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Clinical Study: Role of Bmp-15 Gene in the Pathogenesis of Premature Ovarian Insufficiency (POI)

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Abstract

Premature Ovarian Insufficiency (POI) is a heterogeneous syndrome from an etiological point of view, as different causes work to determine this kind of hormonal disorder. Although many typologies have been recognized, currently for most cases the real causes are uncertain; therefore we come up against forms defined as Idiopathic. Nevertheless, other literature has described the different alterations of most candidate genes responsible for this disorder including the BMP-15 gene.

In this study, we will specifically examine the BMP-15 gene, which belongs to the Transforming Growth Factor-β Superfamily or TGF- β, to which the Growth Differentiation Factors (GDFs) also belong.

This study examines a sample of Sicilian women affected by POI, especially selected according to their young age, because in these specific cases, the genetic factor truly has a major impact.

Identifying a mutant gene that causes the ovarian failure can be important for making a diagnosis that foresees the possible future development of the disease.

The result of the studies does not show mutations on the gene in question, but this fact can be a direct consequence of the limited number of women on which the study in question has been done; This hypothesis can be disproved by increasing the number of patients.

Keywords: Reduced ovarian function; Premature menopause; Infertility; BMP-15 gene; PO; POF

Introduction and Purpose of the Study

Premature Ovarian Insufficiency (POI) is a heterogeneous syndrome characterized by Hypergonadotropic Hypogonadism which occurs in 1% on women under 40 years of age, while if we observe those cases a reported onset occurs at a rate of 1/1000 of women under 30.

This hormonal disorder is caused by the lack of the ovarian response towards to the pituitary's synthesis of gonadotropins (FSH and LH) which are secreted according to the feedback inhibitory mechanism operating between the hypothalamus, pituitary gland and gonads.

Premature Ovarian Insufficiency refers to the presence of amenorrhea following the cessation of the ovarian function in women younger than 40 years old (secondary amenorrhea) or to a primary ovary defect with total absence of menarche (primary amenorrhea). The last is the most serious form and in 50% of cases it is refers to an ovarian dysgenesis [1,2]. The diagnosis is based on elevated levels of FSH (greater than 40 UI/L) dosed twice consecutively with an interval of a few weeks from each other (eliminate "from each other"). The women affected by POI exhibit anovulation and hypoestrogenism, primary (or secondary) amenorrhea or secondary amenorrhea, infertility, deficiency in steroidal hormones deficiency and elevated levels of gonadotropins.

The heterogeneity at the base of this syndrome reflects the multiplicity of possible causes that contribute to can it.

POI has a strong genetic component associated with an alteration of the genes that are mapped on the X Chromosome, developing a primary role in the causes of ovarian dysgenesis [3,4].

Although the description of most candidate genes are described, the causes of POI are still undetermined in most cases of Idiopathic forms [5,6].

Different causes of POI are:

- Iatrogenic (surgical interventions, chemotherapy, radiotherapy);
- Autoimmune, including the autoimmune polyglandular syndrome (APECED) caused by mutation in the AIRE gene;
- Infections (CMV, Herpes Zoster);
- Alteration X chromosome (Turner syndrome, fragile X);
- Monogenetic defects: syndrome by glycosylation defect of glycoprotein CDG-1, galactosemia, Blepharophimosis Epicanthus Inversus Syndrome BPES, pseudohypoparathyroidism type 1; isolated defects (receptor mutation for FSH and LH, FOXL2 mutation, BMP-15 mutation);
- Idiopathic [7].

Materials and Methods

The gene examined in our study is BMP-15. The Bone Morphogenetic Proteins (BMPs) belong to the extracellular signaling proteins reentering in the Transforming Growth Factors-beta (TGF-β)

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superfamily, which also include (s) growth and differentiation factors (GDFs).

These proteins are synthesized as pre-propeptides, cleaved and then processed for to create dimeric proteins.

BMP-15 is an (a) GDF oocyte-specific which stimulates the folliculogenesis and the granulose cells growth and it is delivered in oocytes during early folliculogenesis.

The BMP-15 gene maps on the short arm of the X chromosome in Xp11.2 position, inside one of the critical areas of POI.

The gene codifies fBMP-15 protein and is formed by two exons; the first exon codifies 17 amino acids of peptide signaling and the first part of the pre-peptide, while the second codifies the remainder pre-peptide signaling area and for the whole mature dominion formed by 125 amino acids [8].

Indeed, just the other proteins of the TGF-β superfamily, BMP-15 is synthesized primarily as an inactive precursor. After the removal of signal peptide, proprotein is generated that binds covalently to another identical protein, forming the homodimers, or GDF-9 protein, determining instead the formation of heterodimers.

These dimers, in turn are clevated by a specific endoprotease at the pro-region level forming mature peptides. Thes mature peptides that bind to the specific receptors exposed on the granulosa cells activate the cascade of Smad 1/5/8 signal proteins.

Indeed BMP-15, under ligand form, binds and activates its own type II receptor, BMPR-2, which on his turn then interacts with the type I receptor, ALK-6 activate, responsible for the beginning of the intracellular cascade, operated by the Smad complex [9].

The BMP-15 protein is primarily expressed in the oocytes, from the primary follicle stage until ovulation. Therefore, considering its close correlation with the ovarian function, the research conducted later on such gene showed, after the identification of its mutations in patients affected by premature ovarian insufficiency, that BMP-15 is one of the responsible genes for the heterogeneous disorder.

The human Growth Differentiation Factor 9 (GDF-9), like as the BMP-15, is another one of the superfamily TGF-β members. This gene is mapped on the long arm of chromosome 5 (5q31.1). Although located on different chromosomes, GDF-9 and BMP-15 are two very similar genes that share similar structural and functional characteristics; both encoded proteins can form heterodimers and both correlate with ovarian failure.

It is demonstrated that women with ovarian dysfunction, above especially follicular, experience mutations caused by the gene, so in the same way of BMP-15, GDF-9 is cause of POI.

The target of our work was to effectuate the molecular analysis of BMP-15 gene in a small group of Sicilian patients affected by POI.

The study is collaboration between the Section of Gynecologic Endocrinology, Department of Microbiological and Gynecological Sciences (S. Bambino Hospital, Catania) and Department of Biomedical Sciences and Department of Animal Biology of Catania, all structures belonging to the University of Catania.

This study examined a sample of twenty women that, even if limited, was highly selected, bringing attention mostly on very young patients, in which the generic causes truly have a major impact. The control group we considered the data in the published literature.

Diagnostic criteria considered for the recruitment of these patients, were extremely selective so in order to exclude in advance cases of ovarian insufficiency due to other causes.

The criteria for inclusion in the study were the following

1. Primary amenorrhea and/or secondary amenorrhea (spontaneous absence of menstruation for at least 12 months);
2. Chronological age less than 35 years of age (average age 26.6 years, minimum 14 years and maximum 40 years);
3. Average levels of serum FSH serum equal or superior to 50 mIU/mL and average serum levels of estradiol less than 30 pg/mL in at least three different doses;
4. Absence of endocrine and/or autoimmune disorders.

The selection was taken keeping the age in mind, [of a normal karyotype, 46XX] and the absence of obvious autoimmune disorders, all necessary conditions to reduce the possibility that the premature ovarian failure would be correlated to different causes from other than those genetic.

Three of the twenty patients examined presented primary amenorrhea, the remaining, secondary amenorrhea; furthermore, all showed high levels of pituitary gonadotropins (FSH > 50 mIU/mL /ml) and low estrogen levels (<30 pg / ml).

The subjects' average age is 27 years old and five of them are under 20; the importance of age is explained by the fact that, in younger patients the genetic component plays a major role in the demonstration of pathology.

The purpose of cytogenetic analysis is to identify numerical and/or structural chromosomal abnormalities. Therefore, considering that chromosomal abnormalities have strong implications for the onset of POI, the preliminary confirmation of a normal karyotype turns out to be am further diagnostic criteria that allow the exclusion of the chromosomal defects as a cause of ovarian failure.

The mutation analysis obtained by the sequencing of exons of the BMP-15 gene enables us to:

1. Identify the presence of mutations and thus confirm the implication role of the gene of the eziopathogenesis in premature ovarian failure;
2. Try to identify a genotype-phenotype correlation in relationship to the founder mutations.

The evaluation of the impact of this gene in cases of familiar POI could allow an early diagnosis of mutation in relatives of patients that are not yet affected, informing them early on the development of future disease risk, to better plan their reproductive choices.

Results

The study conducted on the twenty Sicilian women affected by POI, seemingly idiopathic, has not allowed for the identification of any mutation responsible for the pathology caused by the gene.

The only alteration found in three patients refers to polymorphism. Indeed, A/G heterozygosity in position 308 of the cDNA (c.308 A>G), located in the exon 1 of the BMP-15 gene, although determining an amino acid change (p.Asn 103 ser) is found also in normal subjects (control group) as well as in the cases.

The second polymorphism is to to be attributed to an inclusion on

the level of 2.2 fragment of exon 2 of BMP-15 gene (778 TCT 789), also present in the control group.

Discussion

The menopausal period represents the end of a woman's procreative capacity. Actually, it has been well-known for a long time that the decline of ovarian function begins about 10 years before the appearance and the establishing of a clear menopausal picture [10].

This means that 10 years before the spontaneous cessation of the menstrual cycle, even in the presence of ovulatory-type cycles, the capacity for the oocyte being fecundated decreases significantly, as a consequence of the unavoidable phenomena of cellular aging. Statistically, for a woman destined to enter menopause at a regular age range (48-54 years old), starting already from 38-40 years old, her reproductive potential decreases, in a way directly related to the chronological age increase. This is usually underlined, from a laboratoristic point of view, by a progressive increase of FSH serum FSH levels, which, if equal or superior to 15-20 mIU/mL in the follicular phase of the cycle and if associated with low levels of estradiol, clearly denotes the decline of ovarian reserve [11].

In IVF (*In Vitro* Fertilization) centers, as a matter of fact, the pregnancy rates are significantly reduced in the presence of FSH levels equal or superior to 20 mIU/mL. The most precocious markers of reduced ovarian reserve are represented by the Inhibin B and above all, by the Müllerian inhibitory factor [12].

The transition from normal intraovarian peptides values appears to be earlier than serum FSH(switch) and E2 levels; however, various clinical and laboratoristic trials [13] (also) have recently underlined that also the valuations of these peptides are not able with certainty future decline of ovarian reserve, as – similar to what has been shown in FSH and E2 levels, the modification of Müllerian inhibitory factor levels and, especially of Inhibin B, -- is associated with an already concomitant and inexorable reduction of the ovarian reserve [14].

The very early identification of the loss of ovarian reproductive ability in cases of premature or early menopause (POI) is very important to provide clearly to the patient the "window" of reproductive potential of which she can take advantage.

Nowadays, the hormone replacement therapy (HRT) has reached an absolute perfection and effectiveness. Indeed the HRT, enables a woman headed for an early or precocious menopause to limit, or even to reset the potential and serious consequences that she would face in the presence of 10-20 years premature menopause [15]: vasomotor symptoms, depression, osteoporosis, genitourinary atrophy, dysmetabolism, dermal collagen reduction and increase of vascular diseases.

The issues related to the premature exhaustion of ovarian function are also extended to lipid metabolism, with a tendency to increase in average both total cholesterol levels and LDL-cholesterol levels, with a reduction in the HDL cholesterol fraction [16].

The dysmetabolic lipid manifestations can be associated with mean blood pressure values modifications (both systolic and diastolic), with the modifications being represented by a tendency of the constant increase of values and by occasional blood pressure-raising phenomena.

The lipid dysmetabolism and the early modification of blood pressure parameters involve a higher and/or earlier risk of athero-clerotic vascular diseases at the cardiac level, cerebral and aortic [17], with a significant reduction in global quality-of-life. The reduction of individual psychophysical performances is also consequent from the ponderal growth (more or less substantial according to family characteristics and/or basal metabolism) that the cessation of ovarian function involves. As a matter of fact, the average energy expenditure consequent to ovarian activity during the fertile period of the female life is approximately equal to 300 kcal/day. Consequently, even without increasing daily caloric intake, the cessation of ovarian function is associated with an increased weight gain tendency.

From an eziopathogenetic perspective, an early depletion of ovarian function is usually the result of autoimmune processes (with the formation of anti-ovarian antibodies and perifollicular lymphocytic infiltrates), from chromosomal alterations (mainly with features of mosaicism), or gonadal damage following either chemotherapy or radiation treatment protocols.

The issues are related to profound a hypoestrogenemic state and to its subjective consequences, above all, those metabolic, can be easily overcome by hormone replacement therapy, that if established with appropriate timing and modality of administration, is able to prevent entirely (eminently in a symptomatic side) the almost inevitable subjective and objective consequences that premature cessation of ovarian function involves [18].

Thus, the TOS can overcome and solve clinical metabolic and organ problems related to premature depletion of ovarian function [19].

Unfortunately, the question related to reproductive capacity is very different. The rising levels of FSH and E2 are usually associated with failure of all IVF methods. Also the evaluation of the NHI and/or the Inhibin 2 is often unable and/or inadequate to estimate with predetermined times, the margin of residual reproductive capacity. It seems, therefore, a very interesting opportunity to identify an additional methodology, suitable for the early identification of the so-called "subjects at risk," or rather, of women with increasing possibility to prematurely incur depletion in their ovarian function. Particularly, subjects at risk can be considered all young women with a first or second-degree relative with POI, considering the high incidence of POI in established relatives' groups, even in absence of specific chromosomal abnormalities [20].

The evaluation of the chromosomal map, as a matter of fact, is generally negative in relatives of women affected by POI, resulting normal in the great majority of cases. Instead, it is possible to earlier identify subjects at risk by means of more refined and detailed genetic evaluation.

In our case, finding no mutation can be justified:

- because the small number of the examined samples is not statistically significant;
- because the Sicilian population is not predisposed to a mutation of BMP-15 gene;
- because in these women, the cause of premature ovarian failure is determined by other genes responsible for POI.

The detected polymorphic variant has been described in literature as a reason for 9.8% of Caucasian women with cases of POI, for 10% of women with primary amenorrhea and for none of the patients affected by secondary amenorrhea. Instead, in the control group this polymorphic variant has a frequency of 5%, demonstrating that this variant is mainly present in cases of ovarian failure and of primary amenorrhea. This has led us to think that the c.308 A>G and 788 TCT 789 polymorphisms may be correlated with POI [21-25].

With the intention to continue recruiting highly selected patients affected by POI, we aim to continue to sequence BMP-15 gene, in order to identify mutations against it and to extend this research also towards other genes responsible for premature ovarian failure. Taken together, these studies could contribute to clarify the knowledge concerning ovarian and folliculogenesis development and possibly, in the future to elaborate the molecular tests able to recognize the women at risk of developing POI.

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