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Atypical Fetal Prostate Development is Associated with Ipsilateral Hypoplasia of the Wolffian Ducts in the ACI Rat

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Abstract

For over a half century the ACI (August × Copenhagen) rat has been a primary model for studying renal agenesis and ipsilateral hypoplasia (IHP) of the Wolffian derived structures (WDS). Because the ACI rat is also used as a model for prostate research it is important to examine the relationship of IHP and urogenital sinus development. The prostate is dependent on androgens for proper growth and differentiation. Alteration in androgen production and/or delivery to the urogenital sinus has the potential to perturbate normal development. In this study we investigate whether the ipsilateral loss of the WDS is associated with altered prostate development. Digital images of serial sectioned fetal ACI rat urogenital sinus (UGS) were used to create 3-dimensional surface-rendered models of the developing prostate, seminal vesicle, vas deferens and utericle on gestational day 21. The number and volume of prostate ducts developing from the UGS were calculated from the 3-D model data. Animals exhibiting IHP had a significant decrease in total fetal prostate volume (40%; $p < 0.005$) with significant regional specific differences when compared to normal male ACI rats. Anatomical and histological differences in the utericle, abnormal histology of the ipsilateral testes, and a truncation of the ipsilateral Wolffian ductal mesenchyme were also seen in the animals with IHP. Additional research is needed to further understand the mechanisms and consequences of IHP on prostate growth and development. Alterations to normal prenatal development of the male accessory sex organs can have important consequences for the growth and morphology of the adult gland.

Keywords

ACI rat; prostate development; urogenital abnormalities; Wolffian ducts; utericle

Introduction

The crossbreeding of the August 1561 and Copenhagen 2331 lines in 1926 created the ACI rat strain. Following the first sixteen generations of continuous brother-sister inbreeding it was noted that the ACI rat was prone to the development of urogenital abnormalities (Morgan, 1953; Deringer and Heston, 1956). Numerous studies over the past 50 years have confirmed the observation of these abnormalities with 10 – 25% of the animals affected predominantly on the right side (Deringer and Heston, 1956; Fujikura, 1970; Cramer and Gill, 1975; Maekawa and Odashima, 1975; Shoji and Harata, 1977; Marshall et al., 1978;

Fujita et al., 1979; Marshall et al., 1979; Marshall et al., 1982; McCullough et al., 1984; Solleveld and Boorman, 1986; Kneidl et al., 1995; Shull et al., 2006). The urogenital abnormalities include: ipsilateral hypoplasia (IHP) of the epididymis (ED), vas deferens (VD) and seminal vesicle (SV) in males; the presence of a single uterine horn and in some cases a single ovary in females; and the ipsilateral absence or truncation of the ureter in both sexes (Deringer and Heston, 1956; Marshall et al., 1978). More severe defects of the urogenital tract have also been described including hydronephrosis or the complete absence of a kidney ipsilateral to the urogenital agenesis in both sexes (Marshall et al., 1978). The IHP urogenital abnormalities do not conform to normal Mendelian inheritance patterns and selective breeding of non-afflicted parents does not alter the percentage of afflicted offspring (Cramer and Gill, 1975). Recent work by Shull et al. (2006) has demonstrated that the *Renag1* gene on chromosome 14 in the ACI rat is responsible for the incomplete dominance and incomplete penetrance of urogenital inheritance in cross breeding studies. Kneidl et al. (1995) also indicated that afflicted male ACI rats developed genetic abnormalities of chromosome 8 including trisomy and isochromosome.

The ACI rat is very sensitive to exposure to exogenous estrogen or estrogen-mimicking compounds (Shull et al., 1997; Inaguma et al., 2003) and has a marked propensity for the development of spontaneous tumors (Maekawa and Odashima, 1975). Because of this the ACI rat has made a resurgence into the realm of research in the past decade and has been used in endocrine disruption (Inaguma et al., 2003), breast (Shull et al., 2001; Schaffer et al., 2006; Ravoori et al., 2007), and prostate cancer research (Ward et al., 1980; Varma and Austin, 1990; Kondo et al., 1994; Reyes et al., 2005; Yamashita et al., 2005). ACI rats have been shown to be an applicable model for studying the age related development of prostate cancer (Reyes et al., 2005). At 24 months of age 25% of male ACI rats developed intra-alveolar atypical hyperplasia (similar in histology to human high grade prostate intraepithelial neoplasia), while at 33 months of age nearly 100% of the ACI rats developed extensive alveolar prostatic adenocarcinoma with a limited number developing ductal infiltration (Ward et al., 1980).

An extensive search of the literature indicated that there were very limited data on the effect of IHP on the developing prostate. During fetal development the WD are stabilized by androgens produced in the testes. The prostate is dependent on the diffusion of testicular androgens through the caudal WDS (ED and VD) for development, growth and stabilization. Development of the prostate is initiated through budding of the urogenital sinus (UGS) urothelium in response to an androgen dependent secretion of growth factors by the periurethral mesenchyme (Cunha, 1994). Therefore, any perturbation of the WDS could limit the levels of androgens necessary for induction of development and stabilization of the prostate. Using computer-assisted morphometric reconstruction technology (Winsurf®, University of Hawaii) we have examined the prostate of normal and IHP afflicted fetal ACI male rats. To the best of our knowledge this is the first report of fetal ACI rat prostate reconstruction and illustration of the growth abnormalities in response to altered Wolffian duct development. Our data indicate that a significantly smaller prostate is associated with IHP of the Wolffian ducts in fetal ACI rats. This growth alteration has the potential to alter the postnatal development and growth of the prostate, and creates an additional variable that should be considered when using the ACI rat as a primary rodent model for prostate research.

Materials and Methods

Experimental Animals

Time mated 8-10 week old female ACI/SegHSD rats (Harlan Sprague Dawley, Inc. Dublin, VA) arrived at Harlan Biotech Center on gestational day 3 (GD3). On GD 21, pregnant

females were euthanized by CO₂ asphyxiation, followed by cervical dislocation. Because it is known that intrauterine position can affect hormonal levels within the UGS only male fetuses located between a female and male littermate (FMM, 1M) were selected for this study (Timms et al., 1999; 2005). The 1M male fetuses were removed via cesarean section, decapitated and bisected at the level of umbilicus. The caudal portion of the fetuses was placed in individual 50 ml centrifuge tubes containing 10 ml of 4% buffered paraformaldehyde for 2 hours, transferred to fresh paraformaldehyde overnight prior to dehydration in 50% ethanol, and storage at 4°C in 70% ethanol until transferred to the University of South Dakota for final tissue preparation. Upon receipt at the University of South Dakota the specimens were trimmed of excess material (limbs, tail and visceral organs) to facilitate embedding and sectioning. Specimens were briefly re-fixed in 4% buffered paraformaldehyde, dehydrated through graded ethanol and CitriSolv (Fisher Scientific, Pittsburgh, PA) and embedded in paraffin wax in preparation for 3-D reconstruction.

This study was reviewed and approved by the Harlan Institutional Animal Care and Use Committee (IACUC). Harlan Biotech Center is a fully accredited AAALAC facility. This study complied with all applicable sections of the Guide for the Care and Use of Laboratory Animals (National Research Council 1996).

3-D Reconstruction

As previously published (Timms et al., 2005; Hofkamp et al., 2008), paraffin embedded tissues were serially sectioned at 7µm, floated on a warm water bath, placed on Fisherbrand ColorFrost Plus® slides (Fisher Scientific) and briefly stained with hematoxylin and eosin. Digital photomicrographs were taken using an Olympus DP70 camera attached to an Olympus BX60 microscope (Leeds Precision Instruments, Minneapolis, MN). Histological images of the prenatal prostate and associated secondary sex structures were traced, contoured, and re-aligned as surfaced-rendered 3-D models using Winsurf® software (University of Hawaii). Morphometric and volumetric analysis of normal and IHP animals were performed and compared to evaluate possible effects on fetal prostate morphology.

Statistics

All volumetric data were analyzed using a two-tailed t-test assuming equal variances (homoscedastic) comparing normal to IHP animals using Microsoft Excel® software. The confidence interval for rejecting the null hypothesis was $p < 0.05$. Data are presented as mean $\pm$ standard error of the mean (SE). For all groups $n=4$ with all observations and analysis performed in a blinded fashion to obviate bias.

Results

The developing accessory sex glands (ASG) consist of anatomically separate structures including the coagulating gland (CG), seminal vesicles (SV), vas deferens (VD) and the dorsal, lateral and ventral prostate lobes (Hayashi et al., 1991; Timms et al., 1994; Timms, 2008). Throughout the paper the terms normal and control will be used synonymously to describe fetal male ACI rats without ipsilateral hypoplasia (IHP) of the Wolffian derived structures. The combined dorsal, lateral and ventral lobes (DLVP) will be referred to as prostate. The SV and VD are formed as derivatives of the Wolffian ducts whereas the prostate develops from the urothelium of the UGS during early fetal development (Hannema and Hughes, 2007). The SV and VD join together to form the ejaculatory ducts (EJ) (Figure 1A, 1D, 1E, 1G). Many studies of the adult rodent prostate combine the dorsal and lateral lobes as the dorsolateral prostate. As previously published (Hofkamp et al., 2008), we were

able to separate the dorsolateral prostate into its dorsal and lateral lobes and further differentiate the dorsal lobe into distinct cranial and caudal regions (Figure 1A).

Urogenital Sinus

During the course of the experiment approximately 25% of the male ACI rats sectioned exhibited the anatomical and histological characteristics of IHP including the unilateral absence of one seminal vesicle (SV), vas deferens (VD), epididymis and ureter (Figure 1A, 1B, 1C, 6). The IHP was observed on right side in all cases. The kidneys were not sectioned or examined and therefore no data are available on the prevalence of hydronephrosis.

Accessory Sex Structures

In animals affected with IHP the volume of the single seminal vesicle was significantly less (39%; $p<0.05$) than the mean volume of the two SVs in the unaffected control animals (Figure 2A). The mean volume of the coagulating glands in the IHP animals was also significantly less (33%; $p<0.05$) than that of normal animals (Figure 2A). As in previous publications the volume of the vas deferens was not calculated (Timms et al., 2005; Hofkamp et al., 2008).

Prostate

The total prostate volume (DLVP; Figure 1A, 1F), including the combined dorsal, lateral and ventral lobes, in the IHP animals decreased by 40% ($p<0.005$; Figure 2A) when compared to normal male ACI rats. When the dorsal and lateral lobes of the prostate were examined separately, decreases in volume were detected of 37% ($p<0.001$) and 41%, respectively ($p<0.05$; Figure 2B). In the IHP animals the caudal region of the dorsal lobe was decreased in volume by 32% ($p<0.005$), whereas the cranial region of the dorsal lobe was decreased by over 62% ($p<0.05$; Figure 1A, 1F, 2B).

Testes

Histological analysis of the testes revealed abnormalities ipsilateral to the IHP (Figure 4D, E, F). The interior of the ipsilateral testis was primarily composed of connective tissue and was devoid of normal developing seminiferous tubules (Figure 4C, D). The tunica of the ipsilateral testis appeared normal. The contralateral testis exhibited normal development (Figure 4F) similar to that of control animals (Figure 4A, B, C).

Discussion

The ACI rat has been a model of ipsilateral hypoplasia (IHP), urogenital abnormalities and renal agenesis for over a half century (Deringer and Heston, 1956). Its usefulness as a model for human renal agenesis has been known for over a quarter of a century (Fisher et al., 1982). As the ACI rat ages it consistently develops spontaneous tumors of multiple organs, including but not limited to the testes, pituitary, uterus, mammary and prostate (Maekawa and Odashima, 1975; Ward et al., 1980). Solleveld and Boorman (1986) have previously discussed the problems associated with the use of animals with renal and urogenital abnormalities in long term toxicological or carcinogenesis studies. Therefore, in this paper we hypothesize that there would be anatomical and developmental difference in the prostate and accessory sex glands (ASG) of male animals affected by IHP compared to normal male ACI rats. This was confirmed through the use of 3-dimensional reconstruction analysis.

The prostate (DLVP) in the animals with IHP was significantly smaller (40%; $p<0.005$) in comparison with normal males. As in prior studies (Hofkamp et al., 2008), the dorsal (37%; $p<0.001$) and lateral prostate lobes (41%; $p<0.05$) exhibited significant decreases in volume.

The caudal region of the dorsal lobe exhibited a significant sensitivity in terms of decreases in volume (32%; $p < 0.005$) and the number of ducts (26%; $p < 0.05$), whereas the number of ducts developing from the cranial portion of the dorsal lobe was relatively unaffected (Figure 3). The single SV contralateral to the aberration in the animals with IHP was significantly smaller and the Wolffian mesenchyme ipsilateral to the affected side is severely truncated (tWDM; Figure 1A, 1B, 1C, 1D, 2A). Therefore, we conclude that the volumetric changes seen in the animals are a result of a decrease in the size of the solid epithelial prostate buds coupled with a decrease in the number of prostatic buds.

Examination of the ipsilateral and contralateral testes in normal and IHP animals revealed striking histological differences which may contribute to the potential mechanism of the urogenital abnormalities described in this study (Figure 4). In the unaffected animals the epididymis is present and the testes have histologically normal architecture and seminiferous tubule development (Figure 4A, B, C). In the ipsilateral testis of the IHP affected animal the seminiferous tubules are absent. The body of the testis beneath the tunica albuginea in the IHP animals was composed of loose connective tissue (Figure 4D, E). The contralateral testis of the IHP affected animals exhibit normal testicular histology (Figure 4D, F). In the affected animals ipsilateral to the urogenital abnormalities there is a complete loss of the epididymis (Figure 4D, E) whereas contralateral to the abnormalities the epididymis is present and histologically normal (Figure 4D, F). Prior studies have shown that unilateral castration causes unilateral WD regression (Hannema and Hughes, 2007). The observation of altered development of the testis and epididymis ipsilateral to the abnormalities in this study may be associated with asymmetric development of the urogenital sinus in the animals with IHP. In contrast with our finding of fetal testicular abnormalities, previous publications on ACI rat IHP only reported changes after puberty (Marshall et al., 1979; Marshall et al., 1982; McCullough et al., 1984). Therefore, while ipsilateral changes in the testis and WD derived structures provide a potential mechanism of causality, further research is needed to completely understand and confirm the mechanisms responsible for the prostate development and growth abnormalities seen in the animals with IHP. The origins of the prostatic utricle have been debated for a century (Meyer, 1909; Shapiro et al., 2004). The prostatic utricle was originally thought to be a non-regressed Müllerian remnant. However, Shapiro et al. (2004) concluded that the utricle was of definite UGS origin. In our current study the utricle of animals with IHP exhibited both a histologically (Figure 1E, 1G) and developmentally abnormal morphology (Figure 1A, 1B, 1D, 1F, 1H). Following reconstruction and volumetric analysis, the absolute volume of the utricle within the fetal ACI rat with IHP was found not to differ significantly from that of normal males (data not shown).

Although there was no significant change in the absolute volume of the utricle in IHP ACI rats, there was an interesting anatomical difference in utricle histology and morphology. In the normal animal the utricle joins to the urothelium in two places in the dorsal region of the seminal colliculus (Timms et al., 1994). This connection creates a tissue “bridge” which is characteristic of the utricle in the developing prostate (Figure 1G, 1H; Shapiro et al., 2004). This area is associated with a dense mesenchyme reminiscent of vaginal mesenchyme (Kurita and Cunha, 2001; Shapiro et al., 2004). However, in the IHP animals, there was only a single side entrance contralateral to the affected side (Figure 1G, 1H). The IHP utricle was also curved toward to the side lacking seminal vesicle and vas deferens instead of forming a linear structure flanked by the ejaculatory ducts (Figure 1D, 1E, 1H). The curvature of the utricle in IHP animals gives the initial impression of a truncated ejaculatory duct or uterine horn (Figure 1E). In the female this mesenchyme is essential for the formation of the Müllerian derived vaginal epithelium. Within the male ACI rat there is a dense area of vaginal-like mesenchyme (VLM) dorsal to the utricle “bridge” in the seminal colliculus in the normal ACI rat (Figure 1G top panel). In the IHP animals the histological extent of the

VLM is more diffuse as it surrounds the single entrance of the utricle into the urothelial wall (Figure 1G lower panel).

This study was prompted by the recommendation proposed by the Prostate Cancer Foundation at the Prostate Cancer Models Working Group and their request that all currently available animal models of prostate cancer be characterized with respect to their usefulness to researchers and their analogy to human cancer (Pienta et al., 2008). The ACI rat is a fundamental research model for the study of renal agenesis and age-related cancer of multiple tissues. Its inherent sensitivity to exogenous estrogen exposure and extensive development of breast and prostate adenocarcinoma has made it an invaluable tool for researchers. However, because of the abnormalities seen in the fetal ACI rat prostate, and in agreement with previous publications (Solleveld and Boorman, 1986), we recommend that care should be taken when selecting the ACI rat as the predominant model for endocrine disruption or age-related cancer research on the prostate. Alterations to the developing fetal prostate can have implications far into adulthood (Prins et al., 2008). Further studies are needed to elucidate the molecular mechanisms responsible for the ipsilateral abnormalities.

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Figure 1.

3-D serial section reconstruction of accessory reproductive organs in the male ACI rat during prenatal development (GD 21). Control refers to normal fetal male ACI rats unaffected with IHP. **A.** Right lateral views of the surface-rendered anatomical reconstruction of the developing (GD 21) UGS and ASG structures in the normal (left) and IHP (right) male. The individual structures are identified by color. **B.** Cranial view of the developing UGS, WD derived structures, and the utricle in a normal (top) and IHP (bottom) animal. In the IHP animal there is a unilateral loss of the vas deferens and seminal vesicle, and the utricle is markedly enlarged on the side demonstrating urogenital abnormalities. **C.** Representative histological view of the ASG and WD derived structures. In the IHP animal the Wolffian duct mesenchyme ipsilateral to the urogenital abnormality is truncated (red outline; tWDM) with no development of the vas deferens or seminal vesicle. The level of sectioning is indicated in D (top line). **D.** Reconstruction in dorsal view of the UGS and ASG structures in the ACI rat illustrating developmental differences in the Wolffian duct derivatives. **E.** Histological view of WD derived structures sectioned at the level indicated in D (bottom line). In the IHP animal only a single EJ is present and the utricle exhibits an altered histological outline compared to the normal male. **F.** Left dorsolateral view of prostate and utricle development in the normal (top) and IHP (bottom) ACI rat. Note the differences in utricle and prostate growth in the IHP animal. **G.** Histological representation of the UGS at the level of the seminal colliculus where the utricle joins the urothelium (U*). In the normal animal (top panel) normal development of a dual connection creates a histological “bridge” beneath which is dense VLM (Kurita and Cunha, 2001). In the IHP animal (lower panel) the utricle connects to the urothelium at a single point contralateral to the urogenital abnormality with diffuse VLM surrounding the utricle (arrows). **H.** Inferior dorsal view of the UGS and utricle. In the normal animal the histological “bridge” with symmetrical connections to the urothelium is visible along with normal morphological development of a centrally located structure. However in the IHP animal only a single connection to the urothelium is present. Abbreviations: WDM, Wolffian ductal mesenchyme; tWDM, truncated Wolffian ductal mesenchyme; U*, utricle/urothelium interface; VLM, vaginal like mesenchyme; EJ, ejaculatory duct.

Figure 2.

A. Comparative volume analyses of individual and combined regions of the ASG in prenatal (GD 21) ACI rats with IHP versus unaffected control animals. **B.** Comparative volume analysis of individual and combined regions of the prostate in prenatal (GD 21) ACI rats with IHP versus unaffected control animals. Values shown are mean $\pm$ SE ($n = 4$ for both groups). * $p < 0.05$, ** $p < 0.01$, # $p < 0.005$, ## $p < 0.001$, and @ $p < 0.00005$ compared with normal unaffected control males.

Figure 3.

Effect of IHP on prostate duct development in prenatal (GD 21) ACI rats compared to normal. Values shown are mean $\pm$ SE (n = 4 for both groups). *p < 0.05 and #p < 0.005, compared with normal unaffected control males.

Figure 4.

Representative photomicrographs of the caudal region of serial sectioned (GD 21) ACI rats demonstrating differences in testicular histology between control and IHP animals. **A.** Low power photomicrograph of caudal region in control animals. **B and C.** Higher power photomicrograph of testes illustrating normal histological development. **D.** Low power photomicrograph of caudal region in IHP animal. **E.** Higher power photomicrograph of affected (ipsilateral) testis in the IHP animals revealed altered histology including lack of seminiferous tubules surrounded normal tunica albuginea. The epididymis is also absent. **F.** Higher power photomicrograph of non-affected (contralateral) testis demonstrating normal histology. Abbreviations: SV, seminal vesicle; Vas, vas deferens; Ep, epididymis; U, urethra; TA, tunica albuginea; ST, seminiferous tubules. Scale bars indicate 500 μ m.