Hypospadias is one of the most common birth defects. However, its etiology remains largely unknown. The authors investigated the contribution of genetic and environmental factors to familial aggregation of hypospadias. Using Danish health registers, they identified 5,380 boys diagnosed with hypospadias in a cohort of 1,201,790 boys born in 1973–2005. Using binomial log-linear regression, they estimated recurrence risk ratios of hypospadias for male twin pairs and first-, second-, and third-degree relatives of a hypospadias case, which were 50.8 (95% confidence interval [CI]: 34.2, 75.5), 11.6 (95% CI: 9.75, 13.7), 3.27 (95% CI: 2.47, 4.34), and 1.33 (95% CI: 0.94, 1.88), respectively. Recurrence risk ratios did not differ for family members of a hypospadias case related to the same degree. In addition, the authors found no difference in the recurrence risk ratio for maternal compared with paternal second- and third-degree relatives of a hypospadias case. In conclusion, hypospadias was found to have a strong familial component and also to aggregate within more-distant relatives. Importantly, hypospadias was equally transmitted through the paternal and maternal sides of a family, and recurrence risk ratios for brothers and sons of a hypospadias case were similar. These findings indicate that genetic rather than intrauterine environmental factors have a principal role in causing familial hypospadias.
which are prone to bias; and whereas the familial nature of these anomalies is well recognized, familial aggregation has primarily been defined from studies of first-degree relatives. The primary aim of this large, nationwide, register-based cohort study was to describe the aggregation of hypospadias within same-sex twins and first-, second-, and third-degree relatives and to evaluate the mode of inheritance according to paternal and maternal contributions to the development of hypospadias.

MATERIALS AND METHODS

Study population

Familial aggregation of hypospadias was analyzed in a cohort consisting of all boys born from 1973 to 2005. The boys in the cohort were identified by a unique personal identification number in the Civil Registration System. Information from various sources, including Danish population-based registers, can be linked via the personal identification number. Since April 2, 1968, the Civil Registration System has assigned a personal identification number to all residents who live in Denmark. Furthermore, date and place of birth, sex, and continuously updated information on date of death or emigration are registered in the Civil Registration System.

Identification of relatives

Relatives were identified from the Danish Family Relations Database. This database is based on the parental links as registered in the Civil Registration System. Using this system, one can identify parents by shared address for individuals living at home when the Civil Registration System was created in 1968. Individuals not living at the parent’s home in 1968 generally have no parental link. All individuals born in Denmark in 1968 or later have parental links reflecting the biologic or adoption parents. Consequently, the identities of the father and thereby of half/full brothers and same-sex twins were known for all Danish-born boys in our cohort. For boys in our cohort who are born abroad, the identity of both parents was known for 48 percent. By using the parents’ parental link, one can identify grandfathers and thereby cousins and uncles/nephews. However, because of the above-described construction of parental links, registration of grandparents was almost complete for only those boys born in 1990 or later in Denmark, whereas, for boys born between 1973 and 1989, 47 percent had a known grandfather. For boys born abroad, the figures for the two periods were 9 percent and 51 percent, respectively. It should be emphasized that, although the cohort was restricted to boys born from 1973 to 2005, there was no restriction on the birth cohorts of the relatives besides the one imposed by the way in which the parental links were constructed in the Civil Registration System.

Identification of hypospadias cases

Information on hypospadias status, other congenital malformations, chromosomal abnormalities, and surgeries in the cohort and among relatives was obtained from the Danish Hospital Discharge Register, which contains a nationwide registration of all hospital discharge diagnoses and operations performed from 1977 to 2005 as well as outpatient diagnoses since 1995. Additional information on hypospadias status and other congenital malformations (1973–1986) was available from the Danish Medical Birth Register, in which abnormalities observed at birth and other birth parameters are recorded. Among 6,463 identified hypospadias cases, other congenital malformations were registered for 835 (12.9 percent), and 2,995 (46.3 percent) had been confirmed surgically. Among the identified hypospadias, 339 (5 percent) were listed in only the Danish Medical Birth Register. A total of 5,380 males with a diagnosis of hypospadias born between 1973 and 2005 were identified. Information on hypospadias was not recorded before 1973. Affected probands born prior to 1973 were therefore treated as unaffected in the analyses if they were not registered in the Danish Hospital Discharge Register. Among persons born in the period 1935 to 1972, an average of 22 cases of hypospadias per birth year were recorded in the Danish Hospital Discharge Register, which is 14 percent of the expected number assuming the same average number of hypospadias cases as observed from 1973 onward. This information agrees with our observations that treatment and thus registration of hypospadias were performed in adolescence or later for a large proportion of hypospadias cases. Because hypospadias is a congenital malformation, the date of diagnosis was set to the date of birth.

Statistical analysis

Familial aggregation of hypospadias in first-, second-, and third-degree relatives was evaluated by calculating recurrence risk ratios (RRRs) as the ratio between the risk of recurrence of hypospadias for individuals with a proband (an older affected relative) and the risk for individuals with known (i.e., registered in the Danish Family Relations Database) relatives of the same type, where none of them are a proband. Thus, for instance, if an uncle was affected by hypospadias, the RRR was estimated as the risk for nephews with an affected uncle compared with the risk for nephews with known and only unaffected uncles. Comparing only those individuals with the same type of relatives reduces bias due to incomplete registration of family members in older birth cohorts in the Danish Family Relations Database and furthermore adjusts the RRR for the effect of having a specific relative. For example, the RRR for twins is adjusted for an effect per se of being a twin on the risk of hypospadias. Defining only the older relatives as probands ensures that an affected pair, in which both members are included in the cohort, contributes only once. In male twin pairs, one of them was chosen (at random) to be the “oldest.” First-degree relatives of a proband were defined as offspring (sons) or younger brothers; second-degree relatives as grandchildren, younger half-brothers, or nephews/uncles; and third-degree relatives as younger first cousins. To study the maternal and paternal contributions to the inheritance of hypospadias separately, all probands were further subdivided into maternal and paternal grandfathers, uncles/nephews, half-brothers, and first cousins.
The RRRs were estimated by using binomial log-linear regression with adjustment for birth period (5-year categories). As for twins, we attempted to distinguish between monozygotic and dizygotic twin pairs based on information from the Danish Twin Register (19, 20). The RRR estimates for monozygotic and dizygotic twins were based on tabulations from the Danish Twin Registry. The crude RRR was estimated as the probandwise concordance rate for hypospadias divided by one minus the probandwise concordance rate for not having hypospadias (21). Further adjustment of these data was not possible.

To evaluate the relative importance of genetic and environmental factors for hypospadias, heritability analyses were conducted. These analyses were performed by using the liability threshold model (22), in which liability is considered a latent, underlying trait consisting of both genetic and environmental factors. These two factors are further divided into additive (A) and dominant (D) genetic factors as well as shared (C) and nonshared (E) environmental factors. Four variance component models were run to estimate variance components for these factors. These models are ACE, ADE, AE, and CE, where the AE model consists of an additive genetic component and a nonshared environmental component. The best-fitting model was determined by the use of the Akaike Information Criterion and by the likelihood ratio test. Structural equation modeling techniques implemented in Mx software (23) were used to perform these analyses.

We also evaluated whether a variable length of follow-up might have biased our results by 1) stratifying our cohort by birth period and looking for differences in the RRR for first-, second-, and third-degree relatives’ estimates over time; and 2) using Poisson regression to account for attained age at the end of follow-up in addition to potential confounders.

Ethical approval

The project was approved by the Danish Data Protection Agency.

RESULTS

Of the 1,201,790 males in the study cohort, 5,380 (0.45 percent) had a diagnosis of hypospadias. Among these males, we identified 228 (4.2 percent) who had at least one family member with a history of hypospadias, distributed as follows: 26 male twin pairs (19 of unknown zygosity, five monozygotic, and two dizygotic), 36 father-son pairs, 95 male sibling pairs, six grandfather-grandchild pairs, 10 half-brother pairs, 32 uncles/nephew pairs, and 32 male first-cousin pairs who both had a diagnosis of hypospadias.

Male twin pairs and first-, second-, and third-degree relatives of an older hypospadias case all had a significantly increased RRR of hypospadias. All RRRs were adjusted for birth period (table 1). The overall RRR of hypospadias for twin brothers was 50.8 (95 percent confidence interval [CI]: 34.2, 75.5). We found no significant differences in RRRs between different kinds of first-degree relatives (p = 0.16); that is, the RRR for offspring was 10.4 (95 percent CI: 7.54, 14.3) and for brothers was 13.4 (95 percent CI: 11.0, 16.4) (table 1). The RRR estimates for second-degree relatives were 4.16 (95 percent CI: 1.89, 9.19) for grandchildren, 3.31 (95 percent CI: 1.78, 6.16) for half-brothers, and 3.12 (95 percent CI: 2.21, 4.41) for uncle/nephews. For third-degree relatives (first cousins), the RRR was 1.33 (95 percent CI: 0.94, 1.88).

The overall RRRs adjusted for birth period for first-, second-, and third-degree relatives were 11.6 (95 percent CI: 9.75, 13.7), 3.27 (95 percent CI: 2.47, 4.34), and 1.33 (95 percent CI: 0.94, 1.88), respectively (table 1). We found no difference in the risk of hypospadias for maternal and paternal second- and third-degree relatives; the RRRs were 3.36 (95 percent CI: 2.29, 4.92) versus 3.09 (95 percent CI: 2.04, 4.68) and 1.02 (95 percent CI: 0.58, 1.80) versus 1.63 (95 percent CI: 1.05, 2.53), respectively (table 2). Specifically, the RRR for half-brothers who shared the same mother was 3.60 (95 percent CI: 1.50, 8.65) compared with 3.15 (95 percent CI: 1.31, 7.58) for brothers who shared the same father (table 2).

The overall RRR of 50.8 (95 percent CI: 34.2, 75.5) for twins was subdivided for monozygotic, dizygotic, and twins of unknown zygosity into RRRs of 58.8 (95 percent CI: 22.9, 151), 13.9 (95 percent CI: 3.45, 55.9), and 67.3 (95 percent CI: 38.9, 117), respectively. The rates for monozygotic and dizygotic twins correspond to concordance rates of 33.3 percent (95 percent CI: 12.8, 55.1) and 9.1 percent (95 percent CI: 1.2, 27.8), respectively.

The genetic component in the inheritance of hypospadias was approached by conducting heritability analyses based on twins of known zygosity. The AE model was found to be the best fitting variance component model. The heritability

<table>
<thead>
<tr>
<th>Type of proband</th>
<th>Total no. of probands</th>
<th>No. of affected pairs</th>
<th>RRR† for the relative</th>
<th>95% CI‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Twin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Same-sex twin</td>
<td>78</td>
<td>26</td>
<td>50.8</td>
<td>34.2, 75.5</td>
</tr>
<tr>
<td>First-degree</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brother</td>
<td>1,658</td>
<td>95</td>
<td>11.6</td>
<td>9.75, 13.7</td>
</tr>
<tr>
<td>Father</td>
<td>696</td>
<td>36</td>
<td>13.4</td>
<td>11.0, 16.4</td>
</tr>
<tr>
<td>Second-degree</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Half-brother</td>
<td>663</td>
<td>10</td>
<td>3.31</td>
<td>1.78, 6.16</td>
</tr>
<tr>
<td>Uncle/nephew</td>
<td>1,963</td>
<td>32</td>
<td>3.12</td>
<td>2.21, 4.41</td>
</tr>
<tr>
<td>Grandfather</td>
<td>285</td>
<td>6</td>
<td>4.16</td>
<td>1.89, 9.19</td>
</tr>
<tr>
<td>Third-degree</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First cousin</td>
<td>4,740</td>
<td>32</td>
<td>1.33</td>
<td>0.94, 1.88</td>
</tr>
</tbody>
</table>

* The comparison group in the specific analyses was individuals with no such type of older proband who had at least one registered family member of the type.
† RRR, recurrence risk ratios.
‡ CI, confidence interval.
TABLE 2. Recurrence risk ratios* for hypospadias in relatives of a maternal or a paternal proband, according to type of proband, Denmark, 1973–2005

<table>
<thead>
<tr>
<th>Type of proband</th>
<th>Maternal relative</th>
<th>Paternal relative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RRR†</td>
<td>95% CI†</td>
</tr>
<tr>
<td>Second-degree</td>
<td>3.36</td>
<td>2.29, 4.92</td>
</tr>
<tr>
<td>Grandfather</td>
<td>5.59</td>
<td>2.13, 14.7</td>
</tr>
<tr>
<td>Half-brother</td>
<td>3.60</td>
<td>1.50, 8.65</td>
</tr>
<tr>
<td>Uncle/nephew</td>
<td>3.12</td>
<td>1.94, 5.00</td>
</tr>
<tr>
<td>Third-degree</td>
<td>1.02</td>
<td>0.58, 1.80</td>
</tr>
</tbody>
</table>

* The comparison group in the specific analyses was individuals with no such type of older proband who had at least one registered family member of the type.
† Recurrence risk ratios (RRRs) were adjusted for birth period.
‡ CI, confidence interval.

estimate obtained by this model was 77 percent (95 percent CI: 57 percent, 90 percent).

We performed several additional analyses to assess the robustness of the results. First, we tested different case definitions in the following subanalyses: 1) children of parents born in Denmark and of grandparents not born abroad (approximately 75 percent of the cohort), 2) hypospadias cases with no other congenital malformations or chromosomal abnormalities, and 3) probands who had surgery for hypospadias (according to the registers) in addition to the hypospadias diagnosis. The three analyses did not change the results significantly, but an overall strengthening of the results shown in table 1 was observed when the analysis was restricted to cases without other congenital malformations or to probands operated on for hypospadias. Furthermore, stratified analyses based on the parts of the cohort born before and after 1985 (n = 1,740 and n = 3,640, respectively), as well as results obtained by using a Poisson as opposed to log-linear binomial regression models, differed very little (data not shown), suggesting that period and length of follow-up did not affect our results.

DISCUSSION

In this cohort study, we documented familial aggregation of hypospadias within male twin pairs and first-, second-, and third-degree relatives. These findings extend previous suggestions related to the impact of familial aggregation on the development of hypospadias and underline the significant contribution of genetic inheritance to the development of familial hypospadias. The inheritance appears to be transmitted equally through the paternal and maternal sides of a family.

In studies of twin pairs, Kallen et al. (24) found a concordance rate of 18 percent, whereas another study reported concordance rates of 9 percent and 27 percent for dizygotic and monozygotic twins, respectively (25). These findings are very similar to our data. Previous findings based on multifactorial models of pedigree data have found heritability indices ranging from 54 percent to 74 percent (26, 27). These results are in line with our finding of a heritability of 77 percent, which is in agreement with a strong genetic component in the inheritance of hypospadias. Furthermore, the RRR for dizygotic twin brothers was almost identical to the RRR for brothers of a hypospadias case. Brothers of a hypospadias case have, in smaller descriptive or case-control studies, been estimated to have about a 9 percent chance of developing hypospadias, which corresponds to an RRR of 23–45 depending on incidence in the background population (28, 29). Two studies found that the more severe the hypospadias in an individual, the higher the incidence in first-degree relatives, ranging from 3.5 percent to 16.7 percent (13, 30). In addition, other studies from Scandinavia found that the recurrence risks of hypospadias in siblings were in the range of 4–7 percent, equivalent to an RRR of 10–17.5 assuming a prevalence of 4 per 1,000 liveborn boys (18, 25, 31). Hitherto, to our knowledge only one study has reported recurrence risk in more-distant relatives (32). This particular study included 307 cases who had undergone surgery for hypospadias. The recurrence risks were estimated as 11 percent, 9 percent, 2.2 percent, and 3 percent for younger brothers, fathers, uncles, and cousins of a case, respectively, all higher than the estimates found in the present study.

In general, previous studies report higher estimates of the recurrence risk than we found in our material. This difference may in part be due to inclusion in previous studies of only more severe cases (e.g., those who had undergone surgery) who may have a higher degree of familial aggregation than cases with milder hypospadias (13, 30). Indeed, our overall estimates for first-, second-, and third-degree relatives strengthened when only probands undergoing surgery were included in the analysis (data not shown). Furthermore, most of these studies, except those in the Scandinavian countries, were case-control studies based on questionnaires and interview data, which are especially prone to recall bias and differential misclassification. Such studies also suffered from a varying degree of nonparticipation, which may have led to selection bias toward the more severe cases.

We found that hypospadias in second- and third-degree relatives was inherited equally from the maternal and the paternal sides. It has previously been hypothesized that hypospadias is one symptom of the testicular dysgenesis syndrome, which may be increasingly common because of adverse environmental influences, for example, estrogenic or antiandrogenic substances (33–35). According to this hypothesis, we would expect to find a differential effect of inheritance through the maternal and the paternal sides and, in particular, a difference in the RRR for maternal half-brothers (who share the same intrauterine milieu) and paternal half-brothers. However, the RRR estimates for maternal and paternal half-brothers were almost identical, and we did not find a significantly higher RRR for brothers compared with sons of a hypospadias case. In addition, the RRR for dizygotic twin brothers who share the intrauterine environment during the same pregnancy was not higher than the RRR for brothers, which would have been expected if hypospadias was significantly influenced by environmental exposures in utero.

By examining the pattern of familial RRR, it is possible to evaluate the mode of inheritance. The pattern observed in...
our data regarding hypospadias is most consistent with multiple interacting loci (36).

Introduction of the personal identification number in 1968 and establishment of the Danish Hospital Discharge Register in 1977 combined have provided the opportunity to perform large, population-based studies with good power, a minimum of bias (e.g., recall and selection bias), and high validity (37). One potential limitation of this study was the identification of probands born prior to 1973 because hypospadias was not recorded until 1973. However, treatment and thus registration of hypospadias was performed in adolescence or later for a large proportion of hypospadias cases. Therefore, registration at mainly older ages may be influenced by having an affected relative, which may result in misclassification and, in particular, influence the estimate for grandparents. However, we found no significant differences in the estimates in a subanalysis comparing the parts of the cohort born before and after 1985. Milder forms of hypospadias might not always have been registered in the Danish Hospital Discharge Register, which may have resulted in a tendency toward inclusion of more severe cases; therefore, our estimates may not apply to milder forms of hypospadias with only a cosmetic impact.

In conclusion, on the basis of a large, nationwide, register-based cohort study, we found high heritability of hypospadias that aggregates also within second- and third-degree relatives. Importantly, hypospadias was equally transmitted through the paternal and maternal sides of a family, and twin brothers, brothers, and sons of a hypospadias case had similar RRs. These findings indicate that genetic factors have a principal role in causing familial hypospadias.

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