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Newborn and adult recombinant inbred strains: A tool to search for genetic determinants of target organ damage in hypertension

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Newborn and adult recombinant inbred strains: A tool to search for genetic determinants of target organ damage in hypertension. It has been proposed that one of the primary events in the development of essential hypertension is a growth-related process initiated as early as during fetal development. Differences in kidney size have been observed between most rat models of hypertension and their respective controls. In this study, we analyzed relative kidney size (kidney weight/body wt) in a set of rat recombinant inbred strains (RIS) ($N = 27$) and their progenitors, the spontaneously hypertensive rat strain (SHR/Ola) and Brown Norway congenic strain (BN.lx), at two different ages, at birth and at 15 weeks. In the progenitors, the relative kidney weight was higher in the hypertensive than in the normotensive strain of both the newborn ($P < 0.001$) and adult ($P < 0.001$) animals. In the RIS, a significant correlation was found between the newborn and adult relative kidney weight ($r = 0.49, P = 0.01$), indicating that the two phenotypes share some of their genetic determinants. A total genome search of newborn and adult relative kidney weight was performed with a total of 453 genetic markers. These analyses revealed several suggestive quantitative trait loci (QTL), some of which were, indeed, significant for both newborn and adult relative kidney weight (such as, D3Mit9 on rat chromosome 3; $r = -0.50, P < 0.01$; $r = -0.47, P < 0.01$; respectively). Others, such as the locus on rat chromosome 1 (Rt6; $r = -0.43, P < 0.05$), were significant only for the adult relative kidney size. This QTL was found in close proximity to a region previously related to susceptibility to hypertensive renal disease in the fawn-hooded rat and, similarly to that study, its effect was found to be independent of blood pressure. Furthermore, a growth pattern of the kidneys after birth, evaluated as the difference between the newborn and adult relative kidney weight, was also subjected to total genome scan. Several suggestive QTL were identified. One of the most significant loci was found at the D1a marker on rat chromosome 17 ($r = -0.51, P < 0.01$), which was previously related to the determination of adult heart weight in the RIS. In conclusion, the current study demonstrates the usefulness of RIS in studies of hypertension-related phenotypes, some of which are abnormal before the development of high blood pressure. To better understand their role in the pathogenesis of hypertension, studies at different ages are needed, which are uniquely feasible in RIS.

The pathogenesis of essential hypertension has been proposed to have two components: a primary process, which initially raises blood pressure (BP), and an amplifying process, which progressively magnifies this difference throughout life [1]. Traditionally, distinguishing primary (cause) from secondary (consequence) events has been a difficult task, a "which came first, the egg or the chicken" question. Although several approaches to the identification of primary events exist [2, 3], the most straightforward is the genetic one.

To study the genetic basis of complex traits, such as hypertension, co-segregation of blood pressure (BP) and other hypertension-related phenotypes is tested with genotypes at individual loci. At present, these analyses are most commonly carried out in the F_2 generation of a cross between normotensive and hypertensive inbred strains [4]. It has been demonstrated that several hypertension-related phenotypes are abnormal before the development of high blood pressure. Therefore, to better understand the involvement of these phenotypes in the pathogenesis of hypertension, it is important to study them at different time-points during development and progression of the disease. Since the collection of some phenotypes, such as, organ size, usually requires the experimental animal to be sacrificed, they cannot be analyzed at different ages in the F_2 generation. This is because the F_2 generation represents a set of genetically unique animals that cannot be reproduced, and if sacrificed at a certain age, these animals are lost for analysis at a later age. In contrast, in recombinant inbred strains (RIS), which are an inbred replica of F_2 hybrids and as such are genetically reproducible, phenotypes can be collected at different ages and then related to their stable genotypes. At present, only a single set of rat RIS exists. This set has been derived from a reciprocal cross between a spontaneously hypertensive rat (SHR) strain and Brown Norway (BN) congenic strain [5]. Since the current study was aimed at elucidating the genetic basis of kidney growth and its possible relationship to the pathogenesis of hypertension at two different ages, at birth and at 15 weeks, we employed this set of RIS.

METHODS

The rat RIS used in this study consisted of two reciprocal crosses developed in the Biology Department of the Faculty of Medicine, Charles University (BN.lx × SHR/Ola) and the Institute of Physiology, Academy of Sciences, Prague, Czech Republic (SHR/Ola × BN.lx), respectively. The normotensive progenitor was a congenic strain derived from BN that carries a segment of chromosome 8 from the polydactylous PD/Cub strain [6]. The hypertensive progenitor, SHR/Ola, was originally obtained from OLAC (England, UK).

Neonatal body and kidney weights were determined in animals sacrificed within 18 hours after delivery. For each RIS ($N = 27$),

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Table 1. Relative kidney weight and blood pressure in newborn and adult progenitor strains

	Newborn		Adult	
	BN.lx	SHR	BN.lx	SHR
KW/BW *mg/100 g of body wt*	742 ± 18	1128 ± 17[a]	493 ± 6	636 ± 6[a]
SBP *mm Hg*	Not measured		132 ± 3	215 ± 4[a]
MAP *mm Hg*	Not measured		113 ± 3	182 ± 4[a]
DBP *mm Hg*	Not measured		93 ± 3	149 ± 3[a]

Data are shown as means ± SEM. Abbreviations are: BN.lx, normotensive progenitor strains; SHR, spontaneously hypertensive progenitor strain; KW/BW, relative kidney weight; SBP, systolic blood pressure; MAP, mean arterial pressure; DBP, diastolic blood pressure.
[a] $P < 0.001$ vs. BN.lx

an average value was obtained from at least three litters each consisting of six to nine newborns. To avoid a possible confounding effect of litter size on newborn phenotypes, extremely small (<6) and large (>9) litters were excluded [7]. Adult body weight, kidney weight, and blood pressure were determined at the age of 15 weeks. Blood pressure was measured by direct puncture of the carotid artery as described previously [5].

A total genome search for quantitative trait loci (QTL) of newborn and adult kidney weight was carried out with 453 markers [8, 9]. Strain distribution patterns of these markers were obtained from the Ratmap World Wide Web site (http://ratmap.gen.gu.se). QTL were identified by means of Pearson's correlation analysis. The possible confounding effects of significant covariates (for example, systolic blood pressure) on analyzed phenotypes were evaluated by multiple regression analysis.

RESULTS

In progenitors of the RIS, neonatal relative kidney weight was higher in the hypertensive (SHR/Ola) than in the normotensive (BN.lx) strain (Table 1). This was also true for adult relative kidney weight. In the RIS, average newborn kidney weight values were distributed around BN.lx values but did not reach SHR/Ola values (Fig. 1A). In contrast, the distribution of adult kidney weight values was shifted towards values of the SHR/Ola progenitor (Fig. 1B). This suggests that some factors involved in the determination of newborn and adult kidney weight may be distinct. In addition, there was no significant difference in newborn and adult kidney weight between reciprocal crosses (SHR/Ola × BN.lx, 8.68 ± 0.87; BN.lx × SHR/Ola, 8.31 ± 1.08; $P > 0.3$), which indicates that the Y chromosome does not play an important role in the determination of these phenotypes.

To establish whether neonatal kidney hyperplasia is related to adult kidney weight and adult BP, we estimated correlation coefficients between these phenotypes in the RIS. Our calculations showed that newborn kidney weight correlates with adult kidney weight (Fig. 2), but not with adult BP, and that adult BP is negatively associated with the size of the adult kidneys (Fig. 3). These data suggest that newborn and adult kidney weights share some genetic determinants (24%) and that systolic blood pressure (SBP) appears to have a negative impact on adult kidney size, which explains its 17% variance.

To identify the genetic determinants of newborn and adult kidney weight, a total genome search was performed. For both phenotypes, although no QTL were identified that would satisfy

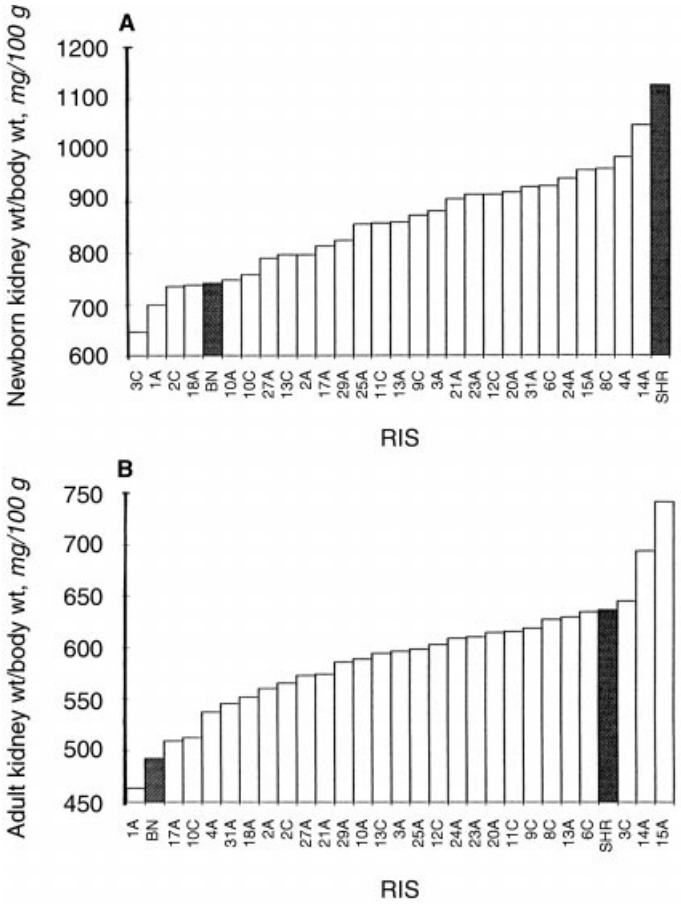

Fig. 1. Distribution of newborn (*A*) and adult (*B*) relative kidney weight values in the recombinant inbred strain (RIS).

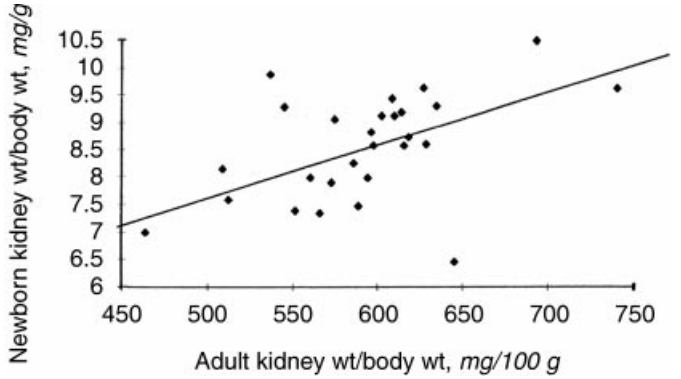

Fig. 2. Correlation between newborn and adult relative kidney weight (KW/BW) in the recombinant inbred strain (RIS). $r = 0.49$; $P = 0.01$.

stringent statistical criteria of the total genome search allowing a claim to linkage were identified [10], several suggestive QTL were demonstrated (Table 2). Some loci appeared to have an effect on both newborn and adult kidney weight (such as, D3Mit9), whereas other loci were significant for only either newborn (for example, B-brown) or adult kidney weight (such as, Rt6 and D8Cebr46 s6). The Rt6 locus on rat chromosome 1 was associated with a negative impact of the SHR allele on adult kidney weight and a

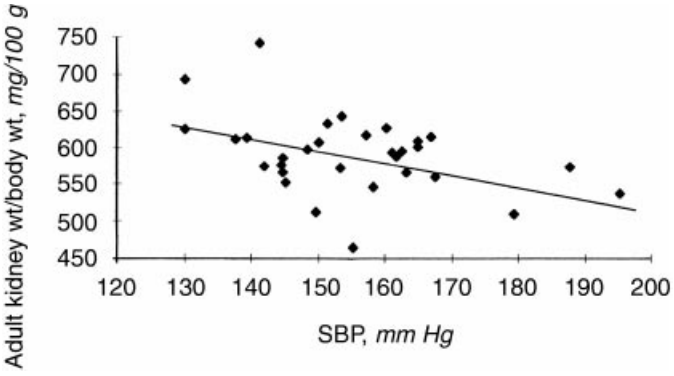

Fig. 3. Correlation between systolic blood pressure (SBP) and adult relative kidney weight (KW/BW) in the recombinant inbred strain (RIS). $r = -0.41$; $P = 0.02$.

Table 2. Total genome search: suggestive quantitative trait loci (QTL) of newborn and adult relative kidney weight (KW/BW)

Chromosome	Marker	*N*	Newborn KW/BW *r*	Adult KW/BW *r*
1	Rt6	25	−0.23	−0.43[a]
3	D3Mit 9	27	−0.50[b]	−0.47[b]
5	D5Cebr312s4	27	+0.20	+0.49[b]
5	B-brown	22	+0.54[b]	+0.10
6	D6Mit5	27	−0.45[a]	−0.28
7	D7Ucsf1	24	+0.58[b]	+0.32
7	D7Mit 10	27	+0.48[a]	+0.30
8	D8Cebr46s6	25	−0.07	−0.45[a]
10	D10Cebp44s3	24	+0.51[a]	+0.25

The results of Pearson's correlation analysis are presented as correlation coefficients (*r*). The statistical significance of these analyses is as follows: [a] $P < 0.05$, [b] $P < 0.01$. *N* indicates the number of RIS available for analysis of an individual marker.

positive impact on SBP. Since in the RIS, there was a negative relationship between adult BP and adult kidney size (Fig. 3), this locus, in fact, could have been one of the genetic determinants of that relationship. However, multiple regression analysis suggested that this was not the case since the effect of the locus on adult kidney weight was independent of SBP ($P > 0.7$). Interestingly, the Rt6 marker lies in close proximity to the Sa gene (Fig. 4), which was originally identified as being expressed at a higher level in SHR kidneys when compared to Wistar-Kyoto (WKY) rats [11] and which has been demonstrated to be a genetic determinant of BP in several rat crosses [12–15]. Recently, QTL of susceptibility to hypertensive renal disease were identified within neighboring regions of the Sa locus and, similarly to our results, the effects of these loci were independent of BP [13].

Furthermore, a decrease of relative kidney weight in adult rats as compared to newborns was observed, which indicates that growth of the kidneys slows after birth relative to the whole body. This growth pattern differed between the progenitors as well as among individual RIS. In the progenitors, the difference between newborn and adult relative kidney weight was greater in SHR/Ola than in BN.lx (Table 1). In the RIS, the values in half of the group were close to those of the BN.lx progenitor, and several animals had values similar to those of SHR/Ola (Fig. 5). When this difference between newborn and adult kidney weights was subjected as a phenotype to total genome scan, several suggestive loci were identified (Table 3). Interestingly, one of the most significant effects was observed at the D1a locus, which has been shown previously to be a significant determinant of heart weight in the RIS [8].

DISCUSSION

It has been suggested that one of the primary defects in the pathogenesis of essential hypertension is a growth-promoting process originating during childhood or, perhaps, as early as fetal development [16]. Based on epidemiological studies, increased systolic blood pressure (SBP) and diastolic blood pressure (DBP) have been related to lower birth weight [16–21]. Furthermore, it has been suggested that impaired fetal growth may have effects on BP regulation. A reduced number of nephrons, frequently found in infants with low birth weight, may compromise the ability of the kidneys to excrete sodium, and this may become particularly significant during salt-overload and/or in the presence of a genetic defect of sodium transport [22].

Previous studies from our [23, 24] and other laboratories [25, 26] have demonstrated, over a decade ago, cardiac and kidney enlargement in newborn SHR when compared to WKY. Increased DNA synthesis was noted in both organs [23], suggesting the existence of cellular hyperplasia in SHR organs. A similar observation was reported for newborn organs from SHRSP and GH (New Zealand) hypertensive strains. On the other hand, in the Lyon hypertensive model, only cardiac and not kidney enlargement was found and, in addition, in the Milan hypertensive strain, both the heart and the kidneys were significantly smaller when compared to their respective controls. Thus, it is evident that growth "programming" of these two very important cardiovascular organs is different, indicating heterogeneity of their involvement in the pathogenesis of hypertension.

An increasing body of evidence supports the critical role of the kidneys in the pathogenesis of hypertension in SHR [27] and humans [28, 29]. Thus, it has been shown that BP can be transferred together with transplantion of the SHR kidney [27, 30] and that parameters of renal hemodynamics co-segregate with BP in the F_2 generation [31]. Furthermore, it has been demonstrated that in F_2 hybrids derived from a cross of SHR and WKY strains, the reduced diameter of the renal afferent arteriole at the age of seven weeks is associated with increased BP at age 23 weeks [32]. This suggests that some phenotypic abnormalities related to the development of hypertension may precede the actual BP changes. In progenitors of the RIS, both newborn and adult relative kidney weights as well as adult BP were higher in SHR than in BN rats. This indicates that the higher kidney weight in newborn rats may predict higher kidney weight and higher BP in adult rats. In the RIS, a significantly positive correlation between newborn and adult kidney weight was observed (Fig. 2), suggesting that large kidneys in newborns predict large kidneys in adults and that, therefore, the two phenotypes may share some genetic determinants. In fact, the total genome search we conducted showed that some of the suggestive QTL are significant for both newborn and adult kidney weights. The use of newborn and adult animals with identical genetic characteristics allowed us to study genes involved in organ size in the newborn (prehypertensive) stage. Furthermore, the use of newborn RIS let us uncover that the decreased relative kidney/total body ratio, which occurs in the transition

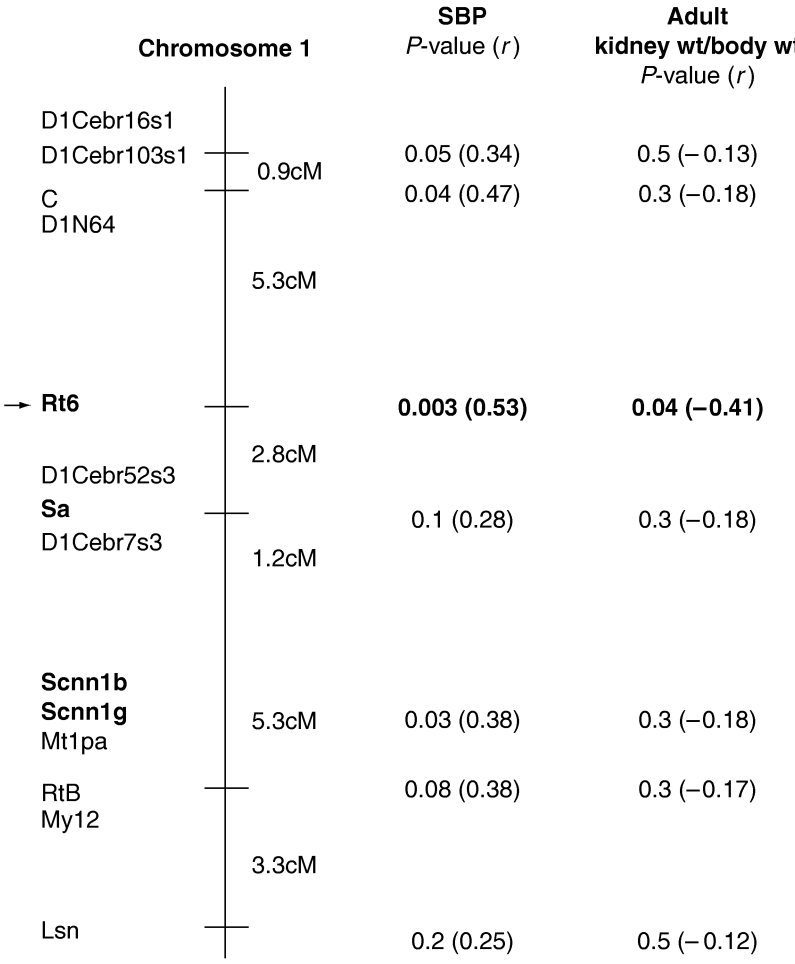

Fig. 4. Quantitative trait locus of adult relative kidney weight (KW/BW) and systolic blood pressure (SBP) on rat chromosome 1.

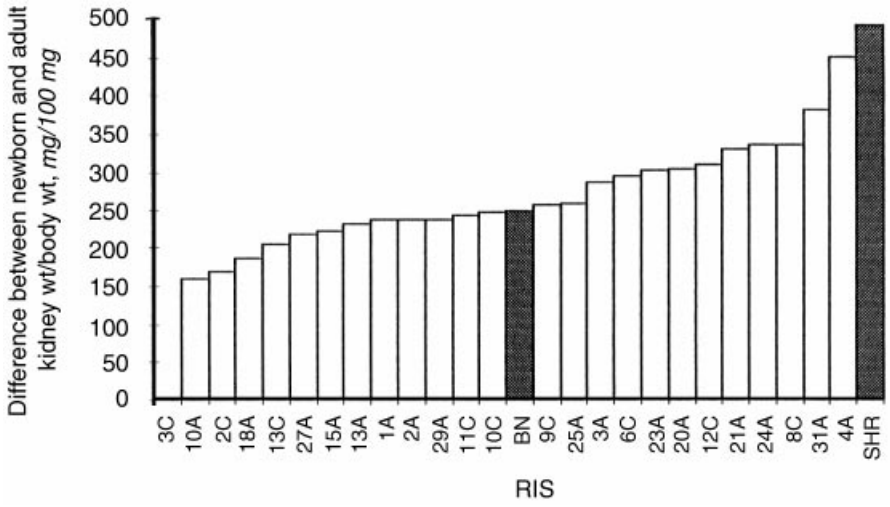

Fig. 5. Distribution of the difference between newborn and adult kidney weight (KW/BW) values in the recombinant inbred strain (RIS).

from the newborn to the adult stage, was twice as big in SHR phenotypes. This developmental change, modulated or not by BP, appears to be controlled by a few candidate loci (Table 3).

In conclusion, we propose that RIS are an unique tool to evaluate neonatal phenotypes and the impact on final outcome, such as hypertension and related target organ damage. This study allowed the distinction between primary hyperplasia and secondarily induced proliferation and permit the determination of genetic loci associated with these events. The identification of positional candidate genes will be one of the paths to uncover loci

Table 3. Total genome search: suggestive quantitative trait locus (QTL) of the difference (Δ) between newborn and adult relative kidney weight

Chromosome	Marker	*N*	Δ of newborn and adult KW/BW *r*
7	D7Ucsf2	27	+0.46[a]
8	D8Mgh9	24	+0.43[a]
10	D10Mit4	26	+0.44[a]
12	D12Mit6	26	+0.46[a]
17	D1a	27	−0.51[b]
19	Es3	27	+0.51[b]

The results of Pearson's correlation analysis are presented as correlation coefficients (*r*). The statistical significance of these analyses is as follows: [a] $P < 0.05$, [b] $P < 0.01$. *N* indicates the number of recombinant inbred strains (RIS) available for analysis of an individual marker.

participating in the pathogenesis of complex traits, such as hypertension and its target organ damage.

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