

Hypogonadism — when does genetic diagnosis help in therapy?

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ABSTRACT

Objectives: The objective of the study was to describe phenotype-genotype correlation in selected cases with infertility and emphasise the importance of genetic testing as useful tool for proper treatment decision making

Material and methods: Genetic tests were performed in four patients as a part of diagnostic procedure by Sanger sequencing or targeted next generation sequencing (NGS gene panel).

Results: We found the genetic causes of hypogonadotropic hypogonadism in 3 males and female with infertility.

Conclusions: Genetic testing is carried out when searching for the genetic causes of clinically identified disorders. Genetic diagnostics may also be extremely helpful in treating hypogonadism but requires the assistance of a clinician endocrinologist or andrologist, as well as a geneticist.

Keywords: hypogonadism; infertility; genetic testing

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INTRODUCTION

Infertility is a social problem. More and more couples are having trouble conceiving a child. In addition, more and more elderly women are trying to get pregnant, advanced procreative age means that diagnosis and treatment should be carried out efficiently and effectively.

In many cases, it is possible to diagnose the cause and help the couple become parents. Often, however, the applied treatment does not bring the desired pregnancy.

In people with fertility disorders, various hormonal disorders are often encountered, but their compensation does not result in pregnancy. It turns out that in many cases, Genetic diagnosis is helpful in unexplained infertility, both in women and men with infertility.

In our work, we present the possibilities of using genetic tests to explain the cause of infertility and enable effective treatment.

The first genetic test recommended in the diagnosis of infertility is the assessment of the karyotype. Further

genetic testing is ordered depending on the history, physical examination, and abnormalities found. Hormonal tests are very helpful in making further decisions, which allow to direct further genetic diagnostics.

In men with azoospermia, *AZF* and *CFTR* gene mutation testing is indicated. In many cases, however, genetic diagnosis needs to be extended. Different abnormalities should also be expected in patients with hypogonadotropic hypogonadism and different in patients with hypergonadotropic hypogonadism. This also applies to women.

Nowadays, genetic diagnostics in infertility is recommended by many scientific societies [1–6], but it is worth paying attention to the need for cooperation between doctors of various specialties (endocrinologist, geneticist, urologist), as well as educating doctors of various specialties in the field of reproductive health, so that they understand the need to perform specific genetic testing and genetic consultations for couples with infertility.

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Objectives

The objective of the study is to describe phenotype-genotype correlation in selected cases with infertility and emphasise the importance of genetic testing as a useful tool for proper treatment decision making.

MATERIAL AND METHODS

Genetic tests were performed as a part of diagnostic procedure by Sanger sequencing or targeted next generation sequencing (NGS gene panel). The NGS panel included genes such as *SOX10*, *BMP15*, *CHD7*, *DUSP6*, *FEZF1*, *FGF17*, *FGF8*, *FGFR1*, *FLRT3*, *IL17RD*, *ANOS1*, *NSMF*, *PROK2*, *PROKR2*, *SEMA3A*, *SPRY4*, *WDR11*, *FSHB*, *CFTR*, *AR*.

RESULTS

Male hypogonadotropic hypogonadism

— case 1

Male, aged 40, presented for an andrological consultation due to unsuccessfully trying to conceive for around 9 years. Azoospermia was confirmed in repeated semen analyses. The patient has a history of obesity, is post-cholecystectomy, post phimosis surgery, has right kidney stones, and symptoms of hypogonadism (reduced libido, erectile dysfunction), with no signs of hyposmia. On physical examination, a gynoid body type, steatomastia, decreased facial and pubic hair, albinism, a small penis, and reduced testicular volume (as determined by ultrasound, right testicular volume was 5.1 mL, left testicular volume was 5.5 mL — normal volume is 15–25 mL per testes [7]) were found. Magnetic resonance imaging (MRI) of the pituitary gland showed heterogenous contrast enhancement of the anterior lobe of the pituitary gland. Hormone tests revealed hypogonadotropic hypogonadism with normal function of the remaining axes: adrenal, thyroid, and normal prolactin levels. Due to the presence of symptoms of hypogonadism and reduced sexual performance, symptomatic treatments were given, that is, testosterone replacement therapy (Tab. 1).

The patient took the recommended testosterone preparation at a dose of 250 mg every two weeks intramuscularly for 8 years. Subsequent semen analyses consistently showed azoospermia.

The patient did not consent to sperm retrieval by a testicular biopsy. Genetic test revealed a hemizygous pathogenic variant of p.Pro366GlnfsTer12 (c. c.1097delC) in *ANOS1* gene (X-linked recessive inheritance pattern). This is a very rare, pathogenic variant previously described in an Asian patient with Kallmann Syndrome [7, 9, 15].

Testosterone replacement therapy was discontinued, hormonal stimulation of spermatogenesis was implemented using human chorionic gonadotropin (hCG) 5000 units biweekly and follicle stimulating hormone (FSH) 2 × 150 units weekly, obtaining an improvement of spermatogenesis.

Table 1. Hormone test results of patient No. 1 with hypogonadotropic hypogonadism and infertility

Parameter	Unit	Result	Normal range
FSH	mIU/mL	0.3	1.5–12.4
LH	mIU/mL	< 0.1	1.7–8.6
Testosterone	nmol/L	5.64	8.64–29
Estradiol	pg/mL	13.5	25.8–60.7
Inhibin B	pg/mL	36	25–325
TSH	uIU/mL	2.0	0.4–4.0
ft4	pmol/L	15.1	12–22
ft3	3.1–6.8	4.31	3.1–6.8
ACTH	pg/mL	25.2	6–56
Cortisol	ug/dL	13.9	2.3–23.3
Growth hormone	uIU/mL	1.2	0.2–10.0
Prolactin	ng/mL	11	5–19

FSH — follicle stimulating hormone; LH — luteinising hormone; TSH — thyroid stimulating hormone; ACTH — adrenocorticotropic hormone

The first spermatozoa appeared in the semen after 3 months of treatment, after a subsequent three months, the number of spermatozoa in the ejaculate was 21 million (normal ≥ 39), and after yet another three months, the sperm count in the ejaculate came to 97 million. The spermatogenesis cycle from the spermatogonium through to the spermatozoa lasts 74 days; the effects of the stimulation cannot be achieved any quicker. The treatment took longer in this patient as we were dealing with pre-puberty hypogonadism. Note that even such a small (pre-puberty) testicular volume is not a contraindication for the hormonal stimulation of spermatogenesis. Full spermatogenesis was successfully restored in the patient despite the small testicular volume and very low inhibin B concentration.

Female hypogonadotropic hypogonadism

— case 2

Female patient, aged 31, presenting at a fertility clinic with unsuccessful attempts to conceive for approximately 2 years. Thus far, treated by various physicians for menses disorders (amenorrhea in the absence of medication). Hormone tests showed hypogonadotropic hypogonadism. Various kinds of hormone treatment were applied (substitution of estradiol progesterone, dydrogesterone, hormone replacement therapy, and ovulation induction with clomiphene, letrozole, and gonadotropins). Ovulation was successfully induced, and ova were collected during *in vitro* fertilisation procedures but the embryo did not develop further and no embryo transfer attempts to the uterine cavity were possible. The patient did not consent to an *in vitro* fertilization (IVF) procedure with a donor's ova. An ultrasound scan revealed a normal image of the reproductive

organ, patent fallopian tubes, and no suspicion of endometriosis was raised. There were no abnormal findings on physical examination and the body mass index was 22.9. The patient was taking 75 microgram per day levothyroxine to treat hypothyroidism.

During the diagnostic tests at the fertility clinic, attention was drawn to the short stature of the patient (measuring 159 cm with a weight of 58 kg). Further diagnostic tests were ordered along these lines (Tab. 2).

Apart from the deficiency of the hypothalamic-pituitary-gonadal axis, a growth hormone deficiency [below the normal ranges for age- and sex-specific serum insulin-like growth factor-1 (IGF-1) levels] was found and confirmed in functional tests. Adrenal insufficiency was also identified [low dehydroepiandrosterone sulphate (DHEAS) concentration and low adrenal reserve in the scope of cortisol secretion]. The DHEAS hormone is essential for the proper maturation of the ovarian follicles.

Genetic testing was carried out and revealed a pathogenic variant in the *PROP1* gene. The patient was qualified for the growth hormone treatment programme in adults. Growth hormone is essential for the growth and maturation of the ovarian follicles. Gonadotropins cannot act normally without the concomitant action of the growth hormone. Growth hormone is key to maintaining normal fertility in women.

Male hypogonadotropic hypogonadism — case 3

Male, aged 37, presented for an andrological consultation due to unsuccessfully trying to conceive for around 6 years. Azoospermia was confirmed in repeated semen analyses. The hormone test results pointed to hypogonadotropic hypogonadism (Tab. 3).

On physical examination, several characteristics of hypogonadism were found almost complete absence of facial hair and gynoid body type. The testicular volume as determined by ultrasound was very small: right testicle 4.6 mL, left testicle 5.6 mL. The patient has been treated with testosterone since adolescence. An MRI of the pituitary gland showed a normal pituitary gland image and normal olfactory bulb. Gonadotropin therapy was implemented [FSH 75 units biweekly, luteinising hormone (LH) 2500 units biweekly]. The testosterone concentration increased above normal levels with higher doses, inhibiting the release of gonadotropin-releasing hormone (GnRH) and, in effect, LH and FSH secretion. Gonadotropin therapy was implemented for a year, but no sperm appeared in the semen.

Genetic test revealed a presence of a rare pathogenic variant p.Glu84GlyfsTer26 in *FGFR1*. This was an indication to continue therapy since patients with a *FGFR1* mutation require prolonged gonadotropin therapy (1–2 years) for the

Table 2. Hormone test results of Patient No. 2 with combined pituitary hormone deficiency

Parameter	Unit	Result	Normal range
FSH	mIU/mL	0.8	1.5–12.4
LH	mIU/mL	0.7	1.7–8.6
Estradiol	pg/mL	< 10	Cycle phase-dependent
Testosterone	ng/dL	17	13–53
Androstendion	ng/mL	1.7	0.3–3.3
ACTH	pg/mL	5.7	5–45
DHEAS	ug/dL	59	74–410
Cortisol	ug/dL	7.9	7–15
Prolactin	ng/mL	7	5–25
SHBG	nmol/L	20.2	19–155
AMH	ng/mL	11.1	1.2–5.0
IGF-1	ng/dL	56	159–478

FSH — follicle stimulating hormone; LH — luteinising hormone; ACTH — adrenocorticotropic hormone; DHEAS — dehydroepiandrosterone sulphate; SHBG — sex hormone binding globulin; AMH — anti-Müllerian hormone; IGF-1 — insulin-like growth factor-1

Table 3. Hormone test results of patient No. 3 with hypogonadotropic hypogonadism and infertility

Parameter	Patient's result	Normal range	Unit
FSH	1.3	1.5–12.4	mIU/mL
LH	0.7	1.7–8.6	mIU/mL
Testosterone	1.2	8.64–29	nmol/L
TSH	2.2	0.4–4.0	uIU/mL
ACTH	22	6–56	pg/mL
DHEAS	234	189–523	ug/dL
Cortisol	12.5	7–15	ug/dL
Prolactin	9.4	5–19	ng/mL

FSH — follicle stimulating hormone; LH — luteinising hormone; TSH — thyroid stimulating hormone; ACTH — adrenocorticotropic hormone; DHEAS — dehydroepiandrosterone sulphate

activation of spermatogenesis. The therapy turned out to be effective and an ejaculate sperm count of 16.5 million was obtained following 12 months of treatment.

Male hypergonadotropic hypogonadism — case 4

Male, aged 34, presented for an andrological consultation due to unsuccessfully trying to conceive for around 3 years. Healthy until the present, no history of chronic diseases, taking various supplements to improve sperm motility and morphology. As a result of repeated semen analyses, oligoasthenotatozoospermia syndrome was detected: a reduced number of spermatozoa, lower motility, mostly abnormal sperm morphology, as well as increased seminal fluid viscosity (Tab. 4).

Table 4. Selected semen analysis parameters in patient No. 4 with infertility

Parameter	Patient's result	Reference value
Liquefaction time	15 min	< 60 min
pH	7.8	≥ 7.2
Viscosity	+++	+
Sperm concentration [mln/mL]	8	≥ 15
Total no. of sperm in ejaculate [mln/ejaculate]	41.6	≥ 39
Progressive motility [%]	1	≥ 32
Sperm with normal morphology [%]	1	≥ 4

In line with the andrology guidelines, the patient's medical history was taken, and a physical examination was performed and no abnormalities or deviations from the norm were found. Further diagnostic tests were ordered: a scrotum ultrasound, hormone tests, and semen microbiology analysis. The presence of the following pathogens was confirmed in semen culture: *Enterococcus faecalis* in titres of > 10E5/mL, Gram-positive cocci in titres of 10E3/mL under aerobic and anaerobic conditions, *Staphylococcus*, coagulase negative, in titres of 10E3 under aerobic conditions, and *Mycoplasma hominis* in titres of > 10E4/mL.

The presence especially of the latter pathogen explained the seminogram abnormalities found. It is evident that urogenital infections may lead to male infertility [8]. *Mycoplasma hominis* is a bacterial infection in men that may cause urinary tract infections, prostatitis, epididymitis, poor semen quality and, ultimately, infertility. This is a sexually transmitted infection and requires concurrent treatment of the partner. The infection may be completely asymptomatic and, with time, may also lead to urinary tract infections, vaginitis, pelvic inflammatory disease, and fallopian tube infertility problems in women. Even an asymptomatic *M. hominis* infection induces a pro-inflammatory immune response in the endometrium and prevents the embryo from implanting within IVF procedures.

Having identified the pathogen, the patient could be treated causally. Antibiotic therapy was implemented in both partners, according to the antibiogram results (ofloxacin and metronidazole in the man, and doxycycline in the woman).

An ultrasound of the scrotum revealed a normal testicular volume but closer to the lower limit of the normal range (right testicle 16.7 mL, left testicle 18.9 mL — with a normal volume for a healthy man ranging between 15–25 mL per testes), grade 1 and 2 varicoeles, and right-sided epididymal cysts up to 3 mm.

Table 5. Selected hormone tests in patient No.4 with infertility

Parameter	Patient's result	Normal range	Unit
FSH	14	0.95–11.95	IU/L
LH	7.5	0.57–12.07	IU/L
Estradiol	28.4	< 44	pg/mL
Testosterone	237	240–870	ng/dL
TSH	1.8	0.4–4.4	mIU/mL
Prolactin	12.9	3.4–19.4	ng/dL

FSH — follicle stimulating hormone; LH — luteinising hormone; TSH — thyroid stimulating hormone

Hormone tests showed hypergonadotropic hypogonadism [FSH, LH, testosterone, prolactin, thyroid stimulating hormone (TSH), estradiol, and vitamin D3] (Tab. 5).

There were no pathogens at a follow-up smear test of the partner after doxycycline treatment. However, a urogenital infection was once again detected in the man, this time *Klebsiella pneumoniae* and *Escherichia coli* was present in the semen culture. Antibiotic therapy was once again administered but the diagnostics were extended to include internal medicine causes of recurrent infections like, for example, diabetes. Within the clinical study, the patient was referred for genetic diagnostic testing. The p.Ser1235Arg variant in one allele of the cystic fibrosis (*CFTR*) gene was found. This variant is described in the Human Gene Mutation Database (HGMD) and ClinVar databases as a variant of uncertain clinical significance. The patient's partner was also referred for genetic testing and there was no pathogenic variant of the *CFTR* gene.

Antibiotic therapy was once again given but this time with mucus thinners (like in cystic fibrosis): acetylcysteine, ambroxol, and vitamins, obtaining normal sperm liquefaction and pregnancy in his partner after 2 months.

DISCUSSION

Carrying out genetic testing in patients with endocrinopathies and infertility brings specific advantages. The guidelines of scientific societies recommend [6, 7, 12, 13]:

- carrying out a karyotype test for all men with infertility who have a sperm count of under 10 million sperm per mL;
- additional diagnostics for azoospermia factor (AZF) and cystic fibrosis (*CFTR*) gene mutation in men with azoospermia.

When diagnosing and treating patients with infertility, we often refer them for additional genetic tests in the search for the causes of endocrine disorders with the hope that we will be able to implement effective treatment programs.

Based on the cases presented above, we are substantiating the rationale for carrying out additional genetic tests.

In order to minimise the costs and increase the chances of finding the genetic causes, the most purposeful seems to be ordering genetic testing depending on the type of identified endocrine disorder(s).

Lessons from case 1

A genetic test explained the cause of azoospermia. A pathogenic variant in *ANOS1* gene caused hypogonadotropic hypogonadism. It is due to this pathogenic variant that gonadotropins FSH and LH are not being secreted by the pituitary gland, which stimulate the testes to produce sperm and testosterone. FSH stimulates Sertoli cells to produce sperm in seminiferous tubules, and LH causes the Leydig cells of the testes to produce testosterone, also conditioning erection. Normal hormone test results give information on spermatogenic activity: the concentration of gonadotropin FSH and LH as well as inhibin B. The absence of FSH secretion by the pituitary gland causes the absence of stimulation of the receptors for FSH in the seminiferous tubules and the absence of spermatogenesis. The absence of LH secretion by the pituitary gland leads to the absence of stimulation of the receptors for LH and disrupts the production of testosterone by Leydig cells. The concentration of inhibin B is considered a marker of spermatogenesis activity in the seminiferous tubules of the testes.

If the testicular tissue is healthy (*e.g.*, not affected by a neoplastic process), hormonal stimulation of spermatogenesis is a highly effective treatment in hypogonadotropic hypogonadism. Even very low concentrations of FSH, LH, testosterone or inhibin B and a small testicular volume do not disqualify a patient when it comes to the possibilities of recreating spermatogenesis, even if the hypogonadotropic hypogonadism is genetically conditioned (*ANOS1* gene pathogenic variant).

The hormone therapy requires 6–9 months for the effects to be seen if the patient never received gonadotropin treatment earlier, although the first spermatozoa may appear as soon as after 3 months of treatment.

Lessons from case 2

Gene *PROT1* (prophet of Pit1) is specific to the pituitary gland. It is expressed in all progenitor cells of the glandular part of the pituitary gland and its mutations give no other clinical symptoms apart from the effects of anterior pituitary lobe hormone deficiency.

Growth hormone is required for ovarian follicular growth and maturation [10]. It has been demonstrated in an animal model that the ovaries are approximately 40% smaller in species without the growth hormone but exhibit all types of follicles (preantral, and small and large antral). Growth hormone deficiency impairs fertility: the ovarian response to treatment with exogenous gonadotropin is 3 times poorer,

where a reduced frequency of ovulation is essentially caused by growth hormone deficiency and not by pituitary gonadotropin deficiency. The number of mature ovarian follicles is significantly reduced, although all categories of follicles are represented. The LH-, FSH- and IGF-I-binding capacity of the follicles is not reduced. Growth hormone receptors in women are located in the ovaries on granulosa cells and germ cells, as well as on mammary glands, the uterus, fallopian tubes, and the skin.

Our patient was treated with growth hormone from childhood until the age of 16 years, thanks to which secondary sex characteristics developed normally and there was normal development of the uterus and genital tract. The treatment was later completed because the patient achieved the target height and closure of the epiphyseal cartilages was obtained. However, for the ovarian follicles to normally grow and mature, growth hormone is also required besides sex hormones. At present, the patient is once again receiving growth hormone treatment within the growth hormone treatment programme in adults with a deficiency of this hormone. She is also taking dehydroepiandrosterone (an adrenal cortex hormone), which is also required for normal ovarian follicle growth.

An abnormal growth and absence of ovarian follicle maturation leads to reproductive failure even during IVF procedures.

Carrying out NGS analysis and identifying a *PROP1* mutation in this patient explained the aetiology of the detected disorders. Determination of IGF-1 levels in adult female patients with a short stature and infertility is also advisable. In patients with combined pituitary hormone deficiency, mutations in the *PROP1* gene should also be designated.

Lessons from case 3

With reference to literature reports [3], a pathogenic variant in the *FGFR1* gene causes congenital hypogonadotropic hypogonadism with secondary testicular deficiency. Congenital hypogonadotropic hypogonadism, in light of the *FGFR1* gene pathogenic variant, causes central nervous system developmental disorders in the form of GnRH neuronal migration disruptions, which results in disrupted GnRH production or action. This pathogenic variant has a prevalence in the range of 1/5000 to 1/8000 for men. It is manifest by delayed sexual maturation and infertility. If the man is planning to father children, pulsed administration of GnRH or gonadotropin treatment should be introduced to stimulate sperm production in the testicle.

Patients with a pathogenic variant in *FGFR1* require a longer duration of gonadotropin treatment to stimulate spermatogenesis. In a study by Li et al. [9], a sperm count of more than 20 million after one year of treatment was not obtained in any patient, but after 2 years of gonadotropin

treatment, the sperm count exceeded 20 million in 25.9% of the patients. A longer treatment duration was necessary if the initial testicular volume was below 5 mL and if there was a history of cryptorchidism. The likelihood of the achievement of successful spermatogenesis in patients with a *FGFR1* pathogenic variant is good, but the stimulation may last even up to 2 years. The success rate of achieving effective spermatogenesis after one year of patient stimulation was 35.7%, and 75.9 % after 2 years of treatment. The median duration of achieving the first spermatozoa was 16 months.

Carrying out NGS in this patient with infertility provided information on the fact that the azoospermia was caused by p.Glu84GlyfsTer26 *FGFR1* pathogenic variant. Knowledge on the fact that this pathogenic variant requires prolonged hormonal stimulation of spermatogenesis allowed us to make the right treatment decisions. The decision was made to continue the costly treatment, which turned out to be successful.

It should be noted that the recommendations of the European Academy highlight that in some patients with pre-puberty hypogonadism a longer duration of gonadotropin or GnRH treatment may be required (1–2 years).

Lessons from case 4

Pathogenic variant of the *CFTR* gene may cause congenital bilateral absence of the vas deferens (CBAVD) by changing the coding of the CFTR protein, which acts as an ion transporter, regulating the viscosity of the mucus. The numerous *CFTR* pathogenic variants that have been identified have varied effects on the quality and quantity of the created protein, which leads to a varied spectrum of symptoms ranging from cystic fibrosis (bronchitis and pancreatitis) to oligozoospermia, asthenozoospermia, epididymal obstruction, CBAVD, idiopathic ejaculatory duct obstruction, and infertility [9].

Other properties of seminal fluid of patients with cystic fibrosis have been described in literature, such as: higher viscosity, longer liquefaction time, and a growing incidence of leukocytospermia and pyospermia. Treatment in such cases consists of introducing medication that aids semen liquefaction (acetylcysteine, bromhexine, and guaifenesin) and, if necessary, antibiotics. The use of these medications in the treatment of our patients improved sperm motility and led to a successful pregnancy outcome in his partner. The child was born healthy, with normal screening test results for cystic fibrosis.

The current andrology guidelines recommend testing for *CFTR* gene pathogenic variants only in the case of azoospermia [11]. It seems, however, that testing for *CFTR* gene pathogenic variants should not only be considered in the case of azoospermia but also in other clinically relevant cases. Perhaps *CFTR* modulator treatment will be available to such

patients in the future. At present, dornase alfa can be used which, according to the licensed indications of this drug, can also be used to treat primary ciliary dyskinesia.

The *CFTR*-related disease suspected due to the oligoasthenozoospermia and higher sperm viscosity was not confirmed in the patient. A p.Ser1235Arg variant in one allele of the *CFTR* gene was identified in a molecular diagnostic test, which is indicative of being a carrier .

The clinical status of the p.Ser1235Arg variant is controversial. It is described in the Human Gene Mutation Database (HGMD) and ClinVar databases as a variant of uncertain clinical significance. According to CFTR2 database, most individuals with this variant (combined with another *CF*-causing variant) will be healthy, however, a small number of individuals may develop mild symptoms or be diagnosed with a *CFTR*-related disorder (*CFTR*-RD), but symptoms are not expected to be severe enough to meet the definition of *CF*. Recent literature data indicate that p.Ser1235Arg is not a *CF* or CBAVD causing variant and should be considered as benign variant , however, a partial penetrance of this variant cannot be ruled out [14].

Interestingly in our patient, the sperm quality improved and, subsequently, a pregnancy was achieved in his partner after implementing treatment used in cystic fibrosis that liquefies mucus, suggesting that this may indeed be by a *CFTR*-related disease and that there are indications to continue looking for another variant of the *CFTR* gene. Unfortunately, due to lack of DNA of the patient we could not perform extended analysis of *CFTR* gene. Thus, limitation of our study is that we did not exclude the presence of other *CFTR* pathogenic variants in cis/trans with p.Ser1235Arg in non-screed regions of *CFTR*. Such extended analysis should be always performed in cases with p.Ser1235Arg variant found in *CFTR* gene.

The other general remark is that in case of detecting a pathogenic variant of *CFTR* gene in the patient, it is recommended to carry out carrier screening for this gene for his/her partner. This will allow genetic counselling to be offered to patients who have a chance of having offspring with cystic fibrosis or *CFTR*-related disorders.

Summary

Genetic testing can be highly useful in diagnosing and treating infertility and the spectrum of tests currently available is becoming wider and wider. Based on the presented cases, we would like to emphasise how important it is to expand the basic genetic testing options offered for couples with infertility available to date (karyotype and *CFTR* gene analysis) to other genes associated with infertility by targeted sequencing panels or whole exome sequencing (WES) for unsolved cases. However, it is important to note that the greatest chances for a proper diagnosis based on

genetic tests are when there are reasonable grounds to suspect a disease based on detailed clinical data. This is because the classification of genetic variants into categories such as benign, pathogenic, likely pathogenic or variants of uncertain clinical significance) should be always made with correlation to clinical symptoms. If the clinical data are lacking, the interpretation of a genetic result is very difficult or in some cases even not possible. Hence, the cooperation of clinicians (endocrinologists and andrologists) with geneticists and clinical geneticists is so crucial. It would then be possible to tailor a genetic testing package depending on the identified disorders and hormone test results.

CONCLUSIONS

Even an extremely small volume of the testes does not rule out the chances of stimulating spermatogenesis if azoospermia is caused by hypogonadotropic hypogonadism with a pathogenic variant of the ANOS1 gene (patient 1).

Sometimes hormonal stimulation of spermatogenesis with gonadotrophins must be continued for longer — even up to 12–24 months (as in the case of the FGFR1 gene mutation in patient 3).

The detection of the PROP1 gene mutation was an indication for treatment with growth hormone, the action of which is necessary for the growth of ovarian follicles (patient 2).

Sometimes, clarification of the causes of recurrent urogenital infections is crucial for effective treatment.

Effective treatment our patients would not be possible without genetic diagnosis. We need understand the need for genetic consultations for couples with infertility. Mutual education of physicians of various specialties dealing with infertile couples is also necessary.

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Conflict of interest

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