www.jpnim.com Open Access eISSN: 2281-0692
Journal of Pediatric and Neonatal Individualized Medicine 2017;6(1):e060136
doi: 10.7363/060136
Received: 2016 May 19; revised: 2016 Jun 06; accepted: 2016 Jul 23; published online: 2017 Feb 25

Original article

Structural and cellular changes in fetal renal papilla during development

Laura Vinci[1], Annalisa Locci[1], Clara Gerosa[1], Melania Puddu[2], Giovanni Ottonello[2], Vassilios Fanos[2], Gavino Faa[1]

[1]Department of Surgical Sciences, Division of Pathology, University of Cagliari, Cagliari, Italy
[2]Neonatal Intensive Care Unit, Neonatal Pathology and Neonatal Section, AOU and University of Cagliari, Cagliari, Italy

Abstract

The mature renal papilla is characterized by medullary collecting ducts, Henle's loops, vasa recta and the interstitium. Cortical and medullary stromal cells are essential for the regulation of urine concentration and other specialized kidney functions. Mechanisms that direct the renal papilla development are not clearly understood. In recent years, the renal papilla has been identified as a niche for renal stem/progenitor cells in the adult mouse. Studies on experimental animals evidenced a probably common interstitial progenitor for the medullary and cortical stromal cells, characterized by the Foxd1+/PAX2- phenotype. Moreover, Hox10 and Hox11 expression is required for differentiation and patterning of the multiple subtypes of developing medullary interstitial cells. Given the scarcity of morphological and molecular studies on the human renal papilla, this work aimed to evidence morphological changes during human gestation, both in the architecture of the medullary interstitium and in cell types differentiating between the collecting tubules and the Henle's loops. Future immunohistochemical studies are needed to better identify different interstitial cell types giving rise to the mature interstitium of the renal papilla.

Keywords

Human kidney, development, interstitial progenitors, renal papilla, human fetus, newborn.

Corresponding author

Laura Vinci, Division of Pathology, Department of Surgical Sciences, University of Cagliari, "San Giovanni di Dio" Hospital, Via Ospedale 54, Cagliari 09124, Italy; tel. +39.070.6092372; fax: +39.070.6092370; e-mail: lau.vinci@gmail.com.

How to cite

Vinci L, Locci A, Gerosa C, Puddu M, Ottonello G, Fanos V, Faa G. Structural and cellular changes in fetal renal papilla during development. J Pediatr Neonat Individual Med. 2017;6(1):e060136. doi: 10.7363/060136.

Introduction

The urine concentration ability of the kidney is a peculiar function of the renal interstitium and, in particular, of cortical and medullary stromal cells. During mammalian kidney development, a self-renewing progenitor population characterized by the expression of Foxd1 has been identified in the nephrogenic interstitium, giving rise to the medullary interstitial cells [1]. According to these data, Foxd1 expression in nephrogenic interstitium-derived cells might represent a developmental boundary between the non-nephron and the nephron lineage, the latter being characterized by expression of PAX2. In recent years, a major role in the development of the kidney has been attributed to the renin-angiotensin system, particularly in the development of medullary interstitial cells [2]. Angiotensin II and angiotensinogen are expressed in the renal stromal cells and act on the AT1 and AT2 receptors located on the ureteric bud tip cells, favouring branching morphogenesis [3]. In contrast, *in vitro* studies showed that Angiotensin II acting via the AT2 receptor has antiproliferative activity in medullary interstitial cells, down-modulating proliferation and differentiation of the medullary interstitial compartment [4]. Moreover, inhibition of the renin-angiotensin system in experimental animals resulted in severe renal abnormalities, including atrophy of the renal medulla, leading to impairment of urine concentration ability [5]. During nephrogenesis, Foxd1+ interstitial progenitors in the renal stroma are subdivided into two distinct populations: the cortical and the renal stromal progenitors [6]. The former are characterized by the expression of Foxd1 and retinoic acid receptors alpha and Beta-2. On the contrary, cells undergoing the medullary stromal fate lose the expression of Foxd1 and acquire the expression of fibroblast growth factor 7 (FGF7) [7], the transcription factor Pod1 [8] and bone morphogenic protein 4 (BMP4) [9]. Medullary stromal progenitors eventually differentiate into mature medullary interstitial cells [10]. Hox genes have been shown to play a critical role in the development and patterning of the different renal stromal compartments: Hox10 and Hox11 expression is required for the differentiation of medullary stromal cells and for their integration with collecting tubules and Henle's loops of the developing kidney [11]. Given the scarcity of studies on the various developmental steps of the human renal medulla, this work aimed to analyse the morphological changes occurring in the renal medulla during gestation, particularly any changes occurring in the architecture of the medullary interstitium and in cell types differentiating between the collecting tubules and the descending and ascending limbs of Henle's loops.

Materials and methods

Kidneys from 16 human fetuses/newborns, ranging from 9th up to 39th week of gestation, were sampled and histologically studied. All the fetuses/newborns included in this study had no congenital malformations. The newborns were admitted to the Neonatal Intensive Care Unit of the AOU and University of Cagliari, and died due to different causes. Kidneys samples were fixed in 10% buffered formalin, routinely processed, and paraffin-embedded. A 3 μm-thick section was obtained from each paraffin block; after dewaxing and rehydration, the sections were stained with hematoxylin-eosin. All procedures performed in this study are in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments.

Results

9 weeks

In the fetal kidney at 9 weeks of gestation, the renal papilla was not well developed. At this gestational age, the branching collecting ducts were characterized by an immature pseudo-stratified epithelium surrounded by a thin basal membrane. The ureteric bud-derived collecting ducts were separated by the developing glomeruli by a loose undifferentiated mesenchyme. Undifferentiated mesenchymal cells showed an onion-like arrangement around the collecting ducts, and appeared at strict contact with the mesenchymal cells surrounding the neighboring glomeruli (**Fig. 1**).

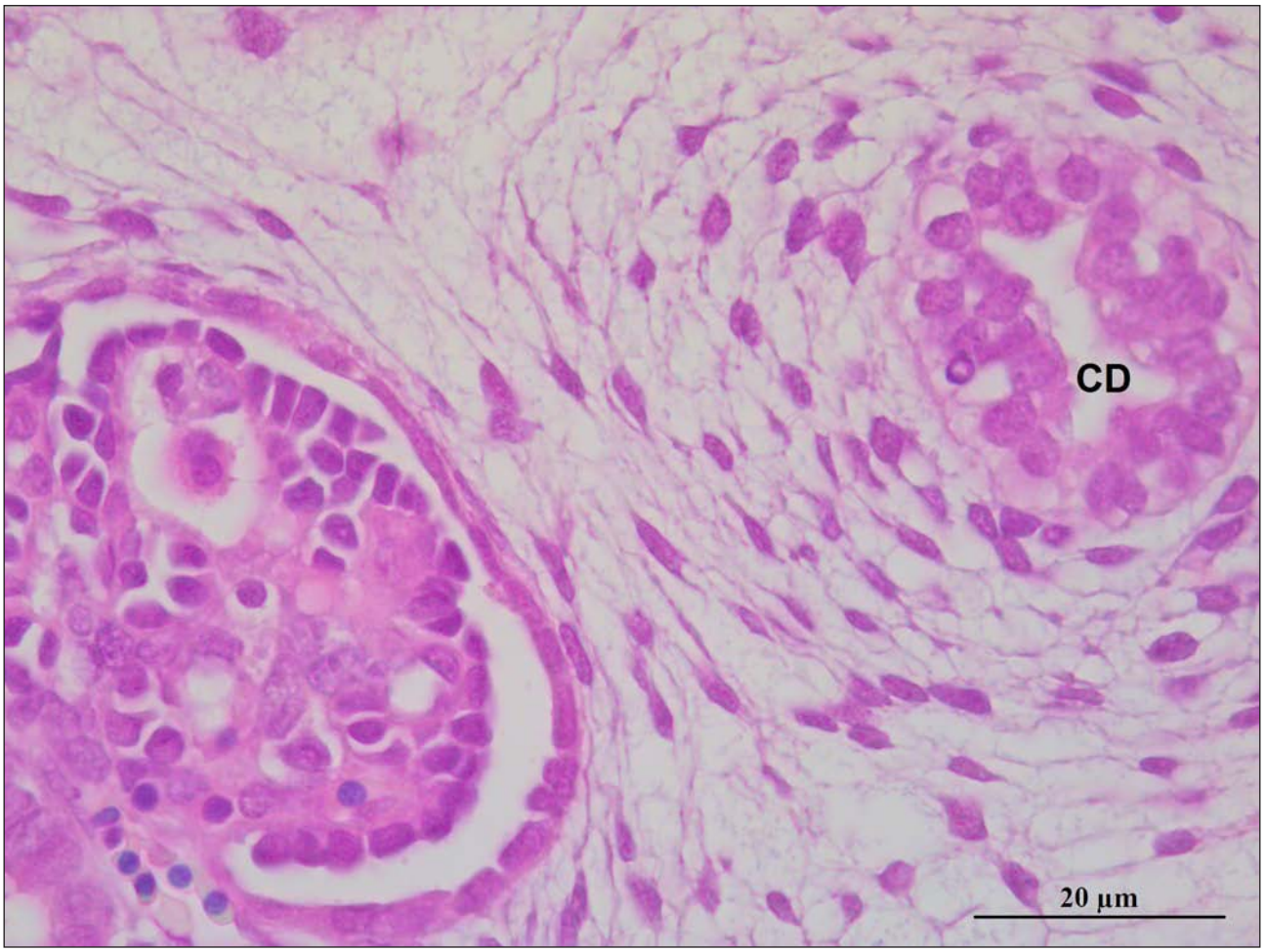

Figure 1. 9 weeks: undifferentiated mesenchymal cells showed an onion-like arrangement around the collecting ducts, and appeared in strict contact with the mesenchymal cells surrounding the neighboring glomeruli.
CD: collecting duct.

12 weeks

At 12 weeks, the developing medulla was characterized by the maturation of the epithelial cells of collecting ducts. The ureteric bud-derived epithelium first showed the typical large basal vacuoles, pushing the roundish nuclei towards the ductal lumen. The mesenchyme intermingled among the ductal structures of the developing papilla was formed by a high number of stromal cells showing different shape and size. At this gestational age, we first noticed the appearance of tubular structures with a dark epithelium in the medulla, putatively representing descending tubules originating from developing glomeruli (**Fig. 2**).

17 weeks

At 17 weeks, a marked decrease in the width of the interstitium was observed (**Fig. 3**). A further maturation of collecting ducts was observed: ureteric bud-derived epithelial cells showed a clear cytoplasm, characterized by the presence of vacuoles both at basal and apical position. At this gestational age, we frequently observed tubular structures characterized by the absence of clear vacuoles, probably representing the descending and/or the ascending limbs of the developing Henle's loops (**Fig. 3**).

21 weeks

A progressive decrease in the interstitial space was evident at 21 weeks of gestation (**Fig. 4**). The regression of interstitial cells was demonstrated by the finding of apoptotic globules in the interstitium. At this gestational age, the fetal medulla was characterized by a dramatic increase in small tubules, putatively representing the descendent and the ascendant limbs of Henle's loop. The proliferation of Henle's loops was confirmed by the occurrence of mitotic figures in these structures (**Fig. 4**).

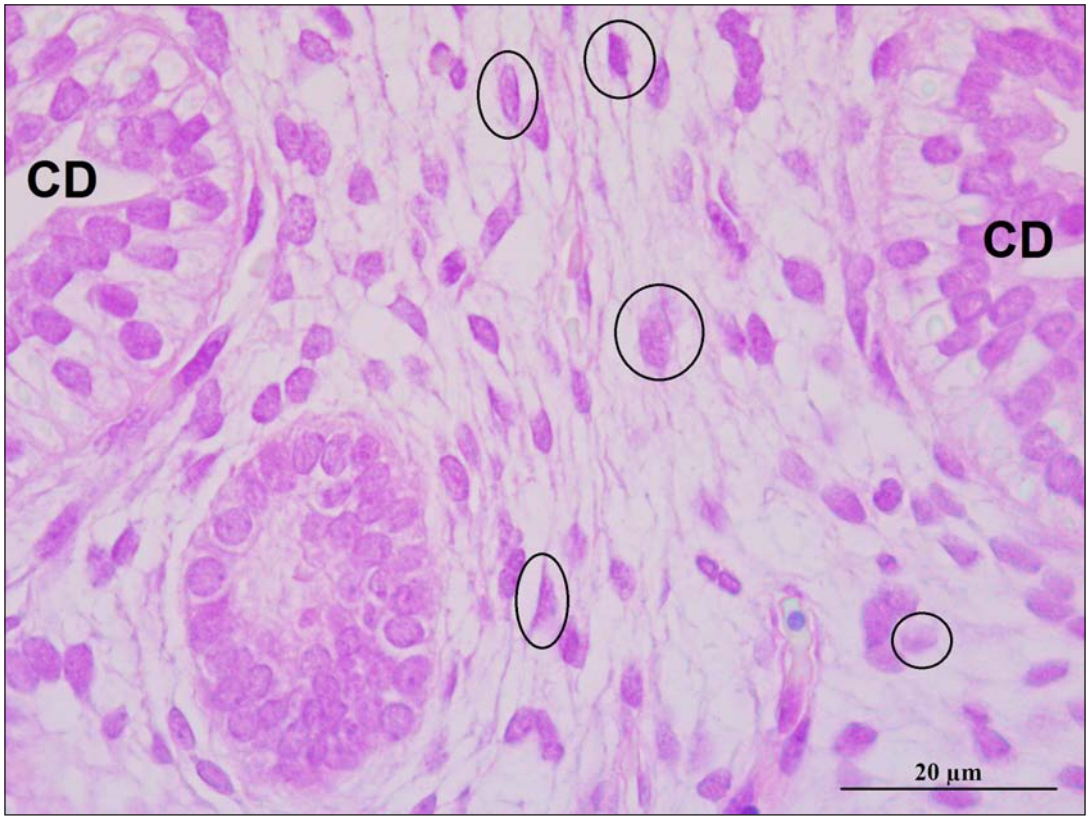

Figure 2. 12 weeks: mesenchymal cells intermingled among the ductal structures of the developing papilla (encircled by a black line) show different shape and size.
CD: collecting duct.

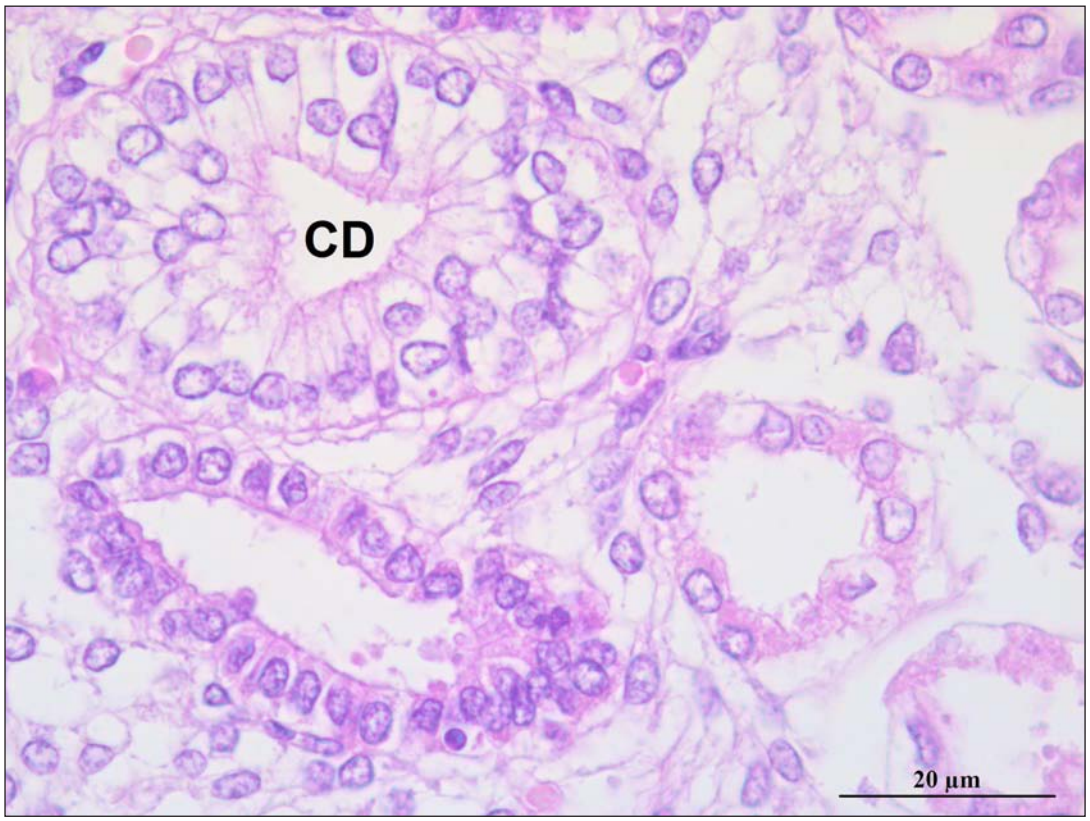

Figure 3. 17 weeks: maturation of tubular structures and decrease in the width of the interstitium.
CD: collecting duct.

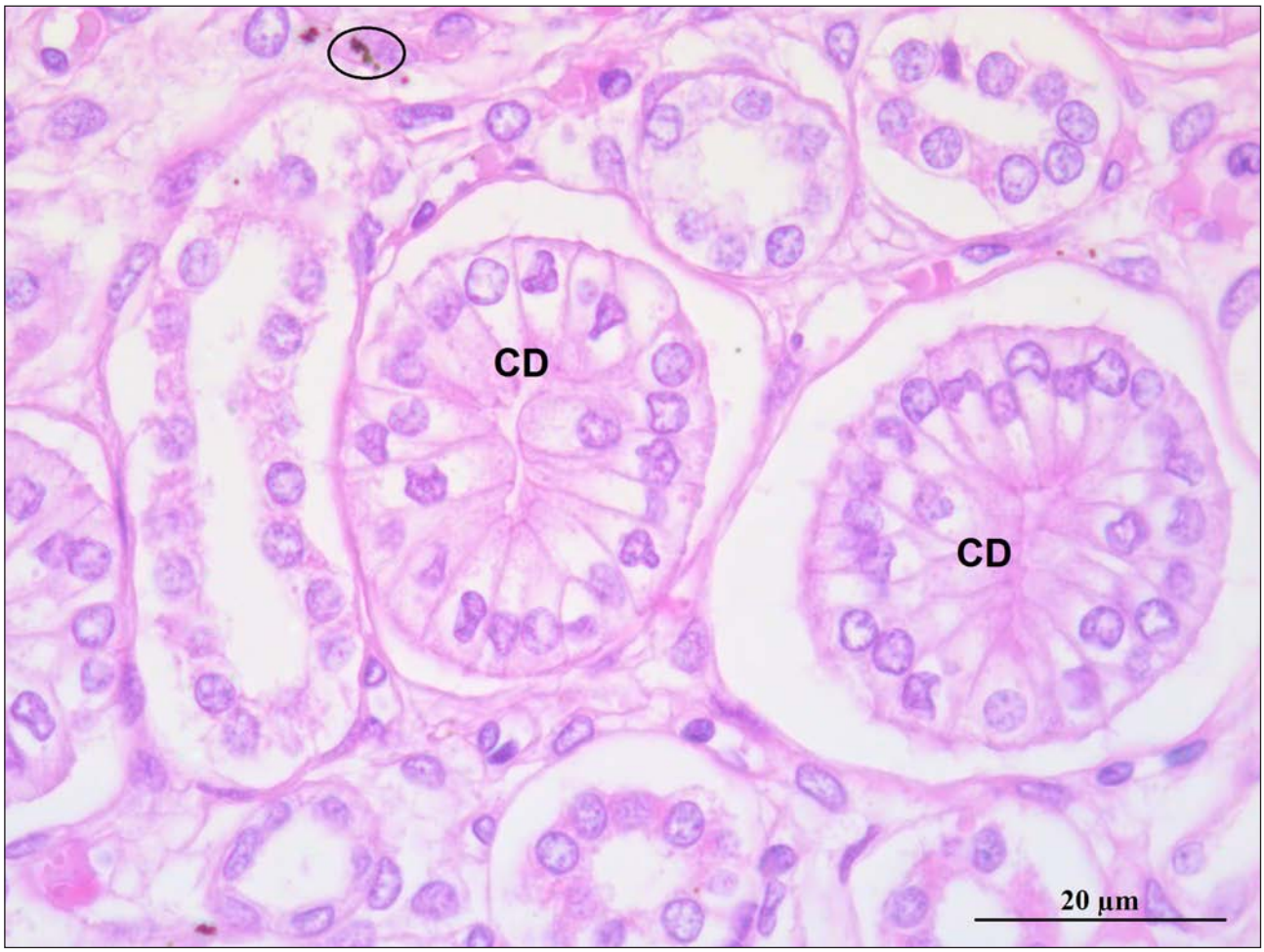

Figure 4. 21 weeks: evidence of progressive decrease in the interstitial space demonstrated by the presence of apoptotic globules in the interstitium (encircled by a black line).
CD: collecting duct.

25 weeks

The developing renal medulla at 25 weeks of gestation was characterized by the predominance of tubular structures on the interstitial cells. At this gestational age, collecting ducts appeared intermingled with the limbs of Henle's loops. At this age, it was not possible to clearly distinguish the descending from the ascending limbs of Henle's loops (**Fig. 5**).

28 weeks

At 28 weeks of gestation, the interstitium of the developing medulla was restricted to elongated, isolated cells separating one tubular structure from the next. At this age, the complexity of the tubular structures localized in the medulla was higher if compared to previous gestational ages. The descending limbs of the Henle's loops were characterized by cuboidal or flat cells, with a scant cytoplasm surrounding a large lumen. The ascending limbs of the Henle's loops was characterized by a prismatic epithelium with an eosinophilic cytoplasm surrounding a narrow lumen (**Fig. 6**).

36 weeks

At 36 weeks of gestation, the components of the Henle's loops appeared as the major component of the renal medulla (**Fig. 7**). At this gestational age, isolated collecting ducts were surrounded by descending and ascending limbs of the Henle's loops. Interstitial cells were characterized by an elongated nucleus with compact chromatin and showed a tendency to surround the Henle's loops (**Fig. 7**).

39 weeks

At the end of gestation (39 weeks), we observed a marked increase in the vascularity of the

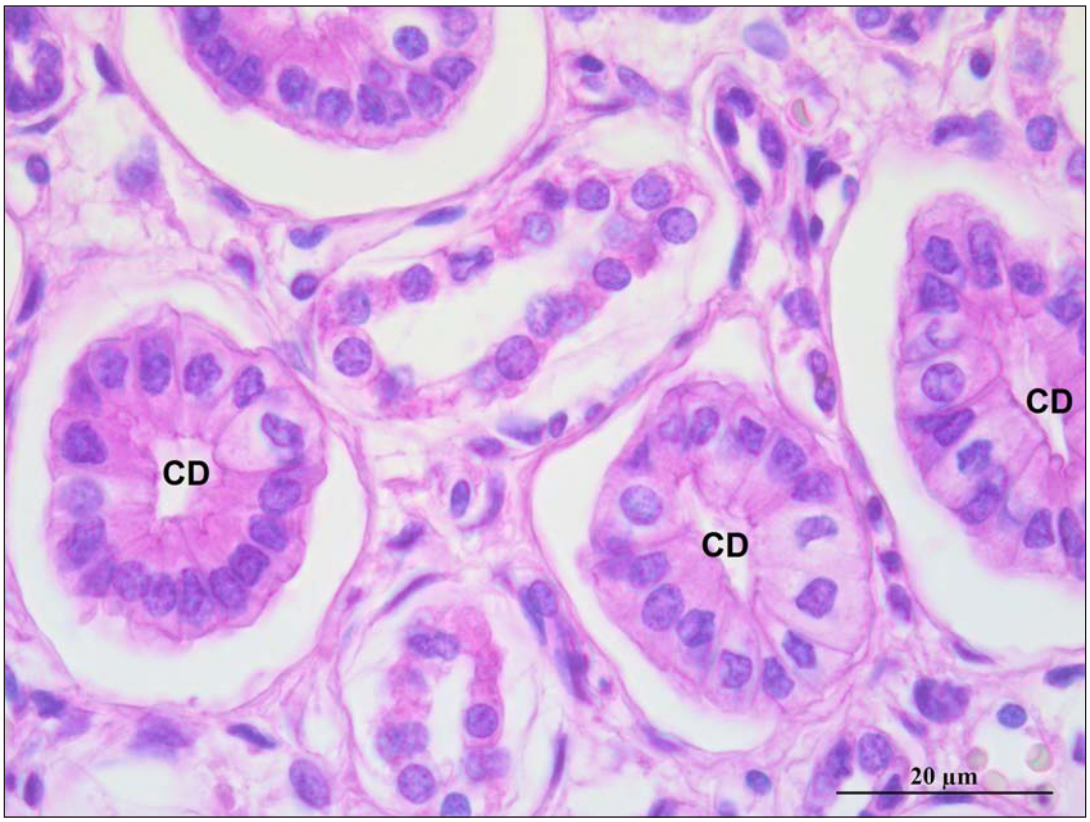

Figure 5. 25 weeks: decrease in interstitial cells and increase in tubular structures.
CD: collecting duct.

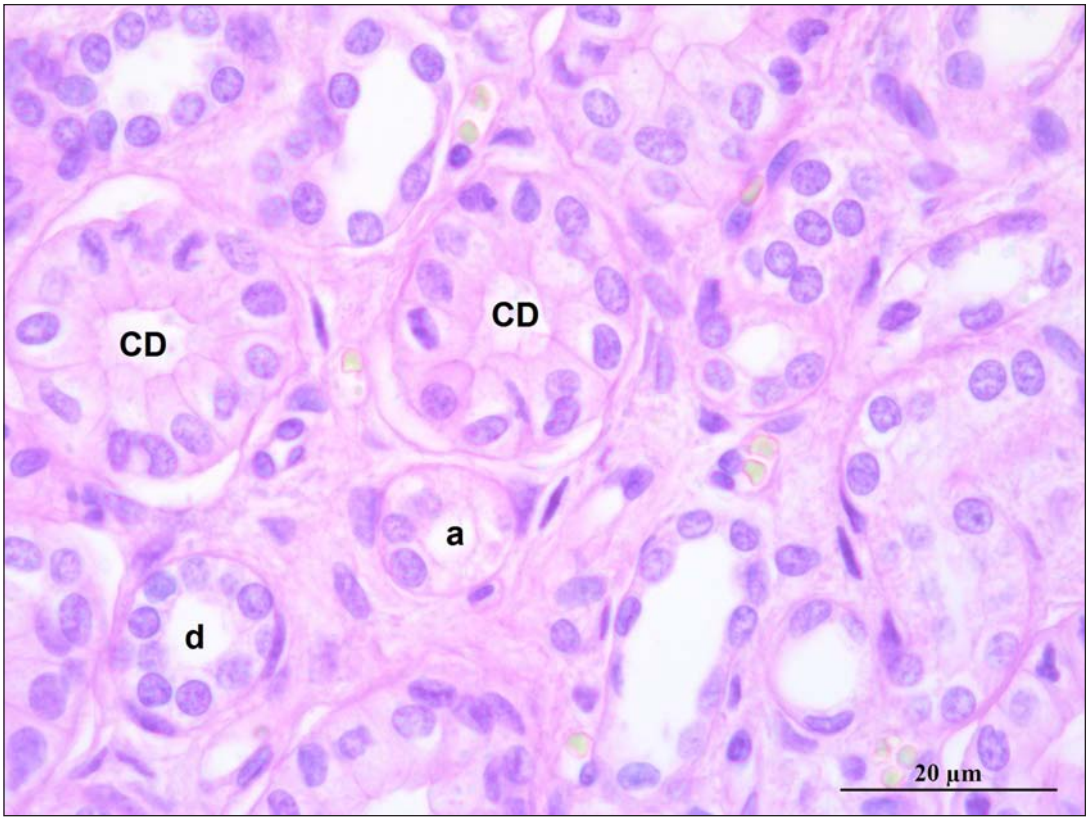

Figure 6. 28 weeks: the interstitium was restricted to elongated isolated cells. The tubular structures show a major complexity if compared to previous gestational ages. It is possible to distinguish ascending and descending limbs of Henle's loops.
CD: collecting duct; a: ascending limb of Henle's loop; d: descending limb of the Henle's loop.

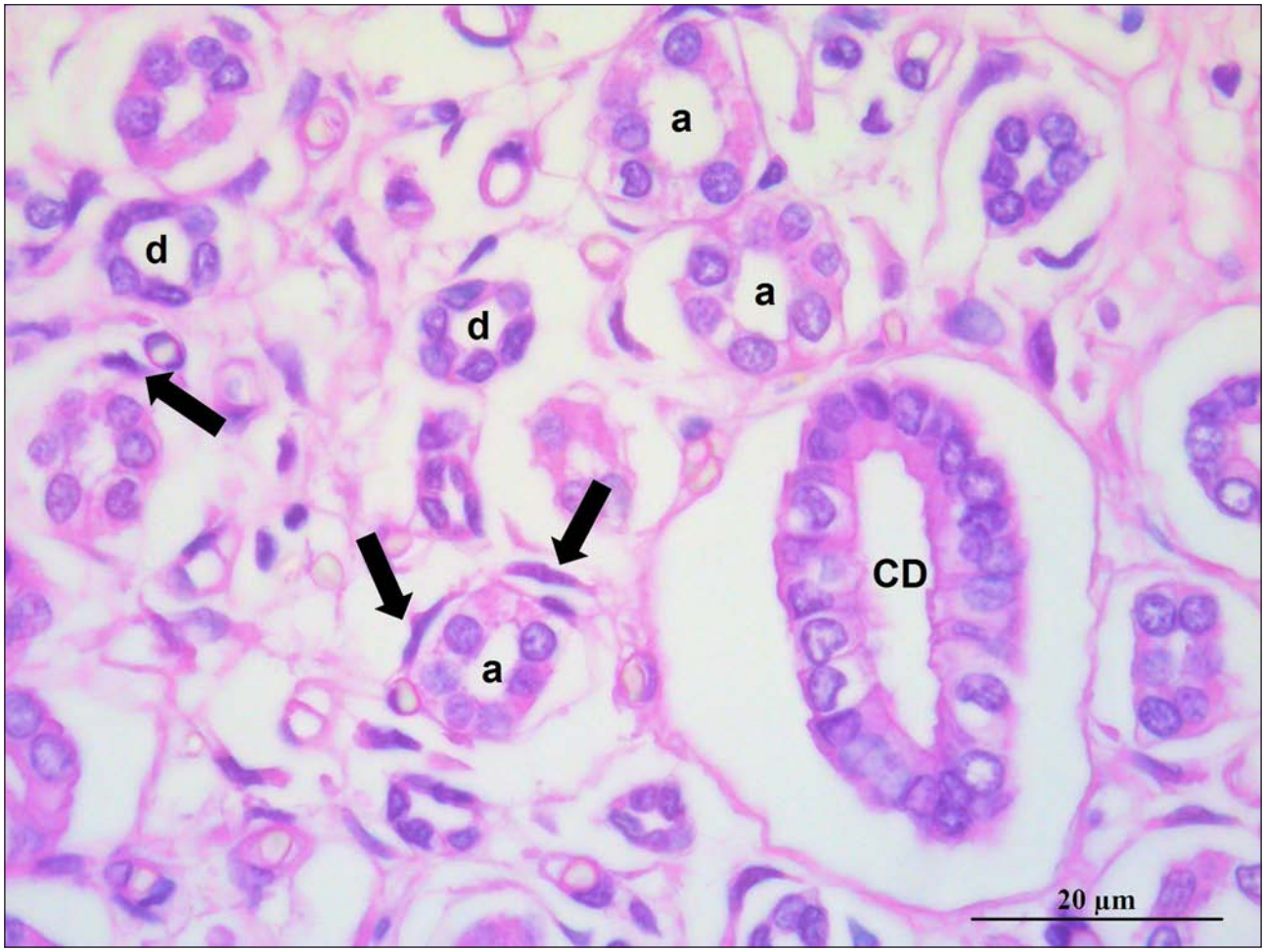

Figure 7. 36 weeks: scattered interstitial cells of renal papilla characterized by an elongated nucleus with compact chromatin surrounding Henle's loops (arrows).
CD: collecting duct; a: ascending limb of Henle's loop; d: descending limb of the Henle's loop.

developing medulla. Scattered collecting ducts with a clear cytoplasm and large lumen were observed surrounded by descending and ascending limbs of the Henle's loops. In the interstitial spaces, we also found a huge number of vascular structures containing blood red cells presumably because the kidney requires a higher blood supply for its correct functioning after birth (**Fig. 8**).

Discussion

The mature renal papilla consists of the medullary collecting ducts, Henle's loops, vasa recta and the interstitium. These components are essential for the regulation of urine concentration and other specialized kidney functions. Mechanisms that direct development of the human renal medulla are not clearly understood. Metanephric mesenchyme-derived medullary stromal cells are required not only for regulation of urine concentration but also for renal morphogenesis, thanks to their ability to generate important signals required for branching morphogenesis of the ureteric bud-derived tubular structures [12].

In recent years, the renal papilla has been identified as a niche for renal stem/progenitor cells in the adult mouse, showing a plastic phenotype of cells co-expressing mesenchymal and epithelial proteins, as well as neuronal markers [13]. In this study, the complexity of the interstitial cells is particularly evident in the early gestational ages and contrasts with the apparent relative monomorphism of these cells at the end of gestation. Furthermore, during the first weeks (weeks 9 and 12 of gestation) a significant difference in the morphology of the various interstitial cells emerged from this study: at histology, these stromal progenitors of the renal papilla appeared as large cells with oval, elongated, flattened or roundish-globular nuclei (see **Fig. 1** and **Fig. 2**). On the contrary, at the end of gestation, the few remaining interstitial cells

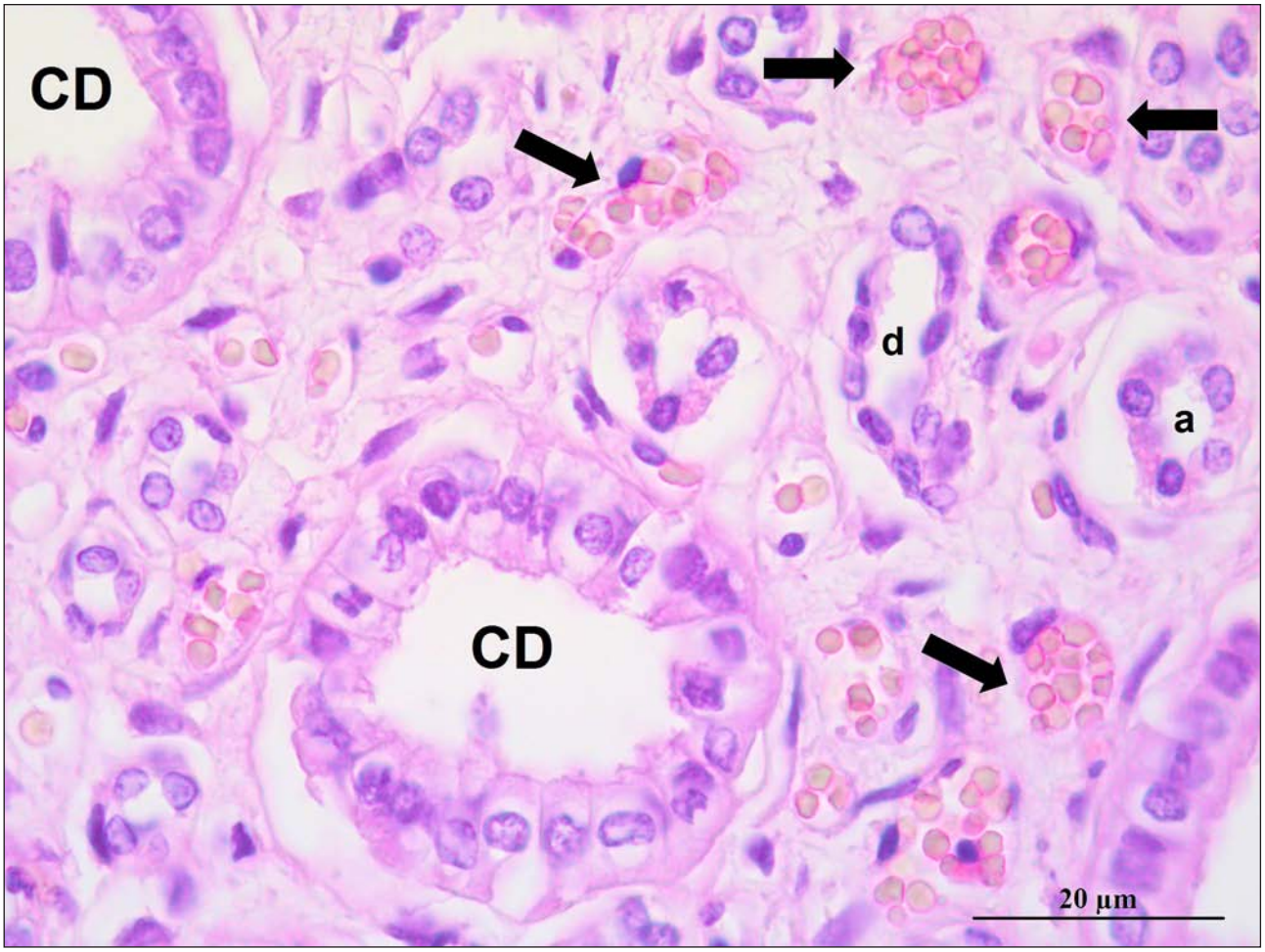

Figure 8. 39 weeks: the end of gestation showed a marked decrease of interstitial cells and a huge number of vascular structures (arrows).

CD: collecting duct; a: ascending limb of Henle's loop; d: descending limb of the Henle's loop.

showed an elongated oval shape (**Fig. 8**). The different morphology of interstitial cells in the metanephric mesenchyme at gestational weeks 9 and 12 may be due to a lack of differentiation. Therefore, these cells represent stem/progenitor interstitial cells that eventually differentiate into mature medullary interstitial cells [10]. With increasing gestational age, we observed a marked decrease in the width of the interstitium due to a decrease of the stem/progenitor cell pool and to the increase of differentiated interstitial cells.

Another peculiarity of the development of the human renal papilla is the progressive maturation of the multiple tubular structures of the medulla. At 9 and 12 weeks of gestation, we observed the prevalence of the collecting ducts in the developing papilla. The descending ducts originating from developing glomeruli appeared at 17 weeks of gestation, while at 21 weeks we observed the increase of small tubules putatively representing the limbs of Henle's loop. Collecting ducts appeared intermingled with structures of Henle's loops at 25 weeks of gestation. At this time point, it was not yet possible to clearly distinguish the descending and the ascending limbs of Henle's loops. At 28 weeks the complexity of the tubular structures localized in the medulla was higher than in previous gestational ages: the collecting tubules were fewer and the descending and the ascending limbs of Henle's loops were clearly identifiable. At 28, 36 and 39 weeks of gestation, the components of the Henle's loops appeared as the major component of the renal medulla. All these data taken together show a previously unreported dynamic picture of renal papilla development, characterized by the following events: i) the ascending branching of the ureteric bud; ii) the descending elongation of the limbs of Henle's loops; iii) the differentiation of the stromal medullary progenitors [14]. The marked changes in the interstitial cells of the developing renal papilla here reported suggest a

major role for the medullary interstitium in the regulation of the interplay between the ureteric bud and the Henle's loops.

In conclusion, this study focused on morphological changes during human renal papilla development, both in the architecture of the medullary interstitium and in the cell types differentiating between the collecting tubules and Henle's loops. Moreover, these data clearly indicate that stromal/interstitial cells represent a major component of the papilla in the early developing human kidney. Future immunohistochemical studies are needed to test different markers, including PAX2 and Foxd1, that allow to identify different interstitial cell types giving rise to the mature interstitium of the renal papilla.

Acknowledgements

The Authors thank Prof. Giacomo Cao (Department of Chemical and Materials Engineering, University of Cagliari) for relevant critical suggestions. Laura Vinci has performed her activity in the framework of the International PhD in Innovation Science and Technology at the University of Cagliari, Italy.

Declaration of interest

The Authors declare that there is no conflict of interest.

References

1. Kobayashi A. PAX2 maintains self-renewing nephron progenitors during mammalian kidney development. 11th International workshop on developmental nephrology; August 24-7, 2010, New Paltz, New York. [Poster Abstract P-9].
2. Moritz KM, Wintour EM, Black MJ, Bertram JF, Caruana G. Factors influencing mammalian kidney development: implications for health in adult life. Adv Anat Embryol Cell Biol. 2008;196:1-78.
3. Yosypiv IV. A new role for the renin-angiotensin system in the development of the ureteric bud and renal collecting system. Keio J Med. 2008;57(4):184-9.
4. Maric C, Aldred GP, Harris PJ, Alcorn D. Angiotensin II inhibits growth of cultured embryonic renomedullary interstitial cells through the AT2 receptor. Kidney Int. 1998;53(1):92-9.
5. Lasaitiene D, Chen Y, Adams MA, Friberg P. Further insights into the role of angiotensin II in kidney development. Clin Physiol Funct Imaging. 2006;26:197-204.
6. Cullen-McEwen LA, Caruana G, Bertram JF. The where, what and why of the developing renal stroma. Nephron Exp Nephrol. 2005;99(1):e1-8.
7. Mason IJ, Fuller-Pace F, Smith R, Dickson C. FGF-7 (keratinocyte growth factor) expression during mouse development suggests roles in myogenesis, forebrain regionalisation and epithelial-mesenchymal interactions. Mech Dev. 1994;5(1):15-30.
8. Quaggin SE, Schwartz L, Cui S, Igarashi P, Deimling J, Post M, Rossant J. The basic-helix-loop-helix protein pod1 is critically important for kidney and lung organogenesis. Development. 1999;126(24):5771-83.
9. Miyazaki Y, Oshima K, Fogo A, Hogan BL, Ichikawa I. Bone morphogenetic protein 4 regulates the budding site and elongation of the mouse ureter. J Clin Invest. 2000;105(7):863-73.
10. Song R, Yosypiv IV. Development of the kidney medulla. Organogenesis. 2012;8(1):10-7.
11. Wellik DM. Hox genes and kidney development. Pediatr Nephrol. 2011;26(9):1559-65.
12. Hatini V, Huh SO, Herzlinger D, Soares VC, Lai E. Essential role of stromal mesenchyme in kidney morphogenesis revealed by targeted disruption of Wingled Helix transcription factor BF-2. Genes Dev. 1996;10:1467-78.
13. Oliver JA, Maarouf O, Cheema FH, Martens TP, Al-Awqati Q. The renal papilla is a niche for adult kidney stem cells. J Clin Invest. 2004;114(6):795-804.
14. Fanni D, Gerosa C, Vinci L, Ambu R, Dessì A, Van Eyken P, Fanos V, Faa G. Interstitial stromal progenitors during kidney development: here, there and everywhere. J Matern Fetal Neonatal Med. 2016;29(23):3815-20.