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De novo 15.5-Mb Interstitial Deletion in 5p in a Male Ascertained by Oligospermia

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Array-based comparative genomic hybridization · Asynapsis · Deletion · Infertility · Oligospermia · 5p13.3p14.3

Abstract
We describe a case of a 34-year-old male presenting with oligospermia and an otherwise normal phenotype. Investigation with array-based comparative genomic hybridization (aCGH) revealed an interstitial deletion of about 15.5 Mb in chromosome 5p13.3p14.3. We compared the phenotype of our patient with recently reported patients studied by aCGH, who show an overlapping deletion. We also analyzed the gene content of the deleted region in order to propose a possible involvement of specific genes in the clinical phenotype.

Deletions in the short arm of chromosome 5 lead to a variety of developmental defects, most of which are classified as cri-du-chat syndrome (CDC, OMIM 123450) [Niebuhr, 1978]. CDC is a well-defined clinical entity with several typical phenotypic features, including the characteristic cry that gives the syndrome its name, facial dysmorphism, speech delay, and mental retardation [Lejeune et al., 1963]. After using array comparative genomic hybridization (aCGH) in a large cohort of patients with CDC syndrome, Zhang et al. [2005] characterized 3 distinct consecutive regions (MRI–III) on 5p with differing effect on mental retardation and overall symptoms. Interestingly, deletions affecting only the most proximal MRIII region, which has a size of 14 Mb and is located in 5p13.3p14.3, produced no discernible phenotype. However, it is worth taking into consideration that mental retardation and other manifestations became worst as deletions that included MRI extended progressively into MRII and MRIII, and they were profound, when all 3 regions were deleted. Although large chromosomal deletions are rather uncommon in healthy individuals, nevertheless more cytogenetically visible unbalanced chromosome abnormalities have been reported in the short arm of chromosome 5 than in any other chromosome arm [Barber, 2005].

Herein, we report the first case of infertility associated with a large 15.5-Mb interstitial deletion in chromosome 5 from p13.3 to p14.3 in a male ascertained due to oligospermia.

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Clinical Report

The patient was a 34-year-old man, physician of profession, while his parents were nonconsanguineous. The patient was referred to our laboratory due to conception failure after 4 years of contraceptive-free intercourse. He had a normal phenotype, and his previous clinical history was unremarkable. His body mass index was 27.8 kg/m^2, height 175 cm and weight 85 kg. Physical examination revealed a normally androgenized male. Ophthalmologic examination was normal. Ultrasound assessment determined a volume of 13 ml of the left testis and a volume of 17 ml of the right testis. The values of follicle-stimulating hormone, luteinizing hormone and testosterone were within normal range. Semen analysis was performed according to WHO guidelines and showed oligoasthenoteratozoospermia.

Materials and Methods

Blood chromosome analysis was performed in the patient, his parents, one of his unmarried brothers, and his wife using high-resolution banding techniques. Twenty metaphases were analyzed from each subject by GTG banding.

Molecular karyotyping was carried out on the DNA extracted from whole blood of the patient and his parents according to standard procedures. All the experiments were conducted through oligonucleotide aCGH platforms (SurePrint G3 Human CGH Microarray, 4x180K; Agilent Technologies, Santa Clara, Calif., USA). Briefly, 500 ng of the proband and of a sex-matched reference DNA (NA10851; Coriell Cell Repositories, Camden, N.J., USA) were processed according to the manufacturer's protocol. Fluorescence was scanned in a dual-laser scanner (DNA Microarray Scanner with Sure Scan High-Resolution Technology, Model G2565CA; Agilent Technologies), and the images were extracted and analyzed through Agilent Feature Extraction Software (v10.5.1.1). Graphical overview was obtained using the Agilent Genomic Workbench (v6.5) software. Changes in test DNA copy number at a specific locus were observed as the deviation of the $\log_2$-ratio value of 0 of at least 3 consecutive probes. The quality of each experiment was assessed by using a parameter provided by Agilent software (QC metric) and on the basis of DNA quality. Copy number changes identified in the samples were evaluated by using the UCSC Genome Browser website (http://genome.ucsc.edu) and the Database of Genomic Variants (http://projects.tcag.ca/variation). The positions of oligomers refer to the Human Genome March 2006 (versions NCBI 36, hg18) assembly.

The DECIPHER database (http://decipher.sanger.ac.uk) was used to support genotype-phenotype correlation.

Y chromosome microdeletions were checked by extraction of genomic DNA from blood lymphocytes using the QIAamp DNA blood mini kit (Qiagen, Hilden, Germany). Then, PCR amplification of sequence tagged sites of the AZFa, AZFb and AZFc regions of the Y chromosome was performed using the Devyser AZF kit (Devyser, Hägersten, Sweden). The patient was screened for cystic fibrosis mutations according to the commercial TagIt CFTR 40 + 4 Assay (Tm Bioscience, Austin, Tex., USA). Genotyping procedures were performed according to the Tm Bioscience's recommended protocol (Luminex Assay).

Results

Cytogenetic analysis showed an interstitial deletion in the short arm of one chromosome 5 (data not shown). The karyotype according to ISCN was 46,XY,del(5)(p13.3p14.3). The karyotypes of the patient's parents, his brother and his wife were normal (data not shown).

Molecular karyotyping characterized the deletion in the short arm of chromosome 5 as involving subbands from 5p13.3 to 5p14.3, a deletion of about 15.5 Mb, which distal and proximal breakpoints fell between 18,451,320–18,497,804 bp and between 33,966,676–33,983,168 bp, respectively (fig. 1).

No deletion was detected after sequence tagged sites analysis of the AZF region of the Y chromosome, and the patient was also negative for cystic fibrosis mutation screening.

Discussion

Cytogenetically visible interstitial deletions containing euchromatin without a detectable abnormal phenotype are rarely reported in literature [Sumption and Barber, 2001; Barber, 2005; Zhang et al., 2005; Hansson et al., 2007; Roos et al., 2008; Filges et al., 2009]. More recently, the advent of genome-wide arrays in the routine practice showed that up to 12% of the genome is constituted of copy number variants, which can arise both meiotically and somatically [Bruder et al., 2008; Hastings et al., 2009; Mkrtchyan et al., 2010]. We describe a man with a 15.5-Mb deletion in the short arm of chromosome 5 with breakpoints in 5p13.3 and 5p14.3. The patient was a normal individual who referred to our laboratory due to oligospermia.

Similar cases with mild or absent clinical features and 5p cytogenetically visible deletions have already been reported in the literature (fig. 2). Walker et al. [1984] described an interstitial deletion in 5p13p15.1 that was found in a mentally retarded woman and 3 of her 4 children. The mother and the 2 chromosomally abnormal children showed mild dysmorphic features and moderate mental retardation. Overhauser et al. [1986] reported an interstitial deletion within band 5p14 that could be traced through 6 individuals in 3 generations without any apparent physical or mental abnormalities. Keppen et al. [1992] communicate an interstitial deletion including region 5p13.3p14.3 which was detected in a 3 generation family affecting 4 family members. Varied manifestations were found among the affected individuals, including micro-

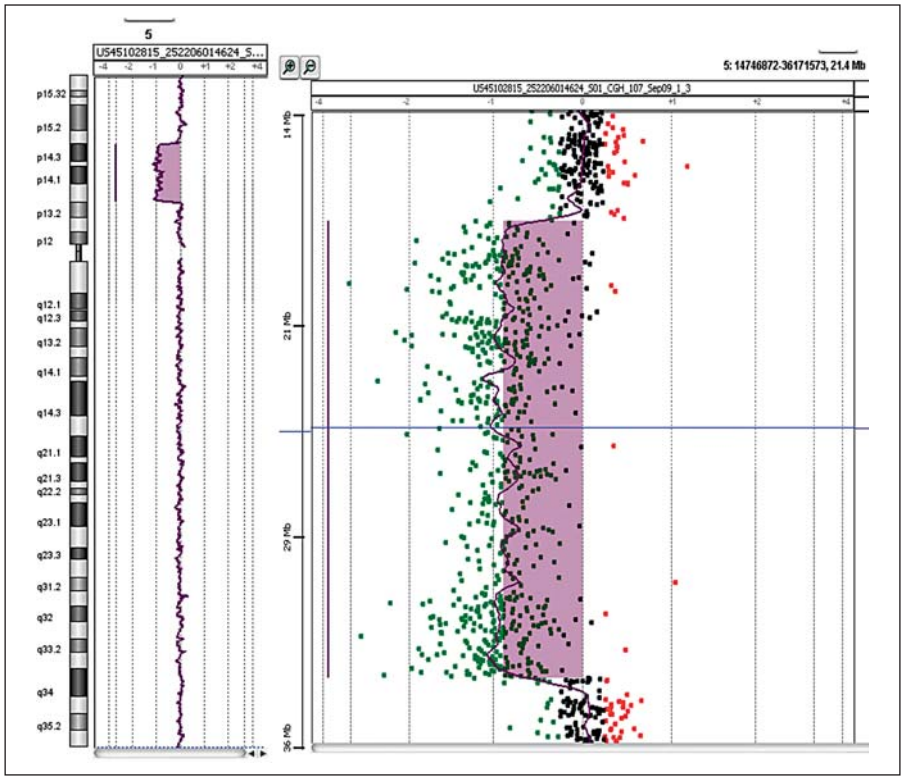

Fig. 1. aCGH profile of chromosome 5 showing an interstitial deletion. To the left, the whole chromosome 5 view, to the right, the enlarged view of the rearrangement as provided by Agilent Technologies, CGH Analytics 3.5.14. The distal deletion breakpoint was between 18,451,320–18,497,804 bp, and the proximal deletion breakpoint was between 33,966,676–33,983,168 bp. The overall size of the deletion was about 15.5 Mb.

cephaly, hypertonia and micrognathia, while mild mental retardation was common to all of them. Hand et al. [2000] described an interstitial deletion (5p14.1p14.3) in an otherwise normal male with a peroxisomal disorder and in his phenotypically normal mother. Johnson et al. [2000] referred to a case of a familial inheritance of a 5p14 deletion from a healthy father to his son. Although the father was clinically and mentally normal, his son had significant clinical manifestations, including microcephaly, seizures and global developmental delay. Zhang et al. [2005] in his effort to localize the region associated with CDC described an asymptomatic individual (patient 51) with a familial deletion in 5p13.3p14.3. The same aberration was found in her mother and grandparent who were both unaffected.

Recently, Barber et al. [2011] reported a family with an interstitial deletion of 5p13.3p14.3 which was 12.23 Mb in size (chr5: 20,352,535–32,825,775). The deletion was found in 6 family members and cosegregated with learning and/or behavioral difficulties but no facial dysmorphisms or other symptoms. After considering the other published benign deletions, the author suggested an 883-kb candidate gene region in 5p13.3 (containing the genes *GOLPH3, MTMR12, ZFR, SUB1*, *NPR3*, and microRNA hsa-miR-579) as responsible for the observed familial intellectual disability. The described deletion overlaps to a great extent with our case. Interestingly, although our patient has also deleted the above suggested region, he displays no learning or behavioral problems.

After searching among DECIPHER patients, we were able to find only 3 smaller deletions (patients 251341, 253161 and 252509) partially overlapping our one (fig. 2). Particularly, patient 251341 has a deletion of 3.46 Mb in 5p14.3 (chr5: 18,738,831–22,195,012), patient 253161 has a deletion of 2.80 Mb in 5p14.3 (chr5: 20,352,476–23,153,755) and finally patient 252509 has a loss of 2.72 Mb in 5p13.3 (chr5: 30,821,965–33,544,656). In patient 251341, the deletion involves 2 cadherin superfamily genes, *CDH18* and *CDH12,* while in patient 253161, only the *CDH12* gene is deleted. Moreover, in patient 252509, only another cadherin gene, the *CDH6*, is absent. All these genes are expressed mainly in the central nervous system and are also deleted in our patient. The patients 251341 and 252509 have mental retardation, although the severity was not reported. Interestingly, patient 253161 has no discernible phenotype. Haploinsufficiency for these genes has not been directly related to mental retardation. Even if their causal link to mental retardation in patients 251341

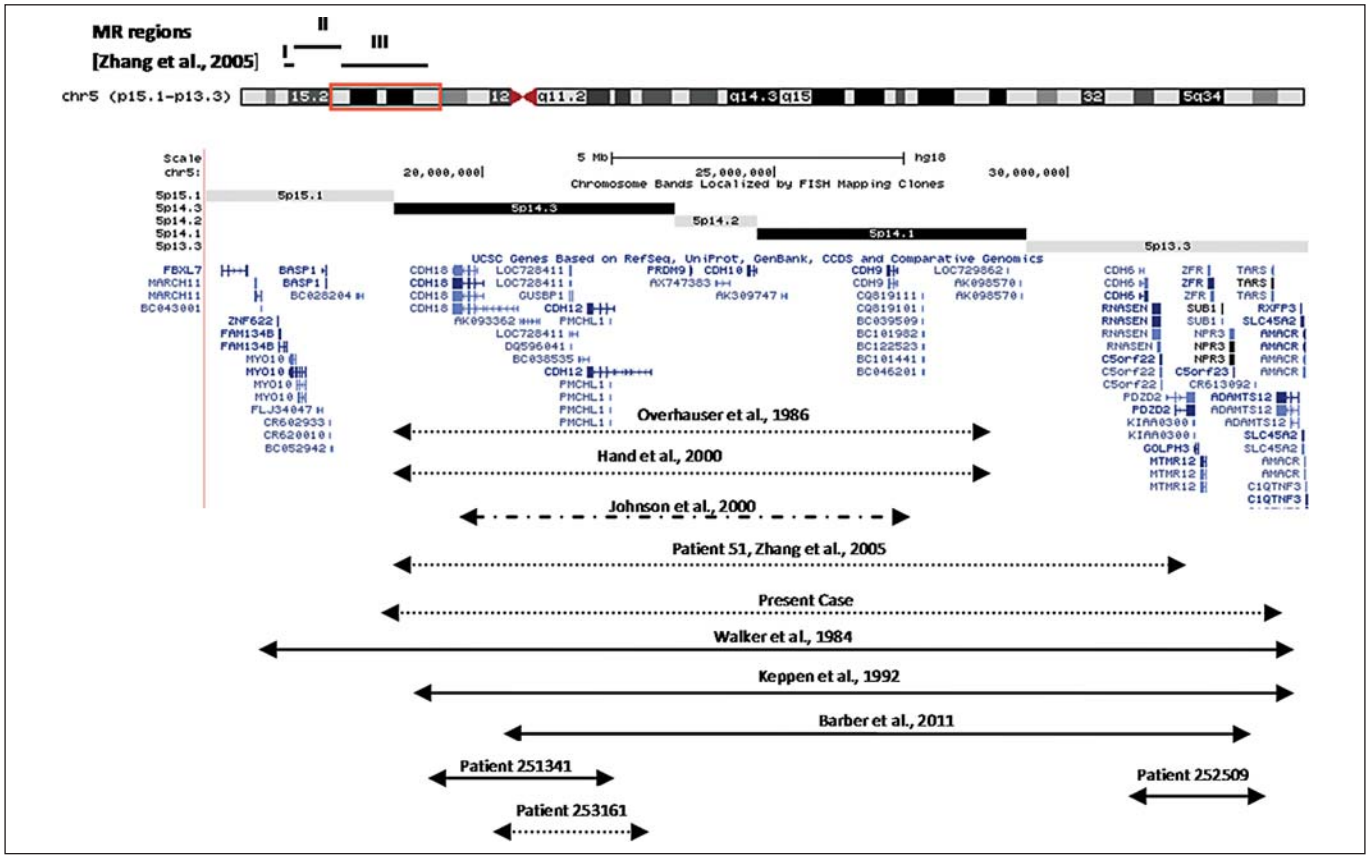

Fig. 2. UCSC genome browser (NCBI 36/hg18) illustration of the short arm of chromosome 5, from base pairs 15,300,000 in 5p15.1 to 34,100,000 in 5p13.3. The region is indicated by the rectangle on the idiogram of chromosome 5 and above the idiogram, the approximate extent of the 3 mental retardation regions MRI (8.0–9.3 Mb), MRII (9.3–18.3 Mb) and MRIII (18.3–31.9 Mb) as proposed by Zhang et al. [2005] are shown. The horizontal double-headed arrows indicate the approximate extent of several deletions: the dotted line of the benign deletions (patient 253161 from Decipher database and our present case) [further patients from Overhauser et al., 1986; Hand et al., 2000; patient 51 in Zhang et al., 2005]; the alternate dotted and dashed line of the affected child with a normal father [Johnson et al., 2000]; the solid line of the transmitted deletions in affected families or individuals (patients 251.341 and 252.509 from Decipher database) [Walker et al., 1984; Keppen et al., 1992; Barber et al., 2011].

and 252509 could be real, we can hypothesize an incomplete penetrance, since our patient has normal intelligence and was referred to our laboratory because of infertility.

According to the NCBI RNA Reference Sequences Collection, the deleted area encompasses 17 transcribed genes, most of which are of unknown function. For none of them, pathogenic variants have been reported, so the genes located in the deleted segment may not be subjected to dosage effect, explaining the normal cognitive phenotype of our patient. Of course, considering all other published cases, we can hypothesize a fragility of the normal phenotype in individuals bearing deletions in region 5p13.3p14.3. Although Overhauser et al. [1986], Hand et al. [2000] and Zhang et al. [2005] described familial cases of deletions in the above region (fig. 2), all family members were unaffected. However, in other reported cases with familial inheritance of similar aberrations, there were affected individuals with varied manifestations and severity [Walker et al., 1984; Keppen et al., 1992; Johnson et al., 2000; Barber et al., 2011]. Additionally, there is a report of a healthy parent with a deletion in 5p14 who had an affected son with the same abnormality [Johnson et al., 2000]. All this variability in the reported phenotypes may indicate that environmental or inheritable factors, which do not affect normal individuals, can adversely affect people with deletions in this region. Moreover, there is always the possibility of the existence of other aberrations in the genome (e.g. point mutations) that were not detected in the reported cases.

It has long been recognized that somatic chromosomal abnormalities are associated with infertility. The frequency of such aberrations in infertile men varies from 3–19%: 3% in the cases of mild infertility and 19% in men with nonobstructive azoospermia [Martin, 2008]. It is well known that male carriers of chromosomal rearrangements like translocations are nearly always presenting transient or permanent asynaptic segments in their spermatocytes [Martin, 2008]. The ultimate physiological consequence of asynapsis consists in male subfertility or sterility due to partial or complete meiotic arrest at the first meiotic prophase [Homolka et al., 2012]. The basic molecular mechanisms, by which unsynapsed regions trigger the meiotic checkpoint machinery to eliminate spermatocytes, remain elusive. Recent studies demonstrated that during pachytene, unsynapsed chromosome parts are transcriptionally silenced, like the sex chromosomes that pair solely in the short pseudoautosomal homologous region with the remaining parts of them naturally undergoing transcriptional silencing and forming the sex body [Homolka et al., 2012]. These studies concluded that interference of meiotic silencing of unsynapsed autosomes with meiotic sex chromosome inactivation leads to disturbance of the later mechanism, a phenomenon which is the most likely cause of asynapsis-related male infertility [Homolka et al., 2012]. In our case, we can hypothesize that the existence of a 15.5-Mb deletion in the 5p arm may trigger asynapsis during spermatogenesis, resulting in oligospermia. Besides, in a recent study, Homolka et al. [2012] showed that spermatogenic impairment is being proportional to the extent of asynapsis. In order to validate this assumption further, other studies of the patient like testicular biopsy should be performed.

There is a possibility that the observed infertility could be related to haploinsufficency of genes located in the short arm of chromosome 5. Although none of these genes is associated directly with spermatogenesis, and infertility has not been reported in any other similar case, this is an adaption that we cannot exclude. Of course, we have always to consider that other factors besides the size and location of the 5p deletion, such as individual genetic background, may modify the clinical manifestations.

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