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Tessellation Analysis of Glomerular Spatial Arrangement in Mice with Heritable Renal Hypoplasia

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Abstract

Renal hypoplasia results from an insufficient kidney volume caused, in part, by a deficient number of glomeruli. The purpose of this study was to apply tessellation analysis to determine whether glomerular point patterns differed between adult normal (WT) and mutant (*Br*) mice with heritable renal hypoplasia and to delineate a spatial distribution accounting for the observed patterns. Kidneys from adult WT and *Br* mice were collected, processed with routine light histology and representative transverse sections were photographed. Cortical area and perimeter were calculated from traced tissue contours and glomeruli were identified and digitized. Voronoi tessellations were constructed and average parameters for Voronoi polygon number, area, perimeter and edge counts as well as spatial metrics comprising nearest neighbor and centroidal distances were calculated and compared. Point distributions were simulated by randomizing glomerular coordinates from each section and plotting the new points utilizing uniform random, Gaussian random, or isotropic functions. Average nearest neighbor distances were generated for each specimen and ranked with respect to corresponding values generated from 1000 iterations for each simulated set. Results showed that WT and *Br* were significantly different for each parameter suggesting that WT kidneys possessed more glomeruli, but these were less clustered compared to *Br*. Simulations suggested that WT and *Br* demonstrated similar, but not identical, underlying glomerular spatial distributions. Defective gene expression in *Br* is important for determining glomerular number and the defective pattern likely results from a heterochronic disturbance consisting of a truncated growth trajectory during embryonic kidney development.

Keywords

Voronoi polygon; glomerulus; *Six2*; *Br* mouse

INTRODUCTION

Morphogenesis of the mammalian kidney is regulated through complex signaling interactions between the ureteric bud and the surrounding metanephric mesenchyme (Dressler, 2006). Mesenchymal and ureteric bud epithelial cells interact resulting in continuous branching of the presumptive collecting duct system as an ever increasing number of glomeruli differentiate and populate the nephrogenic zone of the renal cortex (Saxen, 1987; Herzlinger et al., 1992). Arborization of the ureteric bud occurs through iterative terminal bifurcation events resulting in clusters of nephrons arising from single epithelial tubules (Saxen 1987). Glomeruli become spatially arranged as each emerging terminal projects in close proximity to bilateral counterparts resulting in clusters of nephrons

separated by renal stroma (Cullen-McEwen et al., 2005). Closed lateral branching likely contributes to the glomerular fraction of the kidney by increasing nephron number between terminal nephrons (Oliver 1968; Davies and Bard, 1998; Srinivas et al., 1999). Yet, mechanisms by which nephron differentiation is spatially regulated so that sufficient space intervenes between glomeruli to accommodate tubule elements and additional glomeruli remain unknown.

Proliferation of the renal progenitor cell population and its recruitment to form nephrons relies on numerous signaling molecules and transcription factors (Nishinakamura 2008). These factors promote and maintain the repetitive terminal bifurcation process that results in several thousands of nephrons in the mouse and hundreds of thousands in the human kidney (Costantini, 2006). Spatial regulators are assumed to be present to achieve a sufficient number of cortical glomeruli and tubules ensuring proper water conservation and urine concentrating functions of the kidney (Kriz and LeHir, 2005). Thus, spatial arrangement of an essential number of nephrons becomes a critical achievement for the organism during its embryogenesis.

The *sin oculis* (*Six*) family of transcription factors is associated with mesenchymal differentiation during embryogenesis and is expressed in a number of diverse tissues (Kawakami et al., 2000). *Six2* is one family member that is expressed in the developing mouse kidney throughout the embryonic period, but is particularly prevalent in the metanephric blastema around gestational day 11 (Oliver et al., 1995; Xu et al., 1999; Xu et al., 2003; Ribes et al., 2003; Brodbeck and Englert, 2004). Expression remains high for the next few gestational days but then diminishes with increasing age and finally disappears in the neonatal kidney. Current data from a *Six2* knockout mouse model suggests that this transcription factor is required to maintain the undifferentiated progenitor cell population in the metanephric mesenchyme by opposing inductive and maturation signals from the ureteric epithelial cell population (Self et al., 2006). In the absence of *Six2*, apoptosis of the metanephric mesenchyme occurs, reducing the size of the undifferentiated stem cell pool and causing a decrease in the overall nephron number (Self et al., 2006). Thus, *Six2* appears critical for the establishment of the proper number of renal glomeruli.

Another *Six2* deficient mouse model, called *Br*, arose as a result of a radiation-induced mutation (Searle, 1966). Mice on a C3H/He x H/101 (3H1) background that carry the *Br* mutation are characterized by hypoplastic kidneys inherited as an autosomal semidominant lethal trait (Ma and Lozanoff, 1993; McBratney et al. 2003). The mutant mice display haploinsufficient expression of *Six2* during development with the homozygous mutant mouse lacking *Six2* expression in the developing kidney while the heterozygous condition displays approximately half the amount (Fogelgren et al., 2008). Heterozygous *Br* mice survive to adulthood and reproduce; however, renal function is compromised (Fogelgren et al., 2009). Compared with wild type (WT) littermates, *Br* heterozygous mice maintain significantly higher blood pressures, plasma creatinine and osmolality levels, lower urine creatinine concentrations, lower urine osmolalities, and increased urinary ET-1 values. Physiological features are consistent with a significantly reduced number of glomeruli present in *Br/+* mice compared to WT littermates based on stereological analysis (Fogelgren et al., 2009). These findings also are consistent with the *Six2* knockout data since a reduced number of nephrons could be expected, even though a heterozygous and postnatal phenotype was not described (Self, 2006). Thus, deficiencies in *Six2* expression during embryogenesis could be expected to affect glomerular number and possibly spatial arrangements in the adult.

Planar point distribution analyses have proven useful to examine cellular arrangements in tissue sections (reviewed by Mattfeldt, 2005; Armstrong, 2006). Nearest neighbor distance

(NND) derived from Voronoi tessellations involve one such approach for morphometric characterizations of cellular spatial arrangements in various tissues (Duyckaerts et al., 1994; Duyckaerts and Godefroy 2000; Palanza et al., 2005). We hypothesize that adult mice deficient in *Six2* expression during embryogenesis show a decreased number and altered spatial arrangement of glomeruli. Using a Voronoi tessellation approach and focusing on NND characteristics, we demonstrate that adult WT and *Br* mutant mice display a glomerular spatial arrangement most closely fitting a Gaussian distribution, suggesting that the underlying morphogenetic pattern is consistent between groups. However, mutant mice yield a reduced number of glomeruli that are more clustered compared to WT. Reduced embryonic expression of *Six2* could be related to a heterochronic growth disturbance truncating the renal growth trajectory during prenatal development resulting in few glomeruli and renal hypoplasia.

MATERIALS AND METHODS

Animals

Inbred C3H/He x 101/H (3H1) mice that were either wild type (WT) or heterozygous *Br*+/ (*Br*) were utilized in this analysis. All experiments were approved by IACUC, conducted following state and federal guidelines, and performed in the Animal Laboratory Service Facility at the University of Hawaii. Adult mice were housed under standard conditions with a 12-hour light cycle and were supplied with tap water and food pellets (Agway Prolab Feed, Waverly, N.Y., USA) *ad libitum*.

Histomorphometric analysis of adult kidneys

Adult 3H1 WT and *Br* mice were selected between 12 and 24 weeks of age. Animals were subjected to an overdose of isoflurane, expired, and body weights were immediately recorded. The abdomen was excised, the right kidney (10 WT, 10 *Br*) was removed, blotted dry, weighed and immediately immersion fixed in Histochoice overnight at 4° C. Following this initial fixation, the kidney was placed in increasing concentrations of ethanol and stored. The left kidney was also removed and blotted dry, a wet weight was taken and then dried for at least 48 hours (57°C) and subsequently a dry weight was recorded. Right kidney specimens were embedded in paraffin blocks, exhaustively sectioned in a transverse plane (10 µm), and stained with hematoxylin and eosin (H&E). The sections were viewed using an Olympus microscope (BX41). Images of the kidney were photographed (20x) and saved for subsequent analysis. Tissue shrinkage was assumed to remain consistent between all samples and a correction factor was not applied.

Tessellation and polygon analysis—Sampling of sections for analysis consisted of determining the center section (total number of sections/2). From this central section, the middle 20% of the kidney was calculated (10% above and 10% below this central section) and four equally spaced sections were determined that spanned this region. Thus, a total of 80 sections were analyzed for both groups. All sections were separated by at least 80 µm since this is the average diameter of glomeruli in 3H1 WT mice (Fogelgren et al., 2009), ensuring that no glomerulus was analyzed twice. Each captured image was analyzed with SURFtess (Surface Tessellation Software, version 1.0, www.akuaware.com). Once loaded, the region of interest (ROI) was defined as the cortex bounded externally by the outer edge of the kidney and internally by the corticomedullary junction. This junction was identified where the arcuate arteries took origin as well as the transition from longitudinally oriented collecting ducts and loops of Henle to horizontally oriented tubules. The ROI was manually digitized and area and perimeters were calculated. Glomeruli were subsequently identified and marked at the center using a selection tool.

Voronoi tessellations were generated following (Du et al., 1999). Briefly, for any point (node) z_i on plane N , there exists a set of locations, V_i , that are closer to specified data points z_i than any other set of locations on N . Thus, the area V_i is the Voronoi polygon. Parameters can be established to describe the pattern of polygons comprising the tessellations formed by the data points, z_i (Upton and Fingleton, 1985). Parameters included: number of Voronoi polygons reflecting the number of digitized (glomerular) points; Voronoi polygon area, perimeter, and edge count; and glomerular point metrics including nearest neighbor distance (NND) calculated as the minimum Euclidean distance from one point to its single closest neighbor. An average centroidal distance with corresponding standard deviation was also computed. This value was determined by first calculating the center of mass (c) for each polygon following Bourke (1988)

$$c_{ij} = \frac{1}{6A} \sum_{i,j=0}^{N-1} (x_{ij} + x_{i+1,j+1})(y_{ij} + y_{i+1,j+1}),$$

where A = area of corresponding polygon, N = total number of vertices of the polygon, and therefore $N-1$ = number of line segments of the polygon. A centroidal distance (CD) was determined by calculating the Euclidean distance between the digitized glomerular location and the center of mass for its corresponding polygon. This measure reflects the degree of isotropy occurring within a sample with a smaller value reflecting less point clustering.

Validation of Data Collection Method and Glomerular Spatial Analysis—A test of reliability was undertaken to assess precision of the glomerular digitization process. Three randomly selected WT and *Br* kidney specimens were identified and sections were digitized for each specimen in triplicate. Parameters from polygon patterns were calculated and these values were transferred to Grad Pad (Prism) and Student t tests were conducted to determine whether significant differences existed within a specimen. Using these same values, an intra-group comparison was conducted to determine whether significant differences exists among individuals within a group using the Student t test.

Following assessment of digitization, additional tests were conducted using hypothetical point patterns to assess the usefulness of the selected parameters. For this purpose, 1000 points were placed within an arbitrary cortical region using a uniform random (UR, C++ *rand* function), Gaussian random (GR, www.taygeta.com/random/gaussian.html) or isotropic (IT, Bourke, 1988) functions. Descriptive statistics for each parameter, measured in pixels, were calculated and compared qualitatively to determine whether expected results could be achieved.

Descriptive statistics were computed for each section from each specimen in both groups. Average values and standard deviations were compared using Graph Pad. Subsequently, the three spatial functions (UR, GR, IT) were applied to the digitized glomerular coordinate positions for each section from every specimen in both groups. A buffer zone of 80 μm was applied to each point location to avoid overlap consistent with the average diameter of a typical mouse glomerulus. The observed glomeruli point patterns for each section were sequentially subjected to a UR, GR, or IT transformation and plotted within the bounded cortical region of the corresponding section. Each section was subjected to 1000 randomizations for each function and average NNDs were calculated for each iteration. The observed NND for each section was ranked with respect to the corresponding randomized 1000 average NNDs. The observed value was considered to achieve significance if it fell below 950 of the average NNDs calculated from the randomized sets (lowest 5% of the sample). The number of observed sections achieving this 5% cutoff was divided by the total

number of sections (40) providing a percentage of the sample of observed glomerular spatial patterns that were significantly different from the randomized patterns.

RESULTS

The average age for WT animals used in this study was 165 days (s.d. = 61) while the average age for *Br* animals was 119 days (s.d. = 76) and these values were not significantly different ($p < 0.12$). *Br* mice were not only smaller in absolute body size and kidney weight ($p < 0.001$), but also kidney wet weight ($p < .04$) and dry weight ($p < 0.001$) relative to body weight (Fig. 1), indicating that the mutant *Br* mice displayed renal hypoplasia.

Digitization of glomeruli and subsequent tessellations of representative WT and *Br* kidneys are shown in Figure 2 (A, B). Kidneys were generally too large to photograph in one frame so multiple frames were recorded and tiled together with the SURFtess software. The cortical contour was traced and glomeruli were identified and digitized (Fig. 2, magnified inset). Tessellation edges were depicted as the bisector of the line that connected nearest neighbors with the selected glomerulus. The outer cortical contour established the external polygonal edge for the outermost Voronoi polygons (Fig. 2C, D).

Repeatability values for representative specimens based on triplicate digitization and tessellation processes showed no significant differences for Voronoi polygon area ($p < 0.92$, WT; $p < 0.99$ *Br*), perimeter ($p < 0.97$, WT; $p < 0.98$, *Br*), edge count ($p < 0.91$, WT; $p < 0.96$, *Br*), and NND ($p < 0.97$, WT; $p < 0.98$, *Br*) for within-individual comparisons, indicating that the digitized points shared almost identical locations for triplicates (Fig. 3). Similarly, intra-group comparisons indicated no significant differences for Voronoi polygon area ($p < 0.99$), perimeters ($p < 0.99$), edge count ($p < 0.99$), and NND ($p < 0.97$) (data not shown). Thus, the data collection process was considered highly repeatable and valid.

Simulated spatial patterns based on UR, GR and IT functions are shown in Figure 4 with associated data presented in Table 1. UR and GR patterns showed consistency in parameter values, but generally GR averages and standard deviations were slightly larger. Notably, the standard deviation for GR polygon area was over twice as large as the corresponding UR value. These higher values were due to a GR distribution with a maximum density occurring at the center of the pattern expanding radially. Thus, polygons near the edge of the region displayed much larger areas than more centrally located polygons (compare Fig. 4A, B). The IT distribution displayed homogeneity in spatial arrangement with internal polygons achieving similar geometric features while polygons falling along the boundary, although identical to adjacent neighbors, differed from more centrally located polygons. Data reflected this homogenous pattern showing small or zero standard deviations (table 1). A consistent NND lacking variation occurred with IT spatial arrangement while the average centroidal distance (0 ± 0) indicated that all points lie at the geometric center of the corresponding Voronoi cell.

One representative mid-transverse section from each specimen of each group with corresponding tessellations is shown in Figure 5. Small polygons were positioned along the peripheral border while large polygons spanned the inner cortical regions due to a dearth of juxtaglomerular glomeruli. Generally, more cortical glomeruli occurred in WT animals compared to *Br* specimens since the distinction between renal cortex and medulla was not so evident in the mutant mice (fig 5A,B). Polygon size was variable in WT cortices suggesting that even the cortical nephrons showed clustering (fig 5A,B). The *Br* polygons also demonstrated variable size distribution (Fig. 5C, D).

Quantitative comparisons of tessellation patterns for WT and *Br* specimens are presented in Table 2. The area of the kidney cortex in WT mice (14.12 ± 1.78) was statistically greater (p

< 0.01) compared to the corresponding value of the *Br* mice (5.78 ± 2.02) while the bounding cortical perimeter was also significantly larger in WT (23.86 ± 1.39) compared to *Br* (16.74 ± 3.85). Total Voronoi polygon number for WT specimens (397.6 ± 60.7) exceeded that for *Br* animals (122.4 ± 39.5) by a factor of approximately three, indicating a significantly greater ($p < 0.01$) number of glomeruli in the control. Voronoi polygon area and corresponding perimeters were significantly less ($p < 0.01$) in WT (0.142 ± 0.112 ; 1.513 ± 0.554) compared to *Br* (0.188 ± 0.141 ; 1.772 ± 0.637) even though the WT cortex was larger. The large number of cortical glomeruli in WT specimens thus reflected a large polygon number but with smaller average size. Similarly, the number of polygon edges was significantly different between groups (WT 5.341 ± 1.196 ; *Br* 4.217 ± 1.301 , $p < 0.01$) indicating a large number of smaller polygons. The nearest neighbor distance (NND) was significantly less ($p < 0.01$) for WT (0.180 ± 0.089) compared to *Br* mice (0.222 ± 0.131) indicating that the greater number of glomeruli were positioned more closely together on average than in the *Br* mutant mice. CD for WT (0.104 ± 0.078) was significantly less ($p < 0.01$) than *Br* (0.120 ± 0.082), reflecting less distance between the digitized points in WT and the geometric centers of the corresponding polygons.

Results from the randomization procedure are listed in Table 3 with representative observed and randomized WT spatial plots shown in Figure 6. A WT cortex map showed most glomeruli positioned around the outer border of the kidney with only a few juxtamedullary glomeruli. Some glomeruli appeared to aggregate in clusters while other regions of the cortex were sparsely populated. UR (Fig. 6B) and GR (Fig. 6C) point patterns showed similar arrangements with some glomeruli occurring in clusters while intervening regions showed sparse point populations. The IT map (Fig. 6D) displayed homogeneity of points throughout the section and bore little similarity to the observed pattern. Numeric results indicated that average NNDs from the observed WT group were significantly different from corresponding NNDs generated from the IT patterns for 100% of the sample compared to 55% of the sample from the UR generated pattern, but only 17.5% of the sample generated using the GR function (table 3). The *Br* sample showed the same trend, but with a slightly lower number of samples achieving significance (38% for IT, 27.5% for UR, 5% for GR). Thus, the observed pattern was most similar to the spatial patterns as measured by average NNDs generated by the GR function and least similar to that calculated with the IT function, with the UR-generated pattern falling between.

DISCUSSION

Analysis of spatial point patterns can provide useful information concerning cytoarchitecture resulting from abnormal developmental pathways and pathological changes (Armstrong 2006). Although linear measurements with statistical testing remains the standard approach for morphometric analysis, new methods are being developed and applied for quantifying and comparing spatial relationships and distributions of cells (Duyckaerts and Godefroy, 2000; Cullen-McEwen et al., 2002). Spatial analysis using Voronoi tessellation represents one such approach that is used to examine the cellular positions within a biological tissue (Chang et al., 2007). Following this method, a digitized structure has a set of points closer to it than any other cell within a tissue. A dividing line, defined as a set of points in a tissue plane equidistant from the two contiguous cells, is identified. Spatial relationships are quantitatively expressed by the characteristics of the corresponding Voronoi polygons and summarized to describe and compare morphological features. An additional advantage is that underlying spatial relationships can be examined using simulation techniques as shown in this analysis.

All analyses were performed on a standard 32-bit Dell computer desktop running the Vista operating system. The data collection technique consisted of digitizing glomeruli and then

subjecting the pattern to Voronoi tessellation. Qualitative observations demonstrated that a digitized point could be placed in the center of a glomerulus while the repeatability analysis indicated that tessellations were generated with a high degree of precision. Data extraction from the Voronoi tessellation as well as average parameter computation and statistical comparison were virtually instantaneous. However, the generation of these tessellations, a key component of the randomization process for examining the underlying distribution, was more time consuming. Nonetheless, these procedures can be performed on a laptop or benchtop computer with no specialized computational requirements.

The CD measure established in this study is a novel metric aimed at achieving an equidistant point distribution of glomeruli for the purpose of simulation. As an individual metric descriptor, this parameter reflected the distance between the digitized glomeruli and its hypothetical isotropic position. Thus, an increasing value indicates divergence from isotropy while a smaller value indicates convergence to isotropy. This interpretation was substantiated by an average CD of 0 ± 0 for the isotropic point simulation. CD was also used to achieve an isotropic graphical relationship. The centers of Voronoi polygons are determined and the respective digitized point location is moved to the new center. Successive iterations of the original digitized points provide an isotropic arrangement of all points and the simulated pattern can be compared with the actual point distribution. The isotropic arrangement generally required 10 iterations to achieve positional stasis. Simulating the isotropic pattern required considerably more CPU time than either the uniform or Gaussian random patterns due to the reiteration requirement for each section. Future work will be aimed at minimizing the CD computational routine to reduce the simulation time requirement.

The *Br* mouse displays a lack of expression of the transcription factor *Six2* during embryogenesis (Fogelgren et al., 2008; Fogelgren et al., 2009). Embryonic mice display normal ureteric bud differentiation and initial budding upon contact with the metanephric mesenchyme at gestational day 11.5. However, the proliferative zone remains dispersed, lacking cortical condensations of differentiating nephric tissues subsequently at gestational day 12.0 (Lozanoff et al., 2001). This pattern is consistent with the *Six2* knockout mouse model. Self et al., (2006) proposed that *Six2* activity is required to maintain the proliferative zone mesenchyme in an undifferentiated state. In its absence, a reduced number of glomeruli could be expected, followed by renal hypoplasia resulting from a reduction in overall kidney tissue. Gross anatomical data presented here shows that *Br* mice are significantly smaller based on body weights, but absolute size of the kidneys as well as wet and dry weight/body weight ratios indicate relatively smaller kidney size in the mutant mice consistent with renal hypoplasia as previously reported (Ma and Lozanoff, 1993; Fogelgren et al., 2009).

All parameters derived from the Voronoi tessellations indicated that WT and *Br* mice were significantly different from one another. Data generally reflected a WT kidney that was larger (larger cortical area and perimeter) with more glomeruli (number of Voronoi polygons with more polygon edges), but more densely packed (smaller polygon areas, perimeters and NNDs). Both groups showed similar spatial distribution patterns with differential clustering of glomeruli as opposed to an isotropic pattern. The smaller average CD value for the WT sample suggested that the *Br* mutant mice were less isotropic, and thus more clustered. Visual inspection of representative sections from each kidney confirmed this finding since *Br* cortex contours were consistently smaller with a fewer number of glomeruli, but with more relative intervening tissue between the clusters. Thus, the *Br* mutation appeared to affect the spatial relationships of the glomeruli, but not necessarily the underlying morphogenetic pathway directing glomerular differentiation and initial growth trajectory.

The GR function places points radially from a centroid. Its corresponding simulated pattern generated from an arbitrary sample of 1000 points appears qualitatively different compared to both UR- and IT-simulated patterns. Simulated points fall within a lattice arrangement for UR and IT functions which accounts for this observable difference. Cebian et al., (2002) reported that the renal cortex expands along a circumferential axis as the kidney grows prenatally and glomerular number accounts for a proportionally larger volume than ureteric tips. One would expect a spatial distribution for renal glomeruli most closely reflecting a GR distribution since the differentiation process initiates from a point and radiates outward. Normal kidney cortical size is heavily dependent on nephron number as this process continues. Results from the simulation analysis indicated that both the WT and *Br* samples conformed most closely to the GR distribution while least similar to the isotropic distribution, even though WT was more extreme than the *Br* group in its expression. The murine *Six2* knockout displays initial ureteric bud sprouting, but subsequent ureteric tip branching terminates prematurely (Self et al., 2006). Similarly, *Br* mice display normal ureteric bud differentiation, but subsequent nephrogenic zone condensations are lacking (Lozanoff et al., 2001). Absence of *Six2* expression in *Br* is associated with a reduced nephron number and circumferential growth pattern that could result in a heterochronic growth trajectory disturbance where renal differentiation is initiated normally, but truncated immediately afterward.

The tessellation analysis presented here utilized a planar system of points sampled across the longitudinal axis of the kidney rather than sampling from true three dimensional sample space. This may account for a difference between previous results reported by Fogelgren et al., (2009) who used the Disector method to show an approximate 88% reduction nephron number in adult *Br/+* heterozygotes relative to wild type mice compared an approximate reduction of 70% seen in this study. Renal histomorphometric and physiology parameters among *Br/+* animals show considerably more variation than controls (Fogelgren et al. 2009). Thus, the 18% difference in nephron number might simply reflect greater biological variability within the mutant mouse strain. In both studies, nephron number is significantly reduced. Future work will extend this tessellation method to the three dimensional case for comparison with other spatial assessment morphometric techniques.

The *Six2* gene and associated regulatory elements are not affected in the *Br* mutation (Fogelgren et al., 2009). It is hypothesized that an unknown unit, possibly a transcription enhancer, is defective in *Br* resulting in the phenotypic defects. This could account for the haploinsufficient kidney phenotype. The approach established here to compare point patterns of glomeruli as well as underlying spatial distributions provides an approach to analyze not only an adult spatial pattern established by a developmental defect, but also testing hypotheses concerning the underlying growth trajectory. More generally, this method could prove useful for quantifying most phenotypic features as well as testing hypotheses regarding underlying morphogenetic growth trajectories in mutant mice generated through gene inactivation methods.

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Figure 1. Comparison of average body weight, total kidney weight, percent kidney wet weight to body weight ratio, and percent kidney dry weight to body weight ratio between WT and *Br* mice.

Figure 2. Representative cross-sections of WT (A) and *Br* (B) kidneys with digitized glomeruli (inset) and corresponding tessellations (C,D). Bar = 5.0 mm.

Figure 3. Average Voronoi polygon edge count, area, perimeter and centroidal distance for WT and *Br* triplicates showing no significant differences between values indicating a high degree of repeatability for the digitization process.

Figure 4. Voronoi tessellations for 1000 points based on a uniform random (A), Gaussian random (B) or isotropic (C) function.

Figure 5.

Representative transverse sections from individual WT (A, B) or *Br* (C, D) specimens used in this study. Bar = 5.0 mm

Figure 6. Representative WT section showing the actual distribution of glomeruli (A) and simulated patterns using the same number of digitized points based on a uniform random (B), Gaussian random (C), or isotropic (D) function.

Table 1

Descriptive Statistics derived from Uniform Random (UR), Gaussian Random (GR) and Isotropic (IT) point spatial patterns.

	UR	GR	IT
BR Area	172617	172617	172617
BR Perimeter	1684	1684	1684
Voronoi Polygon #	1000	1000	1000
Polygon Area	172.62 ± 91.90	172.68 ± 206.04	10146.10 ± 519.22
Polygon Perimeter	52.97 ± 12.94	49.59 ± 24.22	379.27 ± 19.69
Polygon Edge Count	5.89 ± 1.35	5.94 ± 1.38	5.88 ± 0.42
NND	6.79 ± 3.42	6.08 ± 4.37	100 ± 0
CD	2.96 ± 1.61	3.05 ± 2.33	0 ± 0

Values represent average ± standard deviation

All measures in pixels

BR: Bounding Region

NND: Nearest Neighbor Distance

CD: Centroidal Distance

Table 2Descriptive statistics derived from WT and *Br/+* glomerular spatial patterns.

	<i>+/+</i>	<i>Br/+</i>	<i>p</i>
Cortical Area (mm^2)	14.12 ± 1.78	5.78 ± 2.02	< 0.01
Cortical Perimeter (mm)	23.86 ± 1.39	16.74 ± 3.85	< 0.01
Voronoi Polygon #*	397.6 ± 60.7	122.4 ± 39.5	< 0.01
Polygon Area (mm^2)	0.142 ± 0.112	0.188 ± 0.141	< 0.01
Polygon Perimeter (mm)	1.513 ± 0.554	1.772 ± 0.637	< 0.01
Polygon Edge Count	5.341 ± 1.196	4.217 ± 1.301	< 0.01
NND (mm)	0.180 ± 0.089	0.222 ± 0.131	< 0.01
CD (mm)	0.104 ± 0.078	0.120 ± 0.082	< 0.01

Values represent average $\pm$ standard deviation*
total number from 4 sections

NND: Nearest Neighbor Distance

CD: Centroidal Distance

Table 3

Number of samples and percentage significantly different ($p < .05$) from the uniform random (UR), Gaussian random (GR) or isotropic (IT) generated spatial patterns based on average NNDs.

	UR	GR	IT
WT	22 (55%)	7 (17.5%)	40 (100%)
<i>Br/+</i>	11 (27.5%)	5 (12.5%)	38 (95%)

Percentage calculated from a total of 40 samples.