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Letter to the Editor

Estrogen receptor mutation in a girl with primary amenorrhea

To the Editor:

Estrogen has been known for its effects on female sexual characteristics, fetal development, protein synthesis, mental health and coagulation. Estrogen receptors (ERs) are members of the nuclear receptor family of transcriptional modulators. Two ERs have been studied extensively: ERα and ERβ. ERβ is the product of the *ESR2* gene on chromosome 14 and is expressed in the granulosa cells of the ovaries, epithelial cells of the prostate, testis and epididymis, colon, bladder, bone marrow and the pituitary gland (1, 2).

Since mutation of the *ESR* in normal tissue was not identified in the past, its mutations were considered to be lethal (3, 4). However, homozygous mutant mice with *Esr1*-disrupted genes appeared healthy and survived to adulthood. Although fertility problems were noted in both female and male mice, prenatal sexual development was not disrupted. Hypoplastic uteri, hypoplastic breasts, cystic ovaries and decreased skeletal mineralization were seen in females. Low sperm counts and decreased skeletal mineralization were noted in males. The only functions completely lost were estrogen functions in the uterus or vagina (5).

An example of ERα deficiency was a tall (204 cm) 28-year-old man with incomplete epiphyseal closure and normal pubertal growth. His serum estradiol, estrone, LH and FSH levels were elevated and his testosterone was normal. He had glucose intolerance and hyperinsulinemia and increased bone turnover. This emphasizes the role of estrogen in bone mineralization and glucose tolerance and shows that disruption of the ER gene is not lethal (4).

Here, we report a 15-year-old girl who presented with primary amenorrhea. She was 149 cm and 49 kg. Her bone age was 13 years. Her external genitalia was normal. Ultrasound evaluation revealed a primitive uterus smaller than normal (29 × 11 mm). The left ovary was multicystic and the right ovary had a 49 mm exophytic cyst. Magnetic resonance imaging showed multiple bilateral ovarian cystic lesions and a hypoplastic uterus.

Endocrinologic evaluation revealed normal thyroid function, normal FSH, normal LH, high prolactin (38 ng/ml, normal range for women: 3.7–23.2), normal DHEA-SO_4, high 17 hydroxy progesterone (3.6 ng/ml, highest normal level: 2.3) and a very high estradiol level (1327 pg/ml, highest normal level: 498).

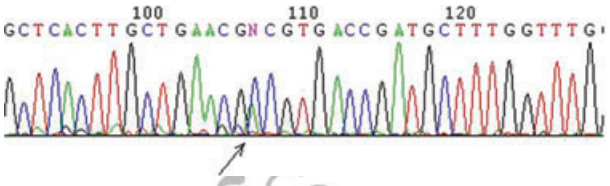

Fig. 1. Mutation detected in patient.

Androstenedione, free testosterone, β-HCG, CEA, AFP, CA125 and CA19-9 were within normal limits.

Genomic DNA was extracted from blood samples of the patient and her mother after informed consent using the standard salting out method. All exons of *ESR2* gene were amplified according to Omrani et al. (6). Polymerase chain reaction products were sequenced using the ABI Prism3130 Genetic Analyzer (Applied Biosystems, Foster City, CA). A mutation in exon 8 of the patient was detected (NM_001040275.1:c. 1295C>A) which was not seen in the corresponding exon of the mother (Fig. 1). The nucleotide change results in amino acid change from alanine 432 to aspartic acid. The 1U9E.pdb file, corresponding to the ERβ ligand binding domain retrieved from the Protein Data Bank (http://www.rcsb.org) was used to model the detected mutation. Mutation, minimization of the structure and visualization of the resulting structure were performed with the use of MOE 2010.10 (Molecular

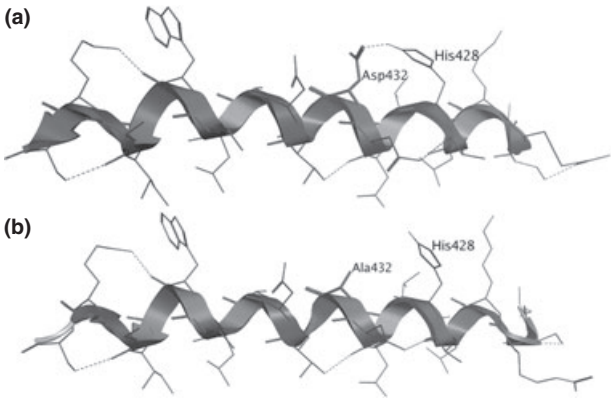

Fig. 2. The helix formed by residues 149–171 is shown as a ribbon. Dotted points indicate hydrogen bonds. (**a**) Asp432 is shown as sticks. Putative hydrogen bond between His428 and Asp432 is shown. (**b**) Ala432 is shown as sticks.

Operating Environment, Chemical Computing Group Inc., Montreal, Canada). An aliphatic residue change to a larger acidic residue is important from a biochemical point of view. *In silico* mutation of this residue in the ligand-binding domain of the protein and consequent minimization of the structure indicates that a potential hydrogen bond may occur between Asp432 and His428 (Fig. 2a) which does not exist in the native structure (Fig. 2b). The computational modeling indicates that hydrogen bond formation in this position may disrupt helix structure while the closer position of Asp to His may result in a tightening of that segment.

Although *ESR1* mutation has been noted in a male patient, this female patient is the first female report of an *ESR2* mutation and provides an example of a non-lethal *ESR2* mutation.

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AQ2. Please provide expansions for the following terms "FSH, LH, β-HCG, CEA, DHEA and AFP."

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