遗传学报 2005 32 (10): 1011-1017 ISSN: 0379-4172 CN: 11-5450/R

人类与医学遗传学

女孩性早熟患者中检出一种新的雌激素受体基因突变

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收稿日期 2004-12-6 修回日期 2005-4-5 网络版发布日期 接受日期

摘要 对收集的16例未见血液雌激素水平升高的临床女孩性早熟患者的外周血样本，利用PCR-SSCP方法筛查了雌激素受体基因编码区的可能突变。结果在1例患者发现：其雌激素受体基因8号外显子编码精氨酸的548位密码子，1个C→T转换导致精氨酸残基被半胱氨酸所替代；这一突变使DNA序列中产生1个Bts I酶切位点，通过PCR-RFLP实验证明此患者为Arg548/Cys548杂合体。为证明该突变在性早熟发生中的作用，构建了一个雌激素受体反应元件报道质粒pGL3-promoter-ERE；成功将野生型ESR1基因定点突变，并克隆于PCR3.1真核表达质粒。报道质粒和表达质粒共转染CMF-7细胞，Cys548突变能够增加萤火虫荧光素酶的产生。结果证明该突变雌激素受体在体外具有高活性特征，因而推测在体内也可能具有相应的过高活性，从而导致女孩的性早熟。

关键词 雌激素受体；基因突变；女孩性早熟

分类号

A Novel Estrogen Receptor Gene Mutation Detected in Girls with Precocious Puberty

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Abstract

Female precocious puberty is caused by premature activation of the hypothalamic-pituitary-gonadal axis, exposure to exogenous sex steroid hormones, and the presence of endogenous sex steroids caused by various factors. Estrogen is the key final factor to start onset of puberty. However, some cases of precocious puberty in girls cannot detect estrogen elevation. The raised sensitivity of estrogen receptor, which may caused by ESR1 mutation or polymorphism, has been frequently mentioned for interpret the etiology of sporodic low estrogen type cases. But no case evidence has been found in clinical practice. For the purpose of screening possible mutations in estrogen receptor gene, leukocyte genomic DNA were collected from 16 girls with precocious puberty of sporodic low estrogen, and exons of ESR1 were amplified and analysized using PCR-SSCP/silver staining method. A single strand conformation change in exon 8 was found in one of the patients(No.14). The suspected fragment were cloned to a T vector and sequenced for analysis. Sequencing of these clones revealed that this conformation change is caused by a C to T transition. This mutation results in the replacement of arginine by cystine at position 548 of ESR1 protein. The mutation created an extra BtsI digest site and made it can be readily identified by PCR-PFLP method. Further detection using this method, and sequencing of cloned exon8 colonies from patients proved that the patient No.14 is Arg548/Cys548 heterozagous in genotype. This mutation increased hydrophobility of the area dramatically. The position and the conservative of this residue in vertebrates suggest Arg548 may play an important role in ESR1 function. For study the role of this mutation in the onset of precocious puberty, a firefly luciferase reporter plasmid pGL3-promoter-ERE was constructed, a pCR3.1-hermut plsimid expressing Cys548 ER was constructed based on wild type pCR3.1-her. Co-transfection of reporter and pCR3.1-hermut in CMF-7 cell strain proved that Cys548 mutant can significantly increases the transcription activity than the Arg548 wild type.

Key words ESR1 novel mutation precocious puberty

DOI:

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