平成17年度研究報告

研究テーマ

小児科外陰部異常症の包括的遺伝子解析：
疾患原因遺伝子変異・疾患感受性遺伝子多型・
環境反応性遺伝子多型・治療応答性多型の解明

－ 小児外陰部異常症の包括的遺伝子解析 －

慶應義塾大学医学部小児科学教室
助教授 長谷川 奉延

現所属：慶應義塾大学医学部小児科学
サマリー

「研究背景・目的」
小児外陰部異常症とは、ミクロペニス・停留精巣・尿道下裂・陰核低形成・陰唇低形成・その他の先天的外陰部異常の総称であり、遺伝的因子と環境因子の相互作用で発症する。本研究期間中の具体的な目的は、以下の2点である。
目的1: 小児外陰部異常症患者において、候補責任遺伝子の遺伝子解析を行い、その分子遺伝学的原因を同定する。
目的2: ミクロペニス、停留精巣、尿道下裂を対象とし、小児外陰部異常症の疾患感受性候補遺伝子多型あるいは環境反応性候補遺伝子多型を同定する。

「研究対象・方法」
小児外陰部異常症患者のうち、本人/家族の同意を得た患者、およびその家族を対象とし、候補遺伝子の変異解析を行う。
本人/家族の同意を得たミクロペニス、停留精巣、あるいは尿道下裂を有する患者において、コントロールより有意に頻度の高いSNPsおよびホプロタイプを同定する。

「研究成果」
成績1: 9例において分子遺伝学的原因を同定した。その内訳は以下の通りである。
アンドロゲン受容体遺伝子異常症 2例
5alpha-reductase 遗伝子異常症 3例（2家族）
5alpha-reductase 遺伝子異常保因者 4例（2家族）

成績2: ミクロペニス、停留精巣、あるいは尿道下裂を有する患者計45例において、疾患感受性候補遺伝子の代表である17-hydroxylaseの翻訳領域内にSNPsを同定した。
現在、コントロールにおけるこれらの遺伝子のSNPsの同定を行っている。

「考察」
今回、同定したアンドロゲン受容体遺伝子異常、および5alpha-reductase遺伝子異常のうち、2つの変異は過去に報告のないnovel mutationsであった。
SNPsに関する本研究をさらに発展させることにより、小児外陰部異常症の新しい疾患感受性遺伝子が特定できること期待される。
A Novel Mutation of Androgen Receptor Gene in Complete Androgen Insensitivity Syndrome

Satoshi Narumi, Naoko Amano, Rumi Hachiya, Tomohiro Ishii, Tomonobu Hasegawa,
Department of Pediatrics, Keio University School of Medicine

Androgen insensitivity syndrome (AIS) is an X-linked recessive disorder caused by mutation in the gene for the androgen receptor (AR) with 46,XY karyotype (OMIM 300068). The clinical phenotype of AIS is complete or partial. Complete AIS is characterized by a consistent phenotype: unambiguous female external genitalia, breast development at pubertal age, blind-ending vagina, absence of uterus, absent or scant pubic and axillary hair, and presence of normally differentiated testes in a girl or woman. However, the clinical features of partial AIS are variable: ambiguous external genitalia in a girl or woman, undervirilized external genitalia in a boy or man, or azospermia with unambiguous male external genitalia in a man (1-4). So far more than 300 mutations in all exons of the AR gene have been reported in complete AIS (5). We report a novel mutation of the AR gene in a patient with complete AIS.

Patient Report

The patient, a Japanese girl, was born after a 39-wk uncomplicated pregnancy and delivery. Her birth weight was 2.90 kg, and length, 45.5 cm. Allegedly, a surgeon found her bilateral abdominal testes at the time of repair of bilateral inguinal hernia in infancy. At the age of 9 years, the patient together with her mother visited our service to consult about the pathogenesis. The maternal uncle, reportedly, has “undervirilized external genitalia.” The mother declined to tell further family history. On examination, the patient’s height was 133.2 cm (≈25th percentile) and weight 44.7 kg (>97th percentile). She had normal female external genitalia. No tumor was palpable around the inguinal area. Breast as well as pubic hair were Tanner I. Operation scars were present on the abdominal wall. Endocrinological studies were as follows: serum LH, 0.3 mIU/mL (normal); FSH 3.7 mIU/mL (normal); testosterone 0.20 ng/ml (normal), which was increased to 1.82 ng/ml by hCG stimulation (3,000 IU/m² / dose i.m. for three consecutive days, blood sampling on day 4). Her karyotype was 46,XY. Pelvic MRI revealed the right testis in the abdominal cavity and the left one in the inguinal canal. The patient was clinically diagnosed as having complete AIS.

Written informed consent for a genetic study of the AR gene for the patient, but not for the parents, was obtained from her parents. This study was approved by the ethical committee of our institution. Genomic DNA was extracted from the patient’s peripheral lymphocytes. The AR coding region and flanking intronic sequences of all the exons were amplified by PCR, followed by direct sequencing as previously described (6). A hemizygous mutation (c.2125G>A, p.E709K) was identified in exon 4
of the AR gene of the patient (Fig). The CAG repeat in exon 1 was 25, within the normal range. This mutation was not found in 50 healthy control male subjects.

**Discussion**

We identified a novel mutation (c.2125G>A, p.E709K) in exon 4 of the AR gene of the patient with complete AIS. Although we did not perform functional study, the AR function of this patient must be severely impaired by this mutation leading to complete AIS for the following reasons. First, the mutation changes the amino acid from acidic (glutamate) to basic (lysine). Second, the site of the mutation is in the amino-terminal portion of the ligand-binding domain, which is critical for AR function (1,7). Third, the affected amino acids are conserved in the mouse, chicken, Japanese Takifugu fish and xenopus, indicating their functional importance (8,9).

More than 300 mutations have been reported in patients with complete AIS. It has not been possible, however, to establish any clear genotype-phenotype (complete or partial) correlation to date, besides total deletion of the AR gene represented by complete AIS (1). Furthermore, an identical mutation could cause different phenotypes in different patients, or even within the same family, which might be the case with this family. Unfortunately, we could not analyze the patient’s mother, who might be a carrier, and maternal uncle, who might be an affected patient, judging by the family history. Modulating factor(s), even genetically or environmentally, might exist causing different phenotypes with the same AR gene mutation.

In conclusion, we here identified a novel mutation of the AR gene in the patient with complete AIS.

**Acknowledgement**

This work was partly supported by the Yamaguchi Endocrine Research Association.
References

8. Available online from: http://www.ensembl.org
9. Available online from: http://www.ebi.ac.uk/clustalw/

【本研究成果の刊行】