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Mapping breakpoints of a familial chromosome insertion (18,7)(q22.1; q36.2q21.11) to *DPP6* and *CACNA2D1* genes in an azoospermic male

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ABSTRACT

It is widely accepted that the incidence of chromosomal aberration is 10–15.2% in the azoospermic male; however, the exact genetic damages are currently unknown for more than 40% of azoospermia. To elucidate the causative gene defects, we used the next generation sequencing (NGS) to map the breakpoints of a chromosome insertion from an azoospermic male who carries a balanced, maternally inherited karyotype 46, XY, inv ins (18,7) (q22.1; q36.2q21.11). The analysis revealed that the breakage in chromosome 7 disrupts two genes, dipeptidyl aminopeptidase-like protein 6 (*DPP6*) and contactin-associated protein-like 2 (*CACNA2D1*), the former participates in regulation of voltage-gated potassium channels, and the latter is one of the components in voltage-gated calcium channels. The deletion and duplication were not identified equal or beyond 100 kb, but 4 homologous DNA elements were verified proximal to the breakpoints. One of the proband's sisters inherited the same aberrant karyotype and experienced recurrent miscarriages and consecutive fetus death, while in contrast, another sister with a normal karyotype experienced normal labor and gave birth to healthy babies. The insertional translocation is confirmed with FISH and the Y-chromosome microdeletions were excluded by genetic testing. This is the first report describing chromosome insertion inv ins (18,7) and attributes *DPP6* and *CACNA2D1* to azoospermia.

1. Introduction

It is estimated that about 15% of couples were troubled with infertility in Western countries (De Kretser and Baker, 1999), and genetic anomaly is the most predominant factor involved. For the infertile males, chromosomal aberration is the most prevalent defects (Foresta et al., 2005). It is widely accepted that the incidence of chromosomal aberration is 10–15.2% in azoospermic patients, and 3–5% in oligozoospermic ones (Yatsenko et al., 2010), and more than 40% of azoospermia are currently categorized as cause-unknown and may be linked to unidentified genetic abnormalities (Hamada et al., 2013).

There are two kinds of chromosomal aberrations, the numerical and the structural. The structural aberration describes altered chromosomes that occur either as intrachromosomal or inerchromosoal events. It is estimated that around 0.5% of the newborn infants carry structural aberration (Jacobs et al., 1992), and the most frequently encountered entities are translocations. Translocation can be further divided into three sub-groups, i.e., reciprocal, centric fusion (Robertsonian) and insertional, and the latter represents the rarest event with an incidence about 1:80,000 in live birth or about 1:10,000 in karyotyped patients (Neill et al., 2011). So far, very few cases have been reported regarding insertional translocation attributed to male infertility.

Chromosomal insertion refers to a portion of one chromosome deleted from its normal location and inserted into another non-homologous chromosome. Because of three breaks involved, it is classified as complex chromosomal rearrangements (CCR). Until now, there are few, if not any, reported data about chromosomal insertion in DNA sequence level.

Abbreviations: aCGH, comparative genome hybridization array; ACTR3B, actin-related protein 3-beta gene; AZF, azoospermia factor; AZFa, azoospermia factor A region; AZFb, azoospermia factor B region; AZFc, azoospermia factor C region; BAC, bacterial artificial chromosome; CACNA2D1, contactin-associated protein-like 2; CNV, copy number variation; CCR, complex chromosomal rearrangements; DPP6, dipeptidyl aminopeptidase-like protein 6; FISH, fluorescence in situ hybridization; FoSTeS, fork stalling/template switching; HERV, human endogenous retrovirus; ISCN, international system for human cytogenetic nomenclature; LINE, long interspersed nuclear elements; NAHR, non-allelic homologous recombination; NGS, next generation sequencing; NHEJ, non-homologous end joining; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism; SRY, sex region of Y chromosome; STS, specific tag sequence.

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Recently, aCGH and NGS have become commercially available and cost affordable, which greatly help scientists to reveal some astonishing facts. For example, the chromosome insertion is found to be 160× more frequent than the previously recognized (1:500 versus 1:80,000) (Kang et al., 2010). Many cryptic imbalances were identified in previously described "truly balanced translocation" including insertion (Sismani et al., 2008). Nevertheless, there are neither reports about chromosomal insertions alleged to azoospermia nor about chromosomal insertion analyzed by NGS in DNA base pair level so far.

Here we report on the NGS characterization of the chromosome breakpoints in an azoospermic male who inherited a balanced insertional translocation 46, XY, inv ins (18,7) (q22.1; q36.2q21.11) from his mother. To the best of our knowledge, this is the first report on an insertional translocation that disrupts *DPP6* and *CACNA2D1* genes and is associated with azoospermia.

2. Case report

The patient was a 23-year old farmer with height of 170 cm, weight of 72 kg and normal libido. His wife carries a normal karyotype with regular menstruation but never got pregnant after marriage for 3 years. His mother at age of 54 has a history of spontaneous abortion. The patient has two sisters, the elder one carries a normal karyotype and has a healthy boy, and the younger one carries the same aberrant karyotype as the proband and has experienced two stillbirths and one ectopic pregnancy. The karyotype of his father is normal. The patient is the third kid of the family.

Semen analysis was performed according to the World Health Organization guidelines (WHO, 2010). About 1.0 mL of semen was for each ejaculation which showed milky color and very thin. No visible sperms could be identified in the semen through all the examinations.

The hormone test was done. The results showed testosterone (T) 17.8 nmol/L (normal range 9.34–59.93 nmol/L); prolactin PRL 0.47 nmol/L (normal range 0–0.68 nmol/L); follicle-stimulating hormone FSH 6.7 IU/L (normal range 0.7–11.1 IU/L); progesterone P 17.66 nmol/L (normal range 8.58–26.62 nmol/L); luteinizing hormone LH 3.4 IU/L (normal range 0.8–7.6 IU/L); and estradiol eE2 135.1 pmol/L (normal range 3.67–205.5 pmol/L). All the values were within the normal ranges.

3. The ethics consideration

This study was performed at Linyi People's Hospital of Shandong Province, China. The institutional ethical review committees (Ethics Committee of Linyi People's Hospital) approved the research protocol, and the proband and his relatives provided written consent.

4. Cytogenetic analysis

G-banding was performed with lymphocytes from peripheral blood. Olympus BX53 microscope (Olympus, Japan) was used for karyotype analysis (Beion Medical Technology, Shanghai, China). At least 50 metaphase cells were analyzed, and 5 metaphases were photographed to determine the patients' karyotype that was named according to ISCN (ISCN, 2009).

5. Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) was performed using bacterial artificial chromosome (BAC) clones as region-specific probes (from State Key Lab of Medical Genetics, Central South University, Changsha, China). Probe RP11-7H17 for human chromosome 18q23 was labeled as color orange, RP11-1005L7 for chromosome 7q21.12 was labeled as color red, and RP11-91C9 for chromosome 7p12.1 was labeled as color green. The Leica CW4000 cytogenetic workstation and Leica Image Processing software (CW4000 Karyo, Leica, Germany) was used through the FISH experiment, and the counterstaining was done with DAPI.

6. Genetic testing of Y-chromosomal microdeletions

Genomic DNA was extracted from the peripheral blood of the patient. Six STSs (sY84, sY86 for AZFa, sY127, sY134 for AZFb, and s254, sY255 for AZFc) were chosen according to EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions (Simoni et al., 2004). SRY gene (sY14) was used as internal control, and the genomic DNA from fertile male and female were served as positive and negative controls respectively. PCR was carried out as described (Li et al., 2013).

6.1. DNA preparation and sequencing

Genomic DNA was extracted from peripheral blood lymphocytes. 20 μg of gDNA was processed and sequenced with pair-end 50 cycle multiplex sequencing on Illumina HiSeq2000 platform according to the procedure as described (Dan et al., 2012).

6.2. Bioinformatics analysis

For alignment and data filtering, the high quality paired-end reads were aligned to the NCBI human reference genome (hg19 or GRCh37.1, here after called hg19). Only unique reads were remained for further analysis (Li et al., 2008). For translocation detection, 3 steps were done in the pipeline. 1. Clusters generation: the mapped paired-end reads that span on 2 different chromosomes would be divided into groups with default setting 10 kb. 2. Filtering by a cohort of control data: 159 Chinese samples with small-insert library were used as control and 49 with mate-pair were used for filtering systematic errors. 3. Filtering by their properties: the number of average mismatches and the number of supported read pairs were taken into consideration for each cluster for filtering. For chromosomal copy number variants (CNVs) detection, aCGH and SNP typing array were employed (http://www.fetalmedicine.hk/en/Fetal_DNA_Chip.asp). Data reporting variations in copy number were released after excluding known non-pathogenic chromosome CNVs. To ascertain the accuracy of MSP-based CNV detection method, two CVS samples were analyzed with HumanOmni2.5-Quad Bead Chip and CNV partition plug-in software.

7. Results

7.1. The azoospermic male has an aberrant karyotype of non-homologous chromosomal translocation with inversion

To understand the possible cause for the patient's sterility, a G-banding analysis was first performed on his peripheral white blood cells. The result revealed a DNA fragment that breaks off from chromosome 7 and inserts into chromosome 18 with inversion (Fig. 1). Carefully analysis finally established a unique karyotype 46, XY, ins (18:7) (18pter → 18q22.1::7q36.2 → 7q21.11::18q22.1 → 18qter;7pter → 7q21.11::7q36.2 → 7qter).

7.2. The aberrant karyotype is inherited from his mother

To explore if the aberrant karyotype is transmitted in a familial pattern, i.e., the proband's karyotype 46, XY, inv ins (18,7) is inherited from his parents, we performed the G-banding analysis on peripheral lymphocytes collected from his parents. The result demonstrated that the karyotype of his mother was exactly the same as the proband's, indicating that the proband inherited the aberrant karyotype from his maternal side.