control expression of STAR, a regulator of intra-mitochondrial cholesterol transport. Using reporter gene constructs, they showed that RIP140 inhibited STAR expression in an SF1-dependent manner. USP25 (ubiquitin specific peptidase 25) is a gene involved in ubiquitination pathways.

Finally, TMPRSS15 encodes a transmembrane protease, serine 15 or enterokinase, an intestinal enzyme responsible for initiating activation of pancreatic proteolytic pro-enzymes.

It is clear that the deletion of these genes could result in a pathological phenotype. So we hypothesize that, even if we did not reveal the 46 chromosome cell line, it is present in mosaic in different tissues. In fact, in our patient, the derivative chromosome seems to balance this complex rearrangement that, as we have seen, is not associated with pathological phenotype. Though, this complex structural chromosomal abnormality exposes the patient to a high risk of offspring affected by unbalanced chromosomal disorder (deletions or duplication of chromosome 18 and 21) and explains her infertility.

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