Aetiological factors in hypospadias

ELISA CALZOLARI*, MARIA RAFFAELLA CONTIERO*,
ELENA RONCARATI†, PIER LUIGI MATTIUZ*, AND SILVIO VOLPATO†

*From *Istituto di Genetica Medica, and †Istituto di Puericultura e Medicina Neonatale, Università di Ferrara, Italy.*

SUMMARY Epidemiological and genetic variables in hypospadias were analysed during the years 1978 to 1983 in a case control study of congenital malformations in the Emilia Romagna region of northern Italy. During the observation period, in a sample of 41 078 male newborns, 168 had hypospadias giving a prevalence at birth of 4.1 in 1000 males. Hypospadias was divided into three types: type I or mild (75.0%); type II or moderate (21.4%); and type III or severe (3.6%). Coexisting malformations were found in 8.9% of cases. The heritability coefficient was 0.669. Maternal risk factors correlated with hypospadias were found to be early age at menarche, threatened abortion, and exposure to progestins. Low birth weight and shorter gestation were also correlated with hypospadias.

Hypospadias is one of the most common urogenital anomalies, with an estimated frequency in males ranging from 2.0 to 4.4 per 1000.^{1–7} In the male embryo fusion of the urethral folds and cannulation of the penile glans occurs between the eighth and 14th week after fertilisation. Hypospadias usually arises from non-fusion of the urethral folds or lack of normal cannulation or both. Since these developmental processes are under the influence of androgenic hormones normally produced by the embryonic testicles, it has been suggested that hypospadias develops as a result of faulty testicular function.^{7–9} In some cases low end organ responsiveness to androgens has been proposed as a cause.^{7–9}

It is generally agreed that both genetic and non-genetic factors are involved in the aetiology of hypospadias.¹⁰ The possibility of autosomal dominant inheritance with low penetrance has been discussed by Kemp,¹¹ Gates,¹² and Lowry and Kliman.¹³ Autosomal recessive inheritance with incomplete manifestation has been proposed by Sørensen,¹⁴ but most authors^{6 15 16} favour a multifactorial model, according to which multiple genetic factors combine with an appropriate intrauterine environment to produce the anatomical defect. According to this model, the threshold for the development of the anomaly is exceeded when a higher than average number of deleterious genes and environmental factors come into play simultaneously. Support for the importance of exogenous

factors in the aetiology of hypospadias has been obtained from studies on various maternal variables associated with the birth of cases of hypospadias, such as maternal drug consumption both before pregnancy (oral contraceptives) and during pregnancy (especially progestins).^{4 6 17–23} In addition, several epidemiological studies of hypospadias have been reported, which indicate an association with low birth weight^{4 6 7 17–19} and, in a few instances, with birth order.^{6 16 23}

The pathogenesis of hypospadias associated with other congenital malformations (18 to 32% of cases)^{7 9 16 17 24} is less well understood. Of interest is the frequent association with cryptorchidism, which is present in 3 to 19% of cases.^{9 15 17} Karyotypes are normal in most cases of hypospadias, but some chromosomal abnormalities have been reported.¹⁶

In the present paper, data on 168 cases of hypospadias delivered in the Emilia Romagna region, northern Italy, during the period 1978 to 1983 are reported. Based on a case control study, the association with genetic and environmental factors has been examined. Heritability of hypospadias in the Emilia Romagna population has been estimated.

Materials and methods

THE EMILIA ROMAGNA REGISTER FOR CONGENITAL MALFORMATIONS

In 1978 a register of congenital malformations was established in Emilia Romagna by IMER (Indagine Malformazioni Congenite in Emilia Romagna).^{25 26}

Received for publication 16 January 1985.

Revised version accepted for publication 27 March 1985.

The register contains information on still- and livebirths from the 28th gestational week to the first week of life. The register is a case control study, the control being the birth closest to the delivery of the malformed baby. The information is collected both for malformed and control subjects by interviewing parents using a specially designed form. In addition, the study provides the basis for descriptive epidemiology in the study area (including prevalence at birth). At present, registration is based on voluntary notification from 42 hospitals covering a population of approximately 80% of the regional births.

POPULATION UNDER STUDY

A total of 168 cases of hypospadias and 378 male controls registered during the 1978 to 1983 period within the IMER study is included. Cases of hypospadias were classified in three types according to the position of the urethral meatus (fig 1).

FACTORS ASSOCIATED WITH HYPOSPADIAS

Among the factors included in the registration forms the following were studied: seasonality of birth, parental age at birth, maternal age at menarche, characteristics of the menstrual cycle (regular versus irregular), oral contraceptives, history of selected maternal diseases (infections and metabolic diseases), threatened abortion, exposure to progestins during pregnancy, and other factors including marital status, parity, previous abortions, and exposure to alcohol, cigarettes, toxins, and radiation before the index pregnancy (table 1).

GENETICS OF HYPOSPADIAS

The presence of hypospadias in first degree male relatives was recorded in the course of parent

FIG 1 Distribution of types of hypospadias. (Adapted from Avellan.¹⁰)

TABLE 1 Factors studied in relation to hypospadias.

Factors	Other studies* (reference numbers)	Present study
Seasonality	6 18	NS
Parental age		NS
Marital status		NS
Consanguinity		NS
Menarche	23	p<0.001
Characteristics of menstrual cycle	23	NS
Oral contraceptives		NS
Threatened abortion	6, 18	p<0.05
Progestins during pregnancy	4 6 17 18 19 21	p<0.05
Parity	6 16 23	NS
Previous abortion		NS
Exposure to toxins		NS
Exposure to radiation		NS
Maternal diseases		NS
Associated anomalies	7 16	NS
Birth weight	4 6 7 16 17 18 19	p<0.001
Length of gestation	7	p<0.001
Twins	6 17 18	NS

* Studies in which significant correlations have been found.

interviews. None of the 166 fathers (only 166 since four cases were twins) or 33 brothers was examined directly. Heritability was estimated according to the method of Falconer.²⁷

STATISTICAL ANALYSIS

Statistical tests included the χ^2 test for the presence or absence of the characteristic under study in index patients and controls and the *t* test for comparison of means.

Results

CHARACTERISTICS OF THE POPULATION UNDER STUDY

During the study period, 168 cases of hypospadias out of a total of 41 078 male births were detected in the Emilia Romagna region, giving an incidence of 4.1 per 1000. The distribution of the three types of hypospadias (fig 1) was 126 (75.0%) of type I (mild), 36 (21.4%) of type II (moderate), and six (3.6%) of type III (severe). There were 153 (91.1%) isolated cases and 15 (8.9%) associated with other malformations (table 2).

FACTORS ASSOCIATED WITH HYPOSPADIAS

Genetic factors

Consanguinity of the parents. There was no instance of parental consanguinity in the 168 cases under study.

Cytogenetics. Karyotypes were obtained in 53 cases; all were normal.

Occurrence in twins. Among our 168 index patients, nine twins were observed. Five of them (three monozygotic and two dizygotic) had an unaffected twin brother and two an unaffected twin

TABLE 2 Associated anomalies; each association occurred once.

Absence of frenum of the tongue
Spina bifida, hydrocephalus
Haemangioma
Ventricular septal defect
Hydrocephalus, polycystic kidney
Cleft lip and palate
Anomalies of foot
Camptodactyly of fifth toe
Hydrocephalus
Syndactyly
Atrial septal defect, haemangioma
Haemangioma
Talipes
Thanatophoric dwarfism
Franceschetti syndrome

TABLE 3 Distribution of age at menarche of mothers of cases of hypospadias and controls.

Age at menarche	Hypospadias		Control group		Expected
	No	%	No	%	
≤10	8	11.94	4	2.86	1.91
11	12	17.91	34	24.29	16.27
12	22	32.84	41	29.28	19.62
13	15	22.39	32	22.86	15.32
≥14	10	14.92	29	20.71	13.88
Total	67		140		

$\chi^2=21.75$, $df=4$, $p<0.001$.

controls. The difference is significant ($\chi^2=5.03$, $df 1$, $p<0.05$).

Progestins. A total of 51 cases out of 160 (31.9%), and 82 out of 371 controls (22.1%) were exposed to progestins during pregnancy. The treatment was for threatened abortion or for a history of previous abortion or both. The difference is significant ($\chi^2=5.68$, $df 1$, $p<0.05$).

Birth weight and length of gestation. The mean birth weight for the index cases was 2968 g and 3392 g for the control group. The difference is significant ($t=6.62$, $p<0.001$). In particular, 45 cases out of 164 (27.4%) were 'small for dates' (fig 2) as opposed to 10% in the controls ($p<0.001$). Of these 45, 17 (37.8%) were also growth retarded, with

sister. Hypospadias was observed in two identical twin brothers.

Environmental factors

Age at menarche. A higher proportion of early menarche (10 years of age or less) was present in the mothers of cases versus controls (table 3). The difference is significant ($\chi^2=21.75$, $df 4$, $p<0.001$).

Threatened abortion. During the index pregnancy, threatened abortion was diagnosed in 28 (17.4%) cases out of 161 and in 39 (10.4%) out of 375

FIG 2 Distribution of weight of cases of hypospadias according to gestational age. ●=type I, ○=type II, ◇=type III.

weight, head circumference, and length below the 10th centile.

However, as shown in table 4, there was no correlation between the reduction of length, weight, and head circumference and the severity of hypospadias. Furthermore, 19 out of 164 (11.6%) index pregnancies were shorter than 36 complete weeks in contrast to 2.1% (seven cases out of 337) in the controls. The difference is significant ($\chi^2=20.27$, df 1, $p<0.001$).

Other factors. There was no demonstrable difference in our study between mothers of newborns with or without hypospadias in respect to the other risk factors considered (seasonality, parental age, characteristics of the menstrual cycle, maternal disease, oral contraceptives before conception, marital status, parity, previous abortions, and exposure to alcohol, cigarettes, toxins and radiation before the index pregnancy).

Combination of environmental factors considered. Table 5 shows the distribution of the three types of hypospadias (mild, moderate, severe) as a function of the presence of one or more of the four environmental risk factors shown to be related to the condition (menarche, progestins, threatened abortion, low birth weight). The data obtained indicate that the severity of hypospadias does not depend on the simultaneous presence of the four risk factors.

TABLE 4 Distribution of the three types of hypospadias as a function of growth retardation.

Type	Degree of growth retardation*				
	0	1	2	3	Not stated
I	62	13	15	9	27
II	17	3	2	8	6
III	3	2	—	—	1
Total	82	18	17	17	34

* The different degree of growth retardation indicates the absence or the presence of one or more of the following variables: birth weight, length, head circumference, all lower than the 10th centile.

TABLE 5 Distribution of the three types of hypospadias as a function of the presence or absence of the four environmental risk factors significantly related to the malformation in the present study (early menarche, progestins, threatened abortion, low birth weight).

Type	No of risk factors*				
	0	1	2	3	4
I	70	37	14	5	—
II	16	10	10	—	—
III	3	1	2	—	—
Total	89	48	26	5	—

* The numbers indicate the presence or absence of one or more of the four risk factors.

TABLE 6 Incidence of hypospadias in relatives of index cases and in the general population*.

	A	N	q	p
General population (males)	—	—	0.00409	0.99591
Relatives of index cases (male probands, male relatives)	10	204	0.04902	0.95098

*Adapted from Chen and Woolley.¹⁶

A=observed number of affected subjects in the sample.

N=total number of subjects in the sample.

q=incidence.

p=1-q.

ESTIMATION OF HERITABILITY IN SIBS AND FIRST DEGREE RELATIVES

It is generally accepted that the best estimate of risk to sibs is to ascertain affected sibs through index cases. Since hypospadias affects only males, only first degree male relatives were considered.

Excluding twins as sibs, the 168 index cases had a total of 33 brothers. Of these, three (9.1%) were found to have hypospadias, with an empirical risk of about 1 in 11. The data needed to estimate the heritability of hypospadias are given in table 6. The 204 first degree relatives included 33 brothers, five unaffected twins, and 166 fathers. In the sample, five fathers and five brothers (three single and two twins) were affected. Assuming multifactorial inheritance, the estimated heritability for first degree relatives, according to the method of Falconer,²⁷ is 66.9%.

Discussion

Both the frequency and the severity of hypospadias seen in Emilia Romagna are similar to those observed in other countries.¹⁻⁷ The frequency of the condition associated with other defects (8.9%) is also close to that reported by Avellan in Sweden (8.6%).¹⁰ No apparent increased tendency to association with other anomalies was found in our population, apart from chordee, meatal stenosis, and undescended testes (present in most cases), as found in other studies.¹⁶

The risk of recurrence is 9.1%, similar to that found by Sørensen¹⁴ (9.6%) and Chen and Woolley¹⁶ (9.7%). Heritability²⁷ in first degree relatives is 66.9%, which is consistent with the data reported in other populations by Czeizel *et al*⁶ (65%), Chen and Woolley¹⁶ (74%), and Neto *et al*¹⁷ (68%).

Among the 18 variables considered in the present study, four are significantly related to the presence of hypospadias, namely menarche, progestins, threatened abortion, and low birth weight. It is worth noting that the severity of hypospadias is not related to the severity of weight reduction nor to

the simultaneous presence of the four risk factors shown to be significantly correlated with the malformation. This could be an indication that the severity of the condition is determined genetically rather than by the action of risk factors.

Alternatively, the degree of hypospadias could depend on the gestational age at which the risk factors act, the more severe condition being determined by factors acting in the earlier phases of the cannulation of the penile glans.

As proposed by others the risk factors identified in the present study could facilitate or determine the appearance of hypospadias through endocrine mechanisms.

This work was supported by Grant MPI 40% 1981, 1982, 1983 and Fondo Regionale. Progetto obiettivo n 3553/6513. We are grateful to Dr Guido Barbuani (Institute of Zoology, University of Ferrara) and to Professor Francesco Conconi (Institute of Biochemistry, University of Ferrara) for their helpful comments during manuscript preparation. We also thank all those institutions in which cases were identified.

References

- ¹ Sorsby A. *Clinical genetics*. London: Butterworths, 1953.
- ² Fishbein M. *Birth defects*. Philadelphia: Lippincott, 1963.
- ³ Chung CS, Myrianthopoulos NC. Racial and prenatal factors in major congenital malformations. *Am J Hum Genet* 1968;**20**: 44-60.
- ⁴ Sweet RA, Schrott HG, Kurland R, *et al*. Study of the incidence of hypospadias in Rochester, Minnesota, 1940-1970, and a case-control comparison of possible etiologic factors. *Mayo Clin Proc* 1974;**49**:52-8.
- ⁵ Avellan L. The incidence of hypospadias in Sweden. *Scand J Plast Reconstr Surg* 1975;**9**:129-39.
- ⁶ Czeizel A, Toth J, Erodi E. Aetiological studies of hypospadias in Hungary. *Hum Hered* 1979;**29**:166-71.
- ⁷ Källén B, Winberg J. An aetiological study of hypospadias in Sweden. *Acta Paediatr Scand [Suppl]* 1982;**239**:1-21.
- ⁸ Aarskog D. Clinical and cytogenetic studies in hypospadias. *Acta Paediatr Scand [Suppl]* 1970;**203**:1-62.
- ⁹ Svensson J. Male hypospadias, 625 cases, associated malformations and possible aetiological factors. *Acta Paediatr Scand* 1979;**68**:587-92.
- ¹⁰ Avellan L. On aetiological factors in hypospadias. *Scand J Plast Reconstr Surg* 1977;**11**:115-23.
- ¹¹ Kemp T. Erbbiologie und erbpathologie des Geschlechtsapparates. In: *Handbuch der Erbbiologie des Menschen*. Vol 4. Berlin: Springer Verlag, 1940;939-41.
- ¹² Gates RR. *Human genetics*. Vol 2. New York: Macmillan, 1948;852-3.
- ¹³ Lowry RB, Kliman MR. Hypospadias in successive generations: possible dominant gene inheritance. *Clin Genet* 1976;**9**:285.
- ¹⁴ Sørensen HR. *Hypospadias with special reference to aetiology*. Copenhagen: Munksgaard, 1953.
- ¹⁵ Bauer SB, Retik AB, Colodny AH. Genetic aspects of hypospadias. *Urol Clin North Am* 1981;**8**:559-64.
- ¹⁶ Chen YC, Woolley PV Jr. Genetic studies on hypospadias in males. *J Med Genet* 1971;**8**:153-9.
- ¹⁷ Neto RM, Castilla EE, Paz JE. Hypospadias: an epidemiological study in Latin America. *Am J Med Genet* 1981;**10**:5-19.
- ¹⁸ Aarskog D. Maternal progestins as a possible cause of hypospadias. *N Engl J Med* 1979;**300**:75-8.
- ¹⁹ Heinonen OP, Sloane D, Shapiro S. *Birth defects and drugs in pregnancy*. Littleton, Massachusetts: Publishing Sciences Group, 1977.
- ²⁰ Janerich DT, Piper J, Glebatis DM. Oral contraceptives and birth defects. *Am J Epidemiol* 1980;**112**:73-9.
- ²¹ Mau G. Progestins during pregnancy and hypospadias. *Teratology* 1981;**24**:285-7.
- ²² World Health Organization. The effects of female sex hormones on fetal development and infant health. *WHO Technical Report Series* No 657. Geneva: WHO, 1981.
- ²³ Polednak AP, Janerich DT. Maternal characteristics and hypospadias: a case-control study. *Teratology* 1983;**28**:67-73.
- ²⁴ Fallon B, Devine CJ, Horton CE. Congenital anomalies associated with hypospadias. *J Urol* 1976;**116**:585.
- ²⁵ Weatherall JAC, De Wals P, Lechat MF. Evaluation of information systems for the surveillance of congenital malformations. *Int J Epidemiol* 1984;**13**:193-6.
- ²⁶ Volpato S, Calzolari E. La sorveglianza delle malformazioni congenite. *Prosp Pediatr* 1980;**38**:145-54.
- ²⁷ Falconer DS. The inheritance of liability to certain diseases, estimated from the incidence among relatives. *Ann Hum Genet* 1965;**29**:51-76.

Correspondence and request for reprints to Dr E Calzolari, Istituto di Genetica Medica, Università degli Studi di Ferrara, Via L. Borsari 46, 44100 Ferrara, Italy.