ORIGINAL RESEARCH

Genetic evaluation in infertility: Case of infertile couple with deletion and duplication of chromosome 9q12

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Abstract

Infertility is defined as the inability of women to ever bear a child or become pregnant after one year unprotected sexual intercourse or carry a pregnancy to a live birth. Genetic counseling can increase a couple's chance of having a healthy baby. Some forms of infertility, particularly sperm abnormalities in males and oligomenorrhoea/amenorrhoea in females have a genetic basis. Couples with these forms of infertility may be at increased risk for transmitting infertility to their children, for having a miscarriage or for having a child with a serious genetic condition. Based on international guidelines for appropriate use of genetic testing in infertility, cytogenetic testing is strongly advised in all cases of infertility. Apart from this, there are several other molecular genetic tests such as Y chromosome deletions, FRAX-A, CFTR gene analysis that are recommended during diagnostic work up of infertility and prior to ART. In the present paper, we report a case of an infertile couple who were referred for genetic evaluation for infertility prior to ART. The husband had a partial trisomy of 9q12 heterochromatin region [46,XY,dup(9)(q12)] while the wife had a partial monosomy of the same 9q12 heterochromatin region [46,XX,del(9)(q12)]. The case discussion emphasizes the role of genetic testing and counseling in infertility cases to determine the etiology of infertility, determination of risk of genetically abnormal offspring. This helps the couple make an informed choice regarding their reproductive choices. Genetic testing and genetic counselling should always be part of an extensive evaluation of infertile couples, especially those opting for ART.

Keywords: Genetic evaluation; infertility; deletion and duplication of chromosome 9q12

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Introduction

Several countries including India have set no national 'best practice' guidelines or recommendations for the appropriate use of genetic testing in infertile males and females. To address this dearth of international guidelines, twelve international scientific societies of different disciplines involved in human reproduction (biology of reproduction, embryology, genetics, gynaecology, andrology, endocrinology and urology) worked together and set up guidelines for the genetic diagnosis of male and female infertility which was published in year 2002 [1].

Chromosomal analysis in blood is used to check for chromosomal aberrations in infertile couples.

Karyotyping evaluates the number and gross structure of the 23 pairs of chromosomes to detect abnormalities by G banding technique. This allows the couples to take an informed decision to elect for specific assisted reproductive techniques using either their own or donor oocytes or spermatocytes. It also permits the health care professionals to offer a prenatal diagnosis to exclude any associated congenital genetic defects in the fetus. Moreover, karyotyping of the couple is mandatory before pre-implantation genetic screening/ diagnosis may be considered for them.

Cytogeneticanalysis(karyotyping)ofperipheralblood is strongly recommended as a first line test during the diagnostic workup of men with azoospermia or severe oligozoospermia. It is also recommended in those cases in which sperm parameters are within the normal ranges or only slightly abnormal as some karyotypic abnormalities may cause male infertility associated with an apparent normozoospermia (such as 47,XYY, translocations and other structural aberrations). The overall incidence of chromosomal anomalies in infertile males ranges between 2% to 8%, and increases to 15% in azoospermic males, being largely contributed by patients with 47,XXY aneuploidy (Gekas et al, 2001 and multiple studies reviewed in Foresta et al., 2002). Notably, the corresponding figure in the normozoospermic infertile men is reported as 3.0% [2].

Karyotyping is recommended during the diagnostic workup of women presenting with amenorrhoea (primary/ secondary including premature ovarian failure), oligomenorrhoea with hypergonadotropinism or recurrent miscarriages/ spontaneous abortions. About 30% of females with amenorrhoea have chromosomal abnormality, mostly 45,X – Turner's syndrome.

The cytogenetic screening is made mandatory for both females and males prior to any assisted reproductive technique (ART) including intrauterine insemination (IUI). Karyotyping of both partners is strongly indicated when no result is reached after one year of sexual intercourse aimed at pregnancy.

Apart from karyotyping, there are several other molecular genetic tests that are recommended by international guidelines during diagnostic work up of infertility and prior to ART under specific clinical criterias. These are summarised below in Table 1. Of note, screening for FRAXA expansion in women with oligomenorrhoea or premature ovarian failure has been shown to predict poor response to ovarian stimulation in invitro ART cycles. Almost 6.5% of women with premature ovarian failure carry a FRAXA permutation expanded allele (60-199 CGG repeats) detectable by a molecular test. About 21% of women with a permutated FRAXA allele have premature ovarian failure in comparison to 1% of general population [3]. These women can potentially have male children with mental retardation, and hence can be recommended to opt for pre-implantation diagnosis of Fragile X syndrome.

Table 1: Other molecular genetic tests recommended by guidelines in infertile couples (apart from cytogenetic analysis/ karyotyping).

Genetic tests	***Male infertility***	***Female infertility***
Y chromosome microdeletions	Prior to ART, azoospermia (non-obstructive), severe oligozoospermia	-
CFTR gene analysis (Cystic Fibrosis)	Azoospermia, severe oligozoospermia (congenital absence of vas deferens)	Prior to ART
KAL1 gene analysis (Kallmann syndrome)	Azoospermia (hypogonadotropic hypogonadism)	Hypogonadotropic hypogonadism
AR gene analysis (Androgen receptor)	Suggested during diagnostic workup of azoospermia/ severe oligozoospermia (high androgen insensitivity index)	-
FRAXA expansion analysis (Fragile X-A CGG repeats)	-	Amenorrhoea, oligomenorrhoea, POF, hypergonadotropinism, prior to ART (especially poor responders)

Abbreviations: Severe oligozoospermia = sperm count <10x 106/ml, ART = assisted reproduction techniques, POF = premature ovarian failure

Genetic diagnosis and genetic counselling should always be part of an extensive evaluation of infertile couples, especially those going in for ART. Basic clinical analysis should precede any genetic analysis. However, genetic evaluation is also recommended when infertility is apparently related to other obvious causes, since different causes may coexist.

In the present paper, we report a case of an infertile couple who were referred for genetic evaluation for infertility prior to ART. Through this case study, we try to elucidate how genetic testing and post-test genetic counseling can help a couple with further reproductive decisions.

Case presentation

A rare case of a couple clinically diagnosed with infertility is reported. The couple has been married for five years with no known history of previous conceptions or miscarriages. Moreover, their efforts of assisted reproductive procedures (intrauterine insemination-IUI) had failed four times. During the pre-test genetic counseling session, a detailed personal and family medical history was taken. The husband aged 35 yrs, had severe oligozoospermia (sperm count = 1 to 2 million/ml). The wife aged 30 years, had a history of oligomenorrhoea. No Consanguinity was reported. In the husband, Kallmann syndrome and cystic fibrosis-related infertility were ruled out with the absence of history of recurrent upper respiratory infections, no congenital absence of vas deferens and no reported anosmia.

Four ml of peripheral blood sample (heparin) was collected from both husband and wife for high resolution chromosome analysis (karyotyping) with the intention to identify gross structural or numerical chromosomal anomalies. The software used was applied spectral imaging. Karyotyping revealed the presence of 46 chromosomes with normal sex chromosomes in the couple. However, the husband harbored a duplication of 9q12 (9qh+) while the wife carried a deletion of the same chromosomal segment (9q12/ 9qh-). The chromosome 9 from both karyotypes is shown below in Figure 1. Duplication occurs when a part of the chromosome is duplicated resulting in extra genetic material and deletion refers to a region of the chromosome being deleted. The husband had a partial trisomy of 9q12 heterochromatin region while the wife had a partial monosomy of the same 9q12 heterochromatin region.

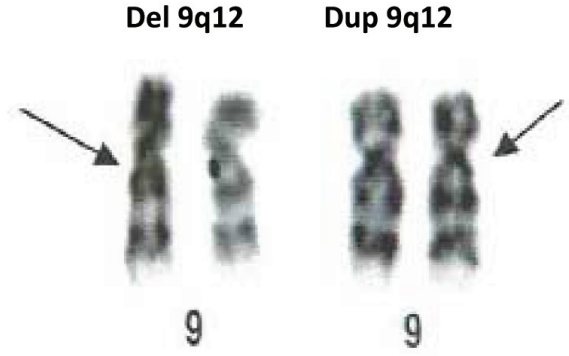

Figure 1: On the left: Karyotype of wife [46,XX,del(9)(q12)]- One normal chromosome 9 and the other with deleted 9q12 region. On the right: Karyotype of husband [46,XY,dup(9)(q12)] – One normal chromosome 9 and the other with a duplication of 9q12 region.

Despite the chromosomal variation in both, the couple did not have any apparent physical or mental disability and no significant dysmorphic features. The morphologic difference between the homologous chromosomes 9 may have been responsible for an error in crossing-over, leading to aberrant spermatozoa and consequently to infertility in the husband and oligomenorrhea in the wife due possibly due to an underlying adverse effect on ovarian or uterine functions.

Such chromosome 9 'polymorphisms' or 'variants' are commonly observed in healthy individuals. However, these have been reported in a higher prevalence among the infertile population and cases of repeated abortions. Open source databases such as DECIPHER v8.6 (Database of genomic variation and phenotype in Humans using Ensembl resources), CYTOD 1.0 (Cytogenetics database by Chang Biosciences) and DGV (Database of genomic variants) have records of several cases with loss or gain in chromosome 9, some of which also encompass 9q12 region along with flanking euchromatin region. These cases have been associated with autism-spectrum disorder, developmental delay, intellectual disability, facial dysmorphism, hearing impairment and multiple congenital anomalies.

Therefore, the parents were counseled that inheritance of either the exact isolated duplication (9q12) or deletion (9q12) is not likely to cause any birth defect in the offspring. However, the clinical

consequences in the progeny remain obscure, if the progeny inherits both of the variant chromosomes or any other chromosomal anomaly (with different breakpoints) caused by abnormal crossing over and segregation of gametes. During the post-test genetic counseling session, the couple was advised to undergo pre-implantation diagnosis to select embryos which have inherited the normal chromosome 9 homologues from both parents.

Discussion

Chromosome 9 is known to be highly polymorphic among the non-acrocentric human chromosomes with a high level of intra- and interchromosomal duplications. Variants like 9qh+, 9cenh+, 9ph+, 9qh-, or inv (9)(p11q13), known as 'heteromorphisms' or 'heterochromatic variants', are commonly found with an overall frequency of approximately 1.5% in the general population [4]. Segmental duplications located adjacent to the centromere and to the heterochromatic block comprise about 7% of chromosome 9 which are thought to predispose and mediate structural rearrangements within the pericentromeric region [5]. In this study, we report a rare case of an infertile couple with a gain of genetic material at 9q12 cytogenetic band (in husband) and loss of genetic material at the same cytogenetic position in wife. The 9q12 region comprises of heterochromatin, hence the polymorphic variant is alternatively named as 9qh+/ and 9qh- respectively. The 9q12 region is composed of heterochromatin which does not house any genes and hence an isolated deletion or duplication of this region is not expected to contribute directly to abnormal gene copy number (Figure 2).

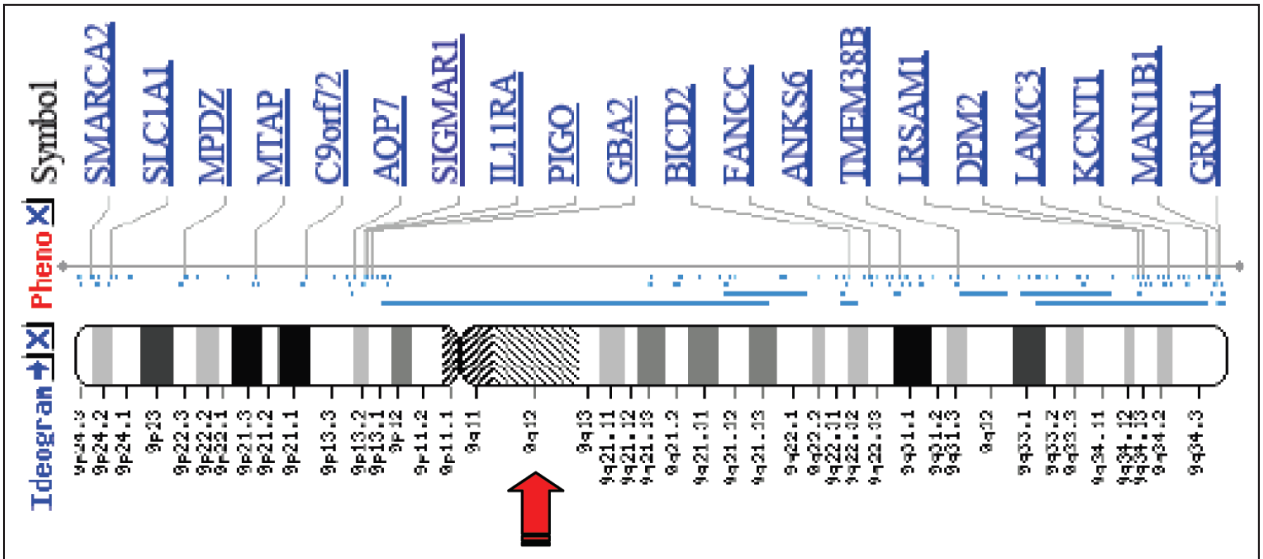

Figure 2: Ideogram of chromosome 9 (pter to qter) and the mapped genes with known phenotypes in OMIM database.

The structure and function of these duplicate and deleted DNA sequences within the heterochromatin are still unknown. Though the densely packed heterochromatin is known to be transcriptionally silent [6], it is thought to be vital to the stability of chromosomes throughout the cell cycle. Variations involving the heterochromatin segments can thereby ultimately lead to defects in chromosome segregation, thus resulting in genome instability. Early studies have also revealed that the heterochromatin in chromosomal polymorphism variations regulates gene expression by an epigenetic mode, that is by, reversible transformation between heterochromatin and euchromatin (expressed DNA sequences) [7].

Several recent studies involving cytogenetic analysis of 1809 infertile individuals reported a statistically significant association with 9qh+ chromosomal polymorphisms [8, 9]. The patho-physiological mechanism of this association is still unknown. However, chromosomal polymorphic variations appear to have no adverse effects on the outcome of IVF–embryo transfer treatment [10].

Conclusion

Genetic counseling can increase a couple's chance of having a healthy baby. Some forms of infertility, particularly male infertility, have a genetic basis. Couples with these forms of infertility may be at

increased risk for transmitting infertility to their children, for having a miscarriage or for having a child with a serious genetic condition. In the present case, based on the genetic anomalies detected in the couple, they were counseled about the option of pre-implantation diagnosis which can enable the selection of embryos with normal chromosome 9 from both parents. Due to the high cost of pre-implantation diagnosis, the couple was also given the option of IVF with donor sperm or donor oocyte to improve their chances of conceiving and probably reducing the risk of genetic anomalies in the fetus. However, the importance of genetic screening of the donor was highlighted as well. The case emphasizes the role of genetic testing and counseling in infertility cases to determine the etiology of infertility, determination of risk of genetically abnormal offspring. This helps the couple make an informed choice regarding their reproductive choices.

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Conflict of interest

The authors declare no conflict of interest.

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