Azoospermia and ring chromosome 9—a case report

Rita J. Laursen · Frank Tüttelmann · Peter Humaidan ·
Helle Olesen Elbæk · Birgit Alsbjerg · Albrecht Röpke

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Introduction

Ring chromosome abnormalities are rare abnormalities potentially involving any chromosome and the vast majority of previously reported cases were seen in patients with various congenital malformations and mental retardation. On the other hand, only few subjects carrying ring chromosomes and with normal phenotype have been described [1]. Until now only 30 cases of ring autosome 9 (r(9)) have been reported in the literature [2]. The prevalence of this pathology is 1 in 50,000 fetuses and in the majority of cases serious malformations were described [3].

The most typical ring formation occurs after terminal deletion and fusion of the p arm and the q arm and under this process genetic material can be lost [4]. The most common breakpoints are found between 9p24–p22 and 9q33–q34, but end-to-end fusion of palindromic telomere sequences have also been reported [5].

The Danish cytogenetic register, which contains data from all cytogenetic testing in Denmark from 1960 onwards (altogether 345,000 cases), has sparse information on two r(9) cases: a female, born in 1946, who died childless and a girl born 2008 for whom no more information is available.

Capsule We report a new case of an azoospermic male with ring chromosome 9.

R. J. Laursen (✉) · P. Humaidan · H. O. Elbæk · B. Alsbjerg
The Fertility Clinic, Skive Regional Hospital, Skive, Denmark
e-mail: rita.laursen@midt.rm.dk

F. Tüttelmann · A. Röpke
Institute of Human Genetics, University of Münster, Münster,
Germany

P. Humaidan
Faculty of Health, Aarhus University, Aarhus, Denmark

P. Humaidan
Faculty of Health Southern University, Odense, Denmark

The most frequent malformations reported in connection with r(9) are related to the deleted regions of both arms and include growth and psychomotor retardation, facial dysmorphism, microcephaly, cardiac malformations, limb and skeletal anomalies [5]. Microdeletions in the telomeric region of the long arm of chromosome 9 were found in patients with Kleeftstra syndrome [6, 7], whereas microdeletions of the telomeric end of the short arm may lead to 46,XY gonadal dysgenesis [2].

The literature regarding genital malformations in males with the presence of r(9) is very sparse; however the following malformations have previously been reported: cryptorchidism, hypospadias and micropenis [7]. Until now it is not clear whether the presence of r(9) affects spermatogenesis, as most cases have been described in young boys. Moreover, the literature is sparse regarding phenotypic details and sperm status. However azoospermia has previously been reported in males with other ring chromosomes, affecting chromosomes 12, 15, 21 and 22 [4, 8, 9].

The distal part of 9p is quite polymorphic and larger deletions of this region are often sporadic, combined with head/facial malformations like trigonocephaly, long philtrum, hypertelorism and micrognathia [2]. However, different gonadal disorders like complete gonadal dysgenesis, ovotestis, cryptorchidism and/or hypoplastic testes have also been described and typically these disorders were caused by deletions of three *DMRT* genes (*DMRT1*, *DMRT2* and *DMRT3*) on the 9p24 region, with *DMRT1* being the prime candidate [10]. These genes are involved in downstream pathways of sex determination; however the exact molecular mechanism is unclear.

The present case describes an azoospermic male with r(9) and this case further highlight that autosomal ring chromosomes can be involved in male infertility.

Case report

A 30 year old healthy male and his spouse were admitted to the Fertility Clinic in Skive, Denmark due to primary infertility. The couple had tried to obtain a pregnancy for 2 years. The medical history was unremarkable with normal psychomotor development; no previous malformations had been recorded and no hereditary diseases were reported in the family. The clinical examination of the genitals revealed a normal penis and both testes located in the scrotum, with a volume of approximately 6 ml each. Moreover, an ultrasound examination of the scrotal contents was normal, without the presence of a varicocele. A single semen analysis with a volume of 2.8 ml revealed azoospermia. The basal endocrine analysis showed an increased level of FSH level of 17.2 IE/L (normal range 1.2–15.8 IE/L) and LH level of 11.1 IE/L (range 1.7–8.6 IE/L), normal testosterone 16 nmol/L (range 11–34 nmol/L) and prolaktin 102 MIE/l (90–580 MIE/l). The chromosome analysis showed the karyotype 46,XY,r(9)(p24.3q34.3)[42]/45,XY,-9[5]/46,XY[3]. No Y chromosomal AZF deletion was detected (analysed according to EAA/EMQN guidelines [11]).

After counselling the couple decided to go for IUI with donor semen to overcome their infertility.

Materials and methods

Karyotyping of Giemsa (GTG)-banded chromosomes from lymphocytes at 550-band resolution as well as fluorescence in situ hybridization (FISH) analysis with telomere specific probes for both arms of chromosome 9 (9pter: D9S917 (ST 9pter), 9qter: D9S1839 (ST 9qter); Kreatech, Amsterdam, Netherlands) was performed according to standard procedures [12].

Genomic DNA was extracted by conventional methods from peripheral blood [13, 14]. Array-CGH (Comparative Genomic Hybridization) with the 400 K SurePrint G3 Human CGH Microarray Kit (Agilent Technologies) was carried out as described previously [15]. Only copy number variations (CNVs) on chromosome 9 were evaluated and described according to the human genome build GRCh37/hg19.

Results

Among 50 metaphases studied in the proband, 42 demonstrated a ring chromosome 9 [r(9)] without any obvious deletion (Fig. 1). A monosomy 9 was seen in 5 metaphases and a normal male karyotype was found in 3 metaphases. The karyotype was 46,XY,r(9)(p24.3q34.3)[42]/45,XY,-9[5]/46,XY[3] and the FISH analysis revealed a signal with the 9qter probe and no signal for the 9pter subtelomeric probe on

the ring chromosome (Fig. 2). These results were confirmed by array-CGH that demonstrated only a loss of genomic material at the telomeric end of the short arm of chromosome 9. The first oligonucleotide with a normal dosage was detected at position chr9:434797. No aberration was determined on the telomeric region of the long arm and, therefore, the position of the breakpoint on 9qter could not be localised. The last oligonucleotide on the Agilent 400 k array was situated at position chr9:141025921. Taken together, the results indicate breakpoints near the telomeric ends of both arms of chromosome 9.

Discussion

We herein describe a case of a male with the presence of autosomal r(9) and azoospermia.

Overall, ring chromosomes are rather uncommon cytogenetic abnormalities. Most patients with a ring chromosome demonstrate typical phenotypes with major dysmorphic features and intellectual disability associated with the amount of deleted euchromatic sequences and the recurring subtelomeric deletion syndromes [16]. However, ring chromosomes with fusion of the telomeres without loss of chromosomal sequences or ring chromosomes with fusion of the subtelomeric chromosomal ends without loss of genes that can be associated with a normal phenotype have been described [17].

Our patient showed mosaicism for the ring 9 chromosome, cells with monosomy 9, and cells with a normal male karyotype. The ring structure itself can be considered unstable and through formation of sister chromatid exchanges during postzygotic mitotic cell divisions may lead to double rings or broken rings finally leading to aneuploidy. This mechanism is called “dynamic mosaicism” in the literature [3]. Our patient demonstrated an apparently normal karyotype in approximately 6 % of the analysed blood cells. However, the proportion of aberrant vs. normal cells may be different in other tissues that were not tested. In general, mosaicism may range from only few (single) cells to the majority of cells analysed [18].

A FISH analysis with telomere specific probes for both arms of the chromosome 9 revealed only a deletion of the 9pter probe on the ring chromosome whereas the 9qter probe showed a normal signal pattern. This constitution was also observed in the array-CGH analysis that demonstrated a loss of approximately 435 Kb of the subtelomeric region of the short arm, whereas no obvious loss was seen of the subtelomeric region of the long arm. Nevertheless, a deletion on the long arm cannot be completely excluded because the distal sequences of the subtelomeric regions are not covered by the array format used. One protein-coding gene, *FAM157B*, the pseudogene *TUBBP5*, and 6 uncharacterised genes

Fig. 1 Aberrant metaphase showing the ring chromosome (karyotype 46,XY,r(9)(p24.3q34.3))

(*LOC101928932*, *LOC102724409*, *LOC102724394*, *LOC102724374*, *LOC102724447*, *LOC102724468*) were located distal of the last oligonucleotide on the 9qter region. Therefore, it is possible that one or more of these genes are

deleted, but the phenotypic consequences of such deletions would be unclear.

The deletion of 435 Kb on 9pter contains 8 genes: *DDX11L5* (pseudogene), *WASH1* (OMIM 613632), *FAM138C* (not protein-coding), *LOC101929157* (uncharacterised), *FOXD4* (OMIM 601092), *CBWD1* (OMIM 611078), *C9orf66* (uncharacterised), and *DOCK8* (OMIM 611432). None of these genes were reported to have an influence on spermatogenesis or testis development.

The 9p24.3-pter was previously described as a sex determining region [6] and especially the *DMRT* genes are of interest. The *DMRT1* gene is located approximately 400 Kb proximal of the *DOCK8* gene. Both genes, *FOXD4* and *DOCK8*, which were also deleted in the reported patient, have been associated with sex reversal in patients with 46,XY DSD [6]. In the case presented here, the patient had azoospermia and small testes, which could be related to the deleted *FOXD4* and *DOCK8* genes.

The identified karyotype of the patient is of special interest because the mosaicism raises important issues of risks for offspring although the proportion of aberrant cells in the gonads remains unknown. In principle, the following karyotypes can be predicted: 1. an entirely normal karyotype, 2. a monosomic karyotype and 3. foetuses carrying the ring chromosome in all metaphases. In the latter case, the ring chromosomes may undergo the “dynamic mosaicism” leading to aneuploidy described above. The latter two cases may be

Fig. 2 FISH analysis using subtelomeric probes for the short (9pter in green) and the long arm of chromosome 9 (9qter in red). On the ring chromosome only the signal of the 9qter probe is present

lethal in utero and lead to spontaneous abortion. The generation of aneuploid cells during embryogenesis influences the stability of developmental pathways [3]. Furthermore, a 4th karyotype may arise from nondisjunction resulting in trisomic cells, which would in case of 47,+r(9) also be lethal in utero.

The ring chromosome may directly influence spermatogenesis by a disturbance in meiotic pairing in spermatogonia containing the r(9). Indeed, in most cases of transmitted ring chromosomes, the mother was identified as carrier indicating that most male carriers of ring chromosomes are infertile [19].

The present case is unique due to the fact that the typical 9pter deletion syndrome was not seen, even though seven genes on the telomeric part of 9pter were deleted; however the patient was azoospermic with small testes without any other genital malformations and, interestingly, the Leydig cell function seemed to be affected as well, as the LH level was moderately increased. A diagnostic testicular biopsy could have provided more information about possible morphological changes in the testes, but a biopsy was not performed as the patient refused to undergo this intervention.

An important issue is to which degree a mosaicism affects the phenotype of an individual. In total 84 % of cells contained the r(9) in the present case, which corresponds well with previous reports on r(12,15,21,22), in which 85–97 % of the autosomal ring chromosomes were found in relation to azoospermia [8, 20–23]. In contrast, some previous cases including fertile men with a lower degree of mosaicism (<50 %) have been described [24, 25].

In conclusion, either the ring chromosome itself may hamper meiosis and cause azoospermia or one or more of the genes on 9p24,3-pter are related to azoospermia in humans. However, the exact encoding proteins and the molecular mechanism of development of the phenotype are unknown, and the degree of mosaicism may be directly related to the phenotype. Additional future analyses in similar patients will be necessary to draw firm conclusions.

Authors Contribution RJL and BA had the clinical contact to the patient. FT and AR performed all genetic analyses. All authors substantially contributed to drafting and critical revising of the manuscript and the final version was approved by all authors.

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