Review paper

Genetic aspects of male infertility

Genetyczne aspekty niepłodności męskiej

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Słowa kluczowe: niepłodność męska, mikrodelecje chromosomu Y, czynnik azoospermii (AZF), geny *CatSper*.

Abstract

Currently, infertility affects up to 140 million people worldwide. It is considered that the male factor is responsible for nearly a half of problems in obtaining pregnancy. Increasingly, infertility treatment clinics, as well as standard examinations, also offer genetic tests in the diagnostics of the male infertility factor, such as: karyotype analysis, detection of Y chromosome microdeletions, and examination of the chromosome composition of sperm by the fluorescent *in situ* hybridisation method (FISH). Genetic factors, defined to date, which cover both chromosomal aberrations and monogenic disorders, are responsible for approximately 10–15% of cases of male infertility. Usually, their phenotypic manifestations are disorders in spermatogenesis, structural changes in the genital organs (e.g. reduced size of the testicles), or sperm dysfunction. Molecular studies intensively carried out in the area of diagnostics and treatment of infertility indicate an increasingly large number of relationships between genetic factors and fertility; however, many genes related with human fertility still remain unidentified.

Streszczenie

Niepłodność dotyka obecnie ok. 140 mln ludzi na świecie. Uważa się, że męski czynnik odpowiada za blisko połowę problemów z uzyskaniem ciąży. Coraz częściej kliniki leczenia niepłodności poza standardowymi badaniami oferują również testy genetyczne w diagnostyce męskiego czynnika niepłodności, takie jak: analiza kariotypu, określanie mikrodelecji chromosomu Y oraz badanie składu chromosomowego plemników metodą FISH (*fluorescence in situ hybridization*). Zdefiniowane obecnie czynniki genetyczne, obejmujące zarówno aberracje chromosomowe, jak i zmiany monogeniczne, odpowiadają za ok. 10–15% przypadków niepłodności męskiej. Fenotypowo przejawiają się one zwykle w postaci zaburzeń spermatogenezy, strukturalnych zmian w obrębie genitaliów (np. zmniejszenie rozmiaru jąder) czy zaburzeń funkcji plemników. Badania molekularne prowadzone intensywnie w dziedzinie diagnostyki i leczenia niepłodności wykazują coraz więcej zależności pomiędzy czynnikami genetycznymi a płodnością, ale wiele genów związanych z płodnością człowieka pozostaje wciąż niezidentyfikowanych.

At present, infertility is a relatively common problem, and it is estimated that according to the definition by the World Health Organization (WHO) it concerns as many as 140 million people worldwide [1]. It is prognosticated that in the near future 1 in 6 couples trying to conceive offspring will have difficulties with obtaining pregnancy. Infertility is a problem concerning a couple, and the diagnostics of the causes cover both the female and male partners [2]. Sometimes, the causes of infertility are obvious and easy to diagnose, e.g. obstruction of the fallopian tubes or spermatic cords. Nevertheless, there still remain a certain percentage of idiopathic cases, in which, according to the present knowledge and available diagnostic methods, it is not possible to determine the cause of infertility [3]. It is considered that the male factor is responsible for nearly a half of the problems with obtaining pregnancy [4]. Approximately 8% of males at reproductive age show infertility or reduced fertility [1]. Standard management in the diagnostics of male infertility covers medical history taking, physical examination, endocrine and viral laboratory, and quantitative and qualitative analysis of sperm, which is crucial for the determination of fertility [5]. In the expanded diagnostics, the presence of the reactive oxygen species (ROS) in sperm is evaluated, biochemical analysis of

seminal plasma performed, and the degree of sperm chromatin damage or the presence of antisperm antibodies assessed [6]. Increasingly, infertility clinics also offer genetic tests in the diagnostics of male infertility factor. At present, genetic tests are commercially performed, such as: karyotype analysis, detection of Y chromosome microdeletions, and examination of the chromosome composition of sperm by the fluorescent *in situ* hybridisation method (FISH) [7]. According to recommendations by the WHO, an indication for genetic diagnostics is a sperm concentration below 10 mln/ml, or total sperm number in the ejaculate below 10–15 mln. It is an interesting fact that the lower the number of spermatozoa in semen, the larger the number of various chromosomal aberrations observed in genetic tests [8].

Analysis of the karyotype detects large-scale genetic changes, such as deletions of large chromosome fragments or whole chromosomes, or translocations. In this test, abnormalities are observed in 15% of patients with azoospermia; however, karyotype changes may be related only to reduced sperm parameters (approx. 5% of cases of reduced fertility). Klinefelter syndrome (47 XXY) is a numerical chromosome aberration most often diagnosed in this test – up to 10% of cases of azoospermia [5]. The presence of an extra X chromosome causes damage to the seminal epithelium and in consequence, a lack of sperm cells in the semen [9, 10]. Clinical symptoms are unequivocal, these are often healthy males who do not show dimorphic disorders, in whom 47 XXY syndrome is diagnosed as late as in the case of infertility [8].

Chromosomal aberrations, both numerical and structural, may exert a considerable effect on fertility. In addition, the chromosomal factor is considered to play a role in spontaneous miscarriages and aneuploidy in offspring. Aneuploidy occurs surprisingly often in humans, and covers as much as 4% of clinically confirmed pregnancies. According to present knowledge, the majority of cases of foetal aneuploidy are due to maternal nondisjunction (approx. 95%), apart from sex chromosomes, for which aneuploidy is 50–100% of paternal origin. To date little is known concerning the importance of seminal aneuploidy in infertility [3]. While using the FISH method, in all semen a certain number of spermatozoa with aneuploidy are observed. In fertile males with confirmed offspring this percentage is approximately 3–5%. In males with disturbed fertility, a considerable, even a 3-fold, increase in this value is found, for all oligozoospermia, asthenozoospermia, or teratozoospermia. In addition, a simple relationship is observed between the level of seminal aneuploidy and the degree of fertility impairment [11].

Balanced chromosomal translocations are an example of changes, the carriers of which often do not show any somatic symptoms; however, they may demonstrate reduced fertility, vulnerability towards spontaneous miscarriage, or foetal defects [3]. It may happen that such a translocation causes phenotypic changes through position effect. This means that a change of gene location position in the genome may decrease or increase its expression in relation to the same gene in the primary position. However, the majority of balanced translocations do not induce phenotypic changes in the carrier. Disorders may occur only during the meiotic division of germ cells because the changes in chromosome length resulting from translocation hinder an equal dispersion of chromosomes into gametes, which leads to the formation of genetically unbalanced gametes [9]. Chromosomal inversions may have a similar effect. According to studies, the ratio between abnormal gametes in sperm remains within the range 1–54% and differs according to the chromosome engaged in the translocation or inversion [2].

The most frequent structural chromosomal changes affecting fertility are so-called Robertsonian translocations, which are diagnosed in 1 per 1,000 males [1]. These translocations involve two acrocentric chromosomes, which fuse at the centromere region and lose their short arms. Short arms of the five acrocentric chromosomes in the human genome contain many copies of the rRNA genes; therefore, such translocations are not hazardous for the carrier. Nevertheless, they may cause abnormal segregation of chromosomes during meiosis, resulting in fertility impairment [7].

Analysis of karyotype is limited by the microscope resolution and does not detect changes in the DNA sequence or submicroscopic abnormalities, such as Y chromosome microdeletions [4].

On the Y chromosome are located genes that are crucial for the development of the male primary sex characteristics and the process of spermatogenesis [10]. The so-called azoospermia factor (AZF) region, located in the long arm of the Y chromosome, is particularly important for the production of normal Y sperm. Considering its location in relation to the centromere, it has been divided into three sub-regions: AZFa, AZFb, and AZFc [11]. Each sub-region covers a separate gene involved in spermatogenesis. Microdeletions in these areas induce various degrees of sperm dysfunction. These changes are observed, on average, in 4% of patients with oligozoospermia, in 14% of patients with so-called severe oligozoospermia, and in 18% of those with non-obstructive azoospermia [12]. An indication for performing tests is oligozoospermia <5 mln/ml [11]. The vast majority of microdeletions in the AZF region occur *de novo*, and their effect is the loss of one or more genes engaged in spermatogenesis [10]. It is known that from among the 2,000 genes necessary to perform the normal process of spermatogenesis, only 31 are located on the Y chromosome. Despite intensive studies, genes of the AZF region have not yet been identified that are critical for the cell cycle and meiosis in the production of

gametes; however, clear relationships are observed between a genetic change in an individual sub-region and phenotypic changes in the form of spermatogenesis disorders at various stages [13]. The most frequent Yq microdeletions concern the AZFc region (approx. 60%), which may partially be due to its larger size compared to other sub-regions [10]. The majority of these cases result in azoospermia with the possibility of obtaining sperm for *in vitro* fertilisation after testicular sperm extraction. In turn, microdeletions in the AZFa sub-region often cause the Sertoli cell-only syndrome [13].

Increasing emphasis is being placed on molecular studies in the area of diagnostics and infertility treatment, and gradually an increasing number of relationships are being found between genetic factors and fertility. These studies provide information concerning biochemical and molecular mechanisms in the background of male infertility, and provide a chance for future identification of individual genotypes responsible for particular defects in the number and quality of sperm in the semen. Many of these genes have already been identified [14].

Classic examples of genes, the mutation of which affect male fertility, are the *CF* transmembrane receptor gene and the androgen receptor gene [15, 16]. Mutations in the *CFTR* gene may lead to congenital bilateral absence of the *vas deferens* and obstructive azoospermia (observed in approx. 2% of infertile males) [9].

The *CatSper* genes, located in the autosomal chromosomes, encode proteins of the voltage-gated calcium channel proteins and proton pump located in the sperm tail at the site responsible for its hyperactivation. In humans, the level of hyperactivated sperm motility shows a positive correlation with the fertilisation capability of sperm. In males with mutation in the *CatSper1* gene, an abnormally low percentage of sperm showing hyperactivation is found [8, 17]. In studies on mice, it was observed that mutations in *CatSper1-4* genes lead to infertility, despite normal development of the testicles and semen parameters [1].

Mutations in the *AKAP3* and *AKAP4* genes (genes for sperm A-kinase anchoring proteins) cause the immobilisation of sperm. Their protein products are a component of the fibrous sheath of the main part of the sperm tail and play an important role in the regulation of sperm motility, capacitation, and acrosome reaction [18, 19]. The studies showed that in humans and in mice the deletion within one of these genes causes dysplasia of the fibrous sheath, and consequently loss of sperm motility. Mutations in genes that encode for dynein proteins (*DNAI1*, *DNAH1*, *DNAH5* genes), found in patients with Kartagener's syndrome, exert a similar effect on fertility. Mutations in these genes cause primary ciliary dyskinesia and impairment of sperm motility [8]. In rare cases of male infertility associated with globozoospermia, mutations in the *SPATA16*, *PICK1*, or *DPY19L2* genes are observed [20]. An increasing number of genes are being discovered that are engaged at various levels in the normal production and functioning of sperm; thus, this is a domain in which at present studies are being very intensively conducted (Table 1).

Summing up, genetic factors which cover both chromosomal aberrations and monogenic disorders

Table 1. The most frequent genetic disorders in male infertility and their clinical implications [5, 7–9, 13, 17, 18, 20]

Genetic disorder	Clinical implication
Klinefelter syndrome (47XXY)	Low concentration of sperm cells in semen (oligozoospermia) or no sperm cells in semen (azoospermia)
Robertsonian translocations	Genetically unbalanced gametes
Y chromosome microdeletions in regions:	
AZFa	Sertoli cell-only syndrome
AZFb	Meiosis I interruption
AZFc	Hypospermatogenesis (progressing to severe oligozoospermia or azoospermia)
CFTR gene mutations	Bilateral absence of the vas deferens and obstructive azoospermia
CatSper gene mutations	Impairment of hyperactivation in sperm cells
AKAP3, *AKAP4* genes mutations	Impairment of sperm motility (asthenozoospermia)
DNAI1, *DNAH1*, *DANH5* genes mutations	Impairment of sperm motility (asthenozoospermia)
SPATA16, *PICK1*, *DPY19L2* genes mutations	Globozoospermia

are responsible for approximately 10–15% of cases of male infertility. Phenotypically, they are usually manifested in the form of disorders in spermatogenesis, structural changes in the genital organs (e.g. reduced size of the testicles), or sperm dysfunction, as shown in Table 1 [1]. Following the detection of abnormalities in karyotype, clinics should always offer patients genetic counselling, explaining that paternity is associated with the risk of transmitting the abnormalities to offspring, and that it may be recommended to consider a sperm donor or adoption [5]. An indication for the performance of genetic tests is azoospermia and oligozoospermia < 10–15 mln sperm in the ejaculate. According to data from the WHO, at present only approximately 25% of patients with the abovementioned diagnoses have genetic tests performed. It is highly probable that the majority of cases of idiopathic infertility, both male and female, could be explained by the analysis of the whole genome – WSG (whole genome sequencing); however, at present, the interpretation of the data obtained is an exceedingly difficult challenge for clinicians, not least because to-date not all functioning variants of genes related with human fertility have been discovered, and their clinical importance remains unknown [8].

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