Family History is Underestimated in Children with Isolated Hypospadias: A French Multicenter Report of 88 Families

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Purpose: While familial forms of complex disorders/differences of sex development have been widely reported, data regarding isolated hypospadias are sparse and a family history is thought to be less frequent. We aimed to determine the frequency of hypospadias in families of boys with hypospadias, to establish whether these familial forms exhibit a particular phenotype and to evaluate the prevalence of genetic defects of the main candidate genes.

Materials and Methods: A total of 395 boys with hypospadias were prospectively screened for a family history with a standardized questionnaire, extensive clinical description, family tree and sequencing of AR, SF1, SRD5A2 and MAMLD1.

Results: Family history of hypospadias was more frequent than expected (88 patients, 22.3%). In 17 instances (19.3%) familial hypospadias cases were multiple. Familial hypospadias was related to the paternal side in 59.1% of cases, consisting of the father himself (30.7%) as well as paternal uncles and cousins. Premature birth, assisted reproductive techniques, other congenital abnormalities and growth retardation were not more frequent in familial hypospadias than in sporadic cases. The severity of phenotype was similar in both groups. The results of genetic analysis combined with previous data on androgen receptor sequencing revealed that familial cases more frequently tend to demonstrate genetic defects than sporadic cases (5.68% vs 1.63%, $p = 0.048$).

Conclusions: Familial forms of hypospadias are far more frequent than previously reported. Even minor and isolated forms justify a full clinical investigation of the family history. Detecting these hereditary forms may help to determine the underlying genetic defects, and may improve followup and counseling of these patients.

Key Words: hypospadias, causality, disorders of sex development, genetics, mutation

Abbreviations and Acronyms

AR = androgen receptor

EDCs = endocrine disrupting chemicals

SF1 = steroidogenic factor 1

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HYPOSPADIAS is defined as congenital hypoplasia of the ventral aspect of the penis with an ectopic opening of the urethra. This condition is the second most frequent genital defect in male newborns after undescended testis, and its prevalence ranges widely according to ethnic group and

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geographic area.[1] Hypospadias occurs more frequently in the Caucasian population, and prevalence is lower among Asians, Hispanics and African Americans,[2] with an average of 1 in 300 live male births.[3] Severity is defined according to level of the division of the corpus spongiosum and ranges from glanular to perineal. Most hypospadias cases are mild,[4] with 50% of cases being anterior.[5,6] This congenital malformation may interfere with normal urination and future sexual intercourse in adult life and often requires surgery, which has a significant complication rate.[7]

The etiology of hypospadias is multifactorial and is likely at the crossroads of 3 factors that may be involved in under masculinization of the fetus.[8,9] First, the genetic background includes genes of gonadal determination, androgen biosynthesis, and phallic development and responsiveness to these hormones. As the genital tubercle grows under the influence of androgens, any alteration in production of or sensitivity to male hormones may lead to the malformation. The mother and the placenta, with their own hormonal production and possible disorders, also deeply influence the hormonal milieu of the fetus. Lastly the occupational environment and living conditions during gestation may expose the fetus to EDCs,[10] including paints, solvents, adhesives, detergents, pesticides, cosmetics and industrial products.[11] Even if the role of EDCs is highly suspected,[11–13] genetics could have a role in individual susceptibility. Thus, familial clustering is not surprising and is reported in 10% of cases.[5,14] Risk recurrence in the male siblings of an affected patient is about 15%.[15–17]

Family history is usually investigated in case of severe phenotype, or in association with cryptorchidism or micropenis. However, data regarding mild and isolated hypospadias are sparse and family history of hypospadias may be underestimated. To date, no prospective study has been performed investigating family background based on a homogeneous cohort of isolated hypospadias cases. The hypotheses of this study were that 1) the frequency of familial forms of hypospadias has been underestimated to date, 2) some phenotypes are particularly prone to be inherited and 3) the prevalence of genetic defects of the main candidate genes (SF1, SRD5A2 and MAMLD1), in addition to a previous AR study,[18] is greater in familial vs sporadic forms.

PATIENTS AND METHODS

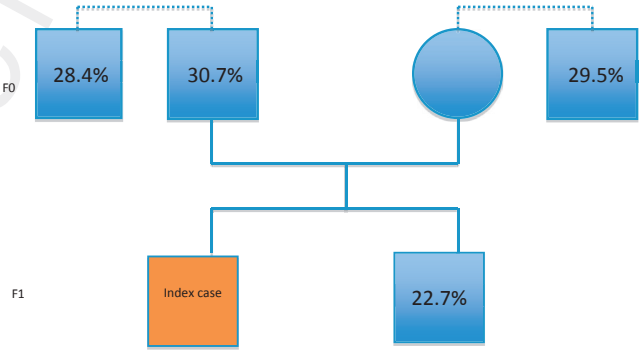
Patients

We prospectively studied 395 consecutive boys presenting with isolated hypospadias (ie no micropenis or cryptorchidism) from January 2010 to December 2015. A pediatric urologist and/or pediatric endocrinologist performed the clinical examination and confirmed the diagnosis. Location of the urethral meatus ranged from glanular to perineal (glanular and penile anterior in 275 patients, mid shaft in 88, penile posterior in 25 and scrotal in 7). Level of division of the corpus spongiosum, which is more reliable for assessing severity of hypospadias, can be evaluated only during degloving of the penis at surgical correction. This method was not used for classification since some of the patients with an anterior meatus did not undergo surgery. Penile length was measured on the dorsal face from the pubis to the top of the stretched penis.

Evaluations of family tree were performed by interviewing the parents with a systematic questionnaire exploring the maternal and paternal sides (see figure). To avoid false-positives results, this history was considered reliable when hypospadias in a relative required a surgical procedure. Ethnic matched and same hospital matched children without urogenital defects or endocrine diseases were included as a control group. The study was approved by the institutional review board (Mediterranean South Personal Protection Center 4, ID RCB 2008-A00781-54), and written consent was obtained from all parents. [F1]

DNA Extraction and Mutation Analysis

Genetic study was performed in all familial and sporadic cases. As previously described, DNA was extracted from peripheral blood with a QIAamp® DNA Blood Mini kit. The manufacturer protocol for DNA isolation was followed with minor modifications. After polymerase chain reaction



Schematic representation of proportion of familial cases in cohort of 88 families. *F0*, generation before index case, ie paternal and maternal levels. *F1*, generation of index case. Squares are for male relatives and circles for female relatives. Left part of schema reveals history of hypospadias in father (30.7%) and paternal uncles or cousins (28.4%). Right part of schema represents maternal side. Sum is greater than 100% since 19.3% of individuals had multiple familial cases. Questionnaire inquired regarding "surgery of urethra in infancy or later for same reason as current case of hypospadias" in different members of family. Question was specifically asked for father and maternal and paternal grandfather of index patient, as well as cousins and uncles (asked of mother for maternal side and of father for paternal side). If brother had hypospadias, he underwent clinical examination. If answer was unknown, it was validated at next appointment after confirmation from parents. Questionnaire was used during 2 consecutive appointments.