Kidneys in Meckel’s syndrome as a model of abnormal renal differentiation

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ABSTRACT. Kidneys of nine human fetuses of 16–22 weeks of gestational age with Meckel’s syndrome were studied. All kidneys were grossly enlarged and cystic. The morphological picture is appreciably different from the kidneys of full term infants with the same syndrome. All kidneys showed distinct subcapsular nephrogenic zone from which the cysts arise. The cyst wall retains characteristics that recall its origins from preformed nephrons and collecting ducts, as can be demonstrated histologically and with lectin and antibody markers. Studies on abnormal fetal kidneys when combined with segment-specific molecular markers of the nephron are invaluable in unraveling the pathogenesis of the various types of human cystic renal diseases.

KEY WORDS: Cystic kidney, Lectins, Meckel’s syndrome, Fetus, Renal dysplasia

Introduction

The organogenesis of the mammalian metanephric kidney and early nephron formation have been thoroughly studied and repeatedly reviewed (see Saxén, 1987). Renal malformations are relatively common. The vast majority of the abnormal fetal renal development is manifested by cystic transformation of the developing ducts of the collecting system and the nephrons of the kidney. The developmental mechanisms leading to the cyst formation are poorly known.

The pioneering microdissection work of Osathanondh and Potter (1964) resulted in a classification of four basic-type cystic kidneys based on the identification of the dilated ducts in relation to the normal renal structures. In this classification, type 1 kidneys show cystic dilatation of collecting ducts, with the nephrons remaining normal. The clinical counterpart of this type is autosomal recessive polycystic kidney disease (ARPKD). Type 2 exhibits primitive ducts and cysts with few or no signs of nephron differentiation. This is the most common type of congenital cystic kidney and it is also known in other classifications as renal dysplasia (Churg et al., 1987). Depending on the size of the kidneys and the cysts, designations such as multicystic dysplasia and aplastic dysplasia are used. Congenial renal dysplasia is often associated with anatomic abnormalities in the upper or lower urinary tract (Churg et al., 1987). Type 3 cystic kidneys show segmental dilatation of a part of the nephron and the collecting duct. The best known clinical example of type 3 cystic kidney is autosomal dominant polycystic kidney disease (ADPKD). Type 4 cystic kidneys show dilations of the subcortical nephrons (the last generations) and this type is associated with lower urinary tract obstruction. In classifications other than that of Osathanondh and Potter, this type is also included in renal dysplasia (Churg et al., 1987).

Many cystic disorders of the kidney, such as those associated with chromosomal abnormalities, tuberous sclerosis, von Hippel-Lindau disease and many others, cannot be satisfactorily fitted into this classification of the four basic types.

Classification and pathogenetic consideration of cystic kidneys on the basis of macroscopic and histological appearance of the abnormal structures as seen in the human kidneys many weeks or even years after the completion of the nephrogenesis may be misleading. Deduction of the ontogenetic events from the abnormal structures cannot unravel the early events of the abnormal development nor elucidate pathologic changes consequent to the primary defect.

Meckel’s syndrome consists of malformations of the central nervous system, postaxial polydactyly, liver abnormalities, cystic kidneys and many other organ malformations in various combinations (Opitz and Howe, 1969; Salonen, 1984; Rapola and Salonen, 1985). The syndrome is inherited as a recessive trait and leads to death before or soon after birth. Prenatal diagnosis in the second or third trimester of pregnancy is possible by demonstration of increased amniotic fluid alpha-fetoprotein due to the open central nervous system defect or by showing the cystic kidneys and brain malformations by ultrasonography (Aula et al., 1977; Shapiro et al., 1977).

The availability human fetal kidneys with Meckel’s syndrome offered the possibility of obtaining insights into the genesis of the cystic transformation of the kidney at the time when the cysts arise from the developing metanephric tissue. Histological and nephron segment specific marker studies of 9 human fetuses with Meckel’s syndrome of gestational ages from 16–22 weeks are reported here.

Results

All kidneys were grossly enlarged and macroscopically cystic. The renal weight exceeded the expected values by the factor of 9–36 times (Table 1.). The kidneys had retained their reniform shape and all showed distinct fetal lobation. Slit-like renal pelvis and ureter was present in all cases. Calyceal system was distorted, but cystic and noncystic elements showing urothelium were found in the medullary parts of all kidneys. Ureters were thin but microscopically patent and the bladder was small but present in all cases.

Histological sections covering renal tissue from the surface to the hilus of the kidney revealed a constant pattern of pathologic changes varying in severity. All kidneys showed a subcapsular zone of undifferentiated renal blastema, and nephrogenesis with immature nephrons in various stages of development (Figs. 1 and 2). Immediately beneath this zone the tubular structures appeared dilated. The first one or two layers of dilated tubules had a diameter of 100 to 300 μm. Some of these slightly
Fig. 1. A. Continuous nephrogenic layer covers the cortex of the kidney. A partially formed column of Bertin separates the lobes. The cysts show a size gradient from cortex to medulla. Case 8.

Fig. 1. B. The nephrogenic zone is interrupted by cysts extending to the renal capsule. Case 5.

Fig. 1. C. A few developing nephrons in otherwise generally cystic kidney. Case 2. H & E, x 25.
dilated structures could be identified as collecting ducts by virtue of branching on sections (Fig. 2). The deeper parts of the kidneys consisted of cysts varying 0.5 to 4 mm in diameter. The cysts were lined by flat or columnar epithelium. Although the epithelium of the cysts appeared undifferentiated there were ribbons of cells suggesting differentiation of proximal and distal tubular epithelium (Figs. 2 and 3). Some hilar cysts and non-cystic structure showed urothelium (Fig. 4) indicating origin of pelvicalyceal system. The large cysts were separated from each other by loose connective tissue. Occasional nondilated immature ducts with concentric fibromuscular collar were detected between the cysts. Glomeruli, some of them associated with cysts, were also found among the cysts (Fig. 3). In the mesenchyme small clusters of nucleated erythrocytes were common. Metaplastic cartilage was not detected in any of the specimens.

Special attention was focused on the variation of the peripheral nephrogenic zone. In three specimens (cases 4, 7, 8) the zone occupied the whole of the peripheral area of the kidney. In three of these cases, a short segment of interlobular column of Bertin could be seen (Fig. 1a).

In cases 1, 3, 5, 6, and 9 the peripheral nephrogenic zone was interrupted by cysts extending to the capsule of the kidney.

In these cases approximately 40-70% of the sub-capsular area was covered by the active nephrogenic zone (Fig. 1b). In case 2 most of the kidney was cystic and only small peripheral islands of immature cells and poorly developed nephric elements were seen under the capsule (Fig. 1c).

The tubules of the nephrogenic zone of the kidneys were readily stained with all of the lectins and antibody markers indicating normal nephron differentiation. Furthermore, the epithelium of the cysts all over the kidneys also showed focal staining with all markers. Lengths of the epithelium showing anti-BB activity suggested proximal tubular differentiation also in HE-stained sections (Fig. 5a and b). The same was not as obvious with other markers, but it appeared that often only part of the cyst wall epithelium was stained with any of the markers (Fig. 5c and d).

**Discussion**

Meckel's syndrome kidneys grow and become cystic early in fetal development. In our material the enlarged cystic kidneys were detected by ultrasound investigation already at the 15th...
week of intrauterine life and the weight of the kidneys was many times more than expected.

Some 20 weeks later, at the end of normal human gestation, the kidneys in Meckel's syndrome have grown in size and often obtain massive dimensions. In the newborn material from our group the renal weight varied from 80 to 1100g, the mean being 336g (Salonen, 1984; Rapola and Salonen, 1985). Similar enlargement of the kidneys has been reported in several recent studies (Andersson, 1982; Moerman et al., 1982; Blankenberg et al., 1987). The renal histology is somewhat different in midterm fetuses than in kidneys studied at term. In the latter the vast majority of the renal parenchyma is occupied by the cysts, embedded in abundant connective tissue stroma and very few or no identifiable nephrons are present. In fetuses in the second trimester of pregnancy a distinct nephrogenic subcapsular zone is evident. Histologically the blastema and the formation of nephrons in this area does not deviate from that seen in normal fetuses of the same age. It is obvious, however, that already in some fetuses the nephrogenic zone is interrupted by cysts intruding into the capsule. The findings indicate that in Meckel's syndrome kidneys the basic nephrogenesis takes place in normal fashion, but the collecting ducts and newly-formed nephrons undergo cystic transformation soon after their formation. It appears that the growing cysts exert inhibition on nephrogenesis by intrusion to the capsule of the kidney. It is possible that the great variation of the renal mass at birth can be explained by the difference in the time of cessation of fetal nephrogenesis in the fetus. There was no correlation between the fetal age of those with abundant nephrogenic zone and those with little nephron formation, but the kidneys with ample nephrogenesis were the least enlarged (Table 1).

The view of cystogenesis from the initially normally-developed nephrons was supported by studies with lectins and segment-specific nephron markers (Holthöfer and Rapola, 1985; Holthöfer, et al., in press). Apart from the newly-formed nephrons, part of the epithelium of the cyst retains its original differentiation markers. The opposite, that is, that dysplastic cysts derived from abnormal branches of the uterine bud would differentiate to show molecular markers of nephrons, is very unlikely.

The classification of cystic kidneys in MS is controversial. The prevailing concept is that kidneys in MS represent cystic dysplasia; i.e. failure of metanephrine differentiation roughly corresponding to type 2 in the classification of Osathanondh and Potter (Bernstein et al., 1974; Bernstein, 1979; Blankenberg et al., 1987). The histological picture of the MS kidneys near or at term makes this view understandable.

Subcapsular nephrogenesis and an increasing gradient of the size of the cysts have been recorded in a few previous studies of MS in midterm fetuses (Rehder and Labbé, 1981; Anderson, 1992; Rapola and Salonen, 1985). All these authors

<table>
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<tr>
<th>Case No.</th>
<th>Gestational age, weeks</th>
<th>Weight of the kidneys, grams</th>
<th>Weight of control kidneys*</th>
<th>Amount of nephrogenic zone, percent</th>
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<td>9</td>
<td>22</td>
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* Control weights from Potter and Craig, 1976.
Fig. 5. The pair A and B shows histological picture and staining with anti-BB antibodies. Note that the anti-BB positive cyst epithelium shows histological differentiation of the proximal tubule. The pair C and D shows histology and anti-TH fluorescence. Only part of one cyst epithelium shows TH activity. x 125.
reached the conclusion that at least part of the cysts arise from previously formed nephrons. The present, and so far largest series, confirms and extends this view. The results of the marker studies lend strong support to this view.

The conclusion from the observations of the fetal MS kidneys is that the initial metanephric differentiation follows the normal course. The cysts arise from the nephrons and collecting ducts after their formation and gradually lose histological landmarks of differentiation. This view is in contrast to the concept of renal dysplasia.

In studies of abnormal human kidneys the developmental events are often deduced from the morphological picture a long time or many months after the first stages of maldevelopment. Secondary changes may modify and confuse the picture to such an extent that the original pathogenesis is no longer evident. The availability of fetal kidneys makes it possible to study the maldevelopment closer to its origin. When these studies are combined with molecular markers a more accurate picture of the cystic kidneys and their pathogenesis may also arise in disorders other than MS.

Materials and Methods

The material consists of 9 fetuses resulting from elective interruptions of pregnancy during the period 1976-1986. The prenatal diagnosis of Meckel’s syndrome was made by determination of amniotic fluid alpha fetoprotein or ultrasonography, or both. The fetuses were autopsied in the pathology unit of the Children’s Hospital, University of Helsinki.

The diagnosis of Meckel’s syndrome was based on the classical triad of central nervous system defect, postaxial polydactyly present in all cases and enlarged cystic kidneys (Optiz and Howe, 1969). In addition to these changes all fetuses showed an increased number of intrahepatic bile ducts at the edges of enlarged portal triads. This constant diagnostic feature of Meckel’s syndrome has been previously emphasized (Rapola and Salonen, 1985).

The pertinent clinical data and renal findings are presented in Table 1. At autopsy the kidneys were inspected, removed, fixed in neutral formalin solution and ample pieces were taken for histological investigation. The sections were stained with hematoxylin and eosin. Sections of kidneys from cases 1, 5, 8 and 9 were also subjected to tubule specific antibody and lectin stainings. Fluorescein isothiocyanate (FITC) labeled lectins of Helix pomata (HPA), Arachis hypogea (PNA) and antibodies to tubular brush border (BB) and Tamm-Horsfall (TH) protein were applied on the deparaffinized sections, as has been described previously (Holthöfer and Rapola, 1985; Holthöfer et al., in press). The sections were studied under a fluorescence microscope equipped with filters for FITC. After photography, the site was marked, the coverslip removed, the section was stained with hematoxylin and eosin and the marked area was re-photographed. The lectins served as markers for collecting ducts and distal tubules (Holthöfer et al., 1981; Holthöfer, 1983) while anti-BB and TH stained proximal and distal tubules of the nephrons, respectively (Ekblom et al., 1980; Ekblom et al., 1981).

Acknowledgements

This study was supported by the Sigrid Jusélius Foundation

References


