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Research article

Microanatomy of the developing nephron in the fetal human kidney during late gestation

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ABSTRACT

Background: Clinical experiences reveal that the kidneys of preterm and low birth weight infants are highly vulnerable. Noxae of various molecular composition can damage the outer renal cortex, resulting in an early termination of nephron formation. However, in contrast to what is known about the rodent kidney, with reference to the damage on the early stages of nephron anlage such as the comma-shaped body, renal vesicles, pretubular aggregate or nephrogenic niche, this information in the fetal human kidney is not available. The few documented pathological alterations in the fetal human kidney during late gestation are glomeruli with a dilated Bowman's space and a shrunken tuft, the reduction in width of the nephrogenic zone and the lack of here contained S-shaped bodies. The latter points out that the shaping, folding or expansion of the developing nephron must be disrupted. Since these specific aspects have been little investigated, the aim of the present microanatomical contribution is to highlight it.
Methods: Firstly, the individual stages of nephron anlage in the fetal human kidney during late gestation were documented by microscopic images. Then, as a stylistic tool for the pointing to specific sites of the running developmental process, a series of true to scale sketches were produced.
Results: The generated sketches depict the spatial expansion of the transiently appearing stages of nephron anlage. These are restricted to the nephrogenic zone and are framed by the inner side of the renal capsule, the related collecting duct ampulla and a perforating radiate artery. Practical hints and a consequent nomenclature explain the developmental course and help us to identify the precise location of the proximal – distal poles, medial – lateral profiles, connecting points, adhesion sites or folds at the developing nephron on microscopic specimens.
Conclusions: The impairment of nephrogenesis in preterm and low birth weight babies is an unsolved biomedical issue. To contribute, by provided true to scale sketches, numerous practical hints and a consequent nomenclature typical features of nephron formation in the fetal human kidney at late gestation are demonstrated.

1. Introduction

The impairment of nephrogenesis can cause early termination of nephrogenesis in preterm and low birth weight babies (Luyckx, 2017; Dyson and Kent, 2019). This is evoked by noxae of various molecular composition including restricted nutrition (Woods et al., 2001), oxidative stress (Sutherland et al., 2014) and drugs (Fryer and Welsh, 2019). As a result, the affected babies develop typical signs of oligonephropathy/oligonephronia, which in turn leads to severe health consequences in later life (Kandasamy et al., 2012).

The mentioned noxae damage the outer cortex of the fetal human kidney during late gestation (Minuth, 2019a). Peripherally, the cortex is protected by the renal capsule and consists of two layers of parenchyma and stroma (Table 1). The outer layer, the nephrogenic zone, contains the transiently appearing stages of nephron anlage. The subjacent layer is called the maturation zone. The functional differentiation of the definitive nephron occurs at this point.

Using the nephrogenic zone of the rodent kidney as an experimental model, it was shown that the nephrogenic niche (Abdel-Hakeem et al., 2008; Awazu and Hida, 2015; Rabadi et al., 2015; Yu et al., 2019), the renal vesicle (Barnett et al., 2017), and the comma-shaped body (Almeida and Mandarim-de-Lacerda, 2002) can be affected by impaired nephrogenesis. However, contrasting data for the fetal human kidney is available. It was shown in gestational controls that the width of the nephrogenic zone is not more than 150 μm, while in the group of preterm babies, it is significantly

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smaller at a width of 100 μm (Sutherland et al., 2011; Ryan et al., 2018). A further study revealed that basophilic S-shaped bodies are absent in preterm infants (Rodriguez et al., 2004). Using premature baboons as a primate model, it was demonstrated that a decrease in glomerular generations correlates with an increase in the renal corpuscle area including the S-shaped bodies (Callaway et al., 2018).

Important to note, frequently mentioned data dealing with the number and spatial distribution of maturing glomeruli in the fetal human kidney, have to be assigned not to the nephrogenic zone but to the underlying maturation zone (Black et al., 2013). Features typical to damaged glomeruli are the dilated Bowman's space and the shrunken tuft (Sutherland et al., 2011). Recently, it was published that prematurity correlates not only with a damage of the developing glomeruli but also causes a reduction in their number (Callaway et al., 2018). This important result points out that, besides the process of glomerulogenesis in the maturation zone, the development of the pre-stages such as the S-shaped body, the comma-shaped body, the renal vesicles and the pretubular aggregate located in the nephrogenic zone may also be damaged.

The thorough analysis of relevant literature revealed, that the potential targets of noxae impairing nephrogenesis and the microanatomical features of the nephrogenic zone in the fetal human kidney have not been explored in great depth. This is also recognized by the fact that microanatomical details about the nephrogenic niche (Minuth, 2018a), the positioning of the nephron anlage (Minuth, 2019b) and the process of nephron shaping (Minuth, 2020) in the fetal human kidney during late gestation have only been published within the past few years. However, data on immunohistochemical profiles, ultrastructure, biochemical processes or the physiological environment is yet is not available. To support the start of such investigations, the aim for this study is to highlight the initial formation of a nephron from the nephrogenic niche to the S-shaped body using true to scale sketches, practical hints and a consistent nomenclature.

2. Materials and methods

2.1. Preparation of the fetal human kidney

The search for cellular and molecular biological traces of impaired nephron anlage focusses on the nephrogenic niche, pretubular aggregate, renal vesicles, comma- and S-shaped body, which are restricted to the nephrogenic zone. This extends along the inner side of the renal capsule. In order to prevent damage during histological preparation, the kidney is held on the hilum, so that the touching of the renal capsule with fine forceps is avoided (Minuth, 2019a). For obtaining comparable perspectives in the histological sections, a fixed kidney is cut from the renal capsule toward the papilla of a lobe. Thereby, the section plane lines perpendicular to the renal capsule and along the axis of vertically running collecting duct (CD) tubules and perforating radiate arteries. When this advice is followed, the nephrogenic zone and the contained stages of nephron anlage become visible underneath the renal capsule in a comparable perspective.

2.2. Microscopy of slides

For the illustrations shown in this paper, specimens of fetal human kidneys between weeks 16 and 18 and some of later gestation were selected from the stock of preparations used for the Course of Microscopic Anatomy for Medical Students at the University of Regensburg. According to routine methods, the samples of renal fetal cortex were fixed in paraformaldehyde solution and embedded in paraffin wax. Then, sections of 5 μm thickness were produced and stained with hematoxylin–eosin solution for analysis by the optical microscope. Screening of stained sections was performed by a Leica DM750 microscope (Leica Microsystems, Wetzlar, Germany). The stages of nephron anlage were analyzed with a HI Plan 63x/0.75 objective lens. Images were taken with a Basler Microscopy Pulse 5.0 camera (Basler AG, Ahrensburg, Germany).

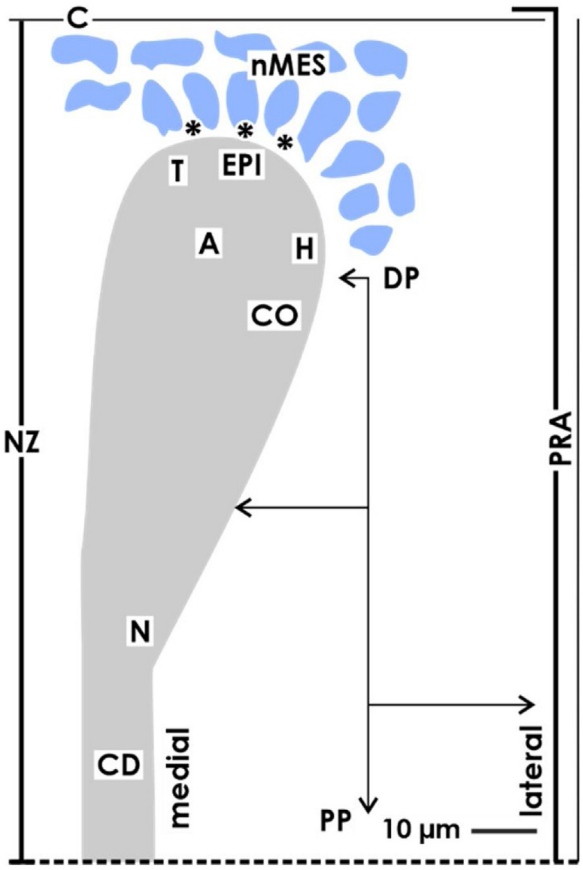

Fig. 1. True to scale sketch of a nephrogenic compartment (framed) as a segment of the nephrogenic zone (NZ) in the fetal human kidney during late gestation. The outer border is the renal capsule (C), while the medial border is the distal end of a collecting duct (CD) tubule dilating into a CD ampulla (A). This consists of a tip (T), head (H), conus (CO) and neck (N). The lateral border is a vertically lining perforating radiate artery (PRA). The inner border (dotted line) crosses the proximal pole (PP) of the S-shaped body transversely. The nephrogenic mesenchymal progenitor cells (nMES), an interface (black asterisk) and the epithelial progenitor cells (EPI) in the tip of a CD ampulla meet in a nephrogenic niche. Induced mesenchymal progenitor cells become angular and first, develop the pretubular aggregate along the tip and then the head of a CD ampulla. The further development of the nephron proceeds perpendicular to the renal capsule from the distal pole (DP) to the proximal pole (PP) of the renal vesicles, the comma- and the S-shaped body. Its medial expansion is directed toward the head, conus and neck of the CD ampulla, while the lateral expansion is bordered by a perforating radiate artery.

2.3. Fabrication of sketches

More than 3000 available images had been analyzed in context with an earlier performed investigation dealing with the shaping of the nephron (Minuth, 2020). From this stock, representative images illustrating important stages during initial nephron development, were selected. Since true to scale sketches of the developing nephron in the late gestational fetal human kidney were not available, the precise positions, sizes and shapes of these individual stages of nephron anlage were documented by blueprints. It was decided to produce these blueprints in an effort to be as accurate as possible and to meet the criteria. Therefore, the eligible

microscopic images were magnified (28 × 18 cm) and the contour line of the developing nephron was marked 1: 1 by hand with a pencil. The marked contour line was then scanned, edited and finally processed by the design program CorelDRAW X7 (Corel Corporation, Munich, Germany). For inserting the necessary labels and to obtain information about metric parameters, the microscopic images and the generated blueprints were analyzed with the same program.

3. Results

3.1. Nephrogenic zone

The outer cortex of the fetal human kidney is covered at the top by the renal capsule. It further consists of the external nephrogenic zone and the underlying maturation zone (Table 1) (Minuth, 2019a). The stages of nephron anlage including the nephrogenic niche, the pretubular aggregate, the renal vesicles, the comma- and the S-shaped bodies are restricted to the nephrogenic zone. In so far, its outer border is in contact with the inner side of the renal capsule. Its inner border was defined earlier by a line, which crosses the proximal (medulla-orientated) pole of developing S-shaped bodies transversely (Sutherland et al., 2011; Ryan et al., 2018).

3.2. Nephrogenic compartment

By vertical lines, the nephrogenic zone can be divided into nephrogenic compartments, which are aligned side by side in a single row (Table 1 and Fig. 1). Within a nephrogenic compartment the development of a nephron from the niche to the late S-shaped body takes place. Meanwhile, each of the nephrogenic compartments is covered by the renal capsule, the medial border is defined by the vertical course of a collecting duct (CD) tubule forming a CD ampulla at its distal end. The lateral border lines along a vertically running perforating radiate artery.

The spatial expansion of a developing nephron within the nephrogenic compartment is complex (Fig. 1). Initially, it extends perpendicular to the renal capsule and in close vicinity of the related CD ampulla. Main trajectories line medial first to the head and then toward the conus of the CD ampulla. The lateral expansion is directed to a vertically lining perforating radiate artery. Later on, each of the nephrogenic compartments can be subdivided by a transverse line into the above positioned district of progenitor cell recruitment and the underlying area of nephron shaping (Table 1 and Fig. 2a) (Minuth, 2020).

3.3. District of progenitor cell recruitment

The district of progenitor cell recruitment has an upper transverse border, which is identical with the outer border of the nephrogenic zone respectively a nephrogenic compartment. This develops along the inner side of the renal capsule and exhibits a length of about 80 μm (Fig. 2a). Both of the vertical borders are 45–50 μm in length. The lower transverse border crosses between the head and conus of a CD ampulla. Regarding orientated histological sections, between the tip of a CD ampulla and the inner side of the renal capsule only 2–3 layers of nephrogenic mesenchymal progenitor cells can be seen. It was recently shown that the nephrogenic progenitors face the tip of the CD ampulla, whilst the stromal respectively interstitial progenitors are aligned near the inner side of the renal capsule (Lindström et al., 2020). Regarding a microscopic slide, within the district of progenitor cell recruitment, a nephrogenic niche at the tip of the CD ampulla, possibly a pretubular aggregate and depending on the developmental stage a primitive renal vesicle is recognized near the head of the CD ampulla.

3.4. Area of nephron shaping

The area of nephron shaping is represented by an extending quadrate whose size goes from being similar to that of a primitive renal vesicle to reach vertical and transverse lengths of up to 100 μm each (Fig. 2a). The upper border lines between the head and conus of the related CD ampulla, meets the attachment site of the mature renal vesicle and the proximal end of the pretubular aggregate. At the lateral side, the border crosses the mesenchymal progenitor cell strand, which connects during subsequent development the proximal end of the pretubular aggregate up to the early comma-shaped body. The lateral border is faced by a vertically running perforating radiate artery. Regarding microscopic specimens, within the area of nephron shaping the attachment site of the presumptive connecting tubule (CNT) on the CD ampulla and either a renal vesicle, a comma-shaped body or a S-shaped body can be seen.

3.5. Nephrogenic niche

The nephron development begins by induction during which the most inner layer of the nephrogenic progenitor cells ($Six2^+/Cited1^+$) and the basal aspect of epithelial progenitor cells contained in the tip of the CD ampulla meet for transient interactions (Minuth, 2018a; Lindström et al., 2018a). During this process different morphogens such as GDNF, Wnts, FGFs and BMPs are exchanged (Little and McMahon, 2012). It can be recognized in the fetal human kidney, that the mesenchymal progenitor cells are separated from the tip of the CD ampulla by a clear interface (Fig. 1) (Minuth, 2020). Upon this, some of the induced nephrogenic mesenchymal progenitor cells increase in size and become prominent, whilst the intercellular spaces between them enlarge. The final step occurs as the cells aggregate along the tip and then head of the CD ampulla to form a pretubular aggregate.

3.6. Pretubular aggregate

From the neonatal rabbit kidney, it is known that a spatial order is maintained by extracellular microfibers, which are found between the renal capsule, the mesenchymal progenitor cells and the tip of the CD ampulla (Minuth and Denk, 2015). In the fetal human kidney, the distal end of the early pretubular aggregate (renal capsule-orientated) remains connected with the most inner layer of the nephrogenic mesenchymal progenitor cells. These begin facing the tip of the related CD ampulla (Fig. 2a). By multiplication of cells at its proximal end (medulla-orientated), an expansion of the pretubular aggregate is observed, whilst its medial part develops near the CD ampulla. At first, between the distal end of the pretubular aggregate and the tip of the CD ampulla, only a clear interface is noticed. However, a close adhesion section is soon seen between the proximal end of the pretubular aggregate and the head of the CD ampulla. Uneven interstices between the cells and a smoothening of the surface at the proximal end of the pretubular aggregate indicate the start of epithelial polarization.

The mesenchymal to epithelial transition (MET) becomes visible in the proximal end of the mid pretubular aggregate. As it is seen in the rodent and human kidney, this process initiates the formation of a renal vesicle (Little and McMahon, 2012; Lindström et al., 2020). On microscopic slides, it can be further observed that a transverse section starts between the distal and proximal end of the pretubular aggregate (Fig. 2b). Meanwhile, the development of an epithelium, as well as a small lumen in the proximal end of the pretubular aggregate becomes visible.

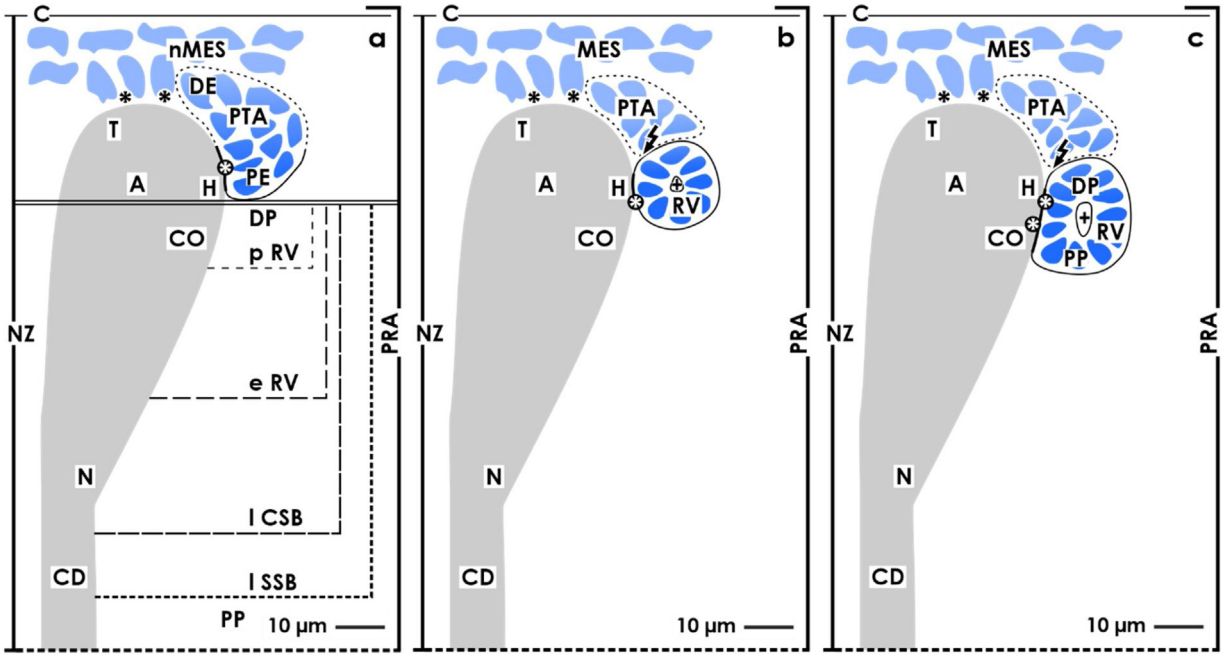

Fig. 2. True to scale sketches of **a** the pretubular aggregate (PTA), **b** the site of mesenchymal to epithelial transition and **c** the formation of a primitive renal vesicle (RV) in the fetal human kidney during late gestation. (**a**) The upper frame in a nephrogenic compartment depicts the district of progenitor cell recruitment. Separated by the transverse double line, the lower frame shows the area of nephron shaping. Here, the successive expansion of the primitive renal vesicle (pRV), extended renal vesicle (eRV), late comma-shaped body (lCSB) and late S-shaped body (lSSB) takes place. The distal end (DE, renal capsule-orientated) of a pretubular aggregate remains connected with nephrogenic mesenchymal progenitor cells (nMES). Separated by a clear interface (black asterisks), it extends along the tip (T) and head (H) of a CD ampulla (A). Between its head and the proximal end (PE, medulla-orientated) of the pretubular aggregate a close adhesion (white asterisks) is forming. (**b**) The interstices between the cells expand, and a smoothening of the surface at the proximal end of the PTA indicate the mesenchymal to epithelial transition. The transition between the clear interface and the close adhesion is consolidating. A lumen (cross) becomes visible near the proximal end. The flash indicates the starting but only partial separation of the RV from the PTA. (**c**) The primitive renal vesicle is still part of the pretubular aggregate. At its distal pole (DP), the partial separation from the PTA proceeds (flash). The epithelium of the renal vesicle in contact with the conus (CO) of the CD ampulla is more prominent than at its lateral aspect. The lumen (cross) extends and a basal lamina covers the proximal pole (PP) of the renal vesicle. C renal capsule, PRA perforating radiate artery.

Although a transverse separation proceeds, a part of the primitive renal vesicle initially stays connected with the late pretubular aggregate at the distal pole (Fig. 2c). The separation is seen at the transition site between the earlier mentioned clear interface and the close adhesion section and extends transversely up to the mid of the pretubular aggregate. However, the lateral part of the pretubular aggregate remains unaffected and for this reason, it stays connected with the distal pole of the renal vesicle via a two-layered progenitor cell strand.

3.7. Change of the location

During further development of a nephron the location is changing. While the primitive renal vesicle arises in the district of progenitor cell recruitment, the development of the extending renal vesicles, the comma- and the S-shaped bodies takes place in the underlying area of nephron shaping (Fig. 2a).

3.8. Extending renal vesicles

Experiments with the rodent kidney revealed that the development of a renal vesicle is complicated. Currently, we differentiate between the primitive, the mature and the extending renal vesicles 1–5 (Georgas et al., 2009; Kao et al., 2012; Yang et al., 2013). Comparable data for the fetal human kidney was recently published (Lindström et al., 2020). As presented here, the current morphological data exhibits that during the formation of the renal vesicle the tubule anlage and then the glomerulus anlage become visible within their specific coordinates. In parallel, the process of nephron shaping is beginning (Fig. 3).

An important site of a mature renal vesicle is at its distal pole. The medial part dissolves from the pretubular aggregate to fix onto the CD ampulla at the border between the head and the conus (Fig. 3a). At this point the tubule anlage, including the later CNT, arises and elongates in a vertical direction. The inner part of the distal pole remains separated from the overlying pretubular aggregate. The lateral part maintains its connection with the pretubular aggregate via a two-layered progenitor cell strand. The contour of the lumen in a renal vesicle is uneven. Due to the commencement of the internal epithelial folding, the lumen rounds off at the proximal pole but develops a v-shape at its distal pole. Between the conus of the CD ampulla and the medial aspect of the renal vesicle, the interface extends in a vertical direction to develop into a cleft.

In the extending renal vesicle the lumen is developing (Fig. 3b). Whilst still being rounded at the proximal pole, at the distal pole it is restricted due to the fact that the tubule anlage is vertically elongating. During further development, the renal vesicle extends by widening and lengthening whilst, at the interface between the conus of the CD ampulla and the medial aspect of the renal vesicle, a vertically lining interstitial cleft can be seen. Since the proximal pole of the renal vesicle is restricted by the CNT of an earlier established nephron, its ongoing extension is radially directed toward the renal capsule. At the same time, an elongation of the CD ampulla in the same direction occurs.

The next developmental event is the fixing of the extended renal vesicle onto the CD ampulla at the section border between its head and conus (Fig. 3c). At this point, the presumptive CNT invades the epithelium of the CD ampulla. During this process, similar to the basal lamina of the CD ampulla, that of the renal vesicle will also dissolve at the site of fixing. Underneath, the cleft between the medial aspect of the renal vesicle and the conus of the CD

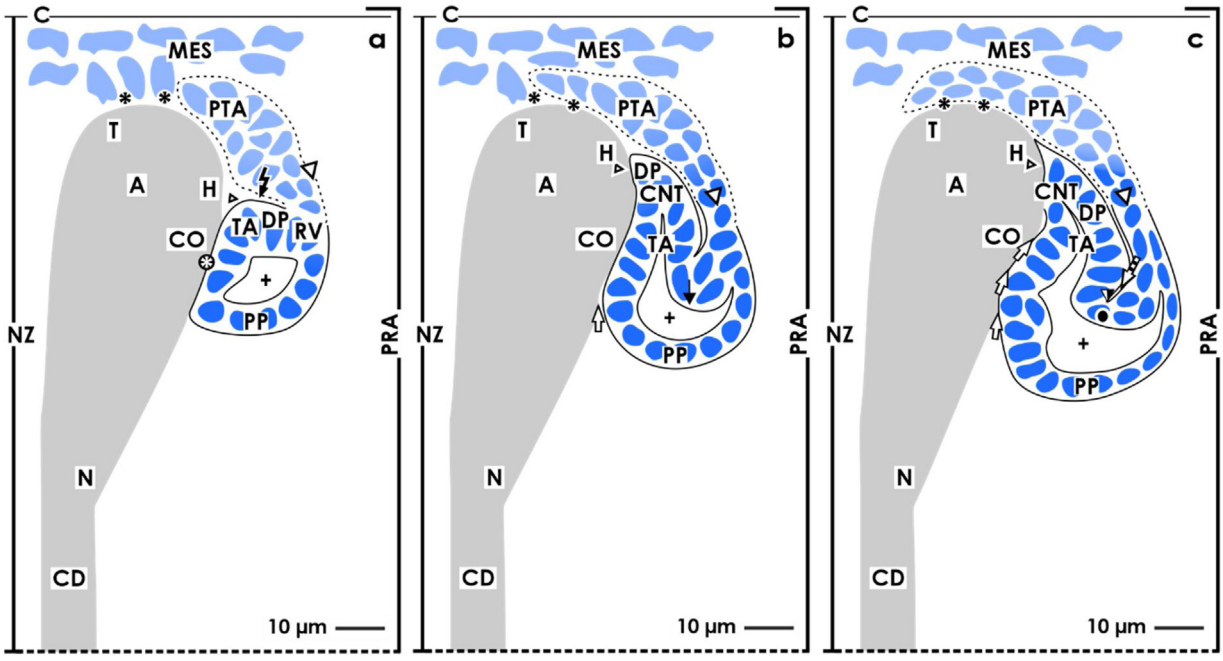

Fig. 3. True to scale sketches of the **a** mature, **b** extending and **c** extended renal vesicles (RV) in the fetal human kidney during late gestation. (**a**) Mature renal vesicle: The medial part of its distal pole (DP, small black/white arrow head) is fixed via the tubule anlage (TA) on the CD ampulla (A) between the head (H) and conus (CO). The mid of it stays separated (flash). The lateral part is connected with the pretubular aggregate (PTA) by a two-layered progenitor cell strand (large black/white arrow head). The proximal pole (PP) is orientated toward the medulla. The lumen (cross) expands unevenly. Between the conus of the CD ampulla and the renal vesicle, the adhesion (white asterisks) elongates vertically. (**b**) Extending renal vesicle: It is fixed via the connecting tubule (CNT) on the CD ampulla. For elongation of the tubule anlage, at the distal pole (DP) the epithelium invaginates (black/white arrow). The progenitor cell strand (white arrow head) at the lateral part of the distal pole is in contact with the PTA. The adhesion between the CD ampulla and the renal vesicle is transforming into a vertical cleft (white arrow). (**c**) Extended renal vesicle: The fixing on the CD ampulla indicates the invasion of the CNT. The distal pole is still connected with the pretubular aggregate via a two-layered progenitor cell strand (white arrow head). The epithelium at the distal pole further invaginates toward the lumen to form an inner fold (black spot). Its medial leg lines to the part of CNT ending at the head of the CD ampulla. The lateral leg lines to the progenitor cell strand (white arrow head), which is connected with the PTA. Between the legs, a vertical cleft (dashed white arrow) arises. The cleft (white arrows) between the renal vesicle and the CD ampulla changes direction from vertical to slightly transverse. C renal capsule, PRA perforating radiate artery.

ampulla elongates. Near the CNT, the direction of elongation begins to change from vertical to slightly transverse. This signals the winding of the renal vesicle. The lateral part of the distal pole is still connected to the pretubular aggregate as the renal vesicle continues to extend via the previously described two-layered progenitor cell strand.

The epithelium of the tubule anlage elongates in a vertical direction to form an inner fold (Fig. 3c). Its medial leg lines up with the epithelial cell strand of the presumptive CNT, which ends at the head of the CD ampulla. The tip of the fold is in the geometric center of the renal vesicle. The lateral leg of the fold is part of the progenitor cell strand, which is connected to the pretubular aggregate. As a result, a vertically lining cleft arises between the two legs of the inner fold. Finally, the lumen reaches a lying C-form, completing the transition from the extended renal vesicle to the early comma-shaped body.

3.9. Comma-shaped body

During early organogenesis, expressed proteins in the early comma-shaped body of the rodent and human kidney were investigated (Kao et al., 2012; Lindström et al., 2015). The current data exhibits that the progenitor cell strand between the early comma-shaped body and the pretubular aggregate dissolves (Fig. 4a). Between the conus of the CD ampulla and the medial aspect of the comma-shaped body, the interstitial cleft expands, so that it lines vertically toward the arising CNT (Fig. 4b). On the lateral aspect, the cleft at the proximal pole of the comma-shaped body changes its course. This announces the fine-positioning of the nephron anlage, the future asymmetrical development and the appearance of the glomerulus anlage at the proximal pole (Fig. 4c). In addition, the process of internal folding determines important morphological features of the developing nephron.

The extended renal vesicle is still connected at its distal pole with the overlying pretubular aggregate via the mentioned two-layered mesenchymal progenitor cell strand (Fig. 3c). However, during the development of the early comma-shaped body, this connection dissolves (Fig. 4a). Henceforth, it can no longer partake in the progressive recruitment of progenitor cells (Lindström et al., 2018b). At the same time point, one can see that the tubule anlage elongates vertically toward the center of the comma-shaped body, resulting in the formation of three internal folds.

The medial fold: This is closest to the point, where the epithelial cell strand of the CNT contacts the conus of the CD ampulla. The tip of the medial fold is the transition between the later parietal cells of Bowman's capsule and the prospective proximal tubule segment (Fig. 4a and b).

The inner fold: This has a medial leg, which lines up with the point, where the epithelial cell strand of the CNT contacts the head of the CD ampulla (Fig. 4a and b). The tip of the inner fold is the transition between the prospective proximal tubule segment (medial leg) and the visceral epithelial cell layer (lateral leg), which transforms into the podocytes.

The lateral fold: This exhibits a medial leg, which contains the layer of visceral epithelial cells developing into the podocytes (Fig. 4a and b). This leg corresponds to the lateral leg of the inner fold. The lateral leg of the lateral fold contains the parietal epithelial cell layer, which further develops into cells of the Bowman's capsule. Consequently, between the medial leg of the medial fold and the lateral leg of the lateral fold, the later Bowman's capsule is expanding.

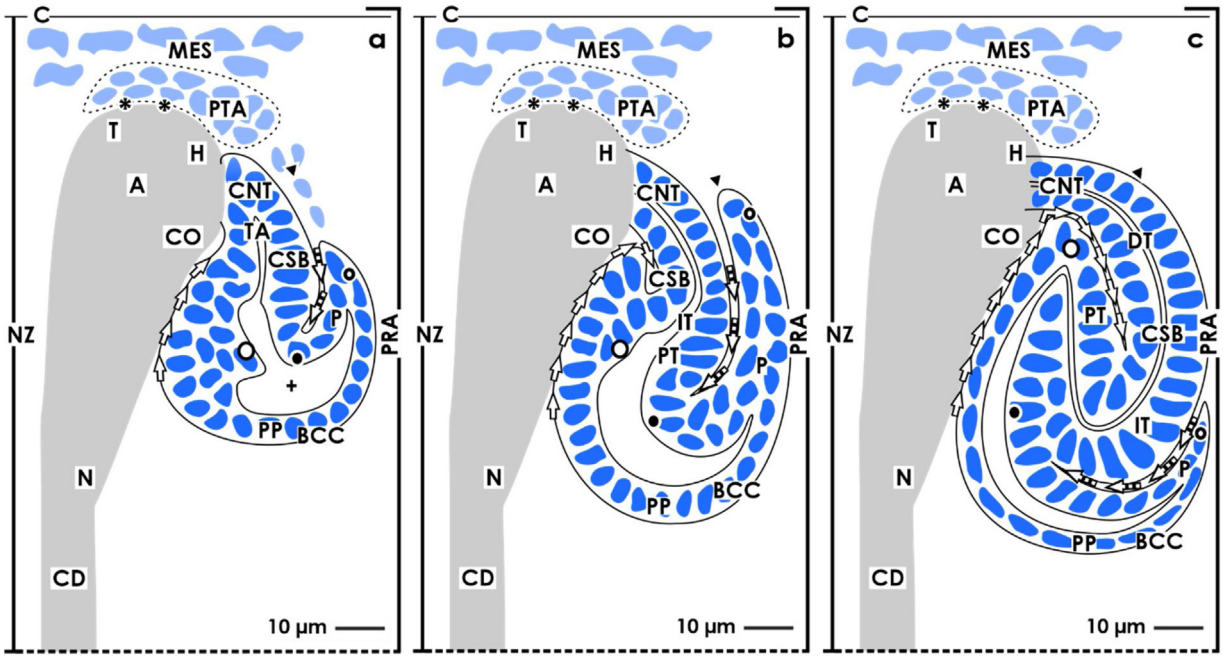

Fig. 4. True to scale sketches of the **a** early, **b** mid and **c** late comma-shaped body (CSB) in the fetal human kidney during late gestation. These are located near the head (H), conus (CO) and neck (N) of a CD ampulla (A). At the proximal pole (PP), the parietal (Bowman's capsule cells, BCC) and the visceral (Podocytes, P) cells form the glomerulus. The tubule anlage (TA) including the proximal (PT), intermediate (IT) and distal (DT) tubule segments protrudes. (**a**) Early comma-shaped body: The progenitor cell bridge (black arrow head) with the pretubular aggregate (PTA) dissolves. The tubule anlage elongates vertically. The cleft (white arrows) between the CD ampulla and the comma-shaped body extends. Internal folds arise. The medial fold (white spot) is near the crotch, where the CNT contacts the conus of the CD ampulla. The point of fold is between the parietal cells of Bowman's capsule and the proximal tubule. The inner fold (black spot) has a medial leg lining to the crotch, where the CNT contacts the head of the CD ampulla. Its tip is the transition between the future proximal tubule (medial leg) and the visceral epithelial cell layer (lateral leg) forming the podocytes (P). The lateral fold (black/white spot) shows a medial leg, which contains the layer of visceral epithelial cells. The tip of the lateral fold lines to the lateral leg containing the Bowman's capsule cells (BCC). (**b**) Mid comma-shaped body: A vertical lengthening of the folds causes extension of the cleft (white arrows) between the CD ampulla and the comma-shaped body. The cleft (dashed white arrows) at the podocytes opens. (**c**) Late comma-shaped body: The cleft (white arrows) between the CD ampulla and the comma-shaped body reaches the center. The cleft (dashed white arrows) at the basal aspect of podocytes changes direction from vertical to transverse. C renal capsule.

In the mid comma-shaped body the connection of its distal pole with the CD ampulla is strengthening (Fig. 4b). The epithelium of the CNT perforates the epithelium of the CD ampulla between the head and conus. In parallel, the comma-shaped body is extending causing an elongation of the medial, inner and lateral folds. As a result, the vertical cleft between the conus of the CD ampulla and the medial aspect of the comma-shaped body increases in length. At the same time, there is elongation of the proximal tubule segment and the visceral epithelial cell layer, which is developing into the podocytes.

While the late comma-shaped body is forming, the contained tubule segments are meandering (Fig. 4c). This leads to a compaction and enables an optimal exploitation of the available internal space. The cleft between the conus of the CD ampulla and the medial aspect of the comma-shaped body is increasing by turning, so that it finally reaches the geometrical center. At the proximal pole of the comma-shaped body an asymmetrical development can be seen. The cleft between the proximal tubule segment and the visceral epithelial cell layer forming the podocytes is altering its direction from vertical to transverse. It opens laterally toward a vertically lining perforating radiate artery to enable the invasion of an afferent arteriole via a short way into the forming glomerular tuft.

3.10. S-shaped body

The last stage of nephron anlage, which develops within the nephrogenic zone, is the S-shaped body. It was investigated in both the rodent and human kidney. A distinction is made between the early, mid and late S-shaped body (Ivanova et al., 2010; Zhang et al., 2019).

The early S-shaped body is positioned perpendicular to the renal capsule (Fig. 5a). It is connected at its distal pole via the CNT with the CD ampulla. At its proximal pole the parietal epithelial cell layer develops into the Bowman's capsule of the glomerulus. Separated by the expanding urinary space, the formation of podocytes can be seen in the overlying visceral cell layer (Fig. 5a). The tip of the fold between the visceral and parietal cell layers is recognized at the lower lateral aspect of the S-shaped body where at the podocyte layer, a transverse cleft is opening toward a vertically lining perforate radiate artery. In the interior of this cleft are forming as well the glomerular tuft, the intra- and extraglomerular mesangium as the capillary network. Numerous cell divisions in the neighboring tubules signal that an elongation of the individual segments is proceeding. The presence of a lumen is a further sign that its physiological differentiation is occurring.

The medial aspect of a mid S-shaped body appears first in the near of the conus and then the neck of the related CD ampulla (Fig. 5b). At its proximal pole, the functional development of the glomerulus proceeds. The cells in the parietal layer become flat to form typical signs of the Bowman's capsule, whilst in the overlying visceral layer the podocytes exhibit a cobblestone-like appearance. The overlying transverse cleft extends, and an immigration of cells with bright nuclei can be observed at its opening on the lateral aspect of the S-shaped body. This signals ongoing development of the glomerular barrier, proceeding mesangiogenesis and a functional connection with the perforating radiate artery via an afferent arteriole. At the urinary pole, the developing brushborder informs that the proximal tubule functionally differentiates. In the distal tubule segment, a lumen is present.

The late S-shaped body extends vertically (Fig. 5c). For this reason, a radial expansion of the nephrogenic zone toward the renal

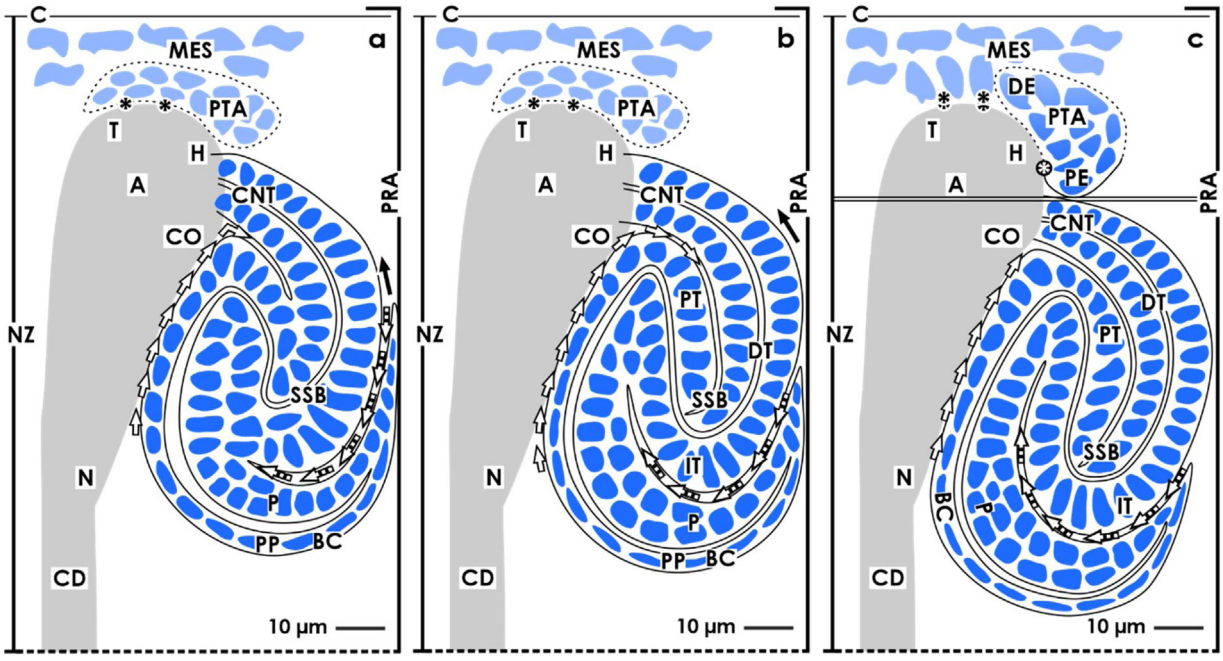

Fig. 5. True to scale sketches of the **a** early, **b** mid and **c** late S-shaped body (SSB) in the fetal human kidney during late gestation. These are forming along the head (H), conus (CO) and neck (N) of a CD ampulla (A) and the collecting duct (CD) tubule, and are separated from it by a vertical cleft (white arrows). At the distal pole, the CNT has a physiological connection with the CD ampulla. The proximal pole (PP) is orientated toward the medulla. Here, the formation of glomerulus including the podocytes (P) and Bowman's capsule (BC) takes place. In the center, the proximal (PT), intermediate (IT) and distal (DT) tubule segments develop. (**a**) Early S-shaped body: The outer cell layer at the proximal pole forms the Bowman's capsule. Separated by the urinary space, the podocytes develop in the overlying layer. The tip of the fold between these layers opens at the lower lateral aspect. The related cleft (dashed white arrows) becomes connected with a perforating radiate artery (PRA). (**b**) Mid S-shaped body: At the proximal pole of the glomerulus, the Bowman's capsule and the podocytes differentiate. The transverse cleft (dashed white arrow) above extends for vessel formation and mesangiogenesis. At the urinary pole, the proximal tubule (PT) develops a brushborder. (**c**) Late S-shaped body: It extends in a vertical direction. The glomerulus is turning, the Bowman's capsule is flat, while the urinary space is broad. The cleft (dashed white arrows) at the basal aspect of podocytes changes direction from transverse to vertical. The formation of capillaries and mesangium is noticed. Its opening points toward a perforating radiate artery. The tubule segments elongate by meandering. The border between the district of progenitor cell recruitment and the area of nephron shaping is indicated by a transverse double line. C renal capsule.

capsule takes place. Typical morphological features of the glomerulus become visible at the proximal pole. The Bowman's capsule is seen as a flat epithelial layer. A further broadening of the urinary space occurs. At the basal aspect of podocytes, a network of capillaries establishes itself to become part of the arising intraglomerular mesangium. The cleft opening toward a vertically lining perforate radiate artery becomes sickle-shaped. Further this first elongates toward the CD ampulla and then changes direction from transverse to vertical. In the mid and upper third of the late S-shaped body, numerous cell divisions point out that the different tubule segments extend in length. The occurrence of a lumen and clear cell borders signal that a functional differentiation takes place. The physiological connection between the CNT and the CD ampulla is complete.

3.11. Different sites of spatial expansion in the nephrogenic compartment

The development of a nephron within a nephrogenic compartment correlates with a spatial expansion (Fig. 2a). To obtain information, as to where and to what degree this takes place, the series of previously described sketches must be compared. First of all, one can recognize that from the starting pretubular aggregate (Fig. 2a) up to the late S-shaped body (Fig. 5c) the district of progenitor cell recruitment maintains a more or less constant size. In contrast, the area of nephron shaping increases (Fig. 2a). It is starting from the size of the mature renal vesicle (Fig. 2c) to transverse and vertical lengths of about 100 µm in the late S-shaped body (Fig. 5c). The expansion extends bidirectionally in a vertical (proximal - distal) direction, and it is asymmetrical. As it was earlier mentioned (Fig. 1), this depends on trajectories, which are directed medially, first to the head, and later to the conus of the CD ampulla and laterally toward a perforating radiate artery. It is remarkable that the spatial expansion takes place stepwise and is most prominent during development of the renal vesicles (Fig. 3a–c), the comma-shaped body (Fig. 4a–c) and the S-shaped body (Fig. 5a–c).

4. Discussion

Regarding the numerous clinical data dealing with the impairment of nephrogenesis in preterm and low birth weight babies (Kandasamy et al., 2012; Stritzke et al., 2017; Monzani et al., 2020), it is striking that so little is known about the cellular and molecular targets of noxae in the outer cortex of the fetal human kidney. This situation is further compounded by the fact that the stages of nephron anlage were previously not systematically investigated and that the damage to the cells, receptors or signaling cascades were not found. It is clear that only a broad search for initial traces left by noxae in the nephrogenic zone will enable us to develop concepts for a therapeutic prolongation of nephrogenesis (Minuth, 2018b; Fanos et al., 2019). A concrete hint for starting the search points to the nephrogenic zone and especially to the here contained nephrogenic compartments (Table 1 and Figs. 1 and 2a).

Previous investigations reveal that in gestational controls the width of the nephrogenic zone is not more than 150 µm, whilst in the group of preterm babies it is significantly smaller at 100 µm (Sutherland et al., 2011; Ryan et al., 2018). However, in this context it was not described, whether the decrease in width is caused by the loss of a single structural element or the sum of several pathological changes. Thus, the proposal is to focus on the nephrogenic compartment (Fig. 1) and to separately assess the district of pro-

Table 1
Schematic view onto the outer cortex in the fetal human kidney during late gestation to allocate marker molecules introduced by Dr. Menon and colleagues (Menon et al., 2018). The borders of a nephrogenic compartment are framed by thick black lines. The separation between the district of progenitor cell recruitment and the area of nephron shaping is indicated by a transverse double line. **d** distal, **p** proximal, **CD** collecting duct.

Stromal lineage		**Renal capsule** **Nephrogenic zone / outer border**		
SFRP1, TNC, DCN, FOXD1, COL3A1, COL1A2, COL1A1, POSTN, CXCL12	**RECRUITMENT**	**Stromal mesenchymal progenitor cells**		**Nephrogenic lineage**
CD lineage		**Nephrogenic mesenchymal progenitor cells**		CITED1, TMEM100, SFRP1, SIX1, SIX2, WT1, HNF1A, ITGA8, EYA1
RET, ETV4, ETV5, DUSP6		Tip	**Niche**	GDNF-RET, WNT, FGF, Notch signaling, Yap
		Head	**Pretubular aggregate (PTA)** **d** **p**	MECOM, POU3F3, GATA3 JAG1, LHX1
CALB1	**NEPHRON SHAPING**	CD ampulla Conus	**Renal vesicle (RV) / -Primitive RV -Mature and Extending RV -Extended RV 1-5** **d** **p**	ETV4, FGF8, SFRP2 HNF4A, EYA1, PAX2
		Neck	**Comma-shaped body (CSB) -Comma-S-Transition** **d** **p**	FGF8, SFRP2
			S-shaped body (SSB) -Early SSB -Mid SSB -Late SSB **d** **p**	POU3F FOXC2, LHX1
		Nephrogenic zone / inner border **Maturation zone**		
CALB1, KRT7, UPK1A, KRT19, SPINK1, GATA3, KRT8, KRT19, Elf5, AqP2, MUC1		Differentiated CD tubule	**Maturing glomeruli**	Parietal: CAV2, PTRF, CLDN1, CLDN3 Visceral: PODXL, NPHS1, NPHS2, CLIC5, PAX8, OLFM3 Mesangium: ACTA2, ANGPT2, TAGLN
			Mature glomeruli	Parietal: CLDN1, NPHS1 Visceral: PODXL, NPHS1, NPHS2, SYNPO, VEGF, NTNG1
			Tubule segments **p** **d**	CUBN, PDZK1, LRP2 UMOD SLC12A1

genitor cell recruitment and the area of nephron shaping (Fig. 2a). The advantage of this necessary separation is that multiple parameters can be screened as opposed to only one. For example, the acquisition of data in the district of progenitor cell recruitment can provide information about the nephrogenic niche (Fig. 1), the pretubular aggregate (Fig. 2a), the mesenchymal to epithelial transition (Fig. 2b) and the primitive renal vesicle (Fig. 2c). In contrast, the parameters analyzed in the area of nephron shaping can inform us about the expansion of the renal vesicles (Fig. 3), the folding in the comma-shaped body (Fig. 4) and the winding of the S-shaped body (Fig. 5). Following this strategy, data from morphometric recordings, immunohistochemical profiles or pathological changes for example can be seen in the sketches provided (Figs. 1–5). This enables transparent, comparable and easy to understand documentation.

As for the basic research, as much as for the assessment in molecular pathology a set of antibodies is needed to obtain detailed information about expressed proteins in the developing nephron. This enabled us to investigate the progressive recruitment of renal progenitor cells (Lindström et al., 2018a) or to record the protein profile in the developing nephron during early organogenesis, for example (Lindström et al., 2020). However, in order to find initial traces left by noxae during late gestation, an extended set of markers is required, not only so that the parenchymal elements such as the single stages of nephron anlage or the CD ampulla can be analyzed, but also that the stromal components such as the interstitium, microvascular system and renal capsule can be considered.

Promising methods for obtaining the necessary information about evolving protein patterns during human kidney development is to isolate RNA and protein from whole kidney lysates (Nüsken et al., 2020). However, the single-cell RNA-sequencing appears more suitable for the targeted search in the nephrogenic zone (Menon et al., 2018). Based on isolated cells, it is possible to identify relevant proteins, which are restricted for example to the stages of nephron anlage. Then, by using the relevant commercially available or personally raised antibodies, it is possible to recognize the up-regulation of stage-specific features, crucial points and/or even transiently expressed proteins in the developing nephron. By comparing the pattern of expressed proteins in normal and damaged kidneys, it should be possible to find initial traces left by noxae, which impaired nephrogenesis. Taking first steps in this direction, data from available and eligible marker molecules for early kidney organogenesis (Menon et al., 2018) were sorted and assigned to the specific structural characteristics of the outer cortex in the fetal human kidney (Table 1).

A first look reveals that numerous marker molecules are available as potential candidates, which recognize various typical stages

in the outer cortex of the fetal human kidney during late gestation nephron development (Table 1). For example, one set of markers indicates maturing glomeruli, establishing nephron segments and differentiating cells in the collecting duct tubule located in the maturation zone. A further set exists comprising of markers for the nephrogenic zone, in particular those suited to analyze the district of progenitor cell recruitment within a nephrogenic compartment. Besides the nephrogenic niche, the stromal and nephrogenic progenitor cell lineages as the epithelial progenitors in the CD ampulla can be also recognized. However, with regards to the extending renal vesicles, the comma- and the S-shaped body within the area of nephron shaping, the selection of markers is poor. So far, no markers could be found, which enable us to recognize recently detected morphological features such as the separation of the renal vesicle from the pretubular aggregate (Fig. 2b and c) or its linking via the presumptive connecting tubule on the related CD ampulla. Furthermore, markers, which indicate the folding of the comma-shaped body (Fig. 4) or the winding in the S-shaped body are lacking (Fig. 5). Finally, one has to also consider that basic steps of nephron formation depend on very individual cell to cell contacts, matricellular communications and highly specific interactions with the extracellular matrix (Kanwar et al., 2004; Combes et al., 2015).

5. Conclusions

The impairment of nephrogenesis in preterm and low birth weight babies is an unsolved biomedical issue. To contribute, the current investigation is focusing on the unexplored target region of noxae. Using the provided true to scale sketches, numerous practical hints and a consequent nomenclature, the site-specific morphology situation of the nephrogenic zone and the here contained stages of nephron anlage in the fetal human kidney during late gestation are highlighted. This again supports the search for the cellular and molecular traces left by noxae impairing nephrogenesis, enables us to refine the pathological assessment and supports the systematic mapping of marker molecules.

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Authors' contribution

The author developed the concept, designed the illustrations and wrote the manuscript.

Ethics standards

The author declares that the manuscript including generated data is in accordance with the Declaration of Helsinki, with national ethical legal regulations and with the ethics committee of the University of Regensburg, Germany.

Conflict of interest

The author declares that he has no conflict of interest.

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