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# Variation in the clinical and genetic evaluation of undervirilized boys with bifid scrotum and hypospadias

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## Summary

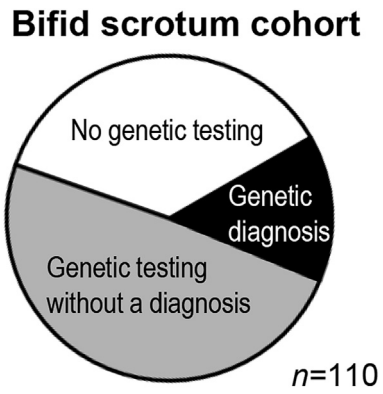
### Background
Bifid scrotum and hypospadias can be signs of undervirilization, yet boys presenting with these findings often do not undergo genetic evaluation. In some cases, identifying an underlying genetic diagnosis can help to optimize clinical care and improve guidance given to patients and families.

### Objectives
The aim of this study was to characterize current practice for genetic evaluation of patients with bifid scrotum, and to identify approaches with a good diagnostic yield.

### Methods
A retrospective study of the Boston Children's Hospital electronic medical records (1993–2015) was conducted using the search term "bifid scrotum" and clinical data were extracted. Data were abstracted into a REDCap database for analysis. Statistical analysis was performed using SPSS, SAS, and Excel software.

### Results
The search identified 110 subjects evaluated in the Urology and/or Endocrinology clinics for bifid scrotum. Genetic testing (including karyotype, microarray, or targeted testing) was performed on 64% of the subjects with bifid scrotum; of those tested, 23% (15% of the total cohort of 110 subjects) received a confirmed genetic diagnosis. Karyotype analysis, when performed, led to a diagnosis in 17% of patients. Of the ten instances when androgen receptor gene sequencing was performed, a pathogenic mutation was identified 20% of the time.

### Conclusion
This study demonstrated that the majority of individuals with moderate undervirilization resulting in bifid scrotum do not receive a genetic diagnosis. Over a third of the analyzed subjects did not have any genetic testing, even though karyotype analysis and androgen receptor (AR) sequencing were both relatively high yield for identifying a genetic etiology. Increased utilization of traditional genetic approaches could significantly improve the ability to find a genetic diagnosis.

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## Introduction

Disorders of male genital development are congenital malformations of the penis and scrotum, and can vary in severity. Fortunately, advances in surgical technique have provided functional improvement for patients, if referred to an experienced surgical team [1,2]. In many cases, however, the underlying etiology of the developmental abnormality is unknown; this lack of a diagnosis may lead to significant distress for patients and their families [3,4]. Androgen receptor mutations provide a clear example of the impact of having a genetic diagnosis. Identification of an androgen receptor mutation allows for targeted clinical care, including the possible use of testosterone, fertility guidance, family testing, and assessment of risk to future children [5,6].

Most disorders of sex development (DSD) conditions can cause a range of phenotypes, from sex reversal to frankly ambiguous genitalia to mild undervirilization resulting in isolated hypospadias. Sex reversal and ambiguous genitalia typically trigger an evaluation for DSD, whereas isolated hypospadias does not, as most cases are thought to be non-hormonal in origin, making a DSD evaluation less likely to identify an underlying diagnosis. However, it is not entirely clear whether a DSD evaluation is warranted for the intermediate phenotype of hypospadias in combination with other signs of potential virilization such as cryptorchidism and/or bifid scrotum.

Although such undervirilization has been well managed surgically, progress in diagnosis has lagged. In many diseases, genetic testing to determine etiology has led to significant progress in understanding pathogenesis and even to targeted treatment, such as in congenital cardiac disease [7], intellectual disability [8], and hyperinsulinism/neonatal diabetes mellitus [9]. Recently, studies have focused on the genetics of DSD that result in frankly ambiguous genitalia [10–13], but relatively few studies have focused on the genetics of milder cases of undervirilization. However, there are recent reports suggesting that a significant number of these milder cases may also have underlying genetic etiologies, including *AR*, *NR5A1*, and *WT1* mutations [14].

Bifid scrotum was chosen as a proxy for undervirilization in this study to evaluate a group of subjects with a relatively narrow range of phenotypes. Bifid scrotum is a midline cleft in the scrotum and can be associated with incomplete fusion of the labioscrotal folds [15]. In the majority of cases, individuals with bifid scrotum also have hypospadias. One potential cause of this phenotype is insufficient testosterone secretion or action: fusion of the labioscrotal folds and urethral localization both take place during the first trimester under the influence of androgens [16]. As such, bifid scrotum with hypospadias may represent a DSD. Individuals with this phenotype are often seen in urology and/or endocrinology clinics and are not consistently classified as having a DSD.

It was hypothesized that few children receive a genetic diagnosis, yet still receive comprehensive surgical and medical management. This hypothesis was based upon clinical experience that only a small fraction of individuals with bifid scrotum, regardless of whether they had additional clinical features, received a diagnosis to explain the underlying mechanism of disease. As syndromic features would potentially point toward possible genetic etiologies, it was also hypothesized that those patients with additional clinical findings were more likely to have additional evaluation and workup. To test these hypotheses, a retrospective chart review of children with bifid scrotum with and without hypospadias was performed to document the characteristics and genetic evaluation of these males at a tertiary care referral center. The review also sought to identify the differences in diagnostic evaluation and outcome between those with isolated genitourinary abnormalities and those with syndromic features.

## Methods

### Cohort

A text-based search system (i2b2) [17] was used to identify subjects seen in the Division of Endocrinology and/or Department of Urology at Boston Children's Hospital between 1993 and 2015 using the search term "bifid scrotum". Additional subjects were prospectively identified through review of Endocrine and/or Urology schedules between December 2014 and May 2015. All subjects in the cohort were actively followed by Endocrinology, Urology, and/or Genetics through at least 2004, with the vast majority being seen more recently. Subject characteristics including gender, race, ethnicity, age at first evaluation, gestational age at birth, severity of hypospadias, genetic evaluation, serum testosterone measurements, and presence of non-genitourinary abnormalities were extracted from review of medical records. Hypospadias was dichotomized as proximal/middle (perineal, scrotal, penoscrotal and penile) and distal (coronal and glanular) to reflect the severity of the hypospadias. Categories of non-genitourinary abnormalities (syndromic features) included cardiac, renal, neurologic, neuro-developmental, gastrointestinal, musculoskeletal, ophthalmologic, endocrine, otolaryngologic, or growth abnormalities.

### Statistical analysis

Study data were collected and managed using Research Electronic Data Capture (REDCap) electronic data capture tools hosted at Boston Children's Hospital [18]. The Boston Children's Hospital Institutional Review Board approved this research. Microsoft Excel (Microsoft, USA), SPSS (IBM, USA), and SAS 9.4 (SAS Institute Inc., USA) software were used for statistical analysis. Groups were compared using Fisher's exact test and two tailed *t*-tests.

### Genetic evaluation

Genetic evaluation was designed to include any of the following: karyotype, chromosomal microarray, or targeted genetic evaluation, including sequencing and deletion testing. None of the patients had whole-exome or whole-genome sequencing. The vast majority of the targeted genetic testing has taken place since 2005 (only four targeted