Y Chromosome Microdeletions in 34 Cryptorchidism Patients
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Abstract

Purpose: In this study, the molecular analysis of Y chromosome in 34 cryptorchidism patients was studied.

Methods: Thirty-four cryptorchid children who underwent orchidopexy were included in this study. Peripheral blood sample of 34 patients was collected and Y chromosome microdeletions were detected by PCR amplification of the Y chromosome specific genes and sequence tagged sites.

Results: Among cryptorchid patients, 19 were affected by bilateral and 15 by unilateral maldescent. Four patients (11.7%) showed a deletion of one or more STS. The prevalence of Y chromosome microdeletions was greater in patients with a history of bilateral cryptorchidism (3 of 19, 15.8%) than that observed in those with unilateral cryptorchidism (1 of 15, 6.7%).

Conclusions: It is important to undergo sperm cryopreservation for assisted reproduction in these patients and the diagnosis of AZF microdeletion should be offered to the couples undergoing assisted reproduction, since it is possible to transmit the genetic anomaly to the male offspring.

Keywords: Cryptorchidism, microdeletion, Y chromosome, PCR

I. Introduction

Cryptorchidism or undescended hidden testis is the most common disorder of sexual differentiation in man with an incidence of 4.5 % at birth and decreases to 0.8 % at 1 year of age until adulthood. Cryptorchidism is associated with impaired spermatogenesis due to high intra-abdominal temperature and increased incidence of testicular cancer (1,2).

It is only in the last few years that the loci involved in the production and differentiation of sperm have been identified using molecular methods (3). The long arm of the Y chromosome contains genes critical for spermatogenesis, the azoospermia factor (AZF)- AZFa, AZFb and AZFc. Deletion of any of these loci is associated with spermatogenic arrest at a particular stage of germ cell development and a characteristic testicular phenotype (1,2).

In this study, we studied the molecular analysis of Y chromosome in 34 cryptorchidism patients.

II. Material and Methods

A. Patients

Between 2002 and 2006, thirty-four cryptorchid children who underwent orchidopexy were included in this study. Each patient was diagnosed by physical and ultrasonographic examination. In each case, the following clinical data were
recorded: age, history, physical examination, affected testis, testis position and testis volume. Peripheral blood cultures were set up for chromosomal analysis and all the patients underwent surgical operation (orchidopexy).

B. Polymerase chain reaction (PCR) Analysis

Peripheral blood sample of 34 patients was collected and DNA was isolated using standard protocols. Y chromosome microdeletions were detected by PCR amplification of the Y chromosome specific genes and sequence tagged sites (STS). Patients were examined for 15 loci of the Y chromosome. The gene sequences and STS primers used were: sY84, sY86 (AZFa); sY127, sY134 (AZFb); sY254, sY255 (AZFc). Samples were subjected to PCR amplification was performed according Simoni et al (4). PCR products were analysed on a 2% agarose gel containing ethidium bromide (0.5 µg/mL). Thirty normal children were used as controls and analyzed with the same methods.

III. Results

Mean patients age was 1.9 year. Among cryptorchid patients, 19 were affected by bilateral and 15 by unilateral maldescent. Of the 19 patients who have bilateral cryptorchidism, 3 had bilateral retractile testes, 6 had one side inguinal and other side retractile testes, and 10 had bilateral inguinal testes. Of the 15 patients who affected unilateral, 7 had retractile testes and 8 had inguinal testes. None of the children had complications as a result of the surgery. Mean follow up period is 22 months.

Of the 34 patients, cytogenetic analyses were revealed as normal karyotype. PCR analysis was done in these patients. Four patients (11.7%) showed a deletion of one or more STS, namely sY254, sY255 in 3 patient (2 bilateral and one unilateral cryptorchid), and sY84 in 1 patient (bilateral cryptorchid). It can be summarized that 3 patient had deletions of AZFc locus and 1 patient had AZFa deletion alone. The characteristics of the patients were shown in Table 1. The prevalence of Y chromosome microdeletions was greater in patients with a history of bilateral cryptorchidism (3 of 19, 15.8%) than that observed in those with unilateral cryptorchidism (1 of 15, 6.7%). We detected Y chromosome microdeletions none in controls patients.

IV. Discussion

Two important consequences of cryptorchidism are an increased risk of developing testicular cancer as well as a decreased sperm count and infertility. The incidence of infertility is about 32% in men with unilateral and about 59% in men with bilateral cryptorchidism (1). In infertile couples, a medical history of cryptorchidism was found to be present in 8% of men (2). The etiology of cryptorchidism is probably multifactorial, related to extrinsic (extragonadal) or intrinsic (gonadal) causes disrupting testicular descent. Extrinsic pathogenesis may be considered when hormonal abnormalities (such as androgen deficiency) or defects in testicular descent (mechanical anomalies) exist. In such cases the testicular damage of the cryptorchid testis is related only to its prolonged permanence in the abdomen. In fact, the normal descended testis in these forms sometimes appears normal or even hypertrophic, and these patients exhibit normal sperm production (5). Although the etiology of cryptorchidism and the testicular damage associated with it remains unknown, some direct and indirect evidence supports a role for genetic causes. In some cases of unilateral cryptorchidism, the contralateral normally descended testis
might be altered too (1); familial cases have been described (2, 3); and animal models have suggested candidate genes, such as INSL3 (4, 5) and GREAT/LGR8 (6).

Cryptorchidism may be associated with a variety of congenital syndromes, due to either chromosomal aberration or monogenic disease; a genetic etiology for cryptorchidism is also suggested by the observation of a familial occurrence in some cases, as it has been reported undescended testis in 1.5–4.0% of fathers and 6.2% of brothers of cryptorchid subjects (6).

Cryptorchidism can be considered as a cause of infertility in 2-9 % of infertile patients, and in testicular cancer, 5-10 % of men have a history of cryptorchidism (3).

About 15 % azoospermic and 5-10 % oligozoospermic men show Y chromosome deletions, which, however, cannot be predicted cyogenetically or based on clinical findings or semen analysis. Thus, PCR based AZF screening for Yq microdeletions is a compulsory preliminary step in management of cases with severe testiculopathy (1). AZF microdeletions are associated with azoospermia, oligozoospermia and with a varied testis histological profile ranging from Sertoli Cell Only (SCO), hypospermatogenesis to maturation arrest. Deleted in azoospermia (DAZ) and RNA binding motif on Y (RBMY) are AZF genes which code for RNA binding proteins and may be involved in the regulation of gene expression (3).

Current data do not permit any final conclusion on the ethiopathogenic role of Y chromosome mutations in cryptorchidism. Finding a high frequency of microdeletions among 40 patients with unilateral cryptorchidism and severe testicular failure (azoospermia or severe oligozoospermia) led Foresta et al. (11) to propose that microdeletions are probably responsible for testicular damage leading to development of cryptorchidism. On the other hand Fagerli et al. (16) reported no microdeletions in 42 patients with a medical history of cryptorchidism (cryptorchid patients), of whom 14 were affected by azoospermia or oligozoospermia. It has been reported that Yq microdeletions was detected in the AZF region in 27.5 % of cryptorchid and 25.4 % of idiopathic severe testiculopathies. They proposed that in cryptorchid with AZF deletion, the testis failed to descend due to altered response to the mechanism regulating testicular descent. Thus AZFb and AZFc deletion may lead to not only spermatogenic arrest but also impaired testicular descent (1,7,8). Furthermore, the frequency of Y chromosome microdeletions in the Slovene cryptorchid infertile population was found as 2,8 %. It has been reported that, Y chromosome microdeletions are not causally related to cryptorchidism and the frequency of cryptorchidism in patients with Y chromosome microdeletions was lower in comparison to infertile patients without microdeletions. Y microdeletion analysis should be limited to azoospermic and severely oligozoospermic men and candidates for intracytoplasmic sperm injection (9-11).

Increased frequency of Y chromosome microdeletions in patients with cryptorchidism compared with the frequency in infertile patients without cryptorchidism would imply an association between the two phenomena, either directly (Y chromosome microdeletions are involved in the control of testicular descent) or indirectly (Y chromosome microdeletions are primarily responsible for a testicular damage, a consequence of which is cryptorchidism). But decreased frequency of Y chromosome microdeletions in cryptorchid patients implies that cryptorchidism per se (cryptorchid location of the testis) is predominantly responsible for infertility in these patients, which is in accordance with the existing experience (9).
In cryptorchid cases, AZF deletions seem not to be directly implicated in the pathogenesis of cryptorchidism itself, but they rather cause only a severe testiculopathy involving the spermatogenic component. Therefore, cryptorchidism is probably the result of altered testicular response to mechanisms regulating testicular descent (12). AZF deletions determine severe spermatogenic impairment and might be found with similar prevalence in men with severe infertility of unknown origin or related to cryptorchidism. Malposition of the testis in these cases probably is not the direct consequence of the deletion, because AZF genes have never been implicated in testicular descent. It can be hypothesized that the strong testicular damage caused by the absence of a number of AZF genes leads to impaired testicular response to mechanisms regulating testicular descent. In this light, it could be speculated that the absence of only some copies of the DAZ gene is not sufficient to lead to cryptorchidism (9,12).

We provide evidence that Y chromosome microdeletions are importantly associated to cryptorchidism. Therefore, screening for Y chromosome microdeletions in cryptorchid men, particularly candidates for intracytoplasmic sperm injection, is recommended. We have confirmed previous observations (10, 12–15) that Y chromosome microdeletions can be found in patients with cryptorchidism. Therefore, it is reasonable to include these patients in the Y chromosome microdeletion screening, because Y chromosome microdeletions may be iatrogenically transferred to male offspring with the increasing use of intracytoplasmic sperm injection (5).

Conclusions

In this study, we found Yq microdeletions in 11.7% of the cryptorchid patients. This rate is similar with Yq microdeletion in infertile population and corroborate that an association may be between the two phenomena, either directly or indirectly. The diagnosis of Yq microdeletion for the patients is important to understand the possibility of transmitting the genetic etiology to male offspring in assisted reproduction practice. It is also important to undergo sperm cryopreservation for assisted reproduction in such patients. Besides, the diagnosis of AZF microdeletion should be offered to the couples undergoing assisted reproduction, since it is possible to transmit the genetic anomaly to the male offspring.

References


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Table 1. The characteristics of the patients.