Chromosomal Mapping of a Major Quantitative Trait Locus Regulating Compensatory Renal Growth in the Rat

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Abstract. Despite extensive research conducted over the past century, the mechanisms of compensatory renal growth (CRG) remain a mystery. Insight into the mechanisms that regulate CRG might be gained by identifying genetic factors that influence this complex phenotype. In a large set of recombinant inbred strains derived from the spontaneously hypertensive rat and the Brown Norway rat, a genome scan for quantitative trait loci (QTL) that regulate CRG was performed. The CRG score was expressed as a ratio of the weight of the remnant right kidney at 8 wk of age to the weight of the left kidney at 5 wk of age, both adjusted for body weight. QTL mapping was performed using Map Manager QT and the strain distribution patterns of more than 600 genetic markers. It was found that CRG after unilateral nephrectomy is a multifactorially determined trait with a substantial genetic component. The heritability of CRG approached 40%. Genome wide scan analysis revealed significant evidence of linkage to a region of rat chromosome 4 designated Crg1 that accounted for more than 50% of the additive genetic variance of CRG in the recombinant inbred strains. The detection of a major QTL influencing CRG in the rat should provide new opportunities for identifying mechanisms that regulate this historically enigmatic phenomenon and may also have implications for research on the pathogenesis of end-stage kidney disease.

The ability of a solitary kidney to undergo compensatory growth has been recognized since the time of Aristotle (1,2). However, despite extensive research conducted over the past century, the mechanisms of compensatory renal growth (CRG) remain a mystery. Given mounting evidence that nephron mass may be a critical factor in the progression of multiple forms of chronic renal failure (3), the search for factors that regulate CRG has taken on renewed importance. Although kidney weight has been reported to be a highly heritable trait, the ability of genetic factors to modulate the phenomenon of CRG is unknown (4). Insight into the mechanisms that regulate CRG might be gained by identifying quantitative trait loci (QTL) that influence this complex phenotype. It has recently been proposed that hypertrophy may be a prerequisite for progression of renal injury and that attenuation of compensatory growth can limit the otherwise relentless progression of kidney damage that occurs in various forms of chronic renal insufficiency (5,6). Thus, identification of factors that modulate CRG could ultimately have implications for the treatment of chronic renal failure and the prevention of end-stage kidney disease. Accordingly, in a large set of recombinant inbred strains, we determined the influence of genetic factors on the phenomenon of CRG in the rat and sought to map QTL that regulate this complex phenotype.

Materials and Methods

Animals

Genetic studies were performed in 29 recombinant inbred (RI) strains derived from spontaneously hypertensive rats (SHR/Ola, referred to as SHR) and normotensive Brown Norway rats (BN-Lx/Cub, referred to as BN) (7,8). The SHR progenitor strain descends from inbred SHR originally obtained from the National Institutes of Health. The BN progenitor is a BN congenic strain that carries a segment of chromosome 8 from the polydactylous PD/Cub strain (7). All strains have been maintained in Prague by inbreeding for more than 15 yr. The RI strains were derived from (SHR × BN)F2 population: The F2 rats were paired off at random, and each of these F2 pairs was used to generate a new inbred strain by repeated brother × sister mating of the offspring for at least 20 generations. Currently, most of the RI strains reached more than 45 generations of brother-sister inbreeding. Results of DNA fingerprint and PCR microsatellite tests have confirmed that the progenitor and RI strains are highly inbred (7,9).

Experimental Procedures

Five-week-old male rats were weighed and anesthetized with ether, and their left kidneys were surgically removed and weighed. Three weeks later, body weights were recorded and the right kidneys were removed and weighed. The CRG score was expressed as a ratio of the weight of the remnant right kidney at 8 wk of age (KW8) to the weight of the left kidney at 5 wk of age (KW5). To adjust for increases in kidney weight associated with normal development, kidney weights were corrected for body weights using an allometric scaling factor of 0.75 in accordance with the results of previous allometric studies of kidney growth in the rat (10–14). The CRG score for each rat was

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calculated as
\[ \text{CRG} = \frac{\text{KW}_5/\text{BW}_5^{0.75}}{\text{KW}_8/\text{BW}_8^{0.75}}. \]
No adjustments were made for any small systematic differences in weight that may occur between right and left kidneys. The CRG score of a given strain was determined by averaging the CRG results of multiple rats from that strain (SHR, \( n = 9 \); BN, \( n = 10 \); RI strains, \( n = 4 \) to 5 per strain).

**Genetic and Statistical Analyses**

Heritability of CRG was estimated according to the method of Plomin and McClearn using the variances in CRG scores between and within the RI and progenitor strains (15). The calculation of heritability corrects for the doubling effects of inbreeding on additive genetic variance and provides an estimate of the narrow heritability that would be expected in an F2 population derived from the SHR and BN progenitor strains (15). QTL mapping was performed using Map Manager QT (version b28) (16) and the strain distribution patterns of more than 600 genetic markers previously mapped in the RI strains (7). The marker data set covers approximately 1200 centiMorgans of the rat genome and has proved effective in genome scanning for QTL regulating a variety of complex traits including BP, insulin resistance, and lipid levels (8,17,18). Map Manager QT was used to test for single locus associations by regression analysis and the significance of each potential association measured using the likelihood ratio statistics (LRS) of Haley and Knott (19). The interval regression method of Map Manager QT was used to test for QTL within marker intervals. The significance threshold for the genome wide scan was empirically determined by the Map Manager QT permutation test, using the informative markers and 1000 permuted data sets as recommended by Doerge and Churchill (20). Significant linkage was defined in accordance with the guidelines of Lander and Kruglyak as statistical evidence occurring by chance in the genome scan with a probability of 5% or less (21). Based on these criteria and the results of the permutation test, an LRS = 15.4 (corresponding to a lod score of 3.3) was established as the threshold for significant linkage in the RI strain data set. This empirically determined threshold is nearly identical to the significance threshold recently recommended by Belknap and colleagues for genome wide scans in RI strains (22). One-half of the fraction of variance attributable to each QTL in the RI strains was used to estimate the QTL effect expected in a comparable F2 population derived from the SHR and BN progenitors (to correct for the doubling effect of inbreeding on additive genetic variance) (15,23). The fraction of genetic variance contributed by each QTL was determined by dividing the estimated QTL effect by the heritability.

**Results**

Initial studies in the progenitor strains revealed a significant difference in the compensatory growth of SHR and BN kidneys following unilateral nephrectomy; the mean CRG score of the SHR progenitor strain, \( 1.42 \pm 0.05 \), was significantly greater than that of the BN progenitor strain, \( 1.28 \pm 0.03 \) (\( P = 0.025 \) by \( t \) test).

In the RI strains derived from the SHR and BN progenitors, the distribution of CRG scores was continuous, suggesting a polygenic mode of inheritance for the trait (Figure 1). The BN progenitor strain exhibited the lowest CRG score, whereas the CRG score of the SHR progenitor fell midway within the distribution of CRG scores of the RI strains (Figure 1). The observation of greater CRG scores in certain RI strains than in either progenitor is consistent with multifactorial inheritance and suggests the possibility of gene-gene interactions. The SHR progenitor was derived by recurrent selective breeding for

![Figure 1. Distribution of means (± SEM). Compensatory renal growth (CRG) scores in the spontaneously hypertensive (SHR), Brown Norway (BN), and recombinant inbred (RI) strains. □, RI strains that inherited the BN allele; ■, RI strains that inherited the SHR allele of the D4Cebr7s17 marker, which was significantly associated with CRG.](image-url)
hypertension; however, no correlation was observed between BP and CRG in the RI strains.

Based on the variances in CRG scores within and between the RI strains, the heritability of CRG was estimated to be 38%. Because of the absence of heterozygotes in the RI strains, this estimate of heritability reflects additive genetic effects on CRG phenotype. Given the finding of a substantial genetic component to CRG in the SHR-BN model, we scanned for QTTL influencing CRG in the RI strains. Genome scanning of the RI strains revealed significant linkage of the CRG phenotype to the D4Cebr7s17 marker on chromosome 4 (LRS = 15.9, lod score = 3.5, \( P = 6.7 \times 10^{-5} \)) (Table 1); the D4Cebr7s17 marker is closely linked to the Cacna1c (D4Arb4) (24) gene coding for Ca channel, voltage-dependent, L type, alpha 1c subunit. Interval mapping across chromosome 4 revealed a distinct peak in the LRS at D4Cebr7s17 and a sharp drop off in the LRS at the adjacent flanking markers (Figure 2). The narrow QTTL peak reflects the enhanced mapping resolution afforded by the fourfold increase in recombination events provided by RI strains (25). This QTTL region, designated Crg1, accounts for 54% of the additive genetic variance of the CRG phenotype and suggests the existence of a major gene regulating compensatory renal growth in the rat. The mean CRG score of the RI strains that inherited the BN allele for the D4Cebr7s17 marker was greater than the mean CRG score of the RI strains that inherited the SHR allele (Table 1). Because neither the SHR nor BN strain was selectively bred for the CRG phenotype, it is conceivable that variants promoting compensatory renal growth are likely to coexist with those that limit compensatory growth in both progenitor strains. No other chromosome region was identified that showed significant evidence of linkage to the CRG phenotype. However, given the known power limitations of RI strains for detecting QTTL with minor effect sizes, it is possible that QTTL exerting more modest effects on CRG are located in other regions of the genome (25). In this regard, it should be noted that suggestive evidence for linkage was obtained for the D6Mit9 marker on chromosome 6 and the D7Mit8 marker on chromosome 7 (Table 1).

<table>
<thead>
<tr>
<th>Marker</th>
<th>BN Allele</th>
<th>SHR Allele</th>
<th>Lod Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>D4Cebr7s17</td>
<td>1.57 ± 0.04</td>
<td>1.41 ± 0.02</td>
<td>3.5(^b)</td>
</tr>
<tr>
<td>D6Mit9</td>
<td>1.36 ± 0.03</td>
<td>1.53 ± 0.02</td>
<td>2.6</td>
</tr>
<tr>
<td>D7Mit8</td>
<td>1.40 ± 0.02</td>
<td>1.53 ± 0.03</td>
<td>2.4</td>
</tr>
</tbody>
</table>

\(^a\) Means (± SEM) of CRG scores in RI strains that inherited BN and SHR alleles of respective genetic markers. BN, Brown Norway; SHR, spontaneously hypertensive rat; CRG, compensatory renal growth; RI, recombinant inbred.

\(^b\) Statistically significant association as determined empirically by the Map Manager QT permutation test (16).

Discussion

CRG has been observed since ancient times and has received serious scientific investigation for over a century, but little is known about its molecular or cellular basis. Although unilateral nephrectomy in newborn rats can promote renal cell hyperplasia, the phenomenon of compensatory renal growth in older animals predominantly involves glomerular and tubular hypertrophy (1,26). A host of growth factors and other biochemical mediators have been implicated in the phenomenon of renal cell hypertrophy, but their relative roles in CRG are unclear (1,2,26). This is partly related to the difficulty in distinguishing between key mechanistic steps in CRG and the plethora of secondary changes that result from the complex physiologic adjustments occurring after unilateral nephrectomy. The current findings demonstrate that in the SHR-BN model, the heritability of CRG may be sufficient to enable the use of genetic dissection techniques to search for key steps in the process and avoid pursuing phenomena that are simply secondary to biochemical or physiologic perturbations that might accompany CRG.

We found that CRG after unilateral nephrectomy is a multifactorial trait with a substantial genetic component. The heritability of CRG approached 40%. Genome wide scan analysis revealed significant evidence of linkage to a region of rat chromosome 4 designated Crg1 that accounted for more than 50% of the additive genetic variance of CRG in the RI strains. Recently, we have mapped the Cacna1c (D4Arb4) gene coding for the Ca channel, voltage-dependent, L type, alpha 1c subunit (24) close to the peak of the QTTL linkage. The gene product is expressed in kidney proximal tubule epithelial cells (27). Furthermore, intracellular calcium has been shown to play an important role in cellular growth and division (28). In addition, it has been reported that administration of calcium channel blockers significantly attenuates the degree of compensatory renal growth seen in mice (29) and in rats with experimental diabetes (30). Taken together, these observations lead us to suggest the Cacna1c as a positional candidate gene in the CRG process. However, it should be emphasized that this suggestion is largely speculative and that additional functional studies would be necessary to establish the role of Cacna1c in CRG.

Based on conserved linkages between rat chromosome 4 and mouse chromosome 6, other genes can also be regarded as positional candidates for the CRG phenotype. For example, the gene for kidney androgen protein (Kap) maps to this region of mouse chromosome 6, and it is well known that androgens are potent stimulators of renal growth in the intact rat (31). The gene for growth and differentiation factor 3 (Gdf3), a member of the transforming growth factor-\(\beta\) superfamily, also maps to the region of mouse chromosome 6 homologous to Crg1 in the rat. Gdf3 is of particular interest because of recent studies showing that another member of the transforming growth factor-\(\beta\) superfamily, myostatin (formerly known as Gdf8), functions as a negative regulator of skeletal muscle growth (32). Disruption of the myostatin gene causes skeletal muscle hypertrophy, suggesting that it normally inhibits the expansion of muscle mass (32). It is conceivable that the kidney also pro-
duces a substance that restrains its own growth and that after unilateral nephrectomy, decreased circulating levels of such an inhibitor allow for hypertrophy of the remaining kidney. It has recently been proposed that hypertrophy may be a prerequisite for progression of renal injury and that attenuation of compensatory growth can limit the otherwise relentless progression of kidney damage that occurs in various forms of chronic renal insufficiency (6). Thus, identification of mechanisms that limit the extent of renal growth could lead to new opportunities for moderating the decline in renal function that typically occurs in patients with renal insufficiency. Accordingly, the genetic dissection of factors that regulate CRG might ultimately have implications for the treatment of chronic renal failure and the prevention of end-stage kidney disease.

Acknowledgments

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References


Figure 2. Interval mapping of Crg1. Likelihood ratio statistics from the Map Manager QT linkage analysis are plotted across chromosome 4. Estimated distances between markers are in centiMorgans determined with the Haldane map function. The horizontal lines indicate the threshold for significance of the likelihood ratio statistic determined by the Map Manager QT permutation test. To convert likelihood ratio statistics to lod scores, divide by 4.6.