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Educational review

Structural and functional links between capsule and nephrogenic zone in fetal human kidney

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Abstract

Little attention received developmental aspects of the human kidney during the late phase of gestation. For healthy newborn babies this subject has no further meaning, since nephrogenesis proceeds unnoticed until birth. Upon delivery, morphogenic activity in the nephrogenic zone is waning and progenitor cell niches aligned beyond the renal capsule disappear by an unknown mechanism. However, a comparable but too early degenerative process takes place in the kidneys of preterm and low birth weight babies. Although born in a period of active nephrogenesis, pathological findings show that they evolve to a high incidence oligonephropathy. Regarding this problematic situation, it is necessary to develop concepts for a therapeutic prolongation of nephrogenesis. However, their realization will be difficult. First, many harming molecules and metabolites, related receptors and disturbed pathways on progenitor target cells have to be identified. Due to the lack of an intact vessel system, a site-specific application of drugs is required. Morphological peculiarities of the nephrogenic zone and positioning of niches must be taken into account. Of current interest are cell biological interactions between the nephrogenic zone and the covering capsule. For example, between gestation weeks 32 and 38, an astonishingly big areal expansion of the capsule and the underlying nephrogenic zone in an order of 30% takes place. Data point out that this little-noticed process also has a special meaning for the control of continuation and cessation of nephrogenesis. Thus, the present contribution likes to inform about this network, and it is simultaneously a call for young scientists to start with investigations in a hardly explored claim.

Keywords

Fetal human kidney, preterm infants, low birth weight babies, impaired nephrogenesis, capsule, nephrogenic zone, niche.

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Introduction

Adaption of a newborn baby to extrauterine life depends on multiple parameters including the intact development of the kidneys. Normally, anlage and setting of the nephron number are completed at the time of birth [1]. However, approximately 10% of babies are born preterm and with low birth weight [2]. Seen from a temporal perspective, kidneys of those babies are still in a period of active nephrogenesis [3]. There is now increasing evidence that delivery of preterm and low birth weight babies is interfering with this process. The resulting oligonephropathy is estimated to be between 8% and 24% of affected babies [4]. Pathologic data illustrate up to 18% morphologically abnormal glomeruli [5]. These exhibit a dilated Bowman's space and a shrunken glomerular tuft [6]. The actual literature further shows that prematurity leads not only to a damage of currently developing glomeruli but also to a decrease in their total number [7]. The site of pathologic changes indicates that the last generation of developing nephrons in the outer renal cortex, in particular the nephrogenic zone, and here contained progenitor cell niches are marred.

The screened literature further exhibits that quite different influences have been associated with impairment of nephrogenesis [8]. First named are restricted nutrition, particular protein or micronutrient intake and poor antenatal perfusion with lack of oxygen [9, 10]. However, eligible molecules harming nephrogenesis are also inflammatory cytokines, reactive oxygen species or antiangiogenic factors [11, 12]. A to date less respected role are playing drugs administered to preterm and low birth weight babies [13, 14]. In many cases it is unknown, whether expected therapeutic benefits are associated with adverse toxic effects of drugs on reactive cells occurring in the nephrogenic zone. An underlying problem in this dilemma is that a suitable *in vitro* test system, which reflects the intact situation of the human nephrogenic zone, is not available [15].

Cell biological changes in the nephrogenic zone

Data for fetal human kidney is missing, but animal models shed some light on physiological and cell biological changes occurring during impairment of nephrogenesis. For example, malnutrition produces murine neonates with a 40% reduced body weight [16]. Renal blood perfusion is reduced by 37%, cells in renal parenchyma exhibit a 2.2-fold increased rate of apoptosis and $Six2^+$ progenitor cells are decreased by 76% in the nephrogenic zone. Moreover, a downregulation of morphogens Wnt9b and Fgf8 involved in initial nephron formation is observed. As a result, 64% less developed renal vesicles and 32% fewer nephrons than in controls were detected. Another study illustrates that maternal food restriction is leading to an up-regulation of mRNA for morphogenic molecules such as WT1, FGF2, and BMP7, whereas Pax2, GDNF, FGF7, BMP4, Wnt4, and Wnt11 mRNAs were down-regulated [17]. A further investigation informs that maternal nutrient restriction inhibits ureteric bud branching but does not affect the duration of nephrogenesis [18]. However, malnutrition is not an exclusive cause. Also, site-specific changes in oxygen delivery [10, 19], suspected disturbance of morphogen transport [20], altered deposition of extracellular matrix [21] and disrupted cell-to-cell interactions [22] within the nephrogenic zone and on the site of niches are presumed to evoke impairment of nephrogenesis. In that regard it is unknown, whether due to toxic influences and physiological changes of environment only districts or the entire nephrogenic zone are affected.

Therapeutic challenge

Although the ultimate causative agents and related receptors on the target tissue are not identified, it is time to think about a site-specific therapeutic approach to buffer harming influences and/or to prolong nephrogenesis in preterm and low birth weight babies [23, 24]. Thinkable is a medication that stimulates morphogenic activity within the nephrogenic zone by a smart drug delivery system [25, 26]. However, the presently available data signal that such a program will be difficult to realize, since it must be adapted to less considered structural and functional features [27].

Due to lack of data, there is also an urgent need of investigations in the next future dealing with areal expansion of the capsule and nephrogenic zone including positioning of niches during growth of the fetal kidney. It includes morphological peculiarities, site-specific physiology, synthesis, secretion and transport of locally operating morphogens maintaining stemness, patterning of niches and triggering of nephron induction [28, 29]. Moreover, to obtain reliable information about distribution and bioavailability of administered drugs, concrete data for a site-specific provision with nutrition and respiratory gas via the incomplete microvascular system in this peculiar area must be generated.

Methodical aspects

The nephrogenic zone of a fetal human kidney extends as a narrow strip along the entire inner side of the capsule (**Fig. 1**) [29]. To prevent damage during histological preparation, one should best hold a fetal kidney on its hilum and should avoid touching the capsule with fine forceps.

Histological sections cut incidentally for microscopic analysis do not allow comparable views on the nephrogenic zone. To obtain repeatable perspectives, blocks of parenchyma must be orientated before embedding and during histological cutting as it was earlier described [30].

For illustration and morphometric measures, a human kidney of gestational age between week 16 and 18 was 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, specimens were fixed in paraformaldehyde solution and then embedded in paraffin wax. Then sections of 5 µm thickness were produced and stained with hematoxylin-eosin solution for analysis by optical microscopy.

Images of histological sections were taken with a digital camera and processed with CorelDRAW X7 (Corel Corporation, Munich, Germany). To obtain information about morphological coordinates and metric parameters, recordings were analyzed with the same program.

Calculations of the surface (S) of a model sphere and a cuboid body were made by equations found in the coaching program Frustfrei Lernen (https://www.frustfrei-lernen.de/mathematik/geometrie-volumen-oberflaeche-fass-kugel.html):

- model sphere $S = 4 \cdot \pi \cdot r^2$, where r stands for radius;
- cuboid body $S = 2 \cdot (L \cdot W + W \cdot H + L \cdot H)$, where L stands for length, W for width and H for height.

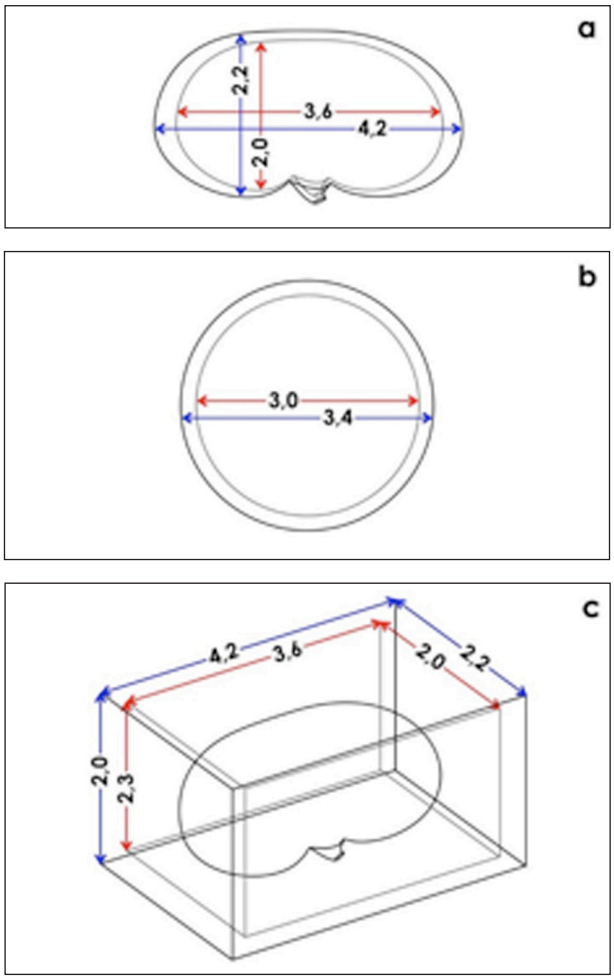

Figure 1. Schematic illustration shows growth of (**a**) human fetal kidney between gestation weeks 32 and 38 according to cited literature [31, 32] and according to **Tab. 1**. Length of a kidney increases from 3.6 to 4.2 cm and the width from 2 to 2.2 cm. The increase in capsule surface including underlying nephrogenic zone is unknown. To obtain an approximation, calculations were performed with (**b**) a model sphere reflecting a minimal surface and (**c**) a model cuboid reflecting a maximal surface.

Expansion of the capsule and nephrogenic zone

Earlier published data shows that between gestation weeks 32 and 38 the length of the kidney increases from 3.6 to 4.2 cm and the width from 2 to 2.2 cm (**Tab. 1**, **Fig. 1a**) [31, 32]. During this time interval, the total renal volume enlarges from 14.5 to 21.6 cm^3. It is perhaps surprising, but concrete information about the increase in surface of a human fetal kidney during this period could not be found in related literature. In this context, one must realize that with a rise of the kidney volume not only the capsule but also the nephrogenic zone is subject to the same areal expansion.

Cited measures of kidney length, width and total kidney volume (**Tab. 1**) can be used to calculate the

Table 1. Approximation to determine the areal expansion of the capsule and nephrogenic zone between gestation weeks 32 and 38 in human fetal kidney. According to the previously published literature [31, 32], the increase in the surface of a fetal human kidney was not presented. However, as seen in **Fig. 1** calculations with a model sphere and cuboid make an approximation possible.

	Parameter	Gestation week	
		32	38
Preterm kidney	Mean kidney length (cm)	3.6	4.2
	Mean kidney width (cm)	2.0	2.2
	Total kidney volume (cm^3)	14.5	21.6
	Kidney surface (cm^2)	?	?
Sphere	Radius (cm)	1.5	1.7
	Diameter (cm)	3.0	3.4
	Volume (cm^3)	14.5	21.6
	Surface (cm^2)	**28.7**	**37.5**
Cuboid	Length x width x height (cm^3)	3.6 x 2.0 x 2.0	4.2 x 2.2 x 2.3
	Volume (cm^3)	14.5	21.6
	Surface (cm^2)	**36.9**	**48.4**

surface of a model sphere (**Fig. 1b**) and the surface of a model cuboid (**Fig. 1c**). Following this approach, the sphere reflects the model with a minimal surface, while the cuboid represents the model with a maximal surface. As a logic consequence, increase in surface of a fetal kidney must be between these two models. The results exhibit that the surface of a model sphere enlarges from 28.7 to 37.5 cm^2, while the surface of a cuboid increases from 36.9 to 48.4 cm^2 (**Tab. 1**). It is surprising so far, since expansion of the surface in the sphere is 30.6%, while it is with 31.1% nearly the same in the cuboid. Paraphrased, between weeks 32 and 38 the areal expansion of the capsule and in parallel the underlying nephrogenic zone increase in the order of 30%. Meant is here the horizontal expansion along the capsule, not meant is the width of the nephrogenic zone. It is unknown, whether the contained cell biological machinery maintaining stemness, triggering branching morphogenesis, induction, anlage and initial development of nephrons is increasing in the same relation.

Microscopic analysis needs a repeatable perspective

Regarding the nephrogenic zone, a basic problem is that randomly cut sections do not help to identify structural details in a reliable manner. For this reasons fetal renal parenchyma has to be orientated during histological preparation and cutting in a microtome so that sections with a comparable perspective and an efficient manner can be produced (**Fig. 2**).

After fixation, a fetal human kidney is cut best from the capsule towards the papilla of a lobus. Following this approach, the section plane shows the nephrogenic zone orientated along the lumen of lining collecting duct (CD) tubules and perpendicular to the capsule (**Fig. 3**). View onto those sections further exhibits that the entire outer side of the nephrogenic zone is covered by the capsule. An imagined horizontal line along the branching sites of CD tubules and necks of CD ampullae points to its inner border. Previous microscopic analysis showed that in gestational controls the width of the nephrogenic zone is not more than 150 µm, while in the group of preterm babies it is with 100 µm significantly smaller [6]. However, not explained is the concrete site of decrease. In recently published literature and in the actual analysis additional metric dimensions of the nephrogenic zone are documented [29, 33].

Developmental vectors in the nephrogenic zone

Regarding a histological section of the nephrogenic zone, one must realize that it reflects only a brief snapshot of a dynamic process during nephrogenesis. For a better understanding, during the later fetal period of kidney development CD tubules elongate straight but fan-like towards the capsule. Each of them shows at its end bifid branching [34]. Paraphrased, an elongating CD tubule reflects a developmental vector with a radial course towards the capsule. The end of it is the point, where radial extension of parenchyma and increase in volume of the kidney takes place (**Fig. 3**). Moreover, each arising branch of a CD tubule is orientated towards the capsule. It dilates to form a CD ampulla consisting of a neck, body and tip (**Fig. 4**) [29]. It is obvious that the formation of each CD ampulla requires space at the lateral side of the related elongating CD tubule. Regarding all arising CD ampullae along the entire inner side of the capsule, it appears inevitably that this process occurs by a horizontal expansion of the nephrogenic zone along the inner side of the capsule.

Within a CD ampulla epithelial progenitor cells are contained, which exhibit a cuboidal shape. Further one can see that the basal aspect at the tip of a CD ampulla is separated from the bodies of neighboring nephrogenic mesenchymal stem cells

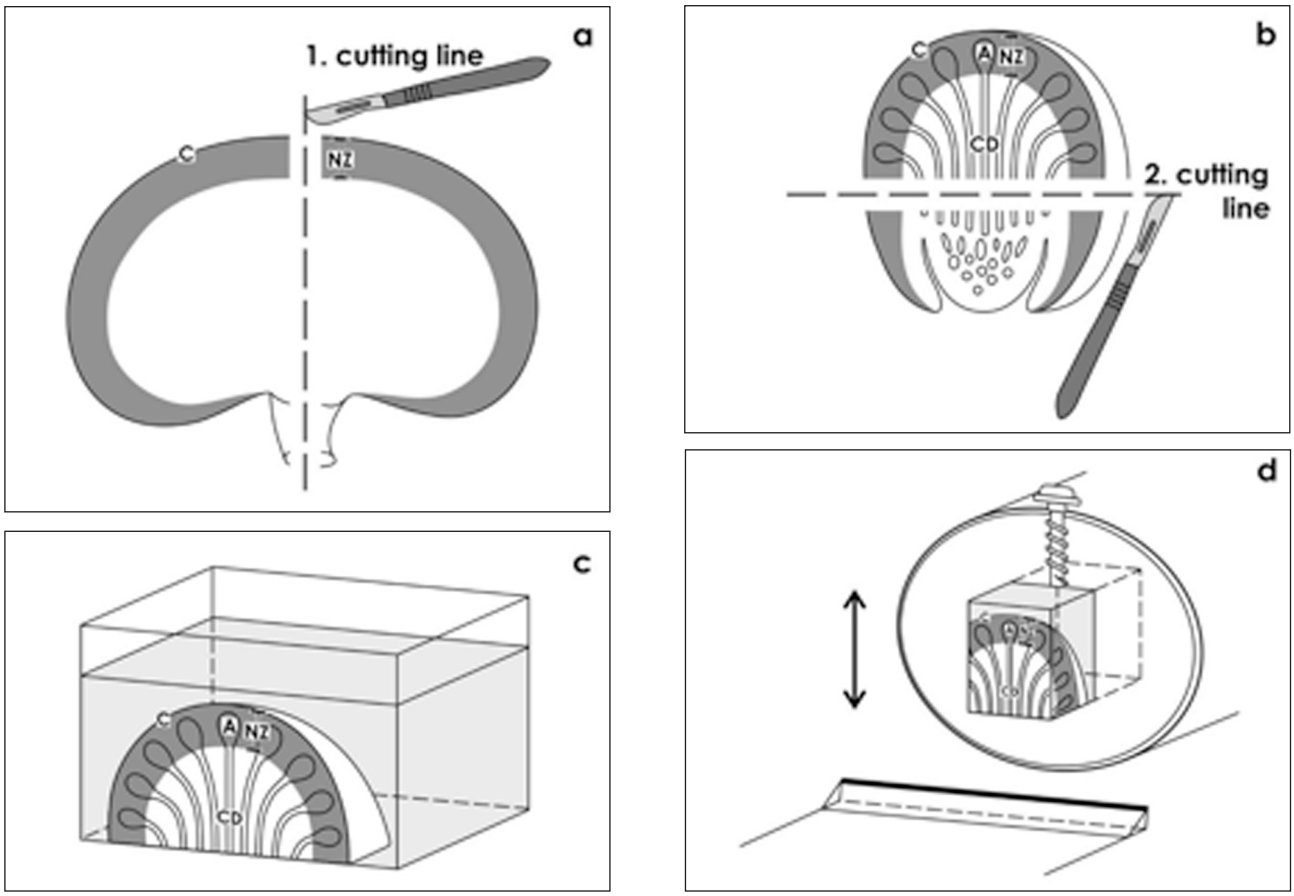

Figure 2. Schematic illustration shows embedding of human fetal kidney for orientated sectioning of the nephrogenic zone (NZ). **a**) First, a fixed kidney is cut from the capsule (C) towards the papilla of a lobus. **b**) Then the medulla is separated from the cortex. **c**) Embedding takes place so that collecting duct (CD) tubules line perpendicular to the capsule. **d**) After trimming the lateral sides of the block, sections with comparable perspective can be produced by a microtome. Following this approach, repeatable views on CD tubules, branching sites, CD ampullae (A) and nephrogenic mesenchyme are possible.

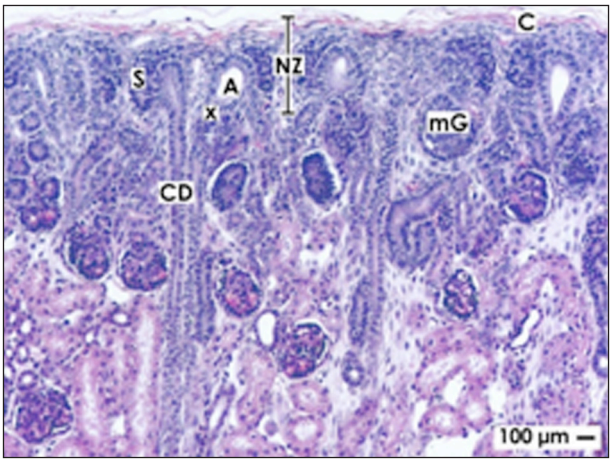

Figure 3. Optical microscopy shows position of the nephrogenic zone (NZ) in the cortex of human fetal kidney. The section lines perpendicular to the capsule (C) and in parallel to the lumen of collecting duct (CD) tubules. Beyond the capsule CD tubules show branching to form a CD ampulla (A). Nephrogenic mesenchymal progenitor cells occur between the tip of a CD ampulla and the inner side of the capsule. At the lateral side of a CD ampulla, an S-shaped body (S) indicates proceeding development of a nephron. A maturing glomerulus (mG) is seen beyond the neck (x) of a CD ampulla.

by an interface [30]. Regarding orientated sections, only two layers of nephrogenic mesenchymal stem cells are seen between the tip of a CD ampulla and the inner side of the capsule. A frequently cited 'cap mesenchyme' known from animal species is not visible in human fetal kidney. Occasionally, three or even four cell layers in the mesenchyme are noticed, when sections are analyzed that line oblique to the axis of a related CD tubule.

Earlier investigations revealed that the spatial separation between the tip of a CD ampulla containing epithelial stem cells and the inner layer of nephrogenic mesenchymal stem cells is not accidental, but is due to a striking interface that was intensively investigated by electron microscopic techniques in neonatal rabbit kidney [30, 35]. It contains textured extracellular matrix and shows projections of mesenchymal cells, which contact epithelial cells at the tip of a CD ampulla via tunneling nanotubes. Functions relevant to biomedicine of these unique structures were recently reviewed [36]. Related ultrastructural data for the human kidney are

lacking. Moreover, it was shown by animal models that the special arrangement of epithelial cells at the tip of a CD ampulla and facing nephrogenic mesenchymal ($GDNF^+/Six2^+/CITED1^+$) stem cells defines the center of an individual niche enabling induction and subsequent formation of a single nephron [22, 28]. It seems that exact positioning of niches including horizontal alignment along the inner side of the capsule is a basic setting for the later endowment of nephrons.

When anlage of a nephron is starting, related morphological changes are observed in close vicinity to the inner side of the capsule (**Fig. 4**). Initial sign is an aggregation of induced nephrogenic mesenchymal cells. This process leads to a pretubular aggregate, which develops by a mesenchymal to epithelial transition into a renal vesicle near the tip of a CD ampulla. Not noticeable, but in parallel, a radial expansion of parenchyma towards the capsule continues. By consequence, stages of advanced nephron formation such as comma- and S-shaped bodies are not seen at the tip but on the lateral side of a CD ampulla. Hence, occurrence of early nephron stages from aggregation until an S-shaped body represents a vertical and centripetal developmental vector, which lines from the inner side of the capsule down to the maturation zone in the outer cortex. At the deep lateral side of a CD ampulla including the differentiating CD tubule and more beyond, advanced processes of nephrogenesis such as glomerulogenesis and nephron segmentation take place [37].

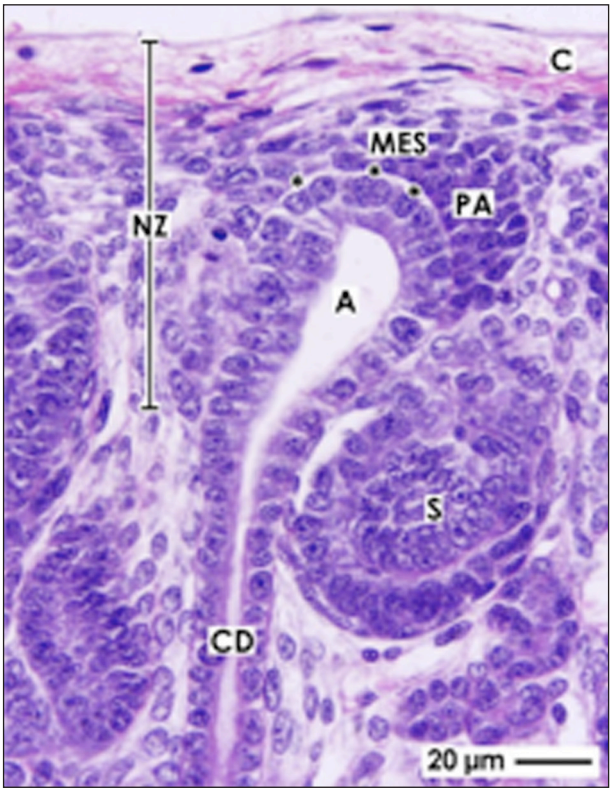

Figure 4. Optical microscopy shows the nephrogenic zone (NZ) in the fetal human kidney. At the end of a collecting duct (CD) tubule, a CD ampulla (A) is seen. Epithelial progenitor cells at the tip of a CD ampulla meet only two layers of mesenchymal stem cells (MES). An interface (asterisks) separates epithelial from mesenchymal stem cell bodies and points to the center of a niche. At the right side of a CD ampulla, tip-induced mesenchymal cells show aggregation to establish a pretubular aggregate (PA). Beyond an S-shaped body (S) is visible.

Coordinates in the nephrogenic zone

In order to obtain information about spatial relations of the nephrogenic zone, morphometric recording was performed according to **Tab. 2** and **Fig. 5a**. The measures revealed that the mean distance between the lateral aspects of neighboring CD ampullae is 68 µm (**Tab. 2.1**, **Fig. 5a/1**). The mean distance between the tips of neighboring CD ampullae is 161 µm (**Tab. 2.2**, **Fig. 5a/2**). The mean distance between the branching site of a CD ampulla and the inner side of the capsule is 106 µm (**Tab. 2.3**, **Fig. 5a/3**). Between the tip of a CD ampulla and the inner side of the capsule is a mean distance of 32 µm (**Tab. 2.4**, **Fig. 5a/4**). The mean length of a CD ampulla is 85 µm (**Tab. 2.5**, **Fig. 5a/5**), while its mean diameter is 71 µm (**Tab. 2.6**, **Fig. 5a/6**). The mean thickness of the mesenchymal cell layer between the inner side of the capsule and the tip of a CD ampulla is 26 µm (**Tab. 2.7**, **Fig. 5a/7**). The mean diameter of a CD tubule is 34 µm (**Tab. 2.8**, **Fig. 5a/8**). The mean horizontal diameter of a bifid CD tubule branch is 120 µm (**Tab. 2.9**, **Fig. 5a/9**).

For a better imagination, according to measures shown in **Tab. 1** beside schematic (**Fig. 5a**) also a pseudo 3D (**Fig. 5b**) illustration true to scale was produced. Finally, in a higher magnification mean position of a renal niche true to scale is shown (**Fig. 5c**). The illustration indicates the mean position of a niche and the spatial relations between the tip of a CD ampulla, the intermediate interface, the facing nephrogenic mesenchymal cells and the covering capsule.

Inconstant horizontal alignment of niches

In animal species, the tip of a CD ampulla and facing nephrogenic ($GDNF^+/Six2^+/CITED1^+$) mesenchymal cells define the center of a niche [22, 28]. Unique for related human progenitor cells is

Table 2. Morphometric parameters of the nephrogenic zone in fetal human kidney depict coordinates of collecting duct (CD) ampullae and mesenchyme in relation to the capsule. The tip of a CD ampulla reflects exact horizontal (**2**) and vertical (**4**) position of a niche.

	Monitoring sites (μm)	Minimum value	Maximum value	Mean	Number of measures
1	Distance between the lateral aspects of CD ampullae	34	104	68	6
2	Distance between the tips of neighboring CD ampullae	107	211	161	6
3	Distance between branching site of a CD ampulla and inner side of the capsule	85	129	106	6
4	Distance between tip of a CD ampulla and the inner side of organ capsule	16	41	32	6
5	Length of a CD ampulla	84	116	85	6
6	Diameter of a CD ampulla	51	86	71	6
7	Thickness of mesenchymal cell layers between the inner side of the capsule and the tip of a CD ampulla	16	29	26	6
8	Diameter of CD tubule	20	42	34	6
9	Horizontal diameter of a bifid CD tubule branch	74	130	120	6

CD: collecting duct.

that Six1 was identified as a Six2 target [38]. To obtain concrete information about the position of niches in human fetal kidney, some morphometric recordings were performed. Data exhibit that the tip of a CD ampulla and the inner side of the capsule have a distance between 16 and 41 μm (**Tab. 2.4**, **Fig. 5a/4**) [29]. The calculated mean of 32 μm signals that the tip of a CD ampulla keeps a relatively close vertical position to the inner side of the capsule (**Fig. 3** and **Fig. 4**). However, regarding the horizontal alignment of niches beyond the capsule, calculation of a mean distracts and leads to incorrect interpretations (**Fig. 5b** and **Fig. 6b**). For example, the distance between the lateral aspects of neighboring CD ampullae is rather variable and can show a minimal distance of 34 μm (**Fig. 6a**) and a maximal distance of 104 μm (**Fig. 6c**, **Tab. 2.1**). The same information about inconstant alignment of niches is obtained, when the distance between the tips of neighboring CD ampullae is regarded (**Tab. 2.2**). The lateral distance from a CD ampulla tip to the next can exhibit a minimum of 107 μm and a maximum of 211 μm. Thus, a minimal lateral distance of a CD ampulla to the next mirrors how many CD ampullae have place in the nephrogenic zone per metric unit (**Fig. 6a**). In contrast, a wide lateral distance signals that the number of CD ampulla tips and the number of niches per metric unit is reduced (**Fig. 6c**).

A wide lateral distance between neighboring CD ampullae (**Fig. 6c**) could indicate decreased ureteric bud branching and impaired nephrogenesis in this district [18]. It may arise by branching disparity caused by mutual suppression of branching at the end of a CD tubule by intrinsic and locally operative mechanisms [39]. A molecule involved in this process is the prorenin receptor, which controls branching morphogenesis via Wnt/β-catenin signaling [40]. However, according to data presented in **Tab. 1** changes in the lateral distance of CD ampullae may also arise by a parallel expansion of the capsule and nephrogenic zone. In this connection, one has to bear in mind that each tip of a CD ampulla is fastened via microfibers at the inner side of the capsule like a puppet on strings [41]. Consequently, active areal expansion of the capsule would inevitably pull apart tissue compounds of the nephrogenic zone in a horizontal direction resulting in an increase in the lateral distance between neighboring CD ampullae. However, more morphometric measures, confocal immunohistochemistry and a close look on related pathological specimens are necessary to confirm this hypothesis.

The capsule represents a complex cover

The capsule, as well as the underlying nephrogenic zone, covers the entire surface of a fetal human kidney (**Fig. 3** and **Fig. 4**). As shown before, during gestation weeks 32 and 38 the total renal volume enlarges from 14.5 to 21.6 cm^3 [31, 32] (**Tab. 1**). At first glance, this result seems rather banal. However, at second sight, the increase in 7.1 cm^3 of kidney volume is paralleled by an astonishing increase in surface including an areal expansion of the capsule and the underlying nephrogenic zone in an order of 30%. In comparison to other organs, such a focused increase of progenitor cell containing tissue at the cover of an organ during development is unique.

Up to date, the capsule was seen only as a simple structural envelope that protects the developing organ to inappropriate exogenous signals. One further associates that it consists of a Tunica fibrosa

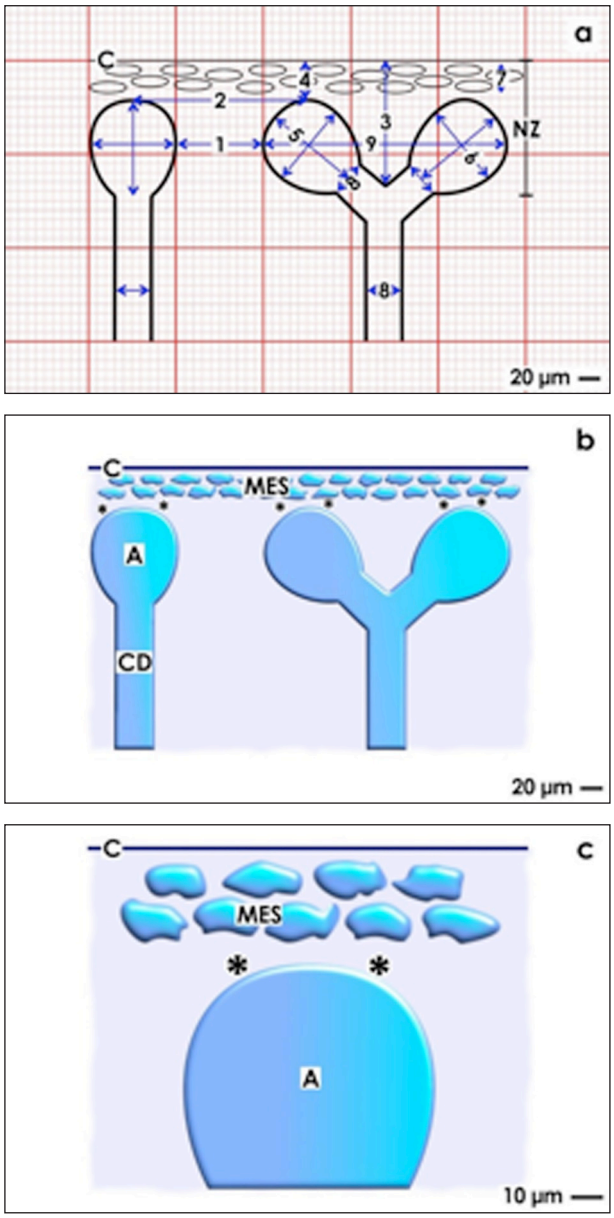

Figure 5. a) Monitoring sites true to scale according to **Tab. 2** and (**b, c**) mean position of niches in the nephrogenic zone (NZ) of the fetal human kidney. **a**) Distances between the lateral aspects (1) and tips of collecting duct (CD) ampullae (2). Distance between branching site of a CD tubule and the capsule (C) (3). Distance between the tip of a CD ampulla and the capsule (4). Length of a CD ampulla (5) and diameter (6). Thickness of mesenchymal cell layers (7), diameter of CD tubules near the branching site (8) and horizontal diameter of branches (9). **b**) Niches in lateral view at the left and frontal view at the right side. Schema shows true to scale position of CD tubules, branching sites, CD ampullae (A) and nephrogenic mesenchyme (MES) beyond the capsule. **c**) View to a niche in higher magnification including the tip of a CD ampulla, the intermediate interface (asterisks) and nephrogenic mesenchymal cells.

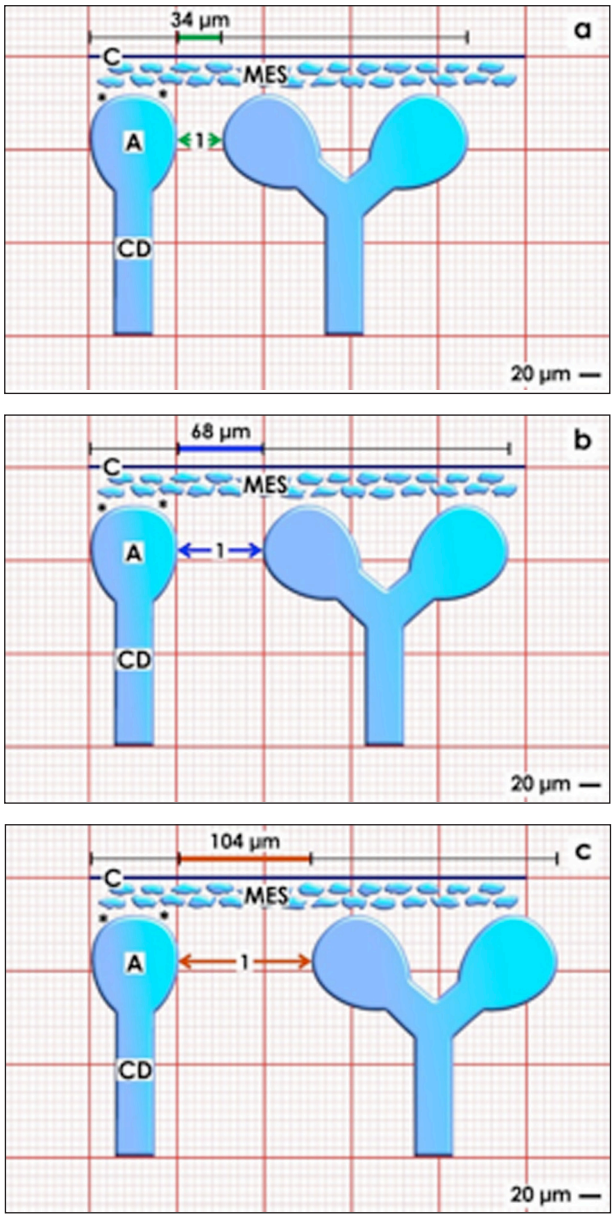

Figure 6. Schematic illustration true to scale shows (**a**) minimal, (**b**) mean and (**c**) maximal horizontal distance between the lateral aspects of collecting duct (CD) ampullae in the nephrogenic zone of fetal human kidney according to data shown in **Tab. 2.1**. For example, (**a**) the minimal distance between the lateral aspects of neighboring CD ampullae is 34 μm. Such a value indicates a dense pattern and reflects how many CD ampullae have principally place in the nephrogenic zone per metric unit. **b**) For comparison, the calculated mean is 68 μm. **c**) The maximal distance is 104 μm. This value signals that the number of CD ampullae per metric unit is decreased.

with several Strata and a Rete capillare capsulare guaranteeing necessary nutrition and respiratory gas supply. Surprisingly, also less attention received its Tunica muscularis. Here atypical smooth muscle cells are contained that show similarities to both smooth muscle cells and fibroblasts [41, 42]. Scarcely known, but described, atypical smooth muscle cells exhibit numerous projections, which establish cell-to-cell contacts. In some of the cases, those projections are not covered by a basal

lamina but show a faint glycocalyx. Further on, between contacting cell projections, a complex tunnel system is established. Physiologically not explained, but one could speculate that it directs the flow of interstitial fluid from the capsule towards the nephrogenic zone to provide here contained cells with necessary nutrition, respiratory gas and morphogenic molecules.

Elongation of a CD tubule includes successive branching, formation of CD ampullae and positioning of niches. Regarding generated data, one assumes that close alignment of niches side by side informs about a rich prospective nephron endowment (**Fig. 6a**). Considering that elongation of CD tubules including branching occurs synchronized along the entire inner side of the capsule in a fetal human kidney, it is obvious that such as process needs coordination and requires step by step an adequate areal expansion of the nephrogenic zone to provide space for correct positioning of niches. One must realize that each bifid branching of a CD tubule needs according to **Tab. 2.9** a mean areal claim of 120 μm. The factual problem thereby is that a more or less non-elastic capsule covers the nephrogenic zone (**Fig. 3** and **Fig. 4**). The resulting but unanswered question is, whether branching of CD tubules and surrounding mesenchymal stem cells produce tension of the capsule or whether a turgor of growing parenchyma and interstitial fluid provoke passive and thereby permanent stretching of the capsule. An alternative consideration is that an actively expanding capsule provides the necessary space.

Functional links between the capsule and nephrogenic zone

Previous and actual investigations exhibit that the number of progenitor cells limits ureteric branching and nephron endowment [43, 44]. Primarily one thinks about progenitor cells that are present in the nephrogenic zone. However, there is now clear evidence that not only the nephrogenic zone but also the capsule represent frameworks for here contained progenitor cells [45]. Focussing on the capsule, as well as the pool of contained mesenchymal progenitor cells, capsule formation as initial steps of nephrogenesis are controlled here [46]. A discrete cell population in the capsule expresses stem cell markers Foxd1, Raldh2 and Sfrp1 [47]. Another investigation demonstrates that these cells also exhibit label for CD29, vimentin, Sca1 and nestin [48]. Most interestingly, progenitor cells in the capsule of mice kidney have the ability to migrate towards the underlying parenchyma with the rate of 30 μm/h. Further illustrated progenitor cells in the capsule of human kidney are not randomly distributed, but show a special patterning and occur in close proximity to capillaries [49].

Pathologic findings inform that in the renal capsule of preterm babies the amount of progenitor cells varies from one case to the next and that their amount does not seem to be exclusively related to gestational age [50]. A previous investigation revealed that by capsule cells the developmental modulator thymosin beta-4 (Tβ4) is expressed [51]. In addition, not only nephrogenic mesenchyme within the nephrogenic zone but also stromal cells in the capsule are a source of morphogen GDNF that is normally directing branching morphogenesis [52]. Surprisingly, also β-catenin is expressed in stroma including the capsule. It modulates morphogen Wnt9b signaling, which in turn drives nephron induction [53]. On the downside, growth factor Midkine expressed in this area promotes expansion of nephrogenic mesenchyme, but at the same time it suppresses expansion of the stromal compartment and branching morphogenesis [54].

Regarding growth of a fetal kidney and expansion of the capsule including the nephrogenic zone, it is worth to have a closer look to the aforementioned Foxd1 cell lineage and correlated downstream functions. For example, ablation of Foxd1-derived stroma cells shows a mispatterning in the nephrogenic zone in the form of altered nephron progenitor caps, areas devoid of nephron progenitors, ureteric branching defects and aberrant vessel formation [55]. Further on, intact prolyl-4-hydroxylation of the hypoxia-inducible factor regulated by prolyl-4-hydroxylase domain (PHD) dioxygenases PHD1, PHD2 and PHD3 is essential in Foxd1 lineage cells for normal nephrogenesis [56]. In addition, evidence is given that crosstalk between Foxd1 and the renin-angiotensin system (RAS) is a prerequisite for intact ureteric bud branching morphogenesis [57]. In this respect and due to its overall presence in the capsule, Foxd1 could belong to a monitoring system, which controls continuity of nephrogenesis not on the site of a single niche, not punctual or regionally but along the entire nephrogenic zone. Thus, due to lack of information, it is of great importance to investigate, whether noxae causing impairment

of nephrogenesis in preterm and low birth weight babies interact with Foxd1 dependent signals.

Conclusions

Impaired nephrogenesis in preterm and low birth weight babies is an unsolved biomedical problem, which urgently requires a reliable therapeutic approach. However, molecular causes, harmed pathways and site of damage in the cortex of a fetal kidney are unknown. To facilitate the entrance in this complex network, data of relevant literature was collected and supplemented with results from the own laboratory. The resulting take-home messages are:

- Previously, it was believed that noxae causing impaired nephrogenesis interfere exclusively with primary steps of nephrogenesis such as anlage and initial nephron formation.
- Actual data force to have a close look not only to harmed initial nephron formation but also to less considered upstreaming processes. This involves areal expansion of the renal capsule and underlying nephrogenic zone.
- Increase in surface of a fetal kidney is remarkable. For example, it is shown that between gestation weeks 32 and 38 the capsule, as well as the nephrogenic zone, is subject to an expansion in the order of 30%.
- Due to lack of knowledge, it must be worked out, whether the process of capsule and nephrogenic zone expansion is affected by mentioned noxae.
- Actual morphometric recordings further exhibit that the lateral distance between niches in the nephrogenic zone can vary. It appears that a small distance indicates regular patterning, while a wide distance points to a minor prospective endowment of nephrons.
- Collected data imply to focus future research on less noticed structural and functional links between capsule, nephrogenic zone and niches. There is now strong evidence that only intact links between them ensure continuation of nephrogenesis.
- Of special interest in this context are Foxd1-dependent signals. A plausible working hypothesis is that disturbance of those signals interacting with capsule synthesis and/or expansion of the nephrogenic zone provokes impairment of nephrogenesis during the late phase of gestation along the entire surface of the developing kidney.

There is very much to do, let us begin with biomedical analysis!

Declaration of interest

The Author declares no conflict of interest.

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