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Review

Non-syndromic monogenic female infertility

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Summary. Infertility is a significant clinical problem. It affects 8-12% of couples worldwide, about 30% of whom are diagnosed with idiopathic infertility (infertility lacking any obvious cause). In 2010, the World Health Organization calculated that 1.9% of child-seeking women aged 20-44 years were unable to have a first live birth (primary infertility), and 10.5% of child-seeking women with a prior live birth were unable to have an additional live birth (secondary infertility). About 50% of all infertility cases are due to female reproductive defects. Several chromosome aberrations, diagnosed by karyotype analysis, have long been known to be associated with female infertility and monogenic mutations have also recently been found. Female infertility primarily involves oogenesis. The following phenotypes are associated with monogenic female infertility: premature ovarian failure, ovarian dysgenesis, oocyte maturation defects, early embryo arrest, polycystic ovary syndrome and recurrent pregnancy loss. Here we summarize the genetic causes of non-syndromic monogenic female infertility and the genes analyzed by our genetic test. (www.actabiomedica.it)

Key words: female infertility, premature ovarian failure, ovarian dysgenesis, oocyte maturation defects, preimplantation embryonic lethality, recurrent pregnancy loss, ovarian hyperstimulation syndrome

Premature ovarian failure and ovarian dysgenesis

Premature ovarian failure (POF) is a frequent and heterogeneous disorder (1-2% of women under age 40 years, 1:10000 women under age 20 years and 1:1000 under 30 years) due to anomalies in follicular development. It is characterized by early functional blockade of the ovary (with respect to menopause which normally occurs after age 45 years) and menstrual cycles can be completely absent (primary amenorrhea) or end before 40 years of age (secondary amenorrhea). The most severe forms are caused by ovarian dysgenesis (50% of cases of primary amenorrhea), whereas postpubertal forms are characterized by disappearance of the menstrual cycle (secondary amenorrhea) (1).

Biochemically, premature ovarian failure is characterized by reduced levels of gonad hormones (estrogens) and increased levels of gonadotropins (LH and FSH) (2). Ovarian dysgenesis is characterized by absence of gonad development, gonadotropin resistance and normal development of the external and internal genitalia (3). Chromosome anomalies (deletions, translocations) and pre-mutation status of the *FMR1* gene are frequent causes of POF (estimated prevalence 10-13%) (4). Several studies have identified genes important for ovarian development and onset of POF (Table 1). Mutations in the *BMP15* gene have been identified in 1.5-15% of Caucasian, Indian and Chinese women with POF (5). One third of patients with POF have mutations in *PGRMC1* while changes

Table 1. Genes associated with primary ovarian failure and ovarian dysgenesis

Gene	Inheritance	OMIM gene ID	OMIM phenotype	OMIM or HGMD phenotype ID	Clinical Features
HFM1	AR	615684	POF9	615724	Amenorrhea
FIGLA	AD	608697	POF6	612310	Small/absent ovaries, follicles absent, atrophic endometrium
FOXL2	AD	605597	POF3	608996	Hypoplastic uterus and ovaries, follicles absent, secondary amenorrhea
MSH5	AR	603382	POF13	617442	Oligomenorrhea, atrophic ovaries, follicles absent
STAG3	AR	608489	POF8	615723	Primary amenorrhea, ovarian dysgenesis
NOBOX	AD	610934	POF5	611548	Secondary amenorrhea, follicles absent
NR5A1	AD	184757	POF7	612964	Irregular or anovulatory menstrual cycles, secondary amenorrhea, dysgenetic gonads, no germ cells
ERCC6	AD	609413	POF11	616946	Secondary amenorrhea
SYCE1	AR	611486	POF12	616947	Primary amenorrhea, small prepubertal uterus and ovaries, no ovarian follicles
MCM8	AR	608187	POF10	612885	Absent thelarche, primary amenorrhea, no ovaries, hypergonadotropic ovarian failure
BMP15	XLD	300247	POF4, OD2	300510	Delayed puberty, primary/secondary amenorrhea, small ovaries, follicles absent, hypoplastic uterus, hirsutism, absent pubic/axillary hair
FLJ22792	XLR	300603	POF2B	300604	Weak teeth, delayed puberty, primary amenorrhea, osteoporosis
DIAPH2	XLD	300108	POF2A	300511	Secondary amenorrhea
FSHR	AR	136435	OD1	233300	Osteoporosis, primary amenorrhea
MCM9	AR	610098	OD4	616185	Short stature, low weight, underdeveloped breasts, no ovaries, retarded bone age and development of pubic/axillary hair, primary amenorrhea
SOHLH1	AR	610224	OD5	617690	Short stature, absent thelarche, primary amenorrhea, hypoplastic/no ovaries, small uterus, retarded bone age
PSMC3IP	AR	608665	OD3	614324	Underdeveloped breasts and absent pubic hair, hypoplastic uterus, primary amenorrhea
AMH	AD	600957	POF	782468699	Primary/secondary amenorrhea
AMHR2	AD	600956	POF	1454100025	Primary ovarian insufficiency
DAZL	AR	601486	POF	782468699	Low ovarian reserves
GDF9	AR	601918	POF14	618014	Primary amenorrhea, no breast development, delayed pubic hair development
LHCGR	AR	152790	POF	1754122511	Primary amenorrhea

(continued on next page)

Table 1 ***(continued).*** Genes associated with primary ovarian failure and ovarian dysgenesis

Gene	Inheritance	OMIM gene ID	OMIM phenotyp	OMIM or HGMD phenotype ID	Clinical Features
INHA	AD, AR	147380	POF	782468699	Primary amenorrhea
PGRMC1	AD	300435	POF	782468699	Hypergonadotropic hypogonadism, amenorrhea
POU5F1	AD	164177	POF	782468699	Small ovaries without follicles
TGFBR3	AD	600742	POF	782468699	Premature ovarian failure
WT1	AD	607102	POF	782468699	Secondary amenorrhea
SGO2	AR	612425	POF	141105721	Ovarian insufficiency
SPIDR	AR	615384	POF	141105721	Hypoplastic/no ovaries
EIF4ENIF1	AD	607445	POF	141105721	Secondary amenorrhea
NUP107	AR	607617	OD6	618078	No ovaries, small uterus, no spontaneous puberty
NANOS3	AD	608229	POF	729748889	Primary amenorrhea

OD=ovarian dysgenesis; POF = primary ovarian failure; HGMD = Human Gene Mutation Database (https://portal.biobase-international.com/hgmd/pro/)

in levels of the encoded protein are known to cause POF through impaired activation of microsomal cytochrome P450 and excessive apoptosis of ovarian cells (6). In 1-2% of cases, mutations in *GDF9*, *FIGLA*, *NR5A1* and *NANOS3* have been identified (6). Whole exome sequencing (WES) in large families has detected mutations in genes important for homologous recombination and meiosis (*STAG3*, *SYCE1*, *HFM1*), DNA repair (*MCM8*, *MCM9*, *ERCC6*, *NUP107*), mRNA transcription (*SOHLH1*) and mRNA translation (*eIF4ENIF1*) (7).

MAGI uses a multi-gene next generation sequencing (NGS) panel to detect nucleotide variations in coding exons and flanking introns of the above genes.

Oocyte maturation defects and pre-implantation embryonic lethality

Oocyte maturation is defined as re-initiation and completion of the first meiotic division, subsequent progression to the second phase of meiosis, and other molecular events essential for fertilization and early embryo development (8). The meiotic cell cycle begins in the neonatal ovary and stops at prophase I of meiosis until puberty, when an increase in luteinizing hormone concentrations re-initiates meiosis and ovulation. Thus the oocyte progresses from metaphase I to metaphase II. Metaphase I is completed by extrusion of a polar body. Mature oocytes are again arrested at metaphase II, the only stage at which they can be successfully fertilized (9).

Microscope observation of mature oocytes shows a single polar body, a homogeneous cytoplasm, a *zona pellucida* (ZP) and a perivitelline space. The *zona pellucida* is an extracellular matrix surrounding the oocytes of mammals and is fundamental for oogenesis, fertilization and pre-implantation embryo development. It consists of four glycoproteins (ZP1-ZP4) and ensures species-specific fertilization and induction of the sperm acrosomal reaction during fertilization. It also contains sperm receptors, contributes to blocking polyspermy and protects early embryos until implantation. Glycoprotein ZP1 connects ZP2 with ZP3. ZP2 is a structural component of the *zona pellucida* and has a role in sperm binding and penetration after the acrosomal reaction. ZP3 is a receptor that binds sperm at the beginning of fertilization and induces the acrosomal reaction (10). Oocyte maturation can be arrested in various phases of the cell cycle. Until recently, the genetic events underlying oocyte maturation ar-

rest were unknown (9). Only in the last few years have pathogenic genetic variations that cause oocyte maturation defects been found. In particular, heterozygous mutations in the tubulin beta 8 gene (*TUBB8*) cause defects in the assembly of the meiotic spindle and in oocyte maturation (11). Pathogenic variations in *TUBB8* can be found in ~30% of cases with oocyte maturation arrest; mutations in *PATL2* and genes that encode ZP proteins are less frequent (12).

Early arrest of embryo development is one of the main causes of female infertility, although diagnosis can be difficult and the genetic causes are largely unknown. Gene-disease is difficult to identify, but studies on animal models suggest that there may be hundreds. A recent study identified a homozygous mutation in *TLE6* in a case of pre-implantation embryonic lethality with reduced female fertility and embryo development arrest at the meiosis II phase of the oocyte (13). Another study found that mutations in *PADI6* cause early embryonic arrest due to lack of activation of the zygotic genome (14). PADI6 may be involved in formation of the subcortical maternal complex, essential for the embryo to go through the two-cell stage in mice as well as humans.

The current list of genes associated with oocyte maturation defects and pre-implantation embryonic lethality includes *ZP1*, *TUBB8*, *ZP3*, *PATL2*, *ZP2*, *TLE6* and *PADI6* (Table 2).

MAGI uses a multi-gene NGS panel to detect nucleotide variations in coding exons and flanking introns of the above genes.

Sporadic and recurrent pregnancy loss

Recurrent pregnancy loss is defined as two or more consecutive miscarriages before the 20th week of gestation (15) and affects 1-5% of women of fertile age. Several other conditions have been associated with recurrent pregnancy loss: chromosome anomalies in parents or embryo, prothrombotic states, structural anomalies of the uterus, endocrine dysfunction, infections and immunological factors. Although there has been progress in the clinical and biochemical diagnosis of the human infertility, it is estimated that 35-60% of cases are still considered idiopathic, suggesting that genetic, epigenetic and environmental factors contribute to the recurrent pregnancy loss phenotype (16).

Fetal aneuploidies are the most frequent cause of sporadic miscarriage and can be detected in 50-70% of miscarriages in the first trimester and 5-10% of all pregnancies. The most frequent chromosome aberrations are trisomy, triploidy and X monosomy. Chromosome anomalies can also be found in the parental karyotype in 4-6% of couples with at least two miscarriages, and are more frequent in women. The most

Table 2. Genes associated with oocyte maturation defect and preimplantation embryonic lethality

Gene	Inheritance	OMIM gene ID	OMIM phenotype	OMIM phenotype ID	Clinical Features
ZP3	AD	182889	OOMD3	617712	Oocyte degeneration, absence of zona pellucida
TUBB8	AD, AR	616768	OOMD2	616780	Oocyte arrest at metaphase I or II; abnormal spindle
ZP1	AR	195000	OOMD1	615774	Absence of zona pellucida
PATL2	AR	614661	OOMD4	617743	Oocyte maturation arrest in germinal vesicle stage, metaphase I or polar body 1 stage; abnormal polar body 1; early embryonic arrest
ZP2	AR	182888	OOMD6	618353	Abnormal of zona pellucida
TLE6	AR	612399	PREMBL1	616814	Failure of zygote formation
PADI6	AR	610363	PREMBL2	617234	Recurrent early embryonic arrest

OOMD=oocyte maturation defect; PREMBL=preimplantation embryonic lethality.

common anomaly found in couples is unbalanced translocation. Carriers are phenotypically healthy, but about 50-60% of their gametes are unbalanced due to anomalous meiotic segregation (17).

Single genes or few genes as the main cause of recurrent pregnancy loss have been less considered. However, in couples with recurrent pregnancy loss, identification of mutations in the *SYCP3* gene, which encodes a fundamental component of the synaptonemal complex involved in meiotic segregation, has demonstrated a correlation between meiosis, aneuploidy and recurrent miscarriages. This suggests that correct segregation of chromosomes is influenced by events that take place in the fertilization phase, during meiosis I (18,19).

Recurrent miscarriage can also be linked to thrombophilia. In fact, mutations in the Leiden factor V gene (*F5*), coagulation factor II gene (*F2*) and annexin A5 gene (*ANXA5* encoding an anticoagulant protein active in placental villi), have been associated with increased risk of recurrent pregnancy loss. Finally, mutations in *NLRP7* and *KHDC3L* have been associated with hydatidiform mole, a disease of the trophoblast. Hydatidiform mole is due to a fertilization defect and is characterized by trophoblast proliferation that prevents normal embryo development. Mutations in the two genes have been reported in 1% of cases of hydatidiform mole.

The current list of genes known to be associated with recurrent pregnancy loss is reported in Table 3.

MAGI uses a multi-gene NGS panel to detect nucleotide variations in coding exons and flanking introns of the above genes.

Ovarian hyperstimulation syndrome

Ovarian hyperstimulation syndrome (OHSS, OMIM phenotype: 608115) is a potentially life-threatening condition. It is a systemic disorder caused by excessive secretion of vasoactive hormones by hyperstimulated ovaries. The physiopathology is characterized by an increase in capillary permeability with leakage into the vasal compartment and intravascular dehydration. Severe complications include thrombophilia, renal and hepatic dysfunction and acute respiratory distress (20).

The syndrome is defined as having early onset when it manifests in luteal phase in response to human chorionic gonadotropin (hCG). It is defined as having late onset when it manifests at the beginning of pregnancy and endogenous hCG further stimulates the ovary. It is often induced by ovarian stimulation used for *in vitro* fertilization, although 0.5-5% of cases are spontaneous. Clinical manifestations may range from benign abdominal distension to massive, potentially lethal ovarian enlargement (21). Pathological features of the syndrome, both spontaneous and iatrogenic, include multiple serous and hemorrhagic follicular cysts surrounded by luteal cells (*iperreactio luteinalis*). The syndrome can arise from high serous levels of hCG caused by multiple or molar pregnancies. It can also be associated with pituitary or neuroendocrine adenomas stimulating follicular hormone (FSH), with hypothyroidism, or with activating mutations of the FSH receptor (FSHR) (22).

Five activating mutations in the *FSHR* gene have been described in pregnant women with OHSS. These

Table 3. Genes associated with recurrent pregnancy loss.

Gene	Inheritance	OMIM gene ID	OMIM phenotype	OMIM phenotype ID	Clinical Features
SYCP3	AD	604759	RPRGL4	270960	Fetal loss after 6-10 weeks of gestation
F2	AD	176930	RPRGL2	614390	Recurrent miscarriage
ANXA5	AD	131230	RPRGL3	614391	
NLRP7	AR	609661	HYDM1	231090	Gestational trophoblastic disease
KHDC3L	AR	611687	HYDM2	614293	

RPRGL=recurrent pregnancy loss; PREMBL=preimplantation embryonic lethality.

mutations increase sensitivity to hCG and/or thyroid stimulating hormone (TSH). By contrast, loss-of-function mutations in *FSHR* can severely upset folliculogenesis, causing ovarian insufficiency. Recent studies reported cases with non-gestational OHSS with new mutations in *FSHR* (23-26).

To date, the only gene known to be associated with OHSS is *FSHR* (OMIM gene ID: 136435) and the phenotype has autosomal dominant inheritance.

MAGI uses a multi-gene NGS panel to detect nucleotide variations in coding exons and flanking introns of *FSHR*.

Conclusions

Infertility is a significant and increasing clinical problem. Several chromosome aberrations have long been known to be associated with female infertility. Only recently have monogenic mutations been found in association with male and female infertility. Genetic tests based on parallel sequencing of several genes are becoming increasingly important in diagnostic practice.

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 female 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).

Knowledge of the exact molecular cause helps clinicians choose the most appropriate treatments and follow-up.

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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