Absence of puberty and estrogen resistance by estrogen alpha receptor inactivation in two sisters: a mutation for variable phenotypic severity

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Introduction

Estrogens play an essential role in reproduction and their peripheral action is mediated via nuclear alfa (ERα) and beta (ERβ) receptors as well as membrane receptors. To date, only 3 females and 2 males from 3 families with a loss of function of ERα have been reported [1,2,3]. The phenotype in these families was strongly suggestive of an estrogen resistance with an absence of a complete puberty, a delay in epiphyseal maturation with high estradiol levels and elevated gonadotropin levels.

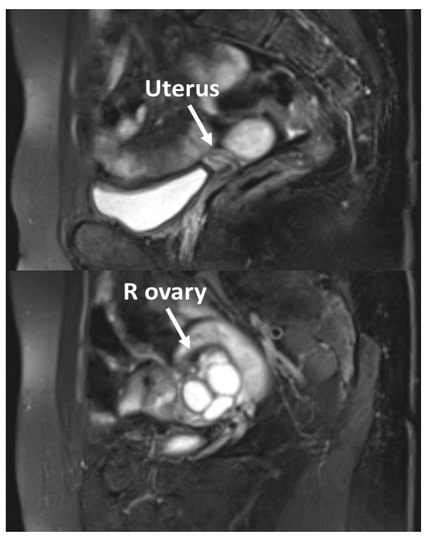
Goal

The objective of this study was to describe a new family in which 2 sisters displayed different levels of endocrine and ovarian defects although they carried the same homozygous loss of function rare variant in the ERα-encoding ESR1 gene.

Materials and methods

A 36-year-old woman with a primary amenorrhea and no breast development (S1), had elevated 17β-estradiol (1497 pg/ml), high FSH (57 IU/L) and LH (21 IU/L) plasma levels (Table 1), and enlarged multifollicular ovaries (11 and 17 ml) (Figure 1). Her 18-year-old sister also had a primary amenorrhea with no breast development and had moderate increases in 17β-estradiol (204 pg/ml) with high FSH (29 IU/L) and LH (22 IU/L) plasma levels. Pelvic MRI shows ovaries of normal size and the uterus was not seen (Table 1). The parents are first cousins.

Fig 1

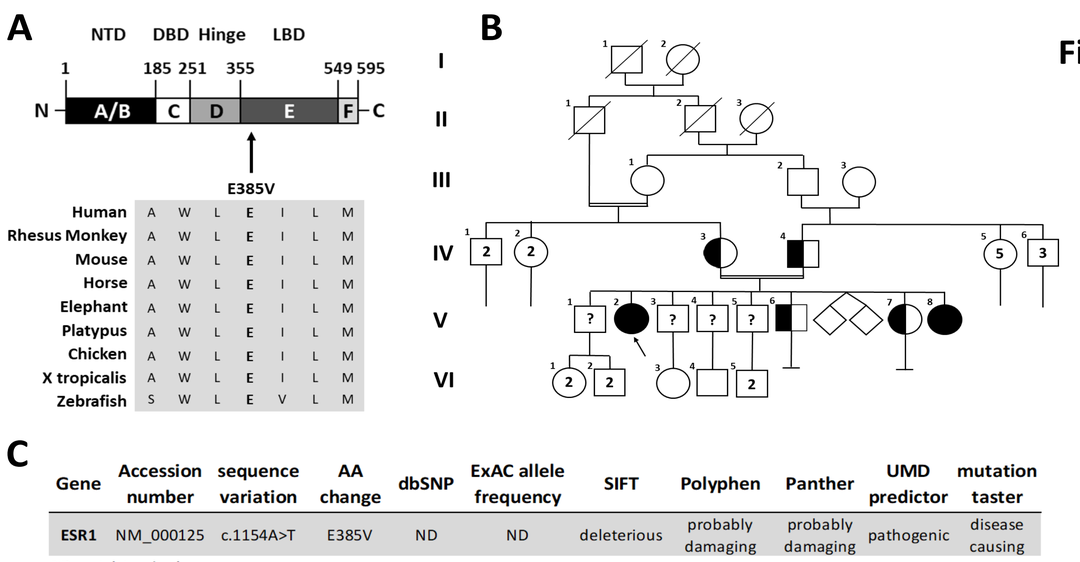


Pelvic MRI of the proband shows small prepubertal uterus and enlarged multifollicular ovaries

Table 1 : Characteristics of the two affected sisters

	V-2	V-8
Clinical characteristics		
Age, y	36	18
Height, cm	179	163
Weight, kg	67	78
BMI, kg/m²	21	27.5
Waist circumference, cm	80	-
Arm span, cm	191	-
Tanner stage	B1P5A5	B1P5A5
Hormonal parameters		
17β oestradiol, pg/mL	1497	204
LH level, UI/L	21	22
FSH level, UI/L	57	29
AMH, pmol/L	31.6	70.9
Testosterone level, ng/mL	0.32	0.18
Delta4 A, ng/mL	1.04	1.07
SDHEA, ng/mL	2604	1837
17 OHP, ng/mL	0.45	0.67
Progesterone, ng/mL	0.10	0.11
11 desoxycortisol, ng/mL	0.13	0.07
11 desoxycorticosterone,	<0.05	<0.05
corticosterone, ng/mL	8.1	2
TSH, UI/L	1.96	2.05
FT4, pmol/L	15.1	13.7
Prolactin, ng/mL	3.3	21
Ovarian ultrasonography		
Right ovarian volume, mL	10.9	3.2
Left ovarian volume, mL	16.9	7.02
Uterus dimension, mm	15x10	NS
Bone evaluation		
Bone age evaluation, y	14	12
Spine bone density mg/cm² (Z score)	0.67 (-4.4)	0.93 (-2.3)
Femoral bone density mg/cm² (Z score)	0.55 (-3.4)	1.06 (-0.3)

Fig 2



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Gene	Accession number	sequence variation	AA change	dbSNP	ExAC allele frequency	SIFT	Polyphen	Panther	UMD predictor	mutation taster
ESR1	NM_000125	c.1154A>T	E385V	ND	ND	deleterious	probably damaging	probably damaging	pathogenic	disease causing

ND : not determined

Results

In both cases, genetic analysis identified a homozygous variant of ESR1 (c.1154A>T) leading to the substitution of the highly conserved glutamic acid at position 385 by a valine (p.E385V) (Figure 2A). Both parents as well as an unaffected sister were heterozygous for the variant (Figure 2B). The E385 is located in the ligand binding domain (Figure 2B) and the in-silico analysis predicted a deleterious effect on the protein function (Figure 2C). Modeling study of the ERα -E385V variant showed a slight displacement of the H4 to H12 helix, suggesting that the E385V replacement might preclude the activation of the receptor (Fig 2D).

A functional analysis was performed by transient expression of WT-ERα or E385V-ERα in HEK293A cells. E385V-ERα transfected cells showed a strong decrease in transcriptional activation by 17β-estradiol of a reporter gene controlled by a standard estradiol-responsive-element (Figure 3) as well as a loss of inhibition of the KISS1 promoter (Figure 4) when compared to WT-ERα. Immunofluorescence analysis showed lower nuclear translocation of E385V-ERα in the presence of 17β-estradiol as compared to WT-ERα (Figure 5 and table 2).

Fig 3

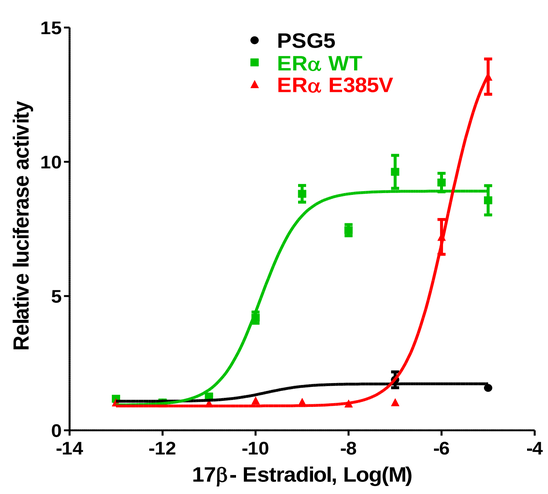

Fig 4

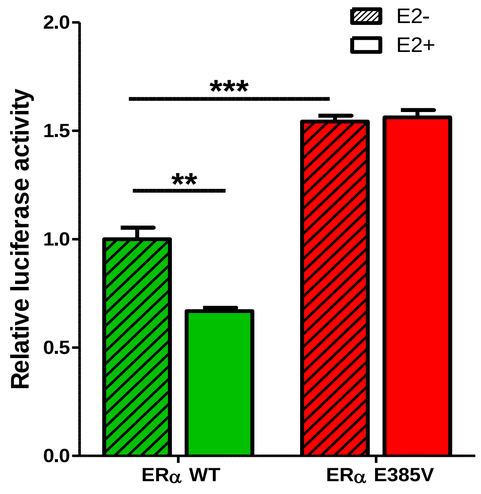

Fig 5

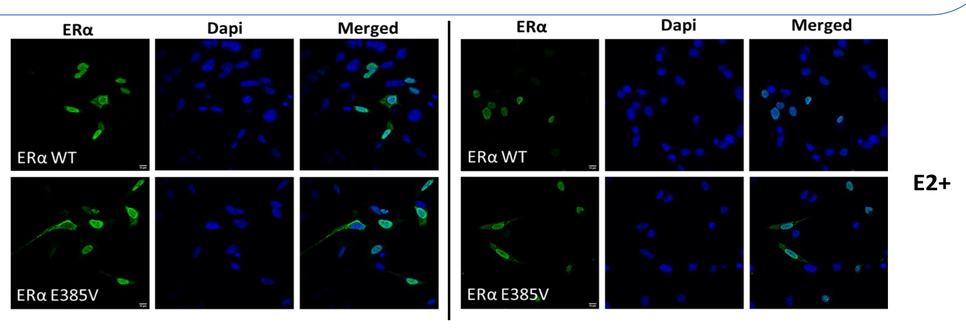

Table 2 : Subcellular location of ERα in HEK cells

	ERα WT		ERα E385V		p*
	E2-	E2+	E2-	E2+	
Nucleus	86% (146/170)	96% (135/141)[a]	67% (84/126)[a]	65% (66/101)	<0.0001
Cytoplasm	2% (3/170)	0% (0/141)[a]	3% (4/126)	6% (6/101)	<0.0001
Both	12% (21/170)	4% (6/141)[a]	30% (38/126)[a]	29% (29/101)	<0.0001

Results are expressed as percentage (nb of cells/total of cells observed)
* P-value for global comparison between the four groups. a. b. c. similar exponent identify significant pairwise post-hoc comparisons after Bonferroni's correction. [a] p<0.05 compare to ERα WT E2-; [b] p<0.05 compare to ERα E385V E2-

Conclusion

These two new cases are remarkable as they are sisters and they display a different level of severity of the ovarian and hormonal phenotypes. This phenotypic discrepancy could be attributable to a mechanism that could partially compensate the ERα inactivation.

Bibliographie

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