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Interindividual variability in L1CAM expression in the human kidney during development: are there implications for fetal programming of kidney diseases presenting in adulthood?

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Abstract. – OBJECTIVE: L1 cell adhesion molecule (L1CAM) is a member of the immunoglobulin superfamily of cell adhesion molecules. The present study investigated the expression of L1CAM during the development in the fetal human kidney at different gestational ages, to reach a better knowledge on the role of L1CAM in renal morphogenesis.

MATERIALS AND METHODS: The immunohistochemical analysis for L1CAM was performed in 24 fetal kidneys of different gestational ages, ranging from 10 to 38 weeks. L1CAM expression was observed in all 24 kidneys examined.

RESULTS: Immunoreactivity for L1CAM was restricted to the collecting tubules, of the developing fetal kidneys. Moreover, L1CAM was detected in the ureteric bud tips, near the subcapsular metanephric mesenchymal stem/progenitor cells. L1CAM was also expressed in the collecting tubules undergoing fusion with the distal tubules of the developing nephrons. L1CAM was mainly expressed along the cell membrane. In fetal kidneys in which the renal pelvis was observed, epithelial cells of the renal pelvis showed strong membranous reactivity for L1CAM.

CONCLUSIONS: Our study shows that L1CAM is expressed in all stages of human kidney nephrogenesis, being restricted to the renal structures derived from the ureteric bud. The expression of L1CAM in the cells of the ureteric bud tips suggests a major role for this adhesion molecule in the induction of metanephric mesenchymal cells to undergo mesenchymal-to-epithelial transition and differentiation into new nephrons. The interindividual variability in L1CAM expression observed in this study might be related to different levels of nephrogenesis, suggesting L1CAM involvement in the fetal programming of adult kidney diseases.

Key Words:
L1CAM, Nephrogenesis, Kidney development, Fetal kidney, Immunohistochemistry.

Introduction

L1 cell adhesion molecule (L1CAM) is a member of the immunoglobulin superfamily of cell adhesion molecules (Igcam), discovered in

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1984 by Rathjen and Schachner[1,2] in the central nervous system in mice. L1CAM is a 200-220 kDa transmembrane glycoprotein, composed of an extracellular region and a cytoplasmic tail[3]. Even though L1CAM should be defined as a transmembrane protein, the intracellular domain may be released and translocate into the nucleus[4]. The extracellular part of the protein may be processed by metalloproteinases and released into the intercellular space[5].

L1CAM is the prototype member of the L1 family, which includes four different members: L1CAM, CHL1 (Close Homolog of L1), NrCAM, and Neurofascin[6]. The cytoplasmic tail of L1CAM can interact with multiple cytoskeletal proteins, including actin, ankyrin, and spectrin[7,8]. Originally detected in the post-mitotic neurons in the cerebral cortex[1], L1CAM has been reported[9] in multiple cancer cell lines, in which it is involved in cell motility, proliferation, invasion, and resistance to chemotherapy. Moreover, L1CAM expression has been associated with epithelial-mesenchymal transition[10] (EMT). In the endometrial cancer, L1CAM has been indicated as an independent poor prognostic factor, with superiority over the other risk scores[11]. A recent paper[12] on L1CAM expression in endometrial cancer reinforced the association of its expression with poor prognosis, poor survival outcome, and adverse clinical course. In physiology, a key role has been assigned to L1CAM in the development of the central nervous system (CNS), a process characterized by tightly regulated intercellular connections facilitated by interactions between multiple adhesion molecules, including L1CAM[13]. Mutations in L1CAM are associated with multiple CNS malformations, collectively known as the CRASH syndrome[14], more recently defined as L1 syndrome. An isoform of L1CAM, expressed in endothelial cells, has been shown to exert high angiogenic functions[15].

In recent years, a role to L1CAM has been assigned in the development of the gastrointestinal tract and the urogenital system[16].

In this article, L1CAM was reported to be expressed in the urinary system, from gestational week 8 to week 12. This work aimed to analyze, at the immunohistochemical level, L1CAM expression in the human kidney during the different phases of development, to reach a better knowledge of the role of L1CAM in renal morphogenesis.

Materials and Methods

Kidneys from 24 embryos and fetuses were obtained at autopsy, at the Division of Pathology, San Giovanni di Dio University Hospital, AOU of Cagliari, Cagliari, Italy. Fetal ages were estimated from the gestational age, ranging from 10 to 38 weeks (10 to 19 weeks, N=12; 20 to 25 weeks, N=8; 30 to 38 weeks, N=3). In the clinical history, no evidence of nephrological malformations was present. None of the kidneys utilized in this study showed macroscopical or microscopical anomalies in the conventional standing preparations (H&E).

Kidney samples were fixed in 10% formalin and embedded in paraffin (according to conventional techniques). Three micron-thick sections were stained with hematoxylin and eosin (H&E) and immunostained with the ultraView Universal DAB Detection Kit (Roche Tissue Diagnostics, Basel, Switzerland). In brief, for detecting primary antibodies, we used a mouse monoclonal antibody (Sigma-Aldrich, mouse IgG1 isotype, clone UJ127) against L1CAM. In brief, slides were incubated for 20 minutes at room temperature with a 1:100 dilution of the monoclonal anti-L1CAM primary antibody. Nervous structures present in every kidney were utilized as internal positive controls. As appropriate negative controls, kidney section processed omitting the primary antibody for L1CAM were used. To better evaluate the degree of immunoreactivity from L1CAM, a semiquantitative scoring system was applied (Table I).

All experimental procedures were approved by the Ethics Experimentation Committee, University of Cagliari. In this context, all protocols have been carried out in full conformity with the rules and guidelines expected for this kind of experiment. Slides were randomized, assigned a study number, and reviewed by two of the authors (GF, FC), while blinded to the clinical history. Classification of each criterion was based on consensus opinion.

Table I. Semiquantitative scoring system for L1CAM.

Score	Immunoreactivity
0	Absent
1	< 50% of cells
2	> 51% < 75% of cells
3	> 75% of cells

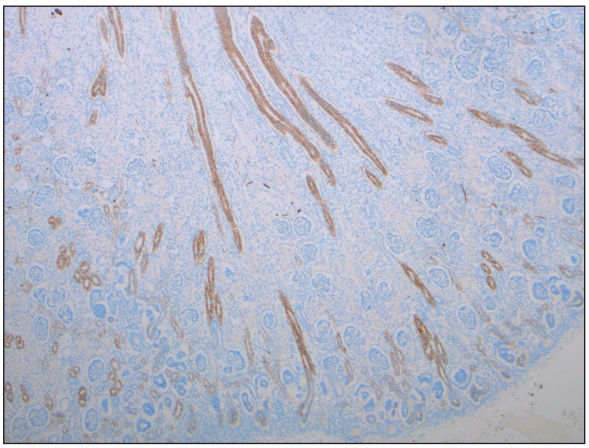

Figure 1. Magnification: (50×), 20 weeks. L1CAM is mainly expressed in the cell membrane of epithelial cells in the collecting tubules of the medulla and of the cortex.

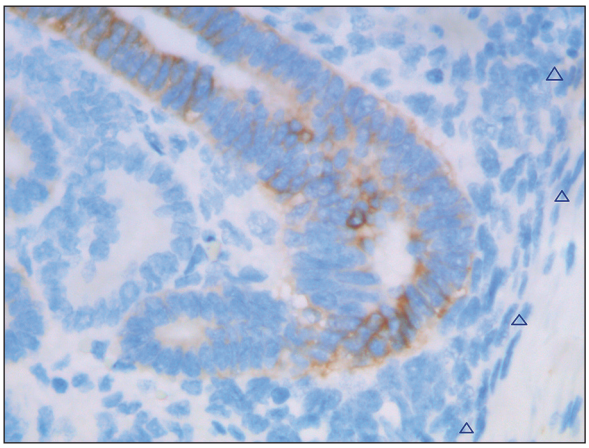

Figure 3. Magnification: (630×), 12 weeks. L1CAM expression in the ureteric bud tip cells (*arrowheads*).

Results

L1CAM expression was detected in all the 24 cases examined (Table I). In all positive cases, immunoreactivity for L1CAM was restricted to the collecting tubules, in the medulla, and the cortex of the developing fetal kidneys (Figure 1). Moreover, immunostaining for L1CAM was detected in the epithelial cells of the ureteric bud tips, near the subcapsular metanephric mesenchymal stem/progenitor cells (Figure 2). The expression of L1CAM was mainly observed along the cell membrane of immunoreactive cells. In the collecting tubules of the medulla, L1CAM was mainly expressed along the whole-cell membrane. On the contrary, in the ureteric bud tip cells, immunostaining for L1CAM was stronger in the section of cell membrane facing the surrounding cap mesenchymal progenitor cells (Figure 3). L1CAM was also expressed in the ureteric bud tips undergoing fusion with the distal tubules of the developing nephrons (Figure 4). In these structures, L1CAM immunostaining was also found in the spaces intermingled between the ureteric bud tip cells and the distal tubular cells (Figure 4). No reactivity for L1CAM was observed inside the nuclei in the ureteric-bud-derived structures. No immunoreactivity for L1CAM was found in the capsular cells, in metanephric mesenchymal cells, in the devel-

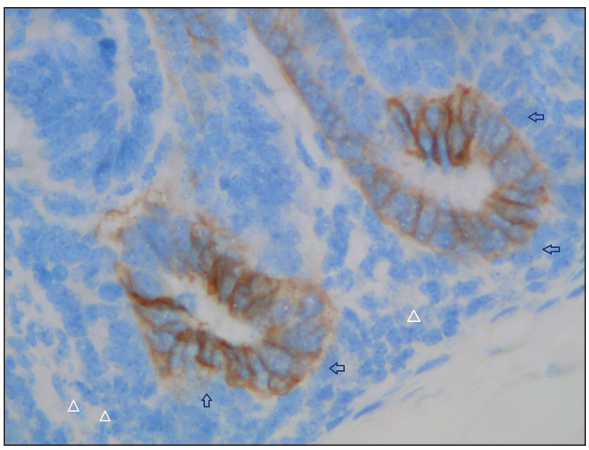

Figure 2. Magnification: (400×), 19 weeks. Immunostaining for L1CAM is detected in the epithelial cells of the ureteric bud tips (*arrows*), in close proximity to the subcapsular metanephric mesenchymal stem/progenitor cells *(arrowheads*).

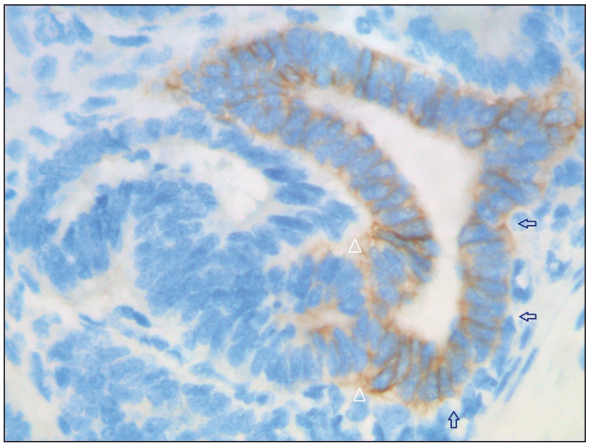

Figure 4. Magnification: (630×) 20 weeks. Immunoreactivity of L1cam in the ureteric bud tips (*arrow*) undergoing fusion with the distal tubule (*arrowhead*) of the developing nephron.

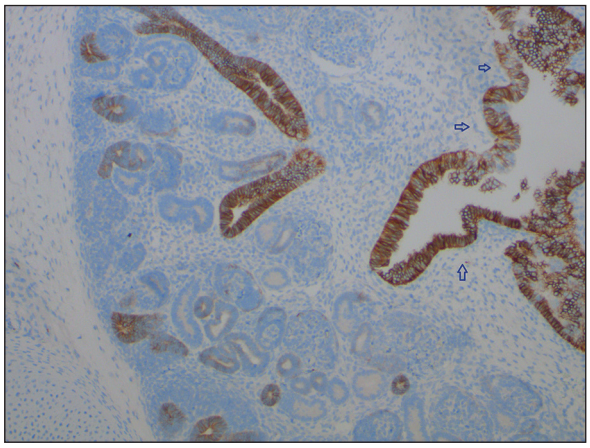

Figure 5. Magnification: (100×), 10 weeks. Strong membranous reactivity of L1CAM in the epithelial cells of the renal pelvis.

oping nephrons, and the interstitial cortical and medullary cells (Figure 1). In a few cases, in which the renal pelvis was observed, epithelial cells of the renal pelvis showed strong membranous reactivity for L1CAM (Figure 5).

Marked interindividual variability in L1CAM expression was observed. The insensitivity of immunostaining (Table I) for L1CAM in medullary collecting tubules changed from case to case. In 20 cases L1CAM was expressed in the vast majority of collecting tubular cells (>75%; score 3) whereas in four cases less than 50% of epithelial cells of the medullary collecting tubules were positive for L1CAM (score 1). A lower degree of L1CAM expression was observed in the collecting tubules in the renal cortex. Strong immunostaining (score 3) was found in 10 out of 24 kidneys (42%). In 9 cases, we observed a mild reactivity for L1CAM (score 2; <75% of cells) (37%); no reactivity was detected in the remaining 5 cases (21%). A marked interindividual variability, regarding immunoreactivity for L1CAM, was evidenced in the ureteric bud tips. A strong diffuse expression (score 3) was found in two cases (8%); an intermediate degree of immunoreactivity (score 2) was observed in three cases (12%); mild reactivity (score 1) for L1CAM was found in 8 cases (33%). In the remaining 11 kidneys, we did not find any significant reactivity for L1CAM in the epithelial cells of the ureteric bud tips. L1CAM expressions were not strictly correlated with the gestational age of fetuses: subjects with the same gestational age showed marked differences regarding L1CAM expression (see the three cases with 17 weeks of gestational age, in Table II). Interindividual variability regarding L1CAM reactivity

Table II. Immunohistochemistry comparison of L1CAM expression in the kidneys at different gestational ages.

21 RIC 135	Medullary collecting tubules	Cortical collecting tubules	Ureteric bud tips	Week of gestation	Gender
1	3	3	1	10	M
2	3	3	3	10	M
3	3	3	2	11	M
4	3	3	2	12	M
5	1	1	0	14	M
6	3	2	1	15	F
7	3	3	1	17	F
8	3	2	2	17	F
9	3	1	0	17	F
10	3	1	0	18	M
11	3	3	1	18	F
12	3	3	3	19	M
13	3	1	0	19	M
14	3	3	1	20	F
15	3	2	0	20	M
16	3	2	0	20	M
17	3	2	1	21	M
18	3	3	1	21	F
19	3	3	1	22	F
20	1	1	0	25	F
21	1	1	0	25	F
22	3	1	0	31	F
23	3	1	0	34	M
24	1	1	0	38	M

was mild in the medullary collecting tubules, increased in the cortical collecting tubules, and appeared marked in the ureteric bud tip cells (Figure 6).

Discussion

Human nephrogenesis is a very complex process, at least in part unknown, characterized by the interplay between multiple stem/progenitor cells, with multiple molecular mechanisms involved in kidney development[17]. Renal development is influenced by multiple epigenetic factors, which are at the basis of marked interindividual variability in renal maturation. Recently, other new potential biological events, like milk mesenchymal stem cells, have been hypothesized to may play a role in post-natal kidney remodeling[18,19]. Epigenetic changes in kidney development give rise to significant differences in nephron burden, which has been hypothesized to range from 200,000 up to 2 million of glomeruli[20]. In previous papers[21,22], immunohistochemistry has been shown to play a major role in the identification of the multiple molecules involved in the different steps of kidney development. Previous immunohistochemical studies[22] carried out on fetal kidneys at different gestational ages allowed a better characterization of the multiple progenitor cells involved in human kidney development. Wilms tumor 1(wt1) appeared to be mainly expressed in the nucleus of metanephric mesenchymal progenitors of the so-called "blue strip[23]", located nearby of the renal capsule[24]. CD44, a transmembrane adhesion molecule involved in uptake and degradation of hyaluronidase, was expressed in a subset of metanephric mesenchymal progenitor cells involved in the early phases of nephrogenesis[25]. The anti-apoptotic protein Bcl 2 has been reported to be highly expressed in the cytoplasm of the cap mesenchymal cells, the progenitor cells which aggregate in the surrounding of the ureteric bud tips[26]. The cap aggregates are also immunoreactive for PAX2 at nuclear level[22]. Expression of MUC1, a transmembrane glycoprotein, has been associated with the process of mesenchymal-epithelial transition (MET), with a putative role in triggering the transition of metanephric mesenchymal cells into epithelial cells[27]. According to a possible key role in human nephrogenesis, MUC1 was found to be expressed in pre-tubular aggregates, in renal vesicles, in comma bodies, and in s-shaped bodies, confirming the strict association of this marker with the early phases of nephron development[28]. Renal vesicles were also immunostained by CD10, a marker of podocyte precursors in the developing glomeruli[29].

A peculiar pattern of immunoreactivity was detected in the developing kidney in previous studies on the expression of thymosin Beta-4 (TB4), a ubiquitous peptide involved in fetal development. TB4 was mainly expressed in the progenitor cells inside the renal capsule, in the stromal cells of the renal hilum, and in stromal

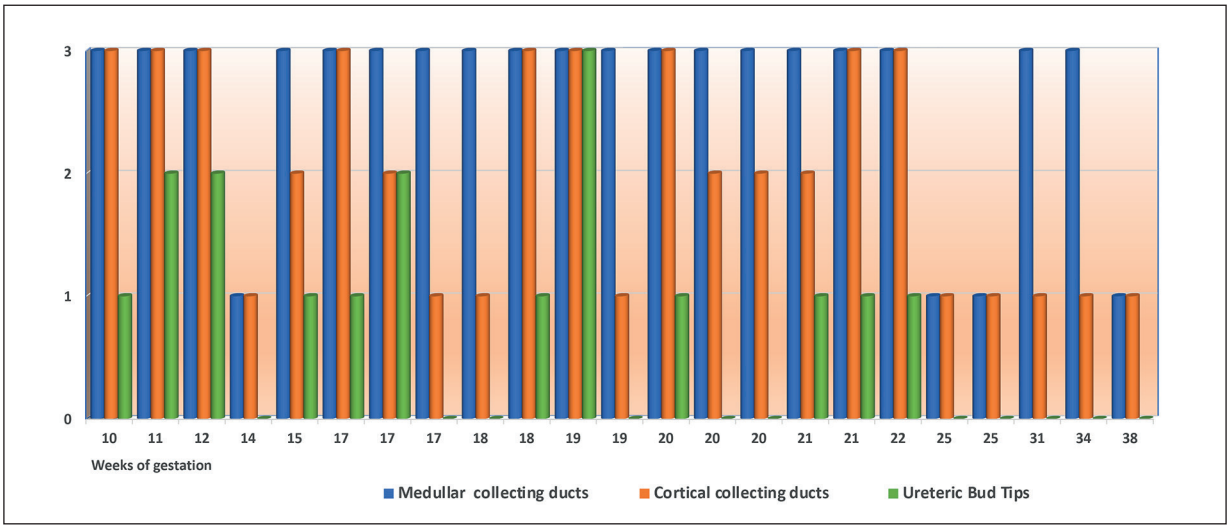

Figure 6. Graphic description of the interindividual variability regarding L1CAM expression in the medullary collecting tubules, in the cortical collecting tubules, and in the ureteric bud tips cells at different gestational ages.

cells encircling the ureter[30]. According to with these findings, it was hypothesized that TB4 might mark two (capsular and hilar) of the multiple renal stem cell niches identified in the fetal kidney during development[31]. Another member of the Beta-thymosin family, Thymosin Beta-10 (TB10) has been reported to be highly expressed during renal embryogenesis, in proximal and distal tubules[32].

In this work, a new actor is reported to have a role in the complex field of human nephrogenesis: L1CAM. The expression of this transmembrane adhesion molecule was restricted, in all the developing kidneys analyzed in this study, to the epithelial structures originating from the ureteric bud. The primary ureteric bud originates from the Wolffian/duct and progressively invades the metanephric mesenchyme. The branching ureteric bud (UB) tip cells induce the subcapsular metanephric mesenchyme to differentiate, giving rise to the cap mesenchyme, the renal vesicles, and new nephrons[33]. The branching ureteric bud-derived tubules subsequently originate the collecting system, including collecting ducts, calyces and the renal pelvis[34,35].

L1CAM expression has been observed to be restricted to the UB-derived structures. The highest levels of reactivity for L1CAM were observed in the medullary section of collecting tubules. In these structures, L1CAM was localized along the cell membrane of epithelial tubular cells. Since L1CAM has been involved in cell motility[36], L1CAM expression in the branching ureteric bud-derived tubules might play a relevant role in the branching morphogenesis that allows the invasion of the metanephric mesenchyme.

The most intriguing finding in this study is represented by L1CAM expression in the UB tip cells. These cells have a major role in nephrogenesis: they induce metanephric mesenchymal stem/progenitor cells to differentiate into cap mesenchyme, starting the process of mesenchymal-epithelial transition that ends with the development of a new nephron[33]. The finding of L1CAM expression along the cell membrane of UB tip cells allows us to hypothesize that L1CAM might be involved in a cell talking between the epithelial and mesenchymal renal precursors. According to with this hypothesis, L1CAM should play a major role in the crucial step of the induction of metanephric stem cells and initiate the mesenchymal-epithelial transition. The decrease or the absence of L1CAM reactivity in the cap mesenchyme, as well as in the renal vesicles and in glomeruli, confirm a crucial role for L1CAM in the initial phase of the induction of metanephric mesenchymal cells.

Another interesting finding emerging from our study is the high expression of L1CAM in cortical collecting tubules undergoing fusion with a distal tubule of a developing new nephron (Figure 4). The fusion of the distal part of a new nephron with a collecting tubule represents a crucial step in the kidney development[37]. The strong immunoreactivity of L1CAM in collecting tubular cells near the fusion with a distal tubule suggests a role for this protein in this complex process, which allows the excretion of the glomerular filtrate through the renal pelvis and the ureter.

Another finding of some interest is the interindividual variability in L1CAM expression in the kidney. Kidneys from fetuses with the same gestational age showed different degrees of L1CAM immunoreactivity. This variability was more marked when the expression of L1CAM in ureteric bud tip cells was considered. Given that the gestational age did not strictly correlate with L1CAM expression, we may hypothesize that epigenetic factors, acting during gestation, might interfere with L1CAM expression, ending with changes in kidney development.

The interindividual variability of L1CAM expression in the ureteric bud tips, whose cells are fundamental for triggering metanephric stem cells to undergo glomerulogenesis, might result in changes in nephrogenesis in the kidneys with low L1CAM levels. According to this hypothesis, L1CAM deficiency in the fetal human kidneys could result in a block of nephrogenesis, ending with a susceptibility to develop kidney disease and renal failure later in life.

Conclusions

Our study shows that L1CAM is expressed in all stages of human nephrogenesis. L1CAM expression in ureteric bud tip cells suggests an important role for this molecule. L1CAM, by its functions well described in the literature, might be involved in the activation of metanephric mesenchymal stem cells. We hypothesize that L1CAM acts as a “director of work” in the transformation, migration, and adhesion of mesenchymal stem cells for orderly nephrogenic development. On the contrary, partial or absent expression of L1CAM in ureteric bud tips could be responsible for renal malformations or delayed

glomerulogenesis. Epigenetic factors can explain the variability of L1CAM expression observed in our cases. According to this hypothesis, L1CAM should be included among the molecules at the basis of the fetal programming of adult kidney diseases[38].

Conflict of Interest

The Authors declare that they have no conflict of interests.

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