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Original article

Morphological changes in the kidney of fetuses with Down syndrome

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Abstract

Background: A variety of renal and urological abnormalities have been reported in subjects with Down syndrome (DS). With increased longevity, it appears that a growing number of these subjects presents chronic renal failure. Definition of underlying cause of renal failure could lead to the prevention of progressive renal dysfunction in these patients. The aim of this study was to improve the understanding of the morphological changes that occur in the kidney of fetuses with DS.

Methods: To this end, 25 subjects were examined. Kidney sections were stained with H&E and digitally scanned. Subjects were subdivided into two groups: fetuses with DS (DS-fetuses, n = 11) with a gestational age ranging from 13 up to 21 weeks, and healthy fetuses (N-fetuses, n = 14) with a gestational age ranging from 9 up to 22 weeks.

Results: DS-fetuses showed slightly larger glomeruli as compared to N-fetuses. Moreover, glomeruli in DS-fetuses group were characterized by an enlarged Bowman's space as compared to glomeruli in N-fetuses ($p = 0.0028$). Differences in the nephrogenic zone width were also observed; DS-fetuses showed a greater width of this zone as compared with N-fetuses.

Discussion: In conclusion, we found relevant morphological differences, which suggests delayed renal maturation. Furthermore, there was a significant increase in glomerular area and several glomeruli were morphologically abnormal. These harmful changes in the glomerular structure may result in a nephron deficit, which may be associated with development of renal diseases and hypertension later in life.

Conclusions: We hypothesize that the observed morphological anomalies could have significant implications for both the short- and long-term renal health of subjects with DS.

Keywords

Fetuses with Down syndrome, renal anomalies, glomerular abnormalities, kidney disease.

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Background

Down syndrome (DS) is the most common genetic disorder in humans associated with the presence of an extra chromosome 21, with a prevalence of 1.72 per 1,000 total births [1]. DS is related with a variety of developmental anomalies [2], including abnormalities of brain [3], heart [4] and gastrointestinal tract [5]. The most common renal and urological anomalies in subjects with DS are represented by urethral obstruction, ureteral dilatation, hydronephrosis, renal parenchymal thickening, renal hypoplasia, renal agenesis, and neurogenic bladder [6-8].

Moreover, a variety of renal diseases including glomerulonephritis [9], immunotactoid glomerulopathy [10], focal and segmental sclerosis [11], and chronic renal failure (CRF) [12, 13] have been reported in carriers of DS. Identifying the main causes of renal failure in these patients could be of help in the prevention of progressive renal dysfunction in this population [14]; for this reason, investigating structural changes during intrauterine life is becoming increasingly important [15]. The aim of this work was to evaluate morphological renal differences between DS fetuses and normal fetuses. We hypothesized that DS might be associated with variations in structure of the fetal kidney, such as glomeruli, in agreement with what it has been observed in adults [16, 17]. In order to assess these variations, in our work we investigated several parameters as glomerular area and the ratio between glomerular area enclosed by Bowman's space, that we defined functional glomerular area (FGA), and total glomerular area (TGA). Moreover, in order to evaluate the nephrogenic potential of developing kidney in DS carriers, we analyzed the width of the subcapsular nephrogenic area [18], recently defined as the "blue strip" zone [19].

Methods

Samples acquisition and preparation

Kidney samples were obtained from 25 fetuses with gestational age ranging from 9 to 22 weeks. Clinical data, including gestational age and body weight, are summarized in **Tab. 1**. Subjects were organized into two groups: fetuses with DS (DS-fetuses, n = 11) and fetuses with normal karyotype (N-fetuses, n = 14).

Portions of the kidney were formalin-fixed and embedded in paraffin, sectioned with a microtome at 5 μm and collected onto glass slides. Complete kidney sections were selected and stained with hematoxylin and eosin. For each kidney, one section was examined.

With the purpose of assessing renal variations between DS-fetuses and N-fetuses, we firstly evaluated any differences in body weight and gestational age between the two groups.

Table 1. Average gestational age, sex ratio and body weight of examined fetuses.

	N-fetuses	DS-fetuses
N	14	11
Gestational age (weeks)	15.9 ± 4.38 (9 to 22)	16.5 ± 2.46 (13 to 21)
Sex ratio (M:F)	9:5	7:4
Body weight (g)	166.1 ± 173.8 (3 to 440)	164 ± 151.1 (36 to 530)

Data are reported as mean ± SD with data range in parentheses. N-fetuses: fetuses with normal karyotype; DS-fetuses: fetuses with Down syndrome.

Qualitative analyses

An observational histological analysis of kidney samples was performed in order to evaluate differences in renal architecture. This evaluation was performed by an experienced pathologist (GF) by analyzing renal structures in glass slides using an optical microscope.

Quantitative analyses

Assessment of glomerular area and functional ratio

We investigated firstly TGA; then, we analyzed FGA, and the ratio between FGA and TGA. We defined this ratio as functional ratio (FR), because

it provides information about the percentage of functional glomeruli compared to total glomeruli, where the functional glomeruli represent the part of glomeruli that allow filtration [20].

According with Hoy et al. [21], 10 glomeruli for each subject were selected along 3 straight lines extending from the renal capsule towards the deepest zone of the cortex [22]. Each straight line was chosen in order to cross at least 3 glomerular profiles. In order to estimate glomerular area, a bounding box approach [23, 24] has been utilized; areas were obtained as the ellipse inscribed within the box. FR was evaluated as the ratio between FGA and TGA:

$$FR = \frac{FGA}{TGA}$$

Rectangles used in the bounding box approach to estimate glomerular areas and FRs are showed in **Fig. 1A**.

Assessment of blue strip width

In order to evaluate the blue strip width, 4 images were acquired in 4 different cortical sub-capsular zones of the kidney [25]. The estimation of the blue strip width was performed using a getline approach [24], which allows to plot a segment with the length of interest, placed at 90 degrees to the renal capsule (**Fig. 1B**). For each image, we plotted 5 segments equally spaced, corresponding to the blue strip width.

All the images were acquired by optical microscopy (Leica Microsystems®). Glomerular images were acquired at 400X magnification; blue strip images were acquired at 200X magnification. Image processing procedures were performed using Matlab® software (Mathworks®).

Statistical analyses

All statistical analyses were performed using Prism version 6.00 (GraphPad Software®).

We firstly analyzed data distribution with D'Agostino-Pearson normality test. Since data were normally distributed, a Student's t-test was used to evaluate the differences in FGA, TGA, FR and blue strip width, between DS-fetuses group and N-fetuses group. The level of significance was accepted at $p < 0.05$ and corrected for multiple comparison testing using a false discovery rate (FDR) approach (Benjamini-Hochberg).

Ethics approval

This study was approved by the ASL 8 Cagliari Research Ethics Committee.

Results

Preliminary analyses (performed to exclude any differences in body weight and gestational age

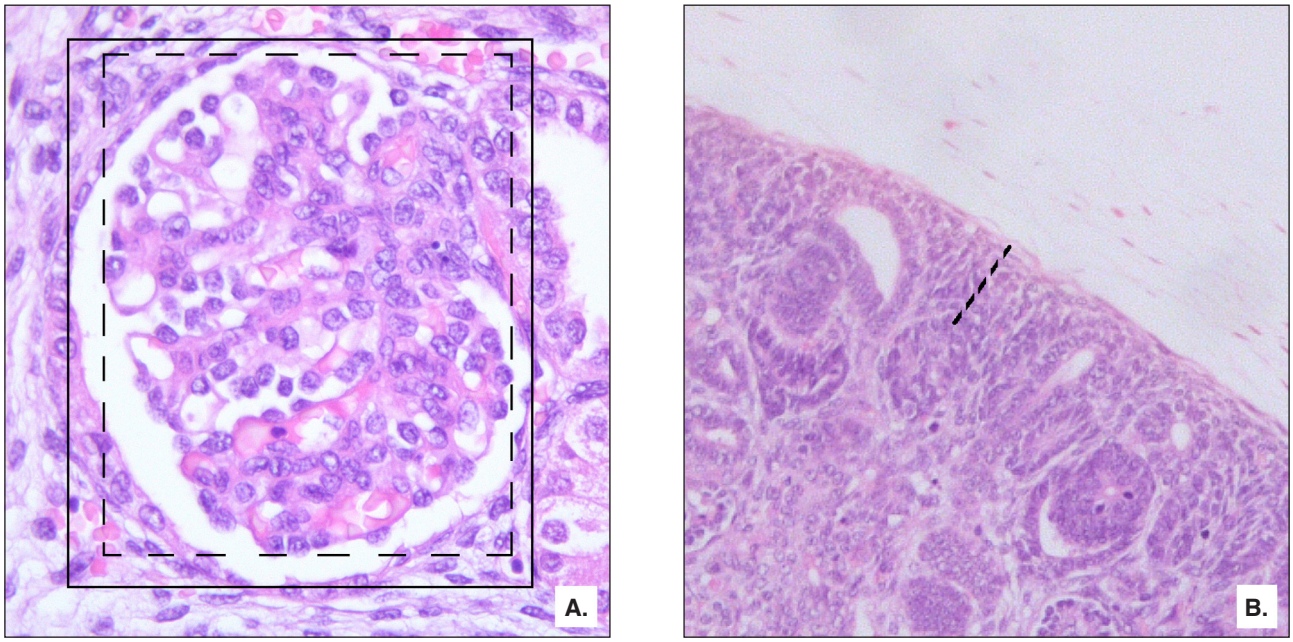

Figure 1. A. An example of a glomerulus enclosed in a bounding box. Total glomerular area (TGA) is enclosed by the solid line; functional glomerular area (FGA) is enclosed by the dashed line. **B.** Getline approach used for the estimation of blue strip width.

between N-fetuses and DS-fetuses) had shown that there were not statistically significant differences in body weight, $t(23) = 0.03236$ and $p = 0.9745$, and in gestational age, $t(23) = 0.4650$ and $p = 0.6463$, between the two groups.

Qualitative analyses

The histological analysis of kidney samples from the 11 subjects affected by DS showed multiple changes in glomerular architecture. Large glomeruli irregular in shape, with a pseudopapillary pattern, were frequently observed (**Fig. 2A**). In some of these large irregularly-shaped glomeruli, architectural changes were associated with a loss of podocyte precursors, well evidenced by the scarcity of rounded dark nuclei at the periphery of the glomerular tuft (**Fig. 2B**).

Other glomeruli showed marked hypercellularity, probably due to the increase in the mesangial component, associated with the scarcity of well-developed capillary lumens (**Fig. 2C**). Marked differences in size were frequently observed among adjacent glomeruli: some were characterized by a small retracted tuft, with an enlarged capsular space containing granular material (**Fig. 2D**).

Quantitative analyses

Assessment of glomerula area

Average TGA in N-fetuses and DS-fetuses was respectively 10,469 ± 3,368 μm^2 and 13,565 ± 4,477 μm^2. There was not a statistically significant difference between the two groups, $t(23) = 2.137$, $p = 0.0580$ (**Fig. 3A**) (**Tab. 2**) (95% CI [104.1, 6,423]).

Average FGA in N-fetuses and DS-fetuses was respectively 8,206 ± 2,685 μm^2 and 9,315 ± 3,289 μm^2. There was not statistically significant

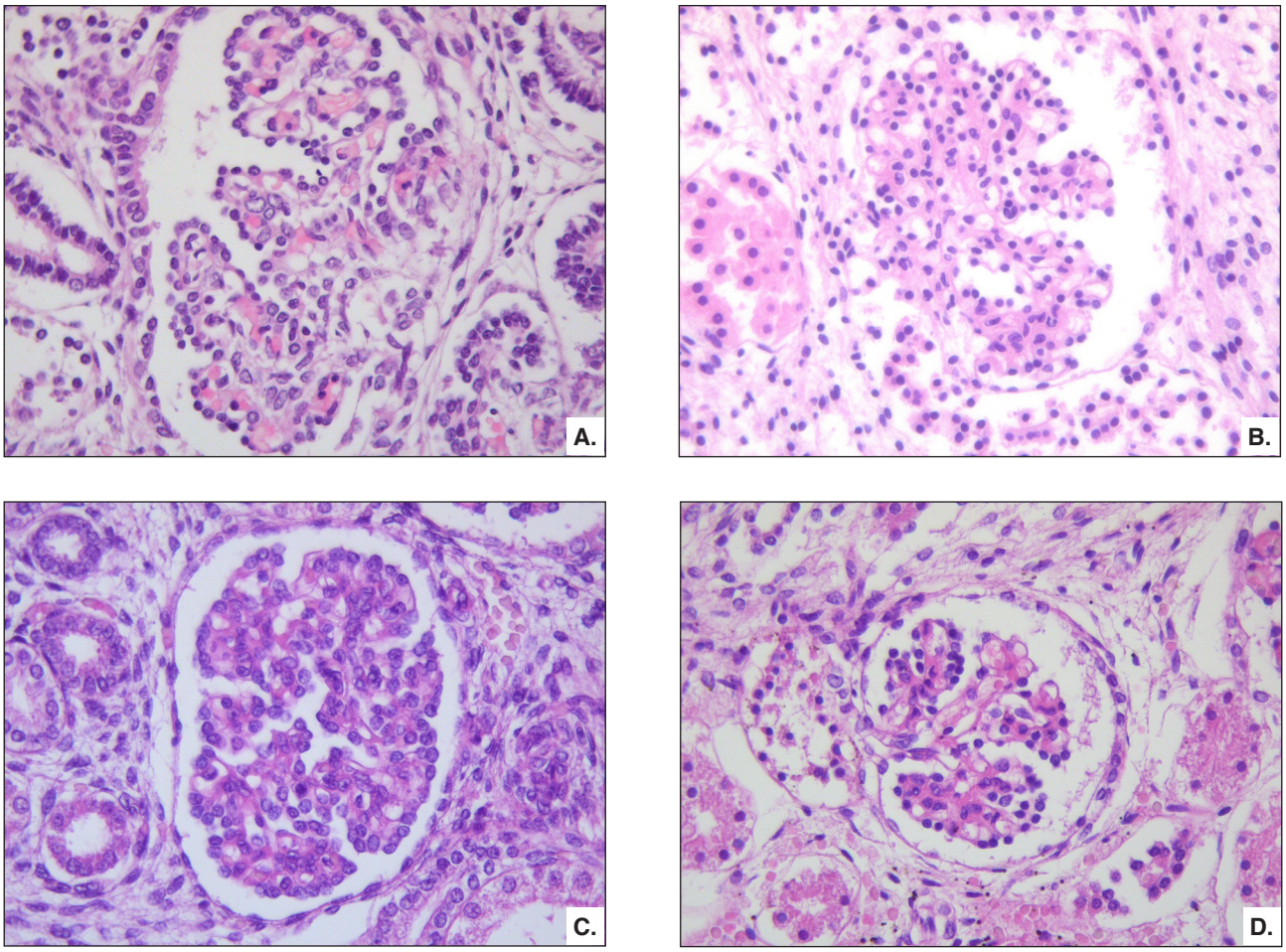

Figure 2. Glomerular changes in Down syndrome (DS): (**A**) large glomerulus irregular in shape with a pseudopapillary pattern; (**B**) glomerulus with a scarcity of podocyte precursors; (**C**) glomerulus with a marked hypercellularity; (**D**) glomerulus with a small retracted tuft, with an enlarged capsular space.

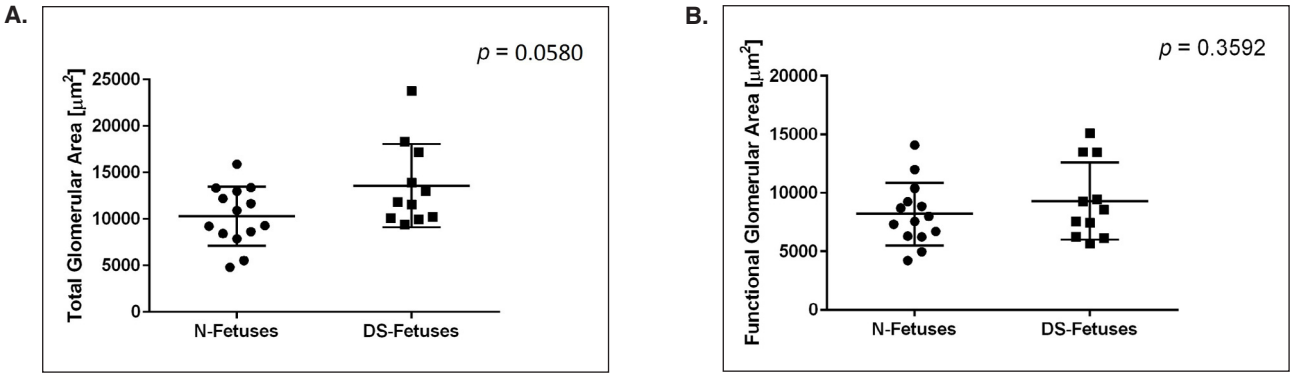

Figure 3. Assessment of glomerular area in normal fetuses and fetuses with Down syndrome (DS). Differences in the dimension of (**A**) total glomerular area (TGA) and (**B**) of the functional glomerular area (FGA). Bars represent mean ± SD. N-fetuses: fetuses with normal karyotype; DS-fetuses: fetuses with Down syndrome.

Table 2. Average glomerular area and blue strip width in the examined fetuses.

	N-fetuses	DS-fetuses	p
TGA	10,469 ± 3,368 μm^2	13,565 ± 4,477 μm^2	0.0580
FGA	8,206 ± 2,685 μm^2	9,315 ± 3,289 μm^2	0.3592
FR	0.792 ± 0.083	0.681 ± 0.066	0.0028
Blue strip width	28.544 ± 3.350 μm	32.285 ± 4.614 μm	0.0552

Data are reported as mean ± SD and as the level of significance (p). The level of significance was accepted at $p < 0.05$, corrected for multiple testing using false discovery rate (FDR).
N-fetuses: fetuses with normal karyotype; DS-fetuses: fetuses with Down syndrome; TGA: total glomerular area; FGA: functional glomerular area; FR: functional ratio.

difference between the two groups, $t(23) = 0.9356$, p = 0.3592 (**Fig. 3B**) (**Tab. 2**) (95% CI [-1,350, 3,578]). A representation of differences in TGAs and FGAs in N-fetuses and DS-fetuses is depicted in **Fig. 4**.

Assessment of functional ratio

Average FR, in N-fetuses and DS-fetuses was respectively 0.792 ± 0.083 and 0.681 ± 0.066. Student's t-test, FDR corrected, showed a statistically significant difference between the two groups, $t(23) = 3.908$, p = 0.0028 (**Fig. 5A**) (**Tab. 2**), with N-fetuses presenting a higher FR than DS-fetuses (95% CI [0.05719, 0.1858]). In **Fig. 4** is it possible to observe the difference in the dimension of Bowman's space between a N-fetus's glomerulus and a DS-fetus's glomerulus.

Assessment of the blue strip width

Average blue strip width in N-fetuses and DS-fetuses was respectively 28.544 ± 3.350 µm and 32.285 ± 4.614 µm. There was not a statistically significant effect between the two groups, $t(23) = 2.351$, p = 0.0552 (**Fig. 5B**) (**Tab. 2**) (95% CI [0.45, 7.034]). **Fig. 6** reports a representative view of differences in the blue strip width between DS-fetuses and N-fetuses.

Discussion

In recent years, a strong link is emerging between our fetal life and kidney diseases occurring in adulthood. This evidence strongly reinforces the theory of the developmental origins of adult kidney diseases [26]. Developmental (or fetal) renal programming [27] is rapidly becoming accepted as a key factor in the etiology of adult kidney diseases, including hypertension, nephrotic syndrome, and chronic kidney injury [28]. Renal diseases have frequently been reported in subjects with DS [29, 30]. With increased survival, it appears that a growing number of DS patients presents with CRF [31]; but when do renal lesions originate? Are they present at birth or do they develop later in life? This study was aimed at giving an answer to these questions.

Our results about renal changes during fetal life showed relevant morphological differences in the renal analyzed structures between DS-fetuses and N-fetuses. In particular, subjects with DS showed glomeruli with larger dimension, as compared to normal subjects. Moreover, glomeruli belonging to DS fetuses were characterized by anomalies in shape. Bigger glomeruli have been previously reported

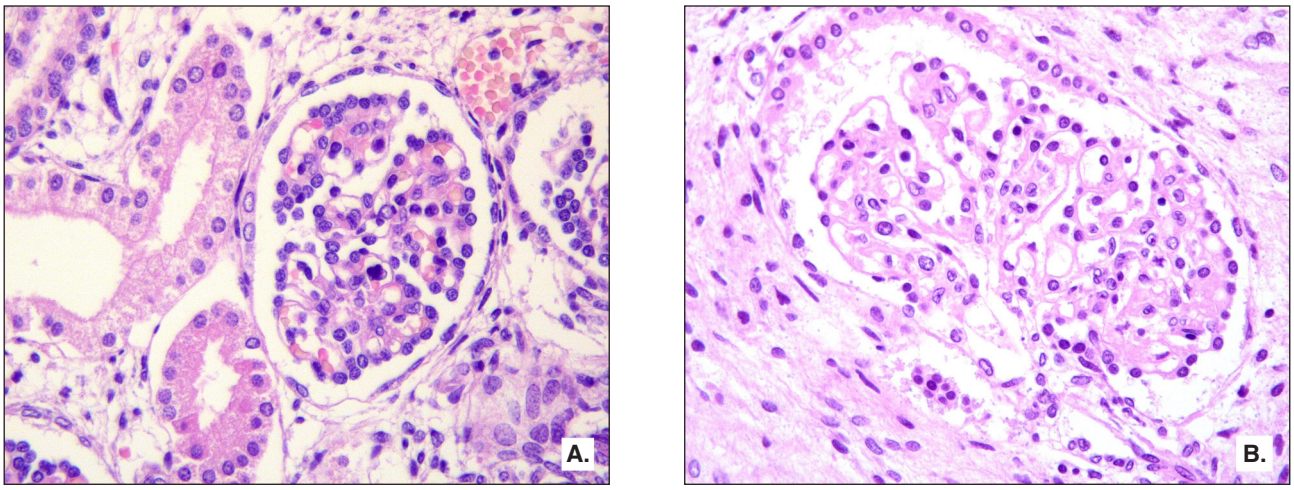

Figure 4. An example of a glomerulus of a normal fetus (**A**) and fetus with Down syndrome (DS) (**B**).

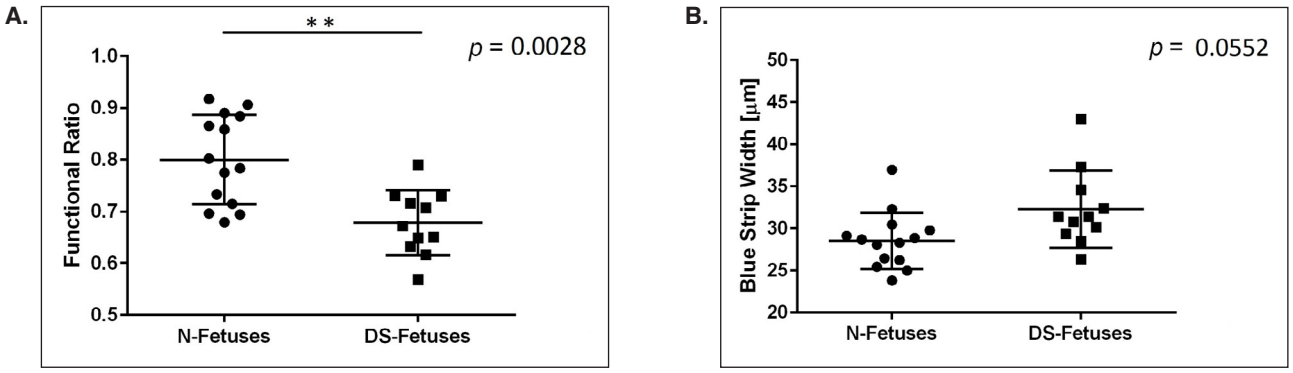

Figure 5. Assessment of (**A**) functional ratio (FR) and (**B**) blue strip width in normal fetuses and fetuses with Down syndrome (DS). Bars represent mean ± SD.

N-fetuses: fetuses with normal karyotype; DS-fetuses: fetuses with Down syndrome.

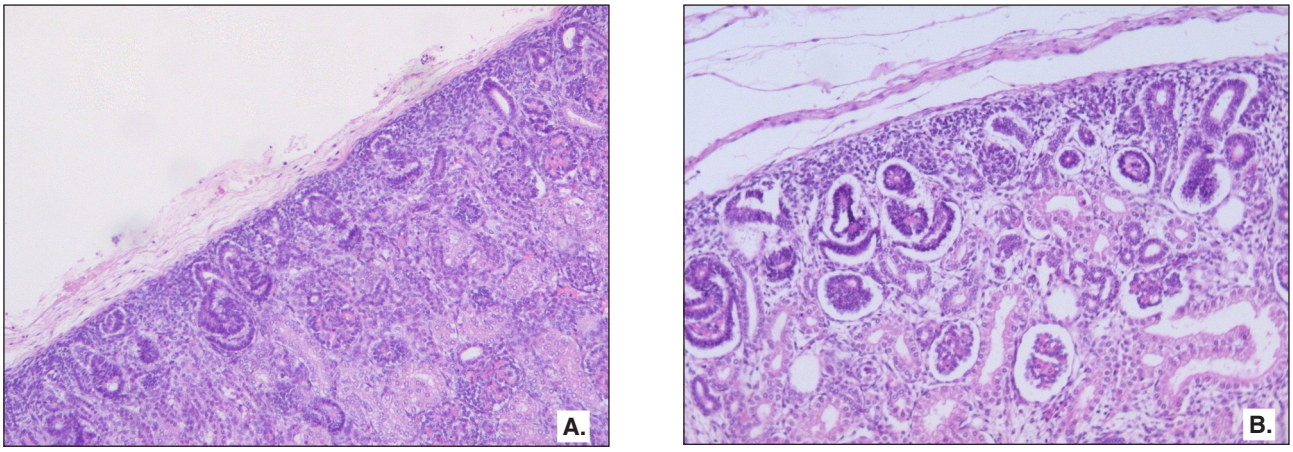

Figure 6. An example of blue strip of a normal fetus (**A**) and of a fetus with Down syndrome (DS) (**B**).

in preterm infants [25]; the production of these larger glomerular structures being probably caused by hypertrophy due to multiple possible causes. However, whether the glomerular enlargement observed in the preterm kidneys is indicative of glomerular hyperfiltration remains to be clarified. We may speculate that immature glomeruli of the preterm kidney may not be able to deal with the

functional demands occurring after birth, resulting in a compensatory glomerular hypertrophy. In our study, glomerular size appeared higher in DS patients than in control fetuses. This finding suggests that DS might represent an additional factor favoring hypertrophy and enlargement of the developing kidney. Moreover, glomerular hypertrophy and hyperfiltration might represent the linkage between DS and the susceptibility of the subjects with DS to develop chronic kidney disease later in life. In fact, in previous studies hyperfiltration and glomerular hypertrophy have been shown to lead to glomerular injury and later nephron loss; these detrimental changes are strongly linked to the development of long-term renal disease [32, 33].

Another hypothesis to explain the larger glomerular size in DS subjects regards a possible anomaly in glomerular development: our findings suggest the hypothesis of a glomerular fusion, due to the confluence of 2 or 3 vascular tufts (**Fig. 7**) [34].

Importantly, our results show a high number of abnormal glomeruli in DS fetuses. Abnormal glomeruli have been investigated in preterm infants in previous studies [35, 36]. These reports suggest that these abnormalities should be related to differences in the postnatal clinical course of the neonates and in particular to medication exposure in the perinatal period.

Our data indicate that other congenital factors, including DS, may be associated with impaired nephrogenesis and abnormal glomerular development.

Another interesting finding emerging from our work is the increased ratio between the FGA and the TGA, that we have defined FR. Analyses concerning the FR showed that the ratio between internal and external glomerular areas is significantly higher in N-fetuses as compared to DS-fetuses. This finding was associated with a greater glomerular Bowman's space in fetuses with DS as compared with normal fetuses.

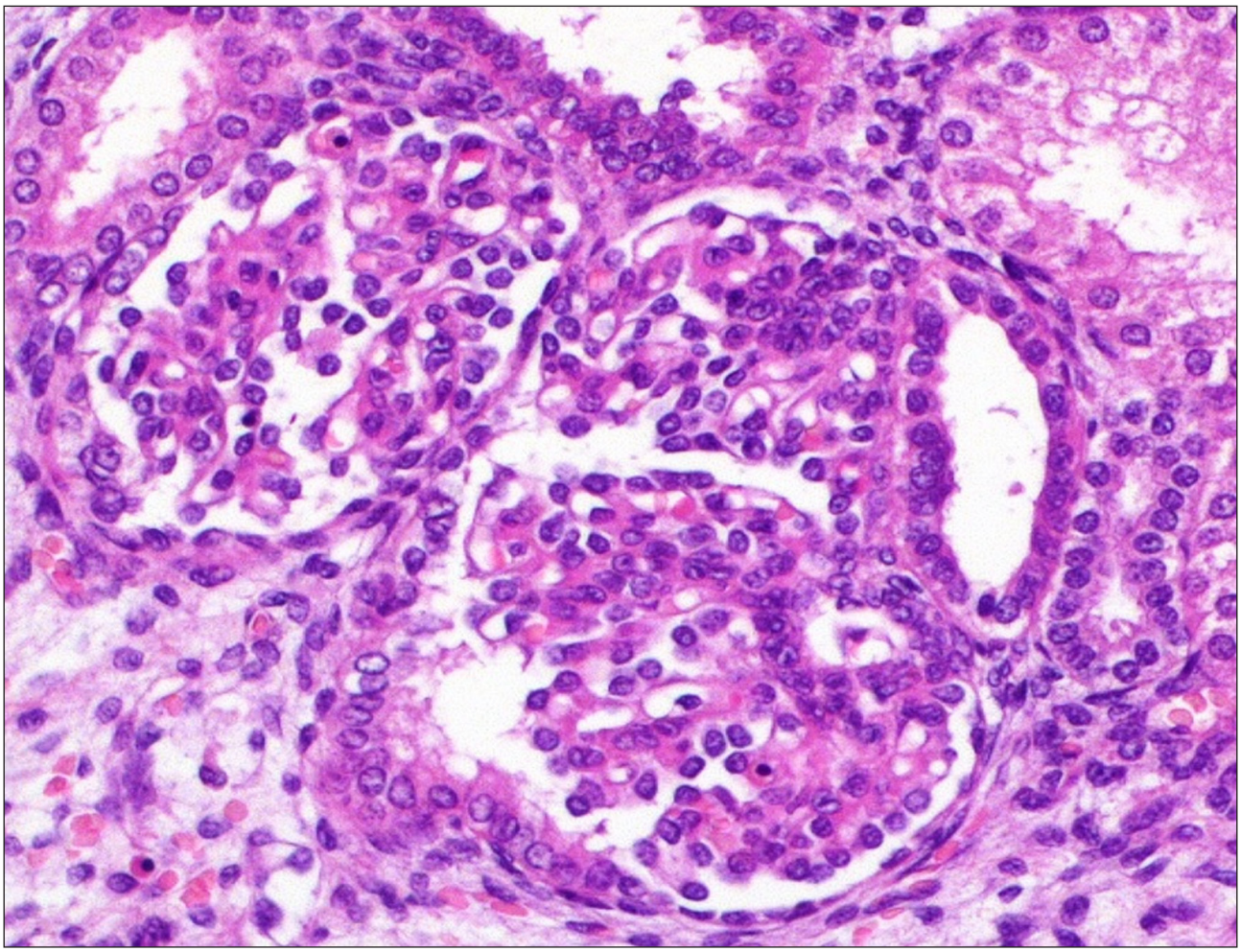

Figure 7. Confluence of glomerular tufts in a fetus with Down syndrome (DS).

An enlarged Bowman's space and shrunken glomerular tuft have been correlated with a functional scarcity of the glomeruli [25]. Kidneys with a large number of abnormal glomeruli are liable to undergo a severe deficit of functional nephrons. This is strongly linked to an increased susceptibility to renal disease in adulthood [37].

The analyses regarding the nephrogenic zone, and in particular the blue strip width, although not significant, have shown a greater width in DS-fetuses as compared with N-fetuses. Sutherland et al. [25] showed a significant reduction in the nephrogenic zone width in the kidney of preterm infants suggesting that this finding might be related to early cessation of nephrogenesis in the postnatal environment and/or accelerated maturation of glomeruli. According to this work, we may speculate that the reason of the expansion of the nephrogenic zone in fetuses with DS could be linked to a series of compensatory renal mechanisms [38] due to the glomerular changes (hypertrophy and malformation) here reported in DS fetuses.

However, caution should be taken in interpreting our results. Inaccuracies may occur in the estimation of glomerular area and blue strip width, due to the approximation of both bounding box method and getline approach [39]. Furthermore, it is important to emphasize that the process of fixation may be responsible for the presence of artifacts [40, 41].

Conclusions

In conclusion, this study comprehensively examines nephrogenesis in the kidney of DS fetuses, and their morphological renal differences as compared to healthy subjects. We found an increased nephrogenic zone width, which suggests delayed renal maturation. Of concern, there was an increase in glomerular area and several glomeruli were morphologically abnormal. Together, these harmful changes in the glomeruli may result in a nephron deficit. These findings, therefore, have significant implications for renal health of subjects with DS.

Further analyses are needed in order to perform a particular monitoring or the identification of specific biomarker to better understand how to intervene to reduce renal diseases and CRF in adulthood.

Abbreviations

CRF: chronic renal failure
DS: Down syndrome
FDR: false discovery rate
FGA: functional glomerular area
FR: functional ratio
TGA: total glomerular area

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Declaration of interest

The Authors declare that they have no competing interests.

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