THE LIMB-MAMMARY SYNDROME (LMS) ASSOCIATED WITH INTERNAL FEMALE GENITALIA DYSGENESIA: A NEW GENOTYPE/PHENOTYPE CORRELATION?

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ABSTRACT

We report on a case of a young women carrying an LMS typical phenotype associated with a severe dysgenesis of internal genitalia. The genotype, a 2-bp deletion (1576-1577DelTT) in exon 13 of the TP63 gene has been described, up to date, only in this subject. Several urogenital anomalies have been reported in literature associated with the EEC syndromes but no data are present on internal female genitalia abnormalities. Our patient presented normal external female genitalia and absence of urinary tract anomalies. A normal karyotype studied by FISH technique excluded disorders associated to sex chromosome defects. Several expression studies on animal models demonstrated the presence of p63 protein in all female reproductive organs and a close association between ectodermal and mesenchymal structures maturation. These data suggest a genotype-phenotype correlation in our index case.

Key words: limb-mammary syndrome; internal female genitalia dysgenesis
INTRODUCTION

The EEC syndrome (ectrodactyly, ectodermal dysplasia and clefting syndrome) is a congenital autosomal dominant disorder involving tissues and systems of epithelia-mesenchyme origine and characterized by ectrodactyly (split-hand/split-foot malformation); anomalies of skin, hair, teeth and nails; cleft palate with or without cleft lip. The LMS syndrome (limb-mammary syndrome) is a EEC-like condition differentiated by ectrodactyly, mammary-gland ad nipple a/hypoplasia, cleft palate without cleft lip, absence of hair and skin defects [Bokhoven et al., 1999]. Other frequent manifestations of the EEC-like conditions include lacrimal duct stenosis, conductive hearing loss and structural urogenital anomalies [Roelfsema et al., 1996; Rinne et al., 2006]. Heterozygous mutations in the TP63 gene mapped on chromosome 3q27 [Bokhoven et al., 1999] are causative for all reported cases of the LMS syndrome and are the major cause of the EEC syndrome. The TP63 gene produces the transcription factor p63 in different isoforms with different functional roles in the developing epidermis. [Yang et al., 1998; Wu et al., 2003]. However, expression of p63 mRNAs is not restricted to the skin but have also been detected in a variety of tissues including placenta, skeletal muscle, heart, thymus, trachea, gonads and uterus [Osada et al., 1998; Dellavalle et al., 2001]. We describe a case of a young female patient who presented an LMS phenotype characterized by absence of mammary gland and nipple and isolate cleft palate associated with a severe dysgenesia of internal genitalia. Several urogenital anomalies and in particular hypoplasia of female anogenital tract [Ince et al., 2002] have been described associated with the EEC syndromes but, up to date, no data are present in literature on internal female genitalia abnormalities.
CLINICAL REPORT

Patient and family medical history

Phenotype

A 14 years old female with a diagnosis of EEC syndrome, arrived to our attention for primary amenorrhea. The girl phenotype characterized by severe ectrodactyly, unilateral isolate cleft palate, mammary-gland and nipple aplasia and absence of hair and skin defects was in reality typical of the LMS syndrome. The clinical examination of the patient and her medical history did not show any relevant problem and in particular no micturition difficulties was referred from the patient. A normal development of external genitalia with regular vaginal and urethral meatus and normal morphology of the vagina tract were present. Also normal for age was the pubic hair development (Tanner stage 4). Growth velocity appeared low for age during the last years in relation to delay of puberty while the bone age was difficult to evaluate because of the severe bone malformations of the hands. The parents of the girl did not show any clinical sign referred to EEC/LMS syndromes. In the family medical history emerged the data of the delayed puberty and miscarriages in the grand-mother died for non- Hodgkin lymphoma.

Molecular and cytogenetic analysis

The molecular study showed a 2-bp deletion (1576-1577DelTT) in exon 13 of the TP63 gene [Bokhoven et al., 2001]. This is a frameshift mutation that introduces a premature stop codon and
affect only the \( \alpha \) isoform of the p63 protein [Bokhoven et al., 2002]. Until now, the mutation has been described only in this patient and it appears as a de novo mutation. The genotype of the parents is not available. They were not studied at the time because of the silent phenotype, but the molecular analysis is planned also for them. Fluorescence in situ hybridization (FISH) analysis was done with X and Y probes to exclude sex chromosomes defects that could explain the uterus and ovaries hypoplasia and it showed a normal female karyotype.

**Radiological and laboratory studies**

The abdominal ultrasound did not show any abnormalities of the urinary tract but could not recognize structures clearly defined as uterus and ovaries. The abdominal RMI confirmed the absence of both reproductive organs. The hormonal studies showed an hypergonadotropic hypogonadism and a very low plasmatic oestrogen levels. The androgenic secretion and thyroid function were normal. A study of bone mineralization was also done by ultrasound technique that showed a normal bone density for age.

**Patient management**

A replacement of estrogens at low doses was started to allow a functional development of the vaginal mucosa and a regular bone mineralization. A clinical follow-up was planned to evaluate the plasma oestrogen levels and bone mineralization.
DISCUSSION

Our patient presented all clinical characteristics of the LMS syndrome, an EEC-like condition characterized by ectrodactyly, mammary-gland ad nipple a/hypoplasia, cleft palate (CP) without cleft lip (CL) and absence of hair and skin defects. The distinction between CLP and CP is relevant because the affected structure, the primary palate and the secondary palate, develop independently of each other [Murray et al., 1995]. The group of the EEC and EEC-like conditions are also called p63 syndromes because the only causative gene found until now is the Tp63 gene located on chromosome 3q27. Urogenital abnormalities are described in literature associated to the p63 syndromes including micropenis and hypospadias, vaginal septum and external female genital hypoplasia but anomalies of the internal female genitalia have never been reported.

The morphogenetic studies performed in p63+/+ and p63-/- mice supported p63-dependent pathways of genital tract development that permit externally, urorectal septation and modelling of external genitalia and internally, epithelial cell differentiation in the vagina, cervix and urinary tract. The \( TP63 \) gene has two promoters and generates two types of transcripts with (TAp63) or without (\( \Delta Np63 \)) a N-terminal transactivation domain. Moreover, TA and \( \Delta N \) transcripts may have three different C-terminal sequences corresponding to \( \alpha \), \( \beta \), and \( \gamma \) forms. Therefore, the p63 protein involved in these syndromes is expressed in different tissues in six different isoforms that may have different transcriptional activity [Yang et al., 1998; Wu et al., 2003]. The studies on animal models showed the expression of the TAp63 isoforms in all female reproductive organs of mouse with the highest concentration in vagina and ovary. The ontogeny of p63 in female reproductive organs is essentially identical in mouse and human. Epithelial cells in the cranial portion of vagina and uterus develop from Mullerian duct. The formation of the urorectal septum subdivides the primitive cloaca and distinguishes the anorectal and urogenital sinus. The Mullerian duct terminates in the urogenital
sinus and the interaction forms the cervix and vagina. In human fetal reproductive tracts p63 is highly expressed in the epithelial cells of urogenital sinus but was still undetectable in the Mullerian duct epithelial cells by post-last-menses (PLM) 11 weeks. In the most of PLM 12-week specimens the Mullerian duct epithelial cells are positive for p63 organized in a gradient from caudal to cranial in the Mullerian tract. At PLM 16 weeks the caudal part of Mullerian duct epithelium is stratified and expressed p63. [Kurita et al., 2005]. In the developing mouse Mullerian duct, p63 expression precedes squamous differentiation of vaginal epithelium and targeted disruption of Tp63 gene transforms Mullerian vaginal epithelium into uterine epithelium [Kurita et al., 2004]. Several studies showed that the physiologic maturation of the ectodermal structures in the epidermis and epidermal derivatives is essential for the normal growing and patterning of the underlying mesenchymal tissues [Headon et al., 1999; Yang et al., 1999]. During the formation of skin appendages epithelium and mesenchyme are inducers and targets of each other [Priolo et al., 2000]. In almost all organs the morphogenic development is regulated by a chain of inductive interactions between the epithelial and mesenchymal tissues. Early signals regulate expression of homeobox genes and/or other transcription factors that regulate molecules at the cell surfaces and in the extracellular matrix contributing to the mesenchymal condensation and to epithelial morphogenesis [Thesleff et al., 1995].

Uterine and vaginal mesenchyme showed an established identity during embryonic period probably due to the Homeobox genes that are found to be expressed in a gradient throughout the female reproductive tracts of both mouse and human foetuses. It appears to be certain that homeobox genes play a key role in the normal epithelial Mullerian duct development in mouse and human, interacting with other factors including p63 protein [Kurita et al., 2005; Yang et al.,1999]. Although the epithelium/mesenchyme interaction mechanism has not been deeply studied in the female reproductive tract we can suggest that the absence of the p63 protein expression in the early development of the ectodermal structure may block the chain of the inductive interactions between the epithelial and mesenchymal tissues and determine a severe damage in the relative organ
morphogenesis. In our patient the morphology of the vagina tract appear normal but histological study of the mucosa to exclude the persistence of the columnar epithelium has not be done. It appear in reality difficult to explain an abnormal development of the Mullerian duct (uterus) with normal development of the urogenital sinus (vagina and external genitalia).

The studies on mouse ovary p63 expression indicate the presence of mRNA encoding the TA and α p63 isoforms in the ovary and the immunohistochemical staining with anti-p63 antibodies demonstrated the expression of p63 proteins in the oocytes [Nakamuta et al., 2007]. Previous results have shown expression of p63 mRNAs also in the mouse testis and nuclear localization of p63 proteins in testicular germ cells [Nakamuta et al., 2004]. Additionally, the p63 proteins expression appear to be different in the quiescent stage of germ cell and in the cell cycle progression both in testes and ovary suggesting that p63 may control the meiosis protecting the germ line during meiotic arrest [Nakamuta et al.,2003; Suh et al., 2006 ]These results imply that p63 is profoundly involved in the regulation of germ cell development but their roles are not fully understood. Finally, both studies on mouse ovary and female reproductive organs indicated that the TA and α isoforms were preferentially expressed in the ovary and in the Mullerian duct epithelium and were present at the earliest stage of the p63 expression. The genetic defect of our patient is described as a frameshift mutation that affect only the α isotype of the p63 protein. This isoform, on the other hand, is present also in the urogenital sinus that not seem to be affected in the morphogenetic evolution of our patient.

In conclusion, the wide expression of p63 proteins in the female reproductive organs and their involvement in both morphologic and functional development together with the unique phenotype described until now associated with the particular mutation of the Tp63 gene carried by our patient are indicative of a genotype/phenotype correlation.

In spite of that, other clinical/molecular observations and biochemical studies on morphogenetic evolution of the female reproductive organs are necessary to clarify the specific involvement of the internal female genitalia abnormalities in the phenotype of the p63 associated disorders.
REFERENCES


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