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Review

Syndromic infertility

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Summary. Infertility due to genetic mutations that cause other defects, besides infertility, is defined as syndromic. Here we describe three of these disorders for which we perform genetic tests. 1) Hypopituitarism is an endocrine syndrome characterized by reduced or absent secretion of one or more anterior pituitary hormones with consequent dysfunction of the corresponding peripheral glands. Deficiencies in all the hormones is defined as pan-hypopituitarism, lack of two or more hormones is called partial hypopituitarism, whereas absence of a single hormone is defined as selective hypopituitarism. Pan-hypopituitarism is the rarest condition, whereas the other two are more frequent. Several forms exist: congenital, acquired, organic and functional. 2) The correct functioning of the hypothalamic-pituitary-gonadal axis is fundamental for sexual differentiation and development during fetal life and puberty and for normal gonad function. Alteration of the hypothalamic-pituitary system can determine a condition called hypogonadotropic hypogonadism, characterized by normal/low serum levels of the hormones FSH and LH. 3) Primary ciliary dyskinesia is frequently associated with infertility in males because it impairs sperm motility (asthenozoospermia). Primary ciliary dyskinesia is a group of genetically and phenotypically heterogeneous disorders that show morpho-structural alterations of the cilia. Adult women with primary ciliary dyskinesia can be subfertile and have an increased probability of extra-uterine pregnancies. This is due to delayed transport of the oocyte through the uterine tubes. (www.actabiomedica.it)

Key words: hypopituitarism, primary ciliary dyskinesia, hypogonadotropic hypogonadism

Genetics of hypopituitarism

Hypopituitarism is an endocrine syndrome characterized by reduced or absent secretion of one or more anterior pituitary hormones with consequent dysfunction of the corresponding peripheral glands. Deficiencies in all the hormones is defined as pan-hypopituitarism, the lack of two or more hormones is called partial hypopituitarism, whereas the absence of a single hormone is defined as selective hypopituitarism. Pan-hypopituitarism is the rarest of the three. Several forms exist: congenital, acquired, organic and functional (1).

Combined pituitary hormone deficiency (CPHD) is characterized by impaired production of several pituitary hormones, such as growth hormone, thyroid-stimulating hormone, prolactin, adrenocorticotropic hormone and gonadotropic hormone, and is caused by mutations in transcription factors involved in pituitary ontogenesis. Congenital hypopituitarism has a low incidence with respect to secondary hypopituitarism due to pituitary adenomas, trans-sphenoidal surgery, or radiotherapy. The incidence of congenital hypopituitarism in the population is 1:3000-4000 (2). Genetic mutations associated with congenital hypopituitarism

mainly affect eight genes encoding transcription factors: *PROP1* (thyroid-stimulating, follicle-stimulating, growth, luteinizing and adrenocorticotropic hormones and prolactin are low or absent), *POU1F1* (growth and thyroid-stimulating hormones and prolactin are low or absent), *HESX1* (thyrotropin, follicle-stimulating, growth, luteinizing and adrenocorticotropic hormones are low or absent), *LHX3* (thyroid-stimulating, follicle-stimulating, growth, luteinizing and adrenocorticotropic hormones and prolactin are low or absent), and *LHX4* (thyroid-stimulating, growth, luteinizing, follicle-stimulating and adrenocorticotropic hormones are low or absent) (2).

The clinical phenotype depends on the affected hormone, the severity of pituitary impairment and age of onset. In childhood, congenital idiopathic forms are the most frequent, and are associated with developmental retardation, delay of puberty and absence of adrenarche. In adulthood, acquired forms are more frequent (3).

Loss-of-function mutations in *PROP1* are the most common cause of sporadic and familial cases of CPHD. This gene is mutated in 11% of cases. The mutation rate, however, varies considerably in relation to geographical area. The prevalence of the mutation is less than 1% in western European, American, Australian and Japanese populations, and higher in Russian and eastern European populations. Patients with mutations in *PROP1* show growth hormone (GH), prolactin (PL), and thyroid-stimulating hormone (TSH) deficiency and variable defects in the secretion of luteinizing (LH), follicle-stimulating (FSH) and adenocorticotropic (ACTH) hormones (4).

Mutations in *POU1F1* are the second most frequent cause of pituitary hormone deficiency. The phenotype associated with *POU1F1* mutations can be inherited by dominant or recessive transmission. The major mutation is the heterozygous p.Arg271Trp, found in ~30% of patients with *POU1F1* mutations. In sporadic cases, mutations in this gene are only found in 1.6% of cases. POU1F1 is a member of the POU family of transcription factors and is expressed in the anterior lobe of the pituitary gland. The phenotype associated with *POU1F1* mutations has severely low levels of GH and PRL, variable levels of TSH, short stature, facial dysmorphism, and dysphagia during infancy (5).

Another gene with occasional mutations is *HESX1*. Mutations in this gene occur in 0.45% of sporadic cases. Single heterozygous mutations causes a less severe disorder with incomplete penetrance, whereas homozygous mutations cause a severe and completely penetrant disorder (6).

Biallelic mutations in *LHX3* cause deficiencies in GH, PRL, TSH, LH, FSH and ACTH. Mutations in *LHX3* are found in 0.3% of sporadic cases and 11.1% of familial cases (7).

The pathological phenotype associated with heterozygous mutations in *LHX4* is inherited as an autosomal dominant trait with variable penetrance. Patients with CPHD have variable reductions in serum levels of GH, TSH, ACTH and gonadotropin. Cranial magnetic resonance imaging shows pituitary gland hypoplasia in most cases. However, there is a wide phenotypic variability within and between families.

Finally, mutations in the *GLI2* gene have been reported in patients with combined pituitary hormone deficiency and ectopic posterior pituitary lobe. For instance, several individuals with truncating mutations in *GLI2* show pituitary anomalies, polydactyly and subtly dysmorphic facial features. The inheritance pattern is dominant with incomplete penetrance and variable phenotype. There are mutations in *GLI2* in 1.5% of CPHD cases.

The genes associated with combined pituitary hormone deficiency are: *PROP1*, *SOX3*, *POU1F1*, *HESX1*, *LHX4*, *LHX3*, *OTX2* and *GLI2* (Table 1). Pathogenic variants may be missense, nonsense, splicing or small indels. MAGI uses a multi-gene NGS panel to detect nucleotide variations in coding exons and flanking introns of the genes listed in the table. Our NGS test has an analytical sensitivity (proportion of true positives) and analytical specificity (proportion of true negatives) of ≥99% (coverage depth ≥10x).

Primary ciliary dyskinesia

Primary ciliary dyskinesia (PCD) is a genetically and phenotypically heterogeneous group of inherited disorders due to morphological and structural alterations of the cilia. It is characterized by chronic bronchorrhea with bronchiectasis and chronic sinusitis and

Table 1. Genes associated with combined pituitary hormone deficiency

Gene	Inheritance	OMIM gene	OMIM phenotype	OMIM phenotype ID	Gene function
PROP1	AR	601538	CPHD2	262600	Paired-like homeodomain transcription factor required for pituitary development
POU1F1	AD, AR	173110	CPHD1	613038	Regulation of expression of genes involved in pituitary development and hormone expression
HESX1	AD, AR	601802	CPHD5	182230	Transcriptional repressor expressed in developing forebrain and pituitary gland
LHX3	AR	600577	CPHD3	221750	LIM-containing domain transcription factor required for pituitary development and motor neuron specification
LHX4	AD	602146	CPHD4	262700	LIM-containing domain transcription factor required for pituitary development
SOX3	XLR	313430	PHPX	312000	Transcription factor required for pituitary function and development of CNS midline structures
OTX2	AD	600037	CPHD6	613986	Homeodomain-containing transcription factor required for brain, craniofacial and sensory organ development
GLI2	AD	165230	CJS	615849	Zinc finger transcription factor required for embryogenesis

CJS = Culler-Jones syndrome; CNS = central nervous system; CPHD = combined pituitary hormone deficiency; PHPX = panhypopituitarism, X-linked; AR = autosomal recessive; AD = autosomal dominant; XLR = X-linked recessive.

is the second most common congenital disease of the respiratory system after cystic fibrosis. The prevalence is estimated at around 1:20000 (8).

Ultrastructural defects of the 9+2 axoneme of cilia and flagella may be: partial or complete loss of internal dynein arms, central microtubule anomalies, and radial spoke defects. These defects cause recurrent sinusitis, bronchiectasis due to immotile cilia in the upper and lower airways, and infertility due to altered cilia in the oviduct as well as altered sperm flagella (9).

Fifty percent of patients show *situs inversus*. The association of *situs inversus,* sinusitis and bronchiectasis is the classical triad known as Kartagener syndrome. It is noteworthy that this syndrome is a subgroup of primary ciliary dyskinesia (10). In fact, we know that *situs inversus* is caused by motility failure of nodal cilia that allow lateralization of organs during early embryogenesis (11).

In some subjects, primary ciliary dyskinesia is associated with other disorders like polycystic kidney, retinitis pigmentosa, Barder-Biedl syndrome and Usher syndrome, the pathogenesis of which is linked to structural defects of the primary cilia (12). Respiratory disorders can appear at birth (neonatal respiratory distress), during infancy and rarely in adulthood, and may include chronic infections of the upper and lower respiratory tract. Bronchiectasis is not present at birth but may be a secondary effect of a chronic lung disease (8).

Severity and progression of the disease are variable among patients and depend on what ciliary substructures are altered. About 50% of male patients with PCD are infertile due to lack of sperm motility (9,13). Adult women with PCD may be subfertile and at risk of extra-uterine pregnancies due to delayed oocyte transport through the uterine tubes (10). The most fre-

quent ultrastructural defects of PCD in spermatozoa are (14,15):

- reduction and/or absence of the outer dynein arm: ~38.5% of all PCD cases;
- reduction and/or absence of both dynein arms (outer and inner): ~10.5% of all PCD cases;
- microtubule (axoneme) disorganization due to absence of the inner dynein arm and defects in the central apparatus: ~14% of all PCD cases;
- absence or interruption of central apparatus (i.e. the pair of central microtubules and/or radial spokes): ~7% of all PCD cases;
- reduction and/or absence of the inner dynein arm (rare);
- oligocilia with or without normal ultrastructure (rare).

Most cases of primary ciliary dyskinesia or Kartagener syndrome have autosomal recessive inheritance, although some cases with X-linked recessive inheritance have been reported. Currently, 39 genes are known to be involved in PCD (Table 2). The most frequent mutations are in: *DNAH5*, *DNAH11*, *CCDC39*, *DNAI1*, *CCDC40*, *CCDC103*, *SPAG1*, *ZMYND10*, *ARMC4*, *CCDC151*, *DNAI2*, *RSPH1*, *CCDC114*, *RSPH4A*, *DNAAF1*, *DNAAF2* and *LRRC6*. Table 2 shows the frequencies of biallelic pathogenic variants in affected unrelated subjects. Pathogenic variants may be missense, nonsense, splicing and small indels. MAGI uses a multi-gene NGS panel to detect nucleotide variations in coding exons and flanking introns of the genes listed in Table 2.

Hypogonadotropic hypogonadism

Correct functioning of the hypothalamo-pituitary-gonadal axis is fundamental for differentiation and sexual development during the fetal period and puberty (16). Hypogonadotropic hypogonadism (HH) is caused by alterations in this axis. Such alterations cause low serum levels of sex hormones associated with normal or low levels of FSH and LH. The prevalence of HH is 1/8000 newborns (17).

Clinically, patients with HH show little or no sexual development, primary amenorrhea (women) and oligoazoospermia (men). Other possible features may be: cleft palate, tooth agenesis, visual impairment, intellectual disability (and other neurological abnormalities), and renal agenesis (18).

Hypogonadotropic hypogonadism may be considered isolated when only the gonads are impaired. There are two forms of the isolated HH: Kallmann syndrome (HH associated with anosmia) is caused by defects in embryonic migration of neurons secreting gonadotropin releasing hormone (GnRH); normosmic HH, in which HH is the only symptom and is due to altered signaling, regulation and secretion of GnRH (19).

The HH may have autosomal dominant, autosomal recessive or X-linked inheritance.

The first gene variation discovered in cases of HH was in *ANOS1* (or *KAL1*). *ANOS1* encodes an adhesion molecule (anosmin), probably involved in migration of olfactory and GnRH-secreting neurons toward the hypothalamus during embryo development. Hypogonadotropic hypogonadism associated with *ANOS1* mutations has X-linked recessive inheritance, so only males are affected. Besides HH and anosmia, patients with mutations in *ANOS1* show renal agenesis and neurological disorders such as intellectual disability, sensorineural deafness and synkinesis (20).

GNRHR was the first gene found to have variations in cases of normosmic HH, a disorder with autosomal recessive inheritance. The gene encodes the GnRH receptor, a protein expressed in the pituitary gland. The associated phenotype is highly variable, ranging from very severe (total absence of puberty) to partial or delayed pubertal development (21).

Since involvement of *ANOS1* and *GNRHR* in hypogonadotropic hypogonadism was discovered, 28 other associated-genes have emerged (Table 3). More than 2% of cases have mutations in *ANOS1*, *CHD7*, *FGFR1*, *GNRHR*, *IL17RD*, *PROKR2*, *SOX10* or *TACR3*. The other genes have only been found in a few families (18).

Pathogenic variants may be missense, nonsense, splicing or small indels. MAGI uses a multi-gene NGS panel to detect nucleotide variations in coding exons and flanking introns of the above genes (Table 3).

Table 2. Genes associated with primary ciliary dyskinesia

Gene	Inheritance	OMIM gene	OMIM phenotype	OMIM or HGMD phenotype ID	Frequency of biallelic variants in affected unrelated subjects (22)
DNAI1	AR	604366	CILD1	244400	2%-10%
DNAAF3	AR	614566	CILD2	606763	<1%
DNAH5	AR	603335	CILD3	608644	15%-29%
HYDIN	AR	610812	CILD5	608647	<1%
NME8	AR	607421	CILD6	610852	<1%
DNAH11	AR	603339	CILD7	611884	6%-9%
DNAI2	AR	605483	CILD9	612444	2%
DNAAF2	AR	612517	CILD10	612518	<1%-2%
RSPH4A	AR	612647	CILD11	612649	1%-2%
RSPH9	AR	612648	CILD12	612650	<1%
DNAAF1	AR	613190	CILD13	613193	1%-2%
CCDC39	AR	613798	CILD14	613807	4%-9%
CCDC40	AR	613799	CILD15	613808	3%-4%
DNAL1	AR	610062	CILD16	614017	<1%
CCDC103	AR	614677	CILD17	614679	<4%
DNAAF5	AR	614864	CILD18	614874	<1%
LRRC6	AR	614930	CILD19	614935	1%
CCDC114	AR	615038	CILD20	615067	<2%
DRC1	AR	615288	CILD21	615294	<1%
ZMYND10	AR	607070	CILD22	615444	2%-4%
ARMC4	AR	615408	CILD23	615451	<3%
RSPH1	AR	609314	CILD24	615481	2%
C21ORF59	AR	615494	CILD26	615500	<1%
CCDC65	AR	611088	CILD27	615504	<1%
SPAG1	AR	603395	CILD28	615505	<4%
CCNO	AR	607752	CILD29	615872	<1%
CCDC151	AR	615956	CILD30	616037	<3%
CENPF	AR	600236	STROMS	243605	<1%
RSPH3	AR	615876	CILD32	616481	<1%
GAS8	AR	605178	CILD33	616726	/
DNAJB13	AR	610263	CILD34	617091	/
TTC25	AR	617095	CILD35	617092	/
PIH1D3	XLR	300933	CILD36	300991	9.5%
DNAH1	AR	603332	CILD37	617577	<1%
STK36	AR	607652	CILD	1147369503	/

CILD = ciliary dyskinesia, primary; STROMS = Stromme syndrome; AR = autosomal recessive; XLR = X-linked recessive; HGMD = Human Gene Mutation Database (https://portal.biobase-international.com/hgmd/pro/)

Table 3. Genes associated with hypogonadotropic hypogonadism

Gene	Inheritance	OMIM gene	OMIM phenotype	OMIM or HGMD phenotype ID	Gene function
KISS1	AR	603286	HH13	614842	Stimulation of GnRH-induced gonadotropin secretion, activation of GnRH neurons
HS6ST1	AD	604846	HH15	614880	Neuron development, neuron branching
IL17RD	AD, AR	606807	HH18	615267	Fate-specification of GnRH-secreting neurons
PROK2	AD	607002	HH4	610628	Chemoattractant for neuronal precursor cells in olfactory bulb
GNRHR	AR	138850	HH7	146110	Receptor for GnRH. Stimulation of LH and FSH secretion
TACR3	AR	162332	HH11	614840	Receptor for neurokinin B. Expressed in hippocampus, hypothalamus, substantia nigra
SPRY4	AD	607984	HH17	615266	Regulation of neurite outgrowth in hippocampal neurons
SEMA3A	AD	603961	HH16	614897	Inhibition of axonal outgrowth, stimulation of apical dendrite growth
FEZF1	AR	613301	HH22	616030	Embryonic migration of GnRH-releasing neurons into brain
FGF17	AD	603725	HH20	615270	Induction and patterning of embryonic brain
GNRH1	AR	152760	HH12	614841	Stimulation of LH and FSH secretion
FGFR1	AD	136350	HH2	147950	Mesoderm patterning, correct axial organization during embryo development, skeletogenesis, development of GnRH neuronal system
CHD7	AD	608892	HH5	612370	Formation of neural crest
NSMF	AD	608137	HH9	614838	Guidance of olfactory axon projections, migration of LHRH neurons
FGF8	AD	600483	HH6	612702	Regulation of embryo development, cell proliferation, differentiation, migration. Brain, eye, ear, limb, GnRH neuronal system, hippocampal neuron development
WDR11	AD	606417	HH14	614858	Regulation of GnRH production
FSHB	AR	136530	HH24	229070	Beta subunit of FSH. Induction of egg and sperm production
TAC3	AR	162330	HH10	614839	Central regulator of gonad function
DUSP6	AD	602748	HH19	615269	Expression regulated by GnRH
KISS1R	AR	604161	HH8	614837	Neuroendocrine control of gonadotropin axis
LHB	AR	152780	HH23	228300	Promotion of spermatogenesis and ovulation by stimulating gonads to synthesize steroids

(continued on the next page)

Table 3 ***(continued)*****.** Genes associated with hypogonadotropic hypogonadism

Gene	Inheritance	OMIM gene	OMIM phenotype	OMIM or HGMD phenotype ID	Gene function
PROKR2	AD	607123	HH3	244200	Induction of tangential and radial migration of olfactory bulb interneurons
FLRT3	AD	604808	HH21	615271	Spatial organization of brain neurons.
ANOS1	XLR	300836	HH1 (KS)	308700	Neural cell adhesion, axonal migration, patterning of mitral and tufted cell collaterals to olfactory cortex
SOX10	AD	602229	WS2E	611584	Development of neural crest, peripheral nervous system, glia
AXL	AD	109135	HH	1734393901	GnRH neuron survival and migration
CCDC141	AR	616031	KS	817012261	Neural radial migration
SEMA3E	AD	608166	KS	817012261	Ensuring synapse formation specificity
SRA1	AR	603819	HH	1734393901	Mediation of transcriptional co-activation of steroid receptors

GnRH = gonadotropin-releasing hormone; HH = hypogonadotropic hypogonadism; KS = Kallmann syndrome; WS = Waardenburg syndrome; AD = autosomal dominant; AR = autosomal recessive; XLR = X-linked recessive; HGMD = Human Gene Mutation Database (https://portal.biobase-international.com/hgmd/pro/)

Conclusions

We created a NGS panel to detect nucleotide variations in coding exons and flanking regions of all the genes associated with infertility. When a suspect of syndromic infertility is present we perform the analysis of all the genes present in this short article. In order to have a high diagnostic yield, we developed a NGS test that reaches an analytical sensitivity (proportion of true positives) and an analytical specificity (proportion of true negatives) of ≥99% (coverage depth ≥10x).

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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