Genetic Studies on Hypospadias in Males

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Hypospadias is one of the most common urogenital anomalies. It is due to a failure of complete fusion of the urethral folds in the early embryonic stage (Norman, 1963). An excellent review on the possible mode of inheritance was presented by Gates (1946). Other investigators (Sørensen, 1953; Ross, Farmer, and Lindsay, 1959; Rodriguez, 1965) also reported the familial occurrence of this condition. This anomaly appears to be inherited, yet the exact mode of inheritance has not been well delineated.

In hypospadias, karyotypes are normal in most cases. However, many types of chromosomal abnormalities in hypospadias, have been reported in the literature: 47 chromosomes with XXY sex complement (Klotz and Sors, 1958; Halbrecht, 1960; Gray, 1961; Passarge and Thompson, 1964); 47 chromosomes with XYY sex chromosome pattern (Milcu *et al*, 1964; Chen *et al*, 1968); a presumably divided Y chromosome translocated to a chromosome of the 13-15 and of the 21-22 group (de Grouchy *et al*, 1963); a translocation between a Y chromosome and a number 21 chromosome (Turpin and Lejeune, 1960); partial deletion of short arms of a number 4 chromosome (Hirschorn, Cooper, and Firschein, 1965; Leao, Neu, and Gardner, 1966); and a deletion of a Y chromosome (Muldal and Ockey, 1962). It appears that these chromosomal abnormalities showed great variations from one hypospadiac to another and no definite aetiological correlations have been established.

This investigation was undertaken to study genetic and non-genetic factors of hypospadias in males, to estimate its heritability assuming the genetic component to be multifactorial, and to analyse the cytogenetic findings.

Materials and Methods

Eighty-five cases of male hypospadiacs have been observed during 1961 to 1967 at the Children's Hospital of Michigan. Seventy-three cases were admitted for operation, and complete necropsy was done in 6 other

cases. The remaining 6 cases were diagnosed in the clinic without surgical correction. Their hospital records were systematically reviewed. A questionnaire was designed to obtain the information about date of birth, birth weight, birth rank, parental ages at birth, associated anomalies, presence of consanguinity, and the affected individuals in the relatives. Of 85 patients sent questionnaires, 50 (59%) replied fully. The parents were invited to bring their children to the clinic for careful physical examination. For those patients who could not be examined, the parents were telephoned to ascertain that the information thus obtained was accurate. We were able to conduct cytogenetic studies in 26 cases (Appendix I).

The general population data for birth weight, maternal age at birth, birth order, and seasonal incidence are those of the State of Michigan in 1964.

Results

The Incidence in the General Population. Steiner (1936) estimated the incidence of hypospadias in the general population to be 1 in 300 to 1 in 1000 male births. The estimate of Sorsby (1953) and that of Fischbein (1963) were very close, being 1 in 330 male births versus 1 in 380 male births. Chung and Myrianthopoulos (1968) reported the incidence of 305 cases of hypospadias per 10,000 births for Caucasian boys and 19.5 for the Negro boys. There are 12 Negro boys (24%) in the present study.

Birth Weights. The observed and expected distributions of birth weights are shown in Table I. There are 19 (38%) premature infants of birth weight less than 2.5 kg; of these, 5 weighed less than 1.5 kg. The birth weight of the smallest one was only 0.9 kg. The mean birth weight for the index cases was 2.7 kg and 3.3 kg for the general population. The difference is statistically highly significant ($t = 3.39$, $df = 98$, $p < 0.001$).

Parental Age at Birth. The observed and expected distributions of maternal age at birth are shown in Table II. The mean maternal age for the

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TABLE I
COMPARISON BETWEEN
OBSERVED AND EXPECTED
DISTRIBUTION OF BIRTH
WEIGHT

Birth Weight (g)	Index Cases	General Population
501-1,000	1	0.24
1,001-1,500	4	0.34
1,501-2,000	8	0.73
2,001-2,500	6	2.44
2,501-3,000	6	8.79
3,001-3,500	18	18.58
3,501-4,000	3	13.82
4,001-4,500	2	4.14
4,501-5,000	1	0.80
5,001-5,500	1	0.12
Total	50	50.00
Mean	2760.00	3312.60
SD	955.60	622.20
t	3.39	
p	< 0.001	

index cases was 24.6 years and 25.9 for the general population. The difference is not significant ($t=1.005$, $df=98$, $0.3 < p < 0.4$). Mean paternal age at birth was 26.80 ± 5.74 . The significance of this figure cannot be determined because of lack of a suitable control population.

TABLE II
COMPARISON BETWEEN
OBSERVED AND EXPECTED
DISTRIBUTION OF
MATERNAL AGE AT BIRTH

Maternal Age (yr)	Index Cases	General Population
15-19	8	6.41
20-24	19	17.91
25-29	15	12.76
30-34	6	7.57
35-39	1	4.14
40-44	1	1.21
Total	50	50.00
Mean	24.60	25.87
SD	6.41	6.23
t	1.005	
p	$0.3 < p < 0.4$	

Birth Orders. The birth order of index cases can be found in Appendix II. The observed and expected distributions of birth order are shown in Table III. The mean birth order for the index cases was 2.08 and 2.77 for the general population. The difference is significant ($t=2.115$, $df=98$, $p < 0.005$).

Seasonal Incidence. The observed and expected quarterly distributions are shown in Table

IV. There was no significant difference between these two groups ($\chi^2 = 2.79$, $df = 3$, $0.25 < p < 0.50$).

TABLE III
COMPARISON BETWEEN
THE OBSERVED AND
EXPECTED
DISTRIBUTION OF
BIRTH ORDER

Birth Order	Index Cases	General Population
1	27	13.90
2	9	11.87
3	7	9.58
4	2	6.60
5	2	4.16
6	1	2.43
7	2	1.46
Total	50	50.00
Mean	2.08	2.77
SD	1.60	1.65
t	2.115	
p	$0.02 < p < 0.05$	

TABLE IV
COMPARISON BETWEEN
EXPECTED AND OBSERVED
QUARTERLY
DISTRIBUTION OF
BIRTHS

Quarter	Index Cases	General Population
First	13	12.16
Second	15	12.43
Third	8	13.08
Fourth	14	12.33
Total	50	50.00

$\chi^2 = 2.79$ with 3 degrees of freedom; $0.25 < p < 0.50$

Associated Anomalies. Thirty-one (62%) index cases had single or multiple associated anomalies (Table V). This is much greater than the expected evidence of anomalies in the general population. Excluding the complications of chor-dee and meatal stenosis, which were present in most cases of hypospadias in various degrees, the most frequent association was genito-urinary anomalies (35%) followed by hernias (15%). This agrees with the study of Ross *et al* (1959) where undescended testes (62%) were most often encountered followed by hernias (32%).

Consanguinity of Parents. There was no instance of parental consanguinity in our 50 index cases.

Twins. Four index cases (4, 6, 11, and 42) had unaffected dizygotic twin brothers and one (5) an unaffected twin sister. Identical coronal hypospadias has been reported by Bourne (1961) in 12-year-old identical twin brothers.

TABLE V
ASSOCIATED ANOMALIES IN 31 (62%)
INDEX CASES

Types	Numbers
Genito-Urinary	18
Cryptorchidism	9
Bifid scrotum	5
Others	4
Hernias	7
Inguinal	5
Umbilical	1
Diaphragmatic	1
Growth and/or mental retardation	5
Congenital heart diseases	4
Gastrointestinal	4
Central nervous system disorders	3
Down's syndrome	1
Miscellaneous	32
Total	74

Risk Figures and the Affected Sibs. The best estimate of risk to sibs is perhaps to ascertain affected sibs through index cases. Excluding twins as sibs, the 50 index cases had a total of 62 sibs. Of these, 6 (9.7%) were found to have hypospadias, ie with an empirical risk of about 1 in 10. Sørensen (1953) estimated it as 9.6%. To calculate the recurrence risk in sibs where the malformation in the index patient was essentially single and not part of multiple malformations, families 1, 2, 6, 7, 9, 16, 18, 20, 25, 29, 30, 31, 36, 41, 45, 46, and 50 were excluded from calculation. This in fact gives a similar recurrence risk of 5 in 48 (10.4%). Though risks for the sons of affected individuals have not been well established, they are probably below these figures. Information on types of hypospadias, presence of associated anomalies, and karyotypes of affected sibs are presented in Table VI. All affected sibs were personally examined at the clinic except in the case of family 23 as the affected sib was not available because he had died of multiple congenital anomalies at four days of age.

TABLE VI
TYPE OF HYPOSPADIAS, ASSOCIATE ANOMALIES,
AND KARYOTYPES OF AFFECTED SIBS

Family No.	Type of	Associate Anomalies	Karyotype
23	Unknown	Multiple congenital anomalies	Not done
24	Glandar	None	Not done
40	Glandar	Umbilical hernia	46,XY
42	Glandar	None	Not done
49	Penile	None	Not done
50	Penile	None	46,XY

In addition to 6 affected with hypospadias among the sibs of propositi, there were two first cousins affected in family 15. All the affected relatives were surgically confirmed.

Estimation of the Heritability. Since the incidence of hypospadias differs between males and females, the sexes of both propositi and the first degree relatives must be treated separately to yield the estimate of the regression. The incidence of hypospadias in males in the general population is assumed to be estimated without error. The following calculations were according to the method of Falconer (1965). The data needed to estimate the heritability of hypospadias in males are given in Table VII. The 117 male first degree relatives include the 62 brothers, the 4 twin brothers and the 50 normal fathers and one normal son. The regression coefficient for the like sex relative is 0.37. Heritability of this anomaly is 74.1% and the standard error of heritability is 12.6%.

TABLE VII
COMPARISON OF INCIDENCE IN RELATIVES OF
INDEX CASES AND IN GENERAL POPULATION

	A	N	q	p	x	a
General population (males)	—	—	0.0028	0.9972	2.770	3.070
Relatives of index cases (male propositi—male relatives)	6	117	0.0513	0.9497	1.632	2.052

A = observed number of affected individuals in the sample.
N = total number of individuals in the sample.
q = incidence.
p = 1 - q
x = normal deviation of affected individuals, and
a = Zq ie, the mean deviation of affected individuals (adopted from Falconer, 1965).

Cytogenetics. Blood cultures were performed on peripheral lymphocytes according to the method of Moorhead *et al* (1960) with some modifications. For culture of skin tissue, a small piece of skin was obtained from the buttocks and cultured according to the technique of Basrur, Basrur, and Gilman (1963). For autoradiographic studies, tritiated thymidine (1.9 c/mM Schwarz Bio Research, 1 μ C/ml medium) was added 4 to 5 hours before fixation. Chromosome analysis was performed on 26 index cases. The following three cases were found to have abnormal karyotypes.

CASE 21. Cytogenetic evaluation of 53 metaphase preparations from peripheral blood cultures showed a modal number of 47 with only few cells deviated from this number. Karyotypic analysis of 15 well-spread metaphases revealed the presence of an extra small

FIG. 1. Metaphase preparation of a peripheral blood cell of case 21 showing chromosome morphology and tritiated thymidine labelling pattern. Note two late replicating Y's and a translocation between long arms of two C group chromosomes probably number 8 and number 12.

FIG. 2. Karyotype of case 29 from skin culture with 46 chromosomes showing XY sex complement.

acrocentric chromosome, which is interpreted as a Y sex chromosome by its morphological characteristics and its late replicating pattern from autoradiographic studies, and a translocation involving two chromosomes of the 6-12 group most probably between number 8 and number 12 (Fig. 1). The chromosome analysis of his parents showed no abnormality.

CASE 29. Forty-six chromosomes were present in all of the 20 cells counted from the peripheral blood culture. Detailed karyotypic analysis of 10 metaphases revealed a normal sex chromosome complement. Skin culture, however, showed a two cell-lines, one with XY and one XX sex complement (Figs. 2 and 3). About 40% XX cell line in the skin culture probably accounts for the

FIG. 3. Karyotype of case 29 from skin culture with 46 chromosomes showing XX sex complement.

FIG. 4. Karyotype of case 50 from peripheral blood culture with 47 chromosomes showing an extra 21 chromosome.

low buccal smear count (9% chromatin positive, of the patient.

CASE 50. Counts were made of 24 cells from the peripheral blood culture. The modal number was 47. The karyotypic analysis revealed a chromosome complement of 47,XY,21+ (Fig. 4) which is compatible with his typical clinical features of Down's syndrome.

Summary

The families of 50 index cases with hypospadias were studied. Of these, 20 (40%) were of glandular type, 23 (46%) of penile type, and 7 (14%) of penoscrotal or perineal type.

The incidence in the general population is estimated to be in the range of 1 in 300 to 1 in 1000 male births. The empirical risks for the siblings is estimated as 1 in 10 in this study. The mean birth weight for the index cases was significantly less than that for the general population. There is no effect of maternal age and seasonal variation. There is evidence of increase of probability of hypospadias in males with an early birth rank. Associated anomalies in hypospadiacs were more than coincidental. The most common anomaly was undescended testes. Parental consanguinity was not found. Five dizygotic co-twins were not affected.

The incidence of hypospadias in relatives of index cases is compared with the estimated incidence in a general population, and the heritability of the anomaly is found to be $74.1 \pm 12.6\%$.

Three cases were found to have abnormal karyotypes. Case 21 had XYY sex complement with a translocation involving two chromosomes of the C group, case 29 had mosaicism for sex chromosome (XX/XY) from skin culture but normal XY sex complement from peripheral blood culture, and case 50 had trisomy 21. Though an increased incidence (6%) of chromosomal abnormalities was found in this study, no definite etiologic relationship between certain chromosomal abnormalities and hypospadias could be established.

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REFERENCES

- Balodimos, M. C., Lisco, H., Irwin, I., Merrill, W., and Dingman, J. F. (1966). XYY karyotype in a case of familial hypogonadism. *Journal of Clinical Endocrinology and Metabolism*, **26**, 443-452.
- Basrur, P. K., Basrur, V. R., and Gilman, J. P. W. (1963). A simple method for short term cultures from small biopsies. *Experimental Cell Research*, **30**, 229-232.
- Bourne, N. W. (1961). Urologist's Correspondence Club Letter, May 20, Cited from Campbell, M. F. (1963). *Urology*, Vol. 2, 2nd ed., W. B. Saunders, Philadelphia.
- Chen, Y. C., Smith, A., Luthardt, F., Nunes-Vas, C., and Woolley, P. V. (1968). Abnormal chromosome constitutions of various congenital syndromes during 1961-1966. *Mammalian Chromosome Newsletter*, **9**, 7-15.
- Chung, C. S. and Myrianthopoulos, N. C. (1968). Racial and prenatal factors in major congenital malformations. *American Journal of Human Genetics*, **20**, 44-60.
- Grouchy, J. de, Josso, N., Lamy, M., Frezal, J., Nezelof, C., Feintuch, G. (1963). Syndrome de Klinefelter chez un nourrisson hypospade. Caryotype à 46 chromosome. *Annales de Pédiatrie*, **10**, 173-177.
- Falconer, D. S. (1965). The inheritance of liability to certain diseases, estimated from the incidence among relatives. *Annals of Human Genetics*, **29**, 51-76.
- Fishbein, M. (1963). *Birth Defects*, Lippincott, Philadelphia.
- Gates, R. R. (1946). *Human Genetics*, Macmillan, New York.
- Gray, J. (1961). Hypospadias with 47/XXY karyotype. *Lancet*, **1**, 722.
- Halbrecht, I. (1960). Nuclear sex determination in azoospermic adults and in newborns with hypospadias. *Fertility and Sterility*, **11**, 112-117.
- Hirschhorn, K., Cooper, H. L., and Firschein, I. L. (1965). Deletion of short arms of chromosome 4-5 in a child with defects of midline fusion. *Humangenetik*, **1**, 479-482.
- Klotz, H. P. and Sors, C. (1958). A propos du syndrome de Klinefelter et de son diagnostic précoce prépubertaire. *Annales d'endocrinologie*, **19**, 128-133.
- Leao, J. C., Neu, R., and Gardner, L. I. (1966). Hypospadias and other anomalies associated with partial deletion of short arms of chromosome No. 4. *Lancet*, **1**, 493-494.
- Milcu, S.-M., Negoescu, I., Maximilian, C., Garoiu, M., Augustin, M., and Iliescu, I. (1964). Biat cu hipospadias si cariotip XYY. *Studii si cercetări de endocrinologie*, **15**, 347-349.
- Moorhead, P. S., Nowell, P. C., Mellman, W. J., Battips, D. M., and Hungerford, D. A. (1960). Chromosome preparations of leukocytes cultured from human peripheral blood. *Experiment Cell Research*, **20**, 613-616.
- Muldal, S. and Ockey, C. H. (1962). Deletions of Y chromosome in a family with muscular dystrophy and hypospadias. *British Medical Journal*, **1**, 291-294.
- Norman, A. P. (1963). *Congenital Anomalies in Infancy*, Ch. 6, Davis, Philadelphia.
- Passarge, E. and Thomson, J. N. (1964). Autoradiography in a boy with XXY karyotype. *American Journal of Diseases of Children*, **108**, 184-188.
- Rodriguez, O. T. (1965). Incidencia familiar de ciertas anomalías congenitas urinarias. *Revista venezolana de urología*, **17**, 321.
- Ross, J. F., Farmer, A. W., and Lindsay, W. K. (1959). Hypospadias: A review of 230 cases. *Plastic and Reconstructive Surgery and The Transplantation Bulletin*, **24**, 357-368.
- Sørensen, R. (1953). *Hypospadias with Special Reference to Etiology*. Munksgaard, Copenhagen.
- Sorsby, A. (1953). *Clinical Genetics*. Butterworth, London.
- Steiner, F. (1936). Zur Erblichkeit der Hypospadias. *Münchener medizinische Wochenschrift*, **83**, 1271.
- Turpin, R. and Lejeune, J. (1960). Le maladies humaines par aberrations chromosomiques. *Revue française d'études cliniques et biologiques*, **5**, 341-347.

APPENDIX I

I.D. No.	Birth Weight (kg)	Birth Order	Age at Birth		Karyotype*	Type of Hypospadias	Associated Anomalies
			Father	Mother			
1	4.2	2	23	21	46,XY	Glandar	Hypertelorism, cryptorchidism, hare lip and cleft palate, mental retardation, aplastic kidney, frontal and facial bone deformity
2	1.9	2	23	21	46,XY	Penile	Micrognathia, low set ears, prominent occiput, inguinal hernia, pulmonary hypertension
3	3.4	3	24	25	Not done	Glandar	None
4	2.3	1	25	24	Not done	Glandar	None
5	1.5	1	24	25	Not done	Penile	None
6	1.9	7	37	29	46,XY	Penile	Low set ears, micrognathia, high arched palate, microphthalmia
7	3.2	1	23	21	46,XY	Penoscrotal	Bifid scrotum, anomalous development of sacrum and coccyx
8	2.3	1	28	24	46,XY	Penile	Cryptorchidism, bronchial asthma, anaemia
9	2.6	1	21	29	46,XY	Penile	Cryptorchidism, growth and mental retardation, club foot, hyperextensibility of joints
10	3.4	3	42	42	Not done	Glandar	Hydrocephalus, convulsive disorder
11	1.4	2	41	34	46,XY	Penile	None
12	3.4	2	30	29	Not done	Glandar	None
13	4.2	1	24	22	Not done	Penile	None
14	3.2	2	28	30	Not done	Penile	None
15	3.1	1	21	16	46,XY	Penoscrotal	Bifid scrotum
16	2.7	3	20	20	46,XY	Glandar	Micrognathia, growth and mental retardation
17	2.9	6	32	25	Not done	Glandar	Nephrosis
18	2.3	1	26	25	Not done	Penile	Tetralogy of Fallot, iron-deficiency anaemia
19	3.4	4	28	28	46,XY	Penile	Umbilical hernia

Appendix I—continued

I.D. No.	Birth Weight (kg)	Birth Order	Age at Birth		Karyotype*	Type of Hypospadias	Associated Anomalies
			Father	Mother			
20	3.0	1	20	18	46,XY	Penile	Phocomelia, bifid scrotum, imperforated anus, hydronephrosis and hydroureter
21	1.6	2	35	25	47,YYY, ?t(Cq-;Cq+)	Penile	Cryptorchidism, haemangioma of chin, hyperbilirubinaemia, growth retardation
22	2.0	2	31	32	Not done	Penoscrotal	Bronchial asthma
23	1.8	4	31	32	46,XY	Penoscrotal	None
24	3.3	2	27	24	Not done	Glandar	None
25	3.4	1	24	22	46,XY	Penile	Dextrocardia, atrial septal defect, diagrammatic hernia
26	3.1	3	35	29	Not done	Penile	None
27	5.2	3	37	36	Not done	Glandar	Convulsive disorder
28	0.9	1	25	22	46,XY	Penile	Cryptorchidism, high arched palate
29	3.8	1	20	19	46,XY (Blood) 46,XX/46,XY (Skin)	Penile	Cryptorchidism, imperforated anus, omphalocele
30	5.0	1	19	17	46,XY	Penile	Polydactyly, hydrocephalus, microphthalmia
31	1.7	1	20	20	46,XY	Penile	Cryptorchidism, inguinal hernia, growth retardation, intestinal obstruction
32	4.0	3	24	20	Not done	Glandar	None
33	3.6	2	27	22	46,XY	Penile	None
34	2.3	7	30	29	Not done	Glandar	None
35	1.9	1	22	17	Not done	Glandar	None
36	2.5	1	29	27	46,XY	Penile	Hypertelorism, low set ears, inguinal hernia, hydrocele
37	3.1	1	22	20	46,XY	Glandar	Pyloric stenosis, bifid scrotum, inguinal hernia, hydrocele
38	3.3	1	20	21	Not done	Glandar	None
39	1.6	1	20	19	Not done	Glandar	Cryptorchidism
40	3.1	3	32	29	46,XY	Glandar	Cryptorchidism, bifid scrotum
41	3.4	1	22	22	Not done	Penoscrotal	Tracheo-oesophageal fistula
42	2.4	5	34	31	Not done	Glandar	None
43	2.9	1	26	22	Not done	Glandar	Hydrocele
44	3.2	1	23	18	46,XY	Penoscrotal	None
45	1.8	1	22	19	Not done	Glandar	Tetralogy of Fallot, cleft palate
46	3.1	1	25	25	Not done	Penile	Ventricular septal defect, coloboma, inguinal hernia, absent radii
47	2.9	1	31	25	46,XY	Penile	Talipes equinovarus, speech delay, malformed ears
48	3.3	1	26	24	Not done	Glandar	None
49	1.8	1	31	22	46,XY	Penoscrotal	None
50	3.1	5	31	33	47,XY,21+	Penile	Down's syndrome

* Karyotype being expressed according to Chicago Conference (1966).

APPENDIX II

SIBSHIPS ARRANGED IN THE ORDER OF BIRTH IN 50 FAMILIES

Families	Sipships	Families	Sibships
1	NH★	26	NNH★
2	NH★	27	NNH★
3	NNH★N	28	H★
4	H★N	29	H★
5	H★N	30	H★
6	NNNNNNH★	31	H★
7	H★	32	NNH★
8	H★	33	NH★
9	H★	34	NNNNNNH★
10	NNH★	35	H★NN
11	NH★N	36	H★
12	NH★	37	H★
13	H★	38	H★
14	NH★	39	H★
15	H★	40	NNH★
16	NNH★	41	H★
17	NNNNNH★N	42	NHNNH★
18	H★	43	H★
19	NNNH★	44	H★
20	H★	45	H★
21	NH★	46	H★
22	NH★N	47	H★
23	NHNNH★NN	48	H★
24	NH★H	49	H★H
25	H★	50	NNNH★N

N = Normal; H = hypospadiac; ★ = index patient; ^ = fraternal twins.