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Original article

Not all renal stem cell niches are the same: anatomy of an evolution

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Abstract

The renal stem cell niche represents the most important structure of the developing kidney, responsible for nephrogenesis. Recently, some Authors have reported, at ultrastructural level, a previously unknown complexity of the architecture of renal stem cell niche in experimental models. This study was aimed at studying, at histological level, the anatomy of renal stem cell niches in the human fetal kidney. To this end, ten fetal kidneys, whose gestational ages ranged from 11 up to 24 weeks, were studied. H&E-stained sections were observed at high power. The study of the anatomy of renal stem cell niches in the human kidney revealed a previously unreported complexity: some niches appeared as a roundish arrangement of mesenchymal cells; others showed the initial phases of induction by ureteric buds; in other niches the process of mesenchymal epithelial transition was more evident; finally, in other stem cell niches the first signs of nephron origin were detectable. These findings suggest the existence of niches with different anatomy in the same kidney, indicating different stages of evolution even in adjacent niches. All stem cell niches were in strict contact with the capsular cells, suggesting a major role of the renal capsule in nephrogenesis. Finally, our study confirms the existence of a strict contact between the bud tip cells and the surrounding mesenchyme in the human developing kidney, giving a morphological support to the theory of intercellular channels allowing the passage of transcription factors from the epithelial to the mesenchymal stem/progenitors cells.

Keywords

Renal stem cell niche, nephrogenesis, human fetus, kidney development, metanephric mesenchymal cells, cap mesenchymal cells.

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Introduction

Kidney development takes place at the periphery of the kidney, and precisely in the subcapsular zone of the fetal organ, in close proximity to the inner side of the renal capsule [1-3]. The identification and characterization of the multipotent progenitors able to differentiate into the multiple cell types of the mature kidney have represented the focus of many researchers in recent years [4]. The most striking features of renal stem/progenitor cells are their considerable capacity of self-renewal and multilineage differentiation, abilities that are at the basis of renal organogenesis during fetal life and of renal repair and regeneration in the postnatal life [5, 6].

Recent investigations revealed that the function of renal stem cells, and in particular the self-renewal capacity, might be regulated within the specific functional structures defined "stem cell niches" by the integration with a peculiar subpopulation of renal stromal cells through the production of extracellular matrix, integrins and growth factors [7]. The stem cell niche represents the main custodian of stemness and differentiation in any developing organ, and the custodian of homeostasis in healthy adult tissues [8]. In recent years the structural organization of the renal stem cell niche during kidney morphogenesis has been analyzed in deep at ultrastructural level, revealing a previously unknown contact between epithelial and mesenchymal renal stem/progenitor cells through cell projections connecting different types of stem cells [9, 10]. Moreover, structural links have been discovered between the stem cell niches and the renal capsule, and microfibers originating from the capsule have been shown to reach the basal lamina of the tip of the ampulla, suggesting a structural link between the ureteric bud-derived structures and the capsular cells [2, 11].

Physical and functional interactions with the interstitial cells of the renal niche are probably essential for the maintenance of renal stem/progenitor cell homeostasis, that depends on the complex micro-architecture of the renal stem cell niche [9]. Within the renal stem cell niche, stem/progenitor cells are quiescent and remain in an undifferentiated state: stromal cells probably have a relevant role in maintaining stem cells in a quiescent state, as well as in inducing stem/progenitor cells to proliferate and differentiate to repair damaged structures.

Preliminary personal data

In order to study the anatomy of the renal stem cell niche, we analyzed multiple fetal kidneys of different gestational ages (ranging from 11 up to 24 weeks) at high power.

Results

The morphological analysis of the renal stem cell niches located in the subcapsular region of developing human kidneys first evidenced a previously unreported complexity of this fundamental structure for nephrogenesis. At low power it was possible to evidence marked differences among stem cell niches, even in the same organ (**Fig. 1**). Some niches were formed by a solid roundish aggregate of stem mesenchymal cells, in the absence of any differentiation. In other niches, the presence of a tubular-like structure was indicative for the ongoing of mesenchymal epithelial transition. Some niches appeared in strict contact with the ureteric bud tip, whereas in others no strict relationship between the mesenchymal and the epithelial structures was evident. In other cases stem cell niches appeared strictly adjacent to the capsular cells, which occasionally gave the impression to migrate into the stem cell niches (**Fig. 1**).

The stem cell niches were not evenly distributed along the subcapsular regions. In some areas, stem cells were absent and differentiated glomerular and tubular structures appeared in strict contact with the renal capsule (**Fig. 2**).

The active stem cell niches were characterized by the presence of a ureteric bud tip (**Fig. 3**). The

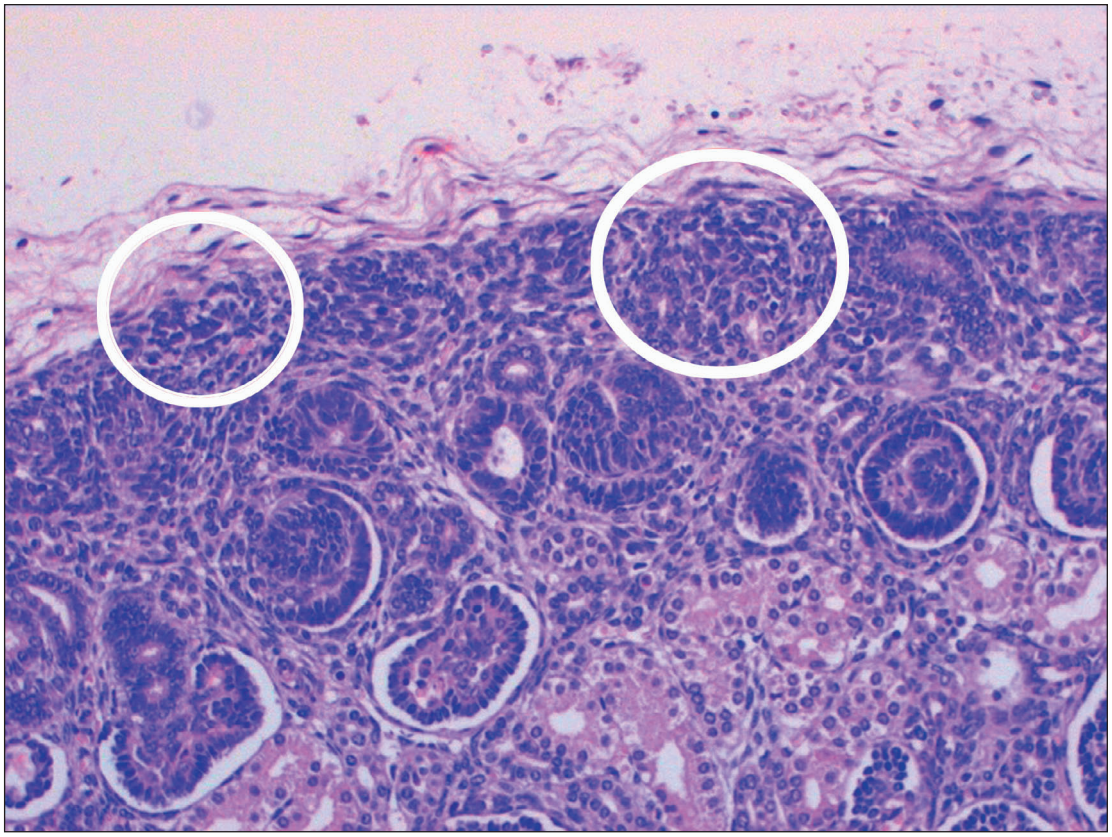

Figure 1. Stem cell niches appeared strictly adjacent to the capsular cells (circles). HE 20x.

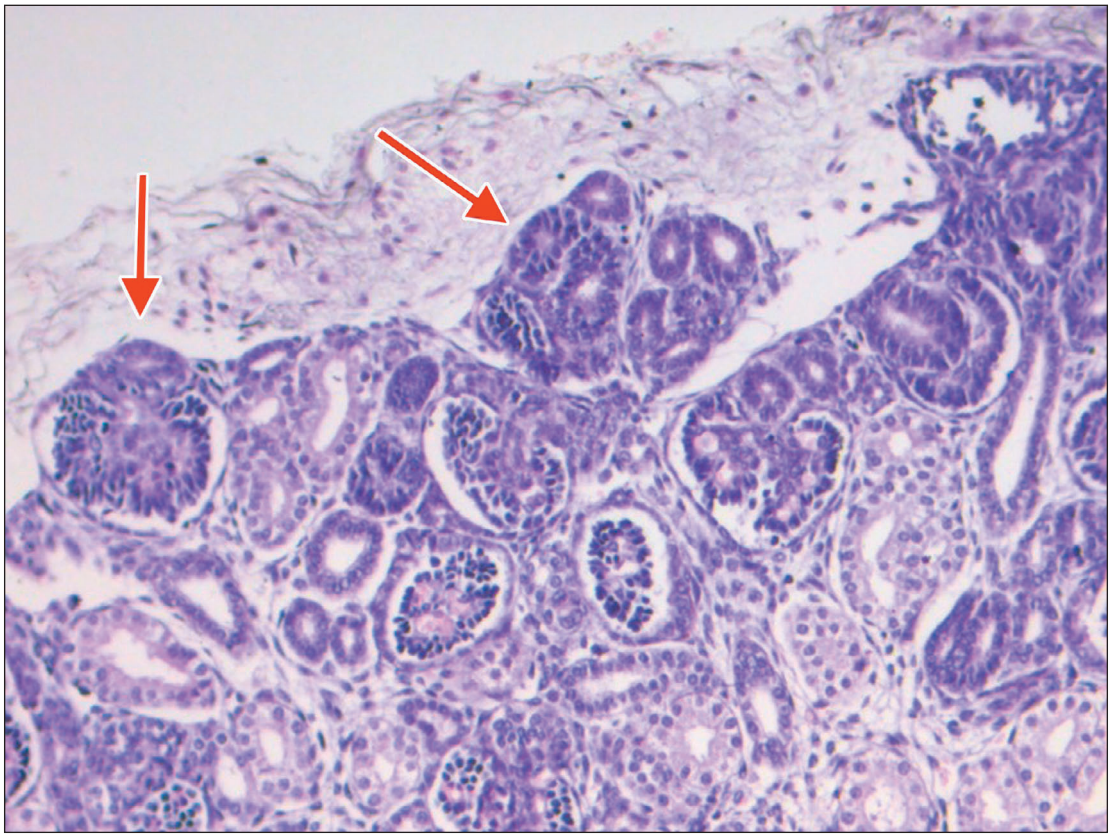

Figure 2. In some areas, stem cells were absent and differentiated glomerular and tubular structures appeared in strict contact with the renal capsule (arrows). HE 20x.

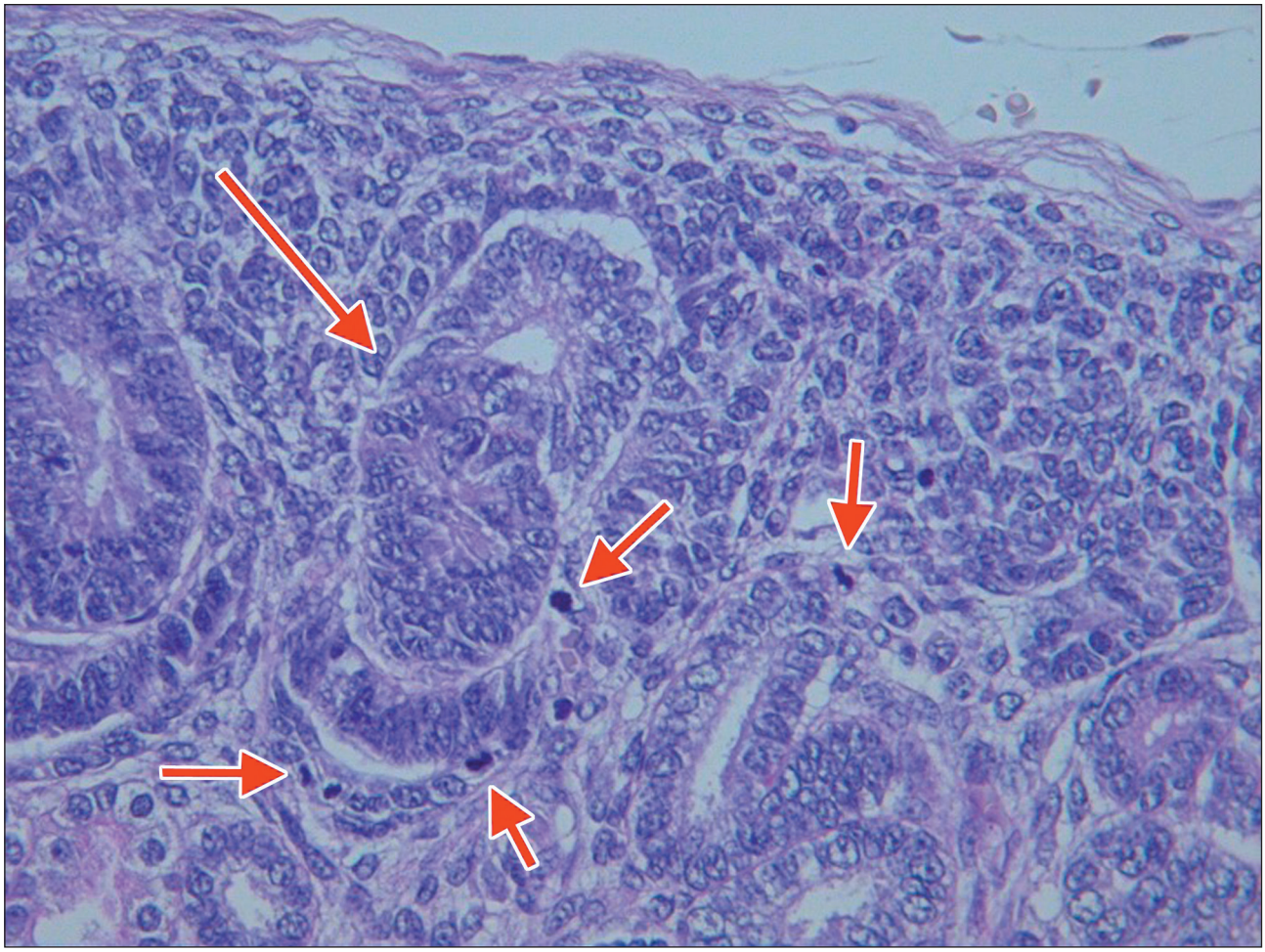

Figure 3. In some niches it was possible to observe the progressive epithelialization of cap mesenchymal cells, ending with the origin of a new glomerulus (arrows); mitosis (small arrows). HE 40x.

contact area between that induced metanephric mesenchyme and the ureteric bud-derived epithelial cells was often underlined by a clear halo (**Fig. 3** and **Fig. 4**). Inside this halo it was possible to evidence some thin filaments suggestive for the existence of intercellular bridges joining the mesenchymal and the epithelial progenitor cells. In some niches it was possible to observe the progressive epithelialization of cap mesenchymal cells, ending with the origin of a new glomerulus (**Fig. 3**). In this areas, mitotic figures were frequently observed (**Fig. 3**).

In some stem cell niches, the relationship between the nephrogenic mesenchyme and the ureteric bud tip was closer. Mesenchymal cells undergoing epithelial transition showed dark nuclei as compared with the clear nuclei of the ureteric bud epithelial cells (**Fig. 4**). Mitotic figures were frequently seen in the newly formed glomerular structures, which remained in strict contact with the ureteric bud cells (**Fig. 5**).

In some stem cell niches it was possible to evidence the initial phases of induction of a nodular aggregate of metanephric mesenchymal cells by infiltrating ureteric derived epithelial cells (**Fig. 6**). As compared to the primitive non-induced nodular aggregates, the mesenchymal cells under induction showed the tendency to a new architectural organization characterized by a concentrical arrangement around the ureteric bud tip cells (**Fig. 6**). Primary and recently induced stem cell niches were always in strict contact with capsular cell progenitors (**Fig. 7**) .

At high power, by PAS stain it was possible to study in deep the strict relationship between the epithelial ureteric bud tip precursor and the metanephric mesenchymal precursors (**Fig. 8**). The basal membrane was very thin and focally it was absent, allowing a direct contact between epithelial and mesenchymal cells. Epithelial precursor of the tip frequently appeared polystratified and organized in small solid aggregates which extended into the surrounding mesenchyme (**Fig. 8**).

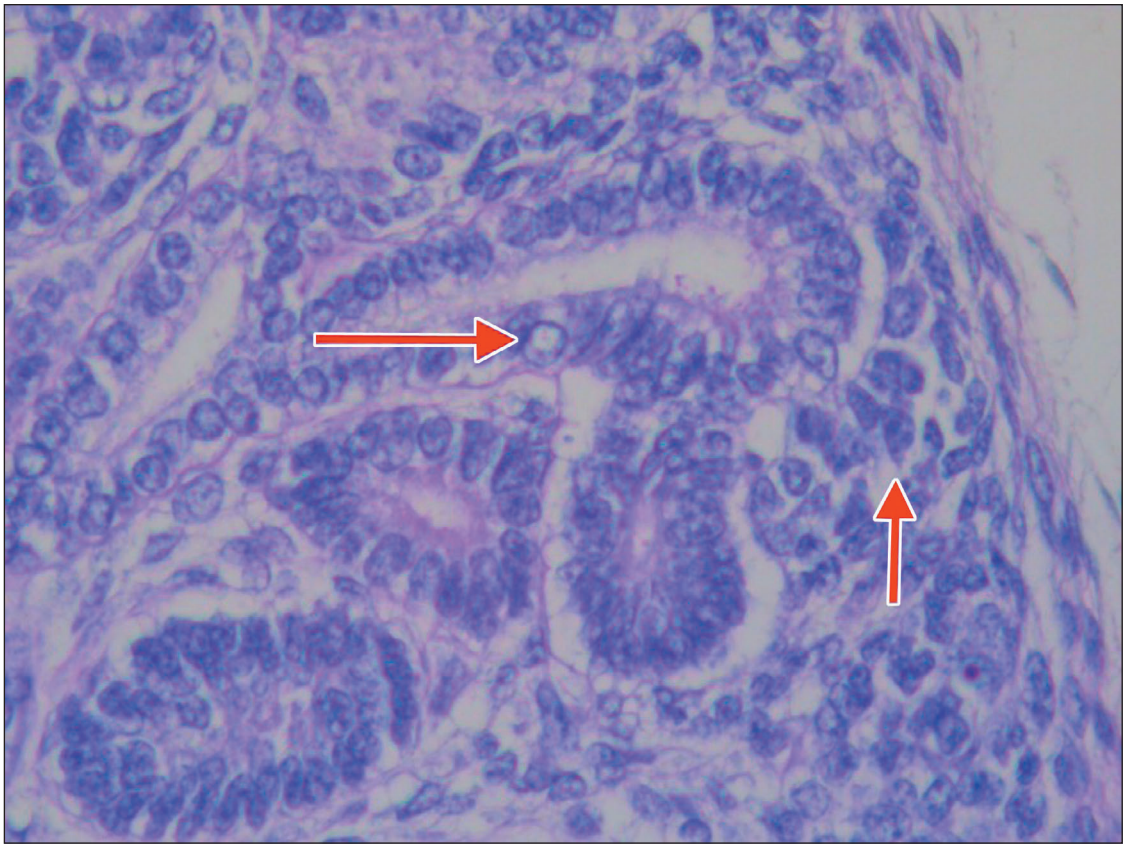

Figure 4. Mesenchymal cells with dark nuclei (arrow) and ureteric bud epithelial cells with clear nuclei (small arrow). HE 40x.

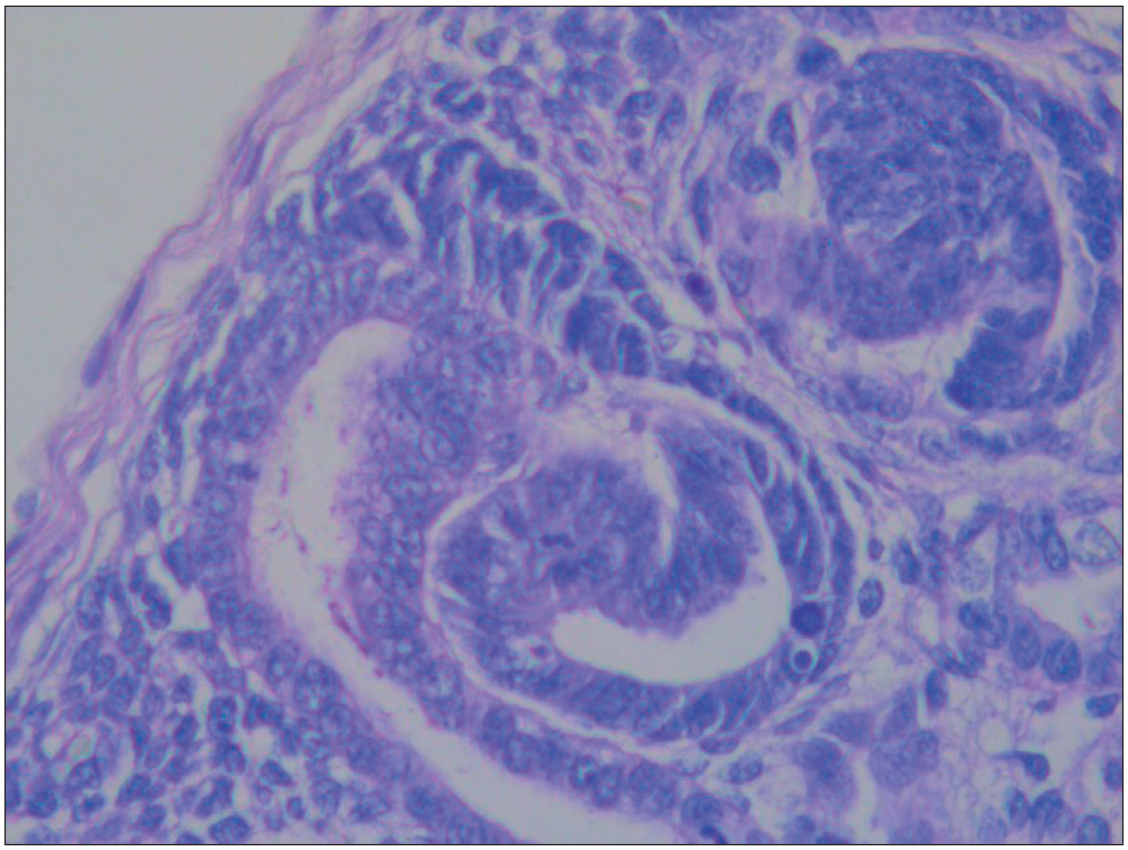

Figure 5. New glomerular structures in strict contact with the ureteric bud cells. HE 40x.

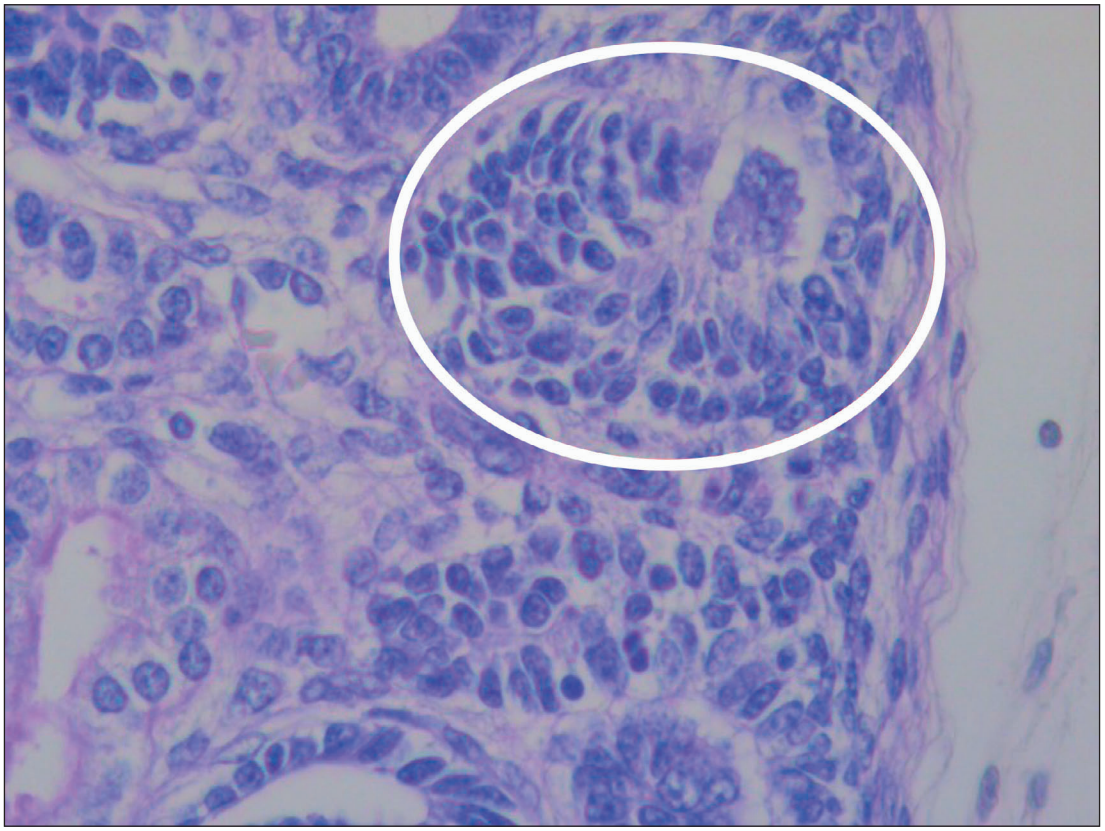

Figure 6. The mesenchymal cells under induction showed the tendency to a new architectural organization characterized by a concentrical arrangement around the ureteric bud tip cells (circle). HE 40x.

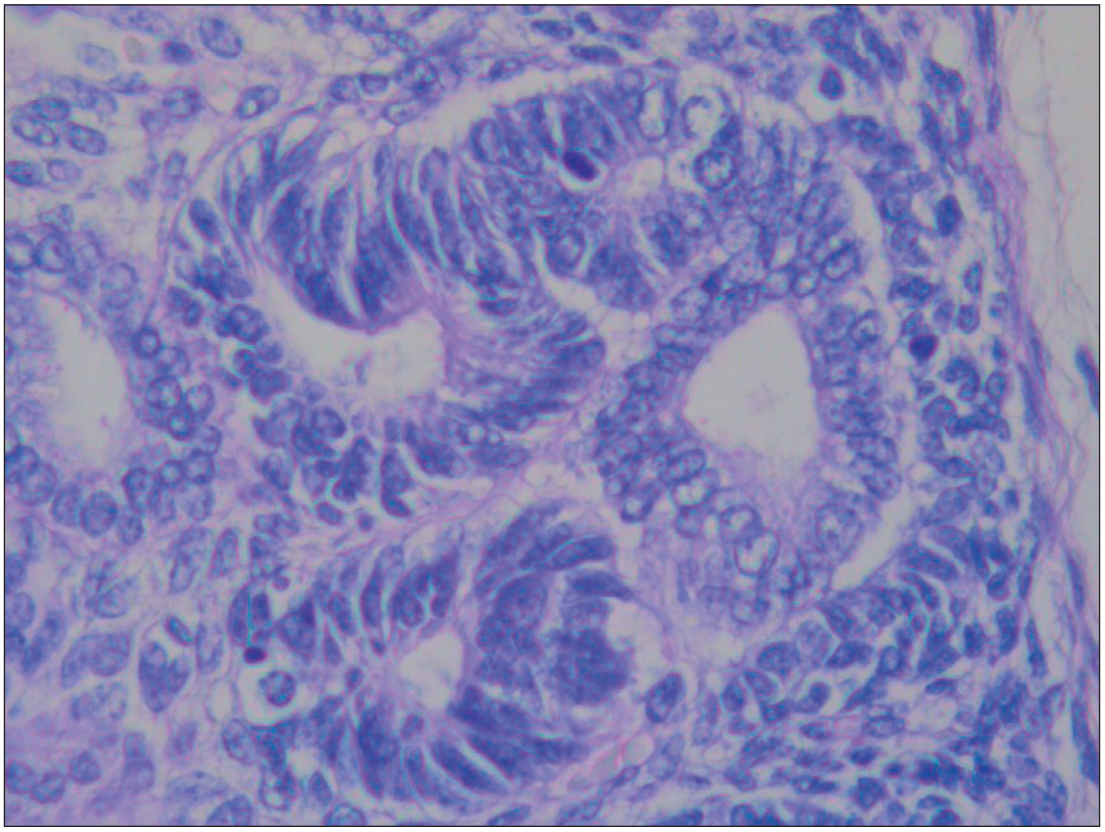

Figure 7. Stem cell niches in strict contact with capsular cell progenitors. HE 63x.

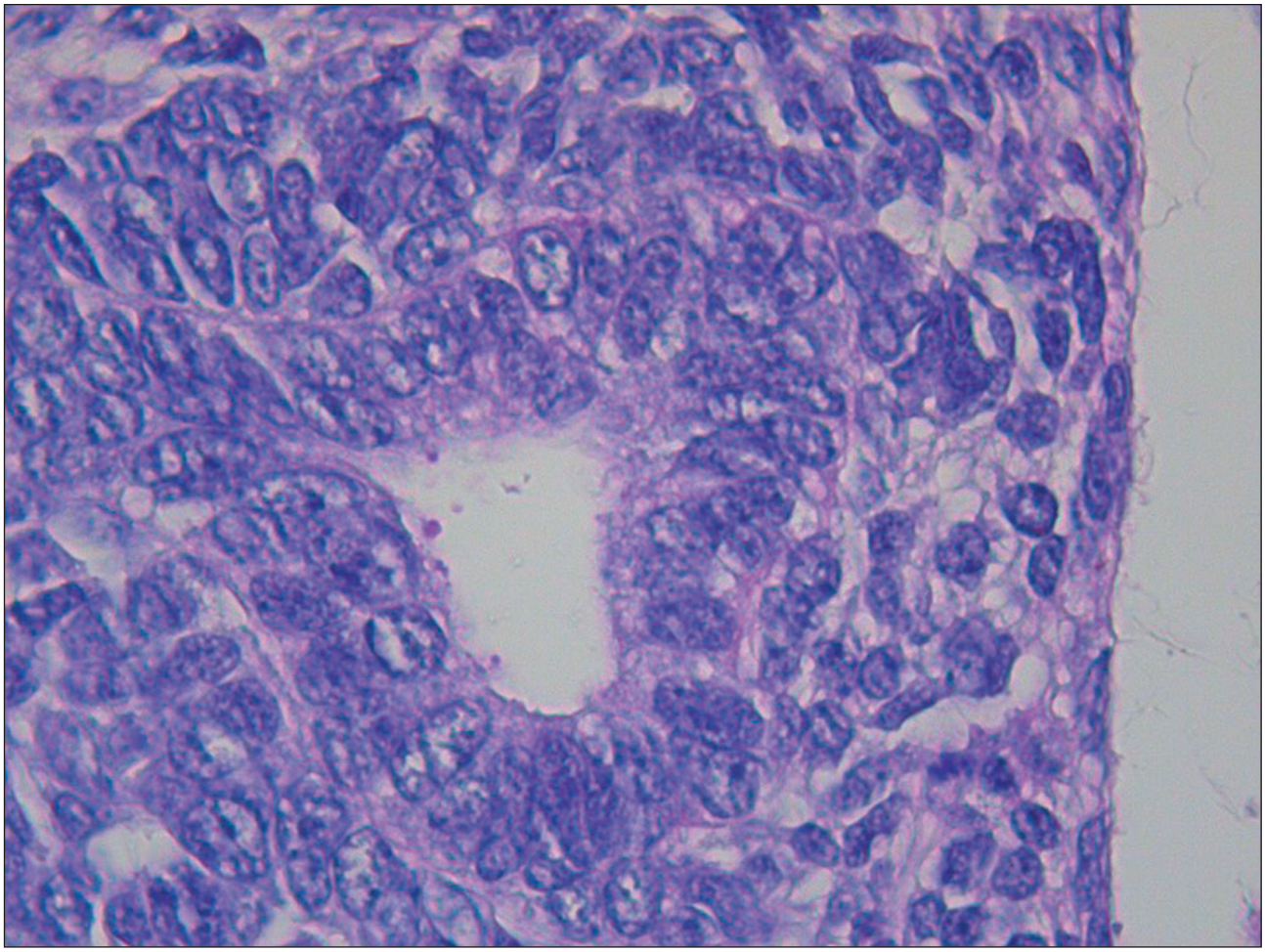

Figure 8. Epithelial precursor of the tip frequently appeared polystratified and organized in small solid aggregates which extended into the surrounding mesenchyme. PAS 63x.

In some activated stem cell niches, at high power it was difficult to differentiate bud tip cells from mesenchymal cells (**Fig. 9**). In these fields the borders between these two structures were not clear.

Activated bud tip cells showed marked nuclear pleomorphism, presenting clear nuclei with clumped chromatin and frequently grooves, giving rise to a pseudotumoral picture (**Fig. 9**). Moreover, the activated epithelial cells showed the tendency to infiltrate the surrounding mesenchyme (**Fig. 9**).

Discussion

Our study shows for the first time the complexity and the variability of the renal stem cell niches in the developing human embryo. The most frequent morphological arrangements of the niches observed in this study are reported in **Fig. 10**.

Regarding the cells types included in the stem cell niches, our study evidences a previously unreported complexity of the human renal stem cell niches. Capsular cells, metanephric mesenchymal cells, cap mesenchymal cells, induced cap cells, ureteric bud-derived cells and stromal cells represent, on the basis of our study, the most important cell types involved in the organization of the renal stem cell niches. Multiple steps of the progressive differentiation from metanephric mesenchyme to the new nephron remain, at the best of our knowledge, unknown. Further studies, mainly based on immunohistochemistry, are needed in order to better define the complexity of differentiation stages of the progenitors involved in the organization of each niche. The complexity here observed underlines the complexity of each attempt to activate nephrogenesis, by the use of exogenous stem cells, in adult kidneys. Our findings indicate that stem cells may not induce nephrogenesis alone.

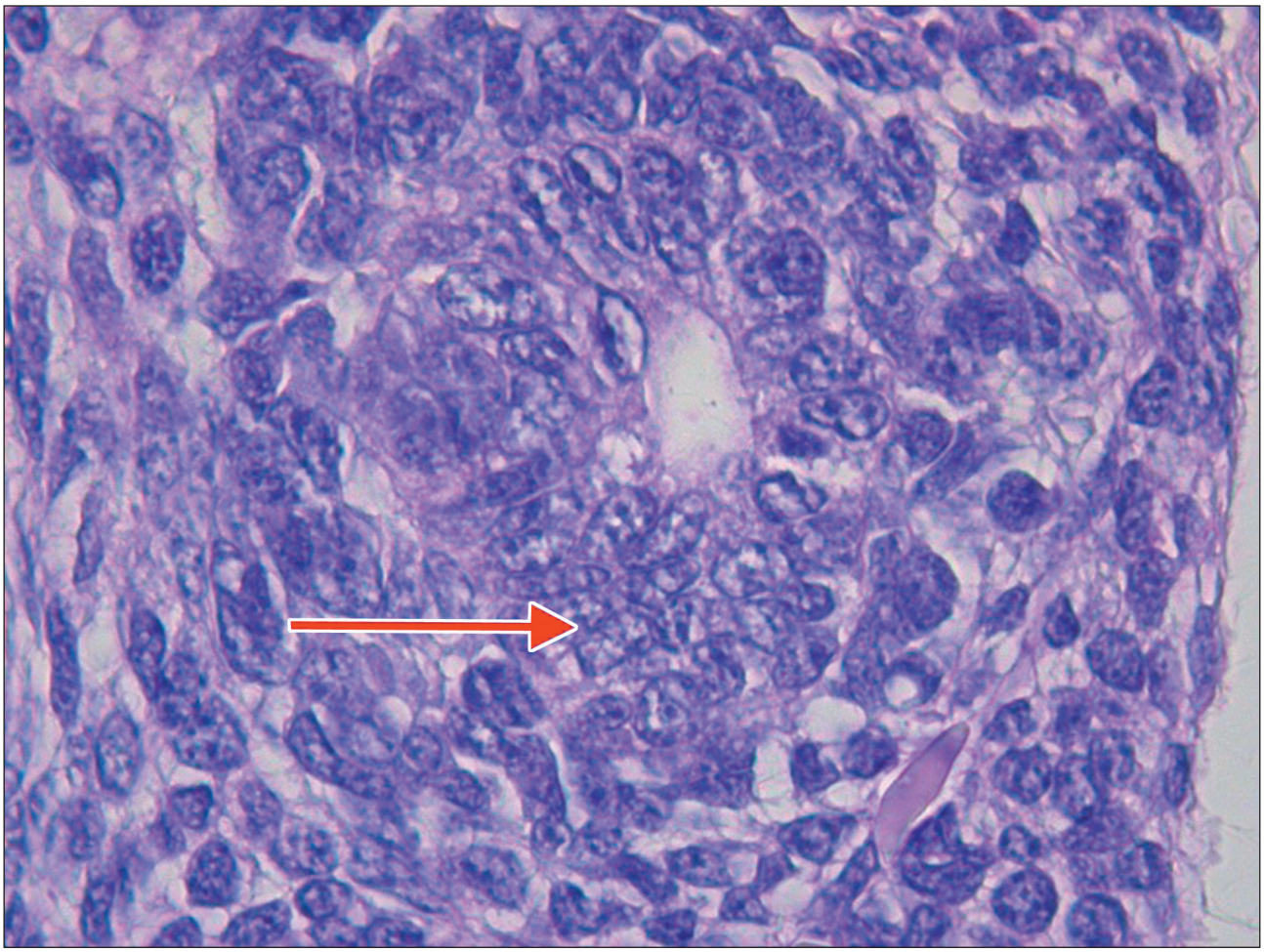

Figure 9. Activated bud tip cells showed marked nuclear pleomorphism, presenting clear nuclei with clumped chromatin and frequently grooves (arrow). HE 63x.

Another interesting finding emerging from our study is the different morphology of adjacent stem cell niches, even in the same kidney, as well shown in **Fig. 1**. This is an intriguing finding, because it demonstrates that stem cell niches have not, at least in the fetal kidney, a peculiar morphological picture. On the contrary, at histology one should be aware that stem cell niches may acquire multiple morphologies.

Differences in stem cell niche morphology probably reflect, in our opinion, different functional states of renal stem cells. According to this hypothesis, the solid roundish arrangement might represent the initial state of activation of the niche and the origin of a new glomerulus might represent the final stage of the stem cell niche. The others morphological forms of stem cell niche probably could be interpreted as intermediated phases of activation of the niche.

This modification of architecture and cell differentiation of renal stem cell niches paralleles, in our opinion, the transformations of lymphoid nodules in the cortex of lymph nodes. Primary follicles, resembling the "solid" stem cell niches, undergo enlargement and architectural changes under antigen stimulation, ending with a small solid nodule at the end of its function.

Another important finding of our study regards the strict relationship between the epithelial cells of the ureteric bud tip cells and the surrounding cap mesenchymal cells undergoing mesenchymal to epithelial transition. Our findings indicate a strict relationship between these two cell types, previously reported exclusively at electron microscopy by Minuth and coworkers [10, 11]. In some fields (see **Fig. 9**) we found overlap between these structures, in the absence of any basal membrane between epithelial and mesenchymal cells. Our data seems to confirm the hypothesis of Minuth regarding the presence of nano-channels joining the epithelial and the mesenchymal cells, allowing the passage of transcription factors from one cell to another.

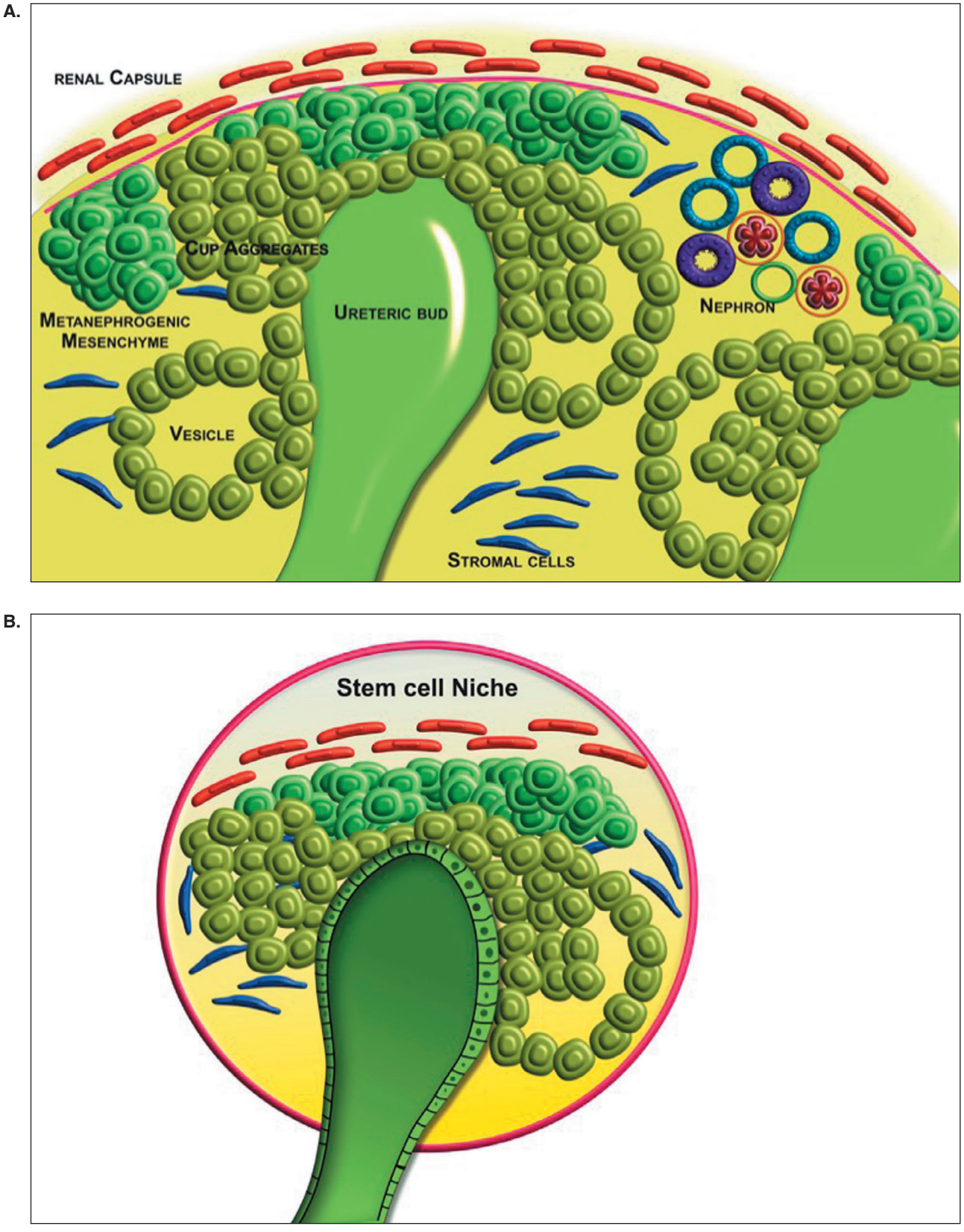

Figure 10. A. Schematic representation of the evolution of a renal stem cell niche. **B**. Renal stem cell niche.

Interesting data emerge even from the strict relationship between stem cell niches and the renal capsule. According with our observations, the multiple undifferentiated cells found in the renal capsule should be considered as an integral part of the stem cell niche.

In conclusion, our study reveals a previously unreported complexity in the anatomy and evolution of the renal stem cell niche in the developing human fetus. This complexity suggests the necessity of further studies aimed at clarifying the role of the multiple subtypes of renal progenitors involved in nephrogenesis. In particular, the strict relationship between the ureteric bud-derived epithelial cells and the metanephric mesenchyme appears in our opinion a fascinating field for future research.

Declaration of interest

The Authors have declared that no conflict of interest exist.

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