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Coexistence of *FOXE1* and *BMP15* gene variants in young females with premature ovarian insufficiency: Evidence of digenic inheritance

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Introduction

Premature ovarian insufficiency (POI) is defined as the occurrence of hypergonadotropic ovarian failure prior to age 40 years. In the presence of normal karyotype, the incidence of POI is 1/100 women under age 40, 1/1.000 under 30 and 1/10.000 under 20 years.

POI may be manifested either as delayed puberty, primary or secondary amenorrhea, infertility or it may constitute a chance finding.

The etiology of POI has not as yet been uncovered in the majority of cases, 90% of the cases still being classified as idiopathic. Nevertheless, it is rather certain that POI represents a **multifactorial disorder** caused by genetic and/or environmental factors. Since up to 30% of POI cases have a familial pattern, a strong genetic background is suspected.

Thus far, genetic variants have been identified in various transcription factors implicated in ovarian ontogenesis and/or function, albeit in a small percentage of cases. Even in these cases only some of the reported variants have been definitely validated as to their relation to POI phenotype.

Among the genes potentially related to POI are the *FOXE1* and *BMP15* genes.

Subjects and methods

Subjects

37 women with POI manifested at a mean age of 17.8±6.1 years either with delayed puberty (n:2), primary amenorrhea (n:10), secondary amenorrhea (n:20) or irregular menses (n:5) were included in the study. All women had normal karyotype and no evidence of a *FMR1* premutation. **75** females from the general population served as controls for the ***FOXE1*** gene and **50** for the ***BMP15*** gene.

Methods

DNA sequencing was carried out in the coding regions and the intron-exon boundaries of the *FOXE1* and *BMP15* genes. MLPA technique was applied when appropriate.

For the validation of detected genetic variants the following tools were used:

1) the frequency of the variants in patients, controls and the European population of the 1000 Genome Project (1000 GP)
2) *in silico* analysis
3) ab initio modeling using the I-Tasser server of the University of Michigan.

Results

Genetic variants identified in the *FOXE1* and the *BMP15* genes are depicted in tables 1 and 2.

The *FOXE1* gene variants in women with POI and in controls were 16.2% and 2%, respectively (p:0.002) [Table 1].

In **four** women with POI, coexistence of *FOXE1* and *BMP15* gene variants were identified (Table 2 and Figure 1).

No variants in the *BMP15* gene were identified in the two control subjects with *FOXE1* alanine tract alterations (Table 1).

Table 1. *FOXE1* gene alanine tract genotypes in women with POI and in controls. *The variants were selected after validation as described in methods.

FOXE1 variants (alanine tract genotypes)	POI n (%)	Controls n (%)
8/16*	2 (5.4)	-
8/14*	1 (2.7)	1 (2)
12/16*	1 (2.7)	-
14/14	16 (43.2)	15 (30)
14/16	10 (27.0)	25 (50)
16/16	3 (8.1)	8 (16)
15/16*	1 (2.7)	-
14/17*	1 (2.7)	-
16/19	-	1 (2)
* Variants possibly related to POI	16.2%	2%
	p: 0.002	

Table 2. Coexistence of *FOXE1* and *BMP15* gene variants in women with POI and the age of POI manifestation. PA: Primary Amenorrhea, SA: Secondary Amenorrhea, IM: Irregular menses

FOXE1 Alanine tract genotype	*BMP15* variants (c.-9C>G and p.N103S) genotype	Age at manifestation (years)	Type of manifestation
†8/16	G/G	15	PA
*8/14	G/G	25	SA
*8/16	C/G	24	SA
12/16	C/C	15	PA
15/16	C/C	15	IM
14/17	G/G + p.N103S/wt	14	PA
*Affected mother: 8/14	C/G	35	SA
†Unaffected mother: 8/14	C/G	-	Normal

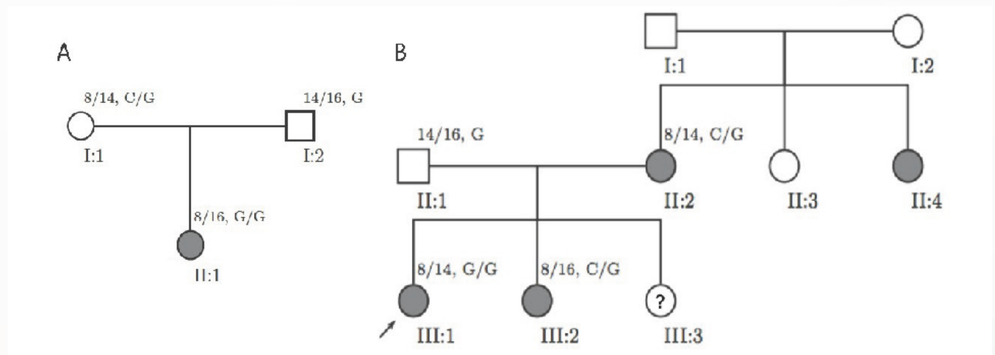

Figure 1. Family A. II:1 has POI and carries the *FOXE1* alanine tract genotype 8/16 and the *BMP15* variant c.-9C>G in homozygosity. I:1 is healthy and carries the *FOXE1* alanine tract genotype 8/14 and the *BMP15* variant c.-9C>G in heterozygosity. **Family B**. III:1 has POI and carries the *FOXE1* alanine tract genotype 8/14 and the *BMP15* variant c.-9C>G in homozygosity. III:2 has POI and carries the *FOXE1* alanine tract genotype 8/16 and the *BMP15* variant c.-9C>G in heterozygosity. II:2 developed POI at 35 years old and carries the *FOXE1* alanine tract genotype 8/14 and the *BMP15* variant c.-9C>G in heterozygosity.

Conclusions

The present data strongly suggest that:

a) *FOXE1* gene variants of altered alanine tract are causatively related to POI

b) Coexistence of variants in POI-related genes (such as *FOXE1* and *BMP15*) are indicative of **digenic inheritance** in this disorder.

The data are in accordance with our previous report on *FOXL2* gene defect, also suggesting **digenic inheritance** in POI (Menopause, 2015).

Given the perplexity of the reproductive axis in its evolution and function, we may hypothesize that either compensatory (protective) mechanisms prevent clinical disease in a given molecular alteration and/or more than one molecular deviation (**digenic inheritance**) with or without environmental contribution is required for the POI phenotype.

It is quite possible that some of the controversies regarding the relation of certain variants to the POI phenotype might be due to the necessity for the presence of more than one molecular aberration in order for the POI phenotype to develop.

We must finally emphasize the importance of identifying the genetic basis in each POI case for:

a) genetic counseling

b) prognosis and

c) planning fertility strategies

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