Genetics of Urogenital Abnormalities in ACI Inbred Rats

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ABSTRACT The frequency of urogenital abnormalities in adult ACI rats was 18.8%. These abnormalities consisted of a spectrum of defects ranging from focal aplasia of the ureter to the complete absence of the ureter, kidney, and tubular genital tract on the affected side. When ACI rats with normal urogenital tracts were crossed with F344 rats the frequency of the defects decreased to a constant level in the F_1, F_2, and F_3 generations. There was no clearly significant decrease in the frequency of the defects in the backcross offspring of (ACI × F344) F_1 × ACI matings, but there was a significant and dramatic decrease in the backcross to F344 rats. Attempts to select against these defects by brother-sister matings among normal ACI rats were not successful: 5 generations of inbreeding did not alter the frequency of the urogenital abnormalities. The frequency of the abnormalities was the same in the offspring of affected parents as in the offspring of normal parents, indicating homozygosity of the factors responsible for the defects. The results suggest that the transmission of these defects is polygenic.

The ACI strain of rats originated in 1926 from a cross between the August line 1561 and the Copenhagen line 2331. Urogenital abnormalities were observed in a subline after approximately 16 generations of brother-sister matings (Deringer and Heston, '56). The abnormalities consistently involved the kidneys and appeared with a variety of other urogenital anomalies (Morgan, '53; Deringer and Heston, '56). Most commonly the right kidney, ureter, and ipsilateral tubular genital tract (uterus or seminal vesicle and vas deferens) were absent. Next most frequently the left ureter was not patent at the bladder, leading to secondary hydroureter, hydronephrosis, and assorted less consistent defects in the genital tract.

The frequency of renal abnormalities in near-term ACI fetuses was 27.9%, and of bilateral renal malformation was 2.0% (Fijikura, '70). The frequency of defects in males and females at this stage was not significantly different. Kidney abnormalities occurred in 15.7% of 650 neonatal animals (Morgan, '53) and in 17.3% of 1561 1–18-day-old animals (Deringer and Heston, '56). These results are similar to the frequency of urogenital defects observed in our laboratory (18.8%) in 533 commercially obtained, adult rats of various ages.

This report describes a series of breeding experiments conducted to investigate the inheritance of these defects. The number and type of defects were recorded; and their relation to possible embryological abnormalities is discussed.

MATERIALS AND METHODS

The ACI strain has been inbred for 102 generations, and the F344 strain for 86 generations (Festing and Staats, '73). The rats were from 3 groups used for a number of experimental purposes: (1) the first group consisted of 533 adult ACI animals (8–24 weeks of age) received from a commercial breeding source (Microbiological, Bethesda, Maryland). They were examined by palpation, and the normal animals separated from those with urogenital abnormalities. At the end of the experimental procedures normal and abnormal animals were examined at autopsy; 417 animals (78.2%) were examined in this fashion, and the results of the physical examination corresponded to those found at the autopsy in all but 3 instances. (2) The second group consisted of F_1, F_2 and backcross animals derived from matings between normal ACI and F344 rats. Those animals were used to examine postimplantation mortality in these strains (Cramer and Gill, '75), and at the end of the experiment they were killed and inspected for renal defects. (3) The third group of animals consisted of the off-

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spring from successive generations of matings between normal ACI male and female rats. At 3 weeks of age the animals were examined, and normal breeding pairs were selected for the next generation; the remainder were killed and autopsied. Several matings between affected parents were also made for comparison with the matings between normal animals. The tissues used for studies of the development of the defects were fixed in Stieve's solution, embedded in paraffin, serially sectioned at 4 μ and stained with HE.

The data were analyzed by the chi-square test. A value of $P \leq 0.01$ was considered significant; $0.01 \leq P \leq 0.05$ of questionable significance; and $P > 0.05$ not significant.

RESULTS

Urogenital abnormalities in commercially derived adult ACI rats

The number and type of urogenital abnormalities are presented in table 1. In 533 animals 17.9% of the females and 22.4% of the males were abnormal. Seventy-five of the 99 abnormal animals were autopsied. The urogenital abnormalities were classified according to the major kidney defect, since the kidney was consistently involved in all animals with urogenital abnormalities. This involvement could be divided into 2 categories: (a) absence of one kidney, or (b) obstructive hydronephrosis of either kidney as the result of segmental aplasia of the ureter. The genital abnormalities were more variable. Aplasia of either kidney was almost always accompanied by aplasia of the ipsilateral genital tract. In males the vas deferens and right seminal vesicle were absent, and in females aplasia of the uterine horn was the most common genital lesion. This series of defects was most frequently right-sided. The adrenals, ovaries, testes, and Fallopian tubes were invariably present on both the affected and unaffected sides. The second major set of abnormalities was the formation of a cystic kidney due to unilateral obstructive hydronephrosis, associated with genital lesions ranging from none to focal areas of aplasia of such structures as the seminal vesicles, vas deferens, or uterine horn on the affected side.

TABLE 1

Urogenital abnormalities in commercially obtained adult ACI rats

Renal status	Number of males	Number of females
Normal	76	358
Absent kidney		
Right	16	49
Left	0	9
Hydronephrosis		
Right	1	7
Left	5	12
Total number with abnormal kidneys	22 (22.4%)	77 (17.9%)

In addition to the major abnormalities described above 2 of the 75 animals autopsied had lesions of an intermediate type. Small remnants of abortive renal formation were present on the right side in 1 animal and on the left side in the other. Both animals were females, and in each the posterior half of the uterine horn on the affected side was absent. The anterior portion of the uterine horn was present and slightly dilated with clear fluid.

Normal ACI breeding

Breeding studies with normal ACI animals were conducted to determine whether selective breeding against the abnormalities could alter their incidence. Selection against the defect was attempted to eliminate the difficulty associated with maintaining breeding lines affected with severe urogenital defects. The results are presented in table 2. No decrease in the frequency of the defects occurred. In addition there appeared to be little environmental selection against abnormal animals during the neonatal period, since the frequency of abnormal 1-day-old F_1 offspring was the same as that of postweanlings.

Hybrid breeding

The frequency of urogenital abnormalities in the F_1 hybrids (AF) of normal ACI $\times$ F344 rats is presented in table 2. Approximately 8% of them had urogenital defects, and there was no significant difference between the reciprocal F_1 crosses (7.1 vs. 8.9%). When normal AF F_1 animals were intercrossed to produce F_2 and F_3 generations the frequency of the abnormalities did not change significantly. This finding stands in contrast with the results of backcross combinations in which the frequency of the defect apparently depended to a great extent on the genotype of the breeding pair (AF/A vs. AF/F).

TABLE 2

Number of urogenital abnormalities in various (ACI × ACI) and (ACI × F344) breeding combinations

Mating combination [1]	Number of offspring	Normal males	Normal females	Abnormal males	Abnormal females	Total abnormal	p [2]
			Normal animals				
(ACI × ACI)							
F_1 (1-day-old)	84	34	36	3	11	16.7	0
F_1	108	49	42	7	10	15.7	0
F_2	203	93	77	12	21	16.3	0
F_3	139	69	51	9	9	12.9	±
F_4	144	58	58	15	13	19.4	0
F_5	59	17	29	7	4	18.6	0
(ACI × F344) F_1							
F_1	211	98	98	7	8	7.1	+
F_2	101	39	53	3	6	8.9	+
F_3	279	135	123	9	12	7.5	+
(F344 × ACI) F_1	79	26	46	2	5	8.9	±
AF/A backcross [3]	202	84	91	14	13	13.4	±
AF/F backcross [3]	115	67	47	1	0	0.9	+
			Abnormal animals				
(ACI × ACI)	50	24	20	2	4	12.0	0
(AF/AF) hybrid [4]	79	34	39	3	3	7.6	+

[1] All animals were examined as adults (8–12 weeks old), except as noted.

[2] The significance of the difference when the results were compared with those observed with commercially obtained adult ACI animals: +, significant ($P \leq 0.01$); ±, questionable significance ($0.01 \leq P \leq 0.05$); and 0, not significant ($P > 0.05$).

[3] AF/A is the backcross of a (ACI × F344) F_1 hybrid female and an ACI male, and AF/F is the backcross of the same female and a F344 male.

[4] The cross of two (ACI × F344) F_1 hybrids (corresponds to the F_1 generation in the similar mating of normal animals shown above in the table).

Abnormal ACI breeding

Matings of ACI parents both of which had urogenital abnormalities produced offspring with the same frequency of urogenital abnormalities as those of normal parents (table 2). The F_2 offspring of abnormal AF F_1 parents had the same frequency of abnormalities as that of offspring of normal AF F_1 parents.

Intermediate type of abnormalities

A variety of urogenital abnormalities of intermediate severity were observed. Two of the 75 commercially derived adult animals autopsied (3.7%) had lesions of this type. The offspring of normal ACI animals had a higher frequency of the intermediate variety of defects (18.2%), but the frequency in each generation was approximately the same and showed no tendency to increase with successive generations. Similarly there was no significant increase in the frequency of animals with the least severe lesion (hydronephrosis) from the initial group of adult animals (18.9%) to the F_4 (33.3%) or F_5 (27.2%) generations. This finding indicates that there is no positive selection favoring the development of less severe defects.

Eighteen of 99 affected offspring from the normal ACI breeding studies had intermediate urogenital abnormalities. Eight females were included in the abnormal offspring. One had a normal genital tract, and 7 were missing a segment of the posterior half of the uterine horn on the side of the affected kidney. The 10 males in this group had small rudimentary kidneys, ureteral aplasia, and variable genital lesions on the affected side. Typically the genital lesions included retention of the ipsilateral testis, complete or segmental aplasia of the vas deferens, and absence of the seminal vesicle.

Eight rudimentary kidneys were serially sectioned and examined histologically. In each there were irregular areas of cortical tissue containing normal glomeruli with proximal and distal tubules (fig. 1). The central medullary portions of the abnormal kidneys consisted of fibrous connective tissue containing reduced numbers of

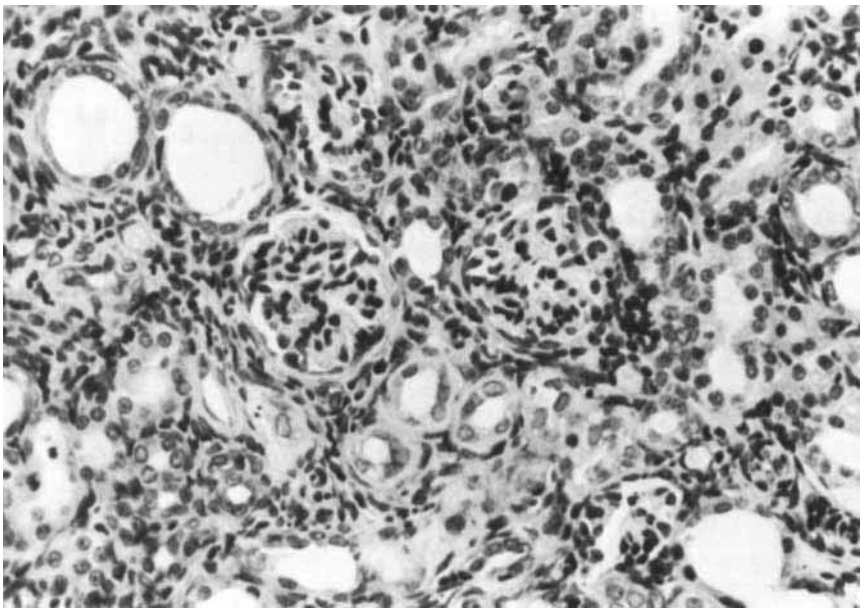

Fig. 1 Primitive cortex of the kidney rudiment including glomeruli and tubules. × 350.

collecting ducts, some of which were dilated and lined by enlarged cuboidal epithelium. In 7 of the kidneys a rudimentary renal pelvis was present, and in 4 of these the pelvis was associated with a short segment of ureter which ended blindly in the tissues immediately surrounding the kidney (fig. 2 A,B).

DISCUSSION

The urogenital abnormalities in the ACI strain of inbred rats involve primarily the tubular structures of the urogenital tract and are probably the result of a common insult to the developing embryological structures including the ureteric bud and the mesonephric (Wolffian) and Müllerian duct systems. All three structures develop in close association, and abnormalities involving one might well produce defects in the others. The variety of abnormalities in this strain of rats may be different expressions of a common basic defect. In severely affected animals there was unilateral renal aplasia and ipsilateral aplasia of the genital organs derived from the mesonephric or Müllerian ducts. In less seriously affected animals variable portions of the caudal aspects of both ductal systems may develop abnormally leading to (a) small rudimentary kidneys with almost normal glomeruli, distal tubules, proximal tubules, and primitive collecting systems and with absent posterior portions of the ureter; (b) defects in the posterior segments of the uterus or its total absence; and (c) aplasia of the vas deferens and seminal vesicle despite the normal development of the efferent ductules of the testis and epididymis. In minimally affected animals the defects consisted solely of segmental absence of the ureter at the entrance to the bladder leading to the development of secondary hydroureter and hydronephrosis.

Unilateral agenesis of the kidney is relatively common in humans, being found in approximately 1 of 500 autopsies (Potter, '72). In man the absence of a kidney is usually associated with the complete absence of the associated ureter, and where portions of the ureter persist the lower segment is more likely to be present (Ashley and Mostofi, '60). This defect has been attributed to the failure of the ureteric bud to develop (Hayman, '71). However, Ashley and Mostofi ('60) observed occasional cases in which small rudimentary kidneys developed in the absence of demonstrable ureteric tissue. They suggested that either the ureter induced kidney development and then re-

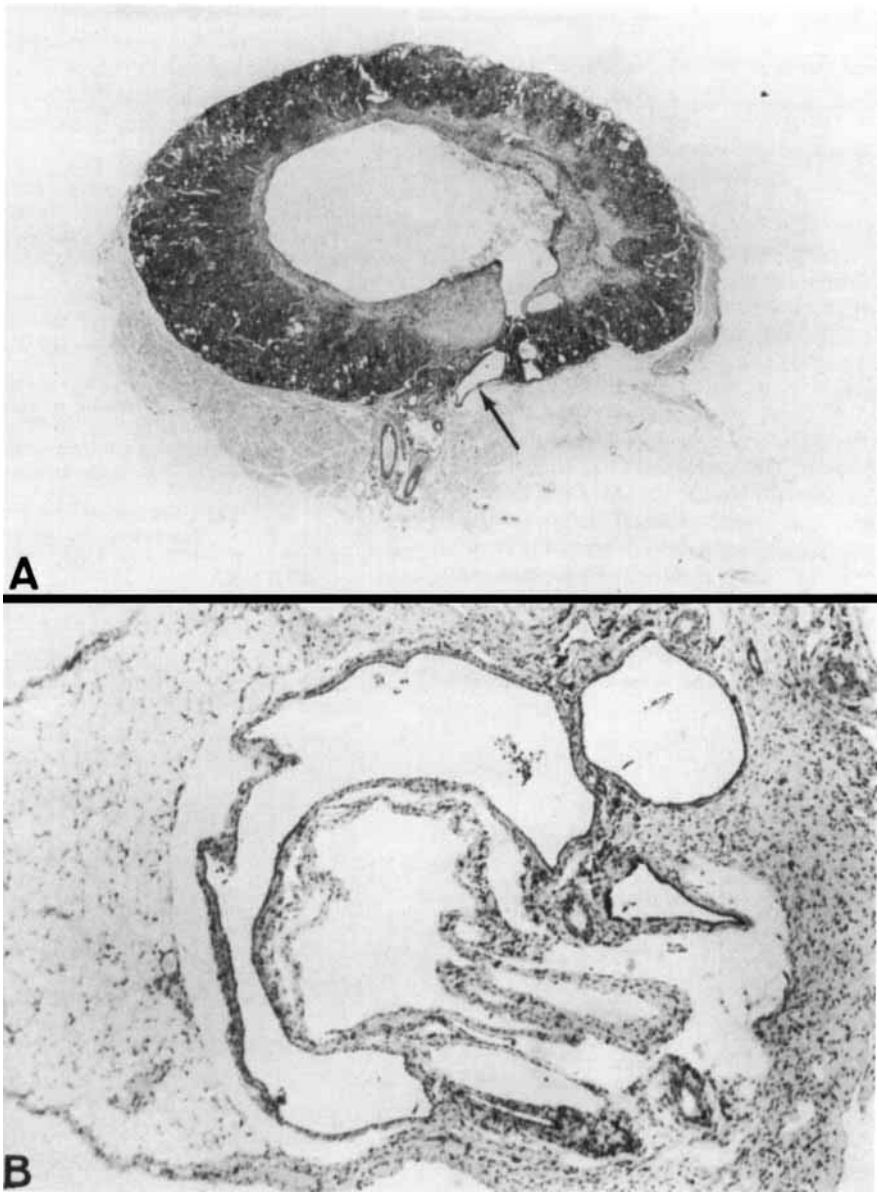

Fig. 2 A. Kidney rudiment with dilated pelvis and emerging ureter (arrow). × 40. B. Emerging ureter ending blindly in the mesentery surrounding the kidney rudiment. × 175.

sorbed or the metanephrogenic blastema was capable of being induced to form a kidney in the absence of the influence of the ureteric bud. They favored the latter hypothesis. In contrast the malformations of the urogenital tract described in this report suggest that the first interpretation may be a more accurate description of the embryological events leading to these defects in ACI rats. The segmental nature of both the urinary and genital lesions with an anterior progression in severity implies that the primary defect is in the failure of the mesenchyme of the urogenital ridge to support

the continued development of these tubular structures.

The mechanism by which these defects are inherited is probably polygenic and may involve a threshold effect. First the frequency of abnormalities in the F_1, F_2, F_3, and backcross offspring from matings of normal ACI × F344 rats (table 2) is not compatible with simple Mendelian transmission. Second the frequency of urogenital abnormalities in the offspring of normal parents was the same as that in the offspring of affected parents (table 2). Third the frequency of the defects in the offspring of the backcross of AF F_1 × F344 rats was significantly and dramatically less than that in the backcross to the ACI strain as well as that in the parental, F_1, F_2 and F_3 matings (table 2). Since the ACI and F344 strains are both highly inbred there should be no significant genetic variation that would be responsive to selective pressures leading to an alteration in the genotype of either strain. Thus the results of this study suggest that multiple genes are involved in the pathogenesis of the urogenital abnormalities in ACI rats.

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