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## Reciprocal congenic lines for a major stroke QTL on rat chromosome 1

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**Rubattu, Speranza, Norbert Hubner, Ursula Ganten, Anna Evangelista, Rosita Stanzione, Emanuele Di Angelantonio, Ralph Plehm, Reika Langanki, Elisabetta Gianazza, Luigi Sironi, Giulia D'Amati, and Massimo Volpe.** Reciprocal congenic lines for a major stroke-QTL on rat chromosome 1. *Physiol Genomics* 27: 108–113, 2006. First published July 11, 2006; doi:10.1152/physiolgenomics.00086.2006.—We previously identified a quantitative trait locus (QTL) for stroke proneness between the kallikrein (*Klk*) and *Mt1pa* markers on rat chromosome 1. To gain functional insights, we constructed congenic strains by introgressing either the whole or selected chromosomal segments from the stroke-prone (SHRsp) onto the stroke-resistant (SHRsr) spontaneously hypertensive rat genome and vice versa. The phenotype was the latency to develop stroke under a Japanese high-salt, low-potassium diet for 3 mo [known as Japanese diet (JD)]. Blood pressure (BP) was measured by tail cuff throughout the experiment. Urinary protein excretion was monitored in all lines under JD. The SHRsp-derived lines carrying the SHRsr allele, and particularly the *D1Rat134-Mt1pa* chromosomal segment, had a significant delay of stroke occurrence and improved survival compared with SHRsp ( $P < 0.001$ ). On the other hand, a significant occurrence of stroke events (20%) was detected in the reciprocal lines by the end of the 3-mo treatment with JD ( $P = 0.003$ ). The stroke phenotype was also associated with increased proteinuria. Our results underscore the functional importance of the Chr 1 stroke QTL. Furthermore, they underscore the utility of stroke/congenic lines in dissecting the genetics of stroke.

genetics; quantitative trait locus; functional genomics

STROKE IS A MAJOR WORLDWIDE health issue with a complex, multifactorial etiopathogenesis (16). To identify genes that contribute to stroke, we previously carried out a total genome scan in hybrid rats derived from the mating between stroke-prone spontaneously hypertensive rat (SHRsp) and stroke-resistant spontaneously hypertensive rat (SHRsr) strains (12). Through this approach we identified three quantitative trait loci (QTLs) on different chromosomes that explained 28% of the genetic variance. The QTL contribution appeared to be blood pressure (BP) independent. In particular, a QTL on chromosome 1 exerted the major contributory effect and accounted for 17% of the overall phenotype variance. This 42-cM region is

located between *Klk* and *Mt1pa* markers. To study this chromosomal segment further, we generated multiple congenic strains. We aimed to provide functional evidence for the role of the chromosome-1 QTL on stroke susceptibility in our rat model. The resulting congenic lines introgressed either the whole region of interest or parts from the SHRsr onto SHRsp genomic background and vice versa.

We assessed the functional significance of the congenic segments by recording the stroke latency under a stroke-permissive dietary regimen (stroke proneness phenotype) as previously reported (12). The BP response to a high-salt diet was registered by a noninvasive procedure. We also measured protein excretion in the urine, since proteinuria usually precedes the onset of cerebrovascular events in the SHRsp strain (5, 21).

### METHODS

#### Animals

All animals were obtained from colonies at the Max Delbrück Center for Molecular Medicine (MDC) in Berlin, Germany, and maintained at the center's animal facilities of the MDC and the Neuromed Institution in strict compliance with the guidelines set forth by the American Physiological Society. Animal protocols were approved by the Institutional Animal Care and Use Committee of the MDC and Neuromed Institution. Climate was controlled, and temperature was set at 22°C. Diurnal 12-h cycles were kept automatically. Animals were housed two or three per cage with free access to regular rat chow (containing 22% protein, 2.7 mg/g Na<sup>+</sup>, 7.4 mg/g K<sup>+</sup>, 0.05 mg/g methionine) and tap water, unless stated otherwise. The Japanese high-salt, low-potassium diet (JD) contained 17.5% protein, 3.7 mg/g K<sup>+</sup>, 0.03 mg/g methionine (Lab. Piccioni, Milan, Italy), and 1% NaCl added to the drinking water.

#### Congenic Lines

To construct our congenic lines, we selected both the whole region included between *Klk* and *Mt1pa* markers (42.3 cM) and the inner areas either between *Klk* and *D1Mit3* (22 cM) or between *D1Rat134* and *Mt1pa* (25 cM). As a result, all lines shared an overlapping area included between *D1Rat134* and *D1Mit3* (4 cM). We designed six congenic lines. Three lines carried the whole QTL, the *Klk-D1Mit3* area, and the *D1Rat 134-Mt1pa* area, respectively, in the SHRsp configuration within the SHRsr background, and they were defined as follows: [SHRsr.SHRsp-(*Klk-Mt1pa*)], [SHRsr.SHRsp-(*Klk-D1Mit3*)], and [SHRsr.SHRsp-(*D1Rat134-Mt1pa*)]. The reciprocal lines carried the corresponding segments in the SHRsr configuration

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within the SHRsp background: [SHRsp.SHRsr-(Klk-Mt1pa)], [SHRsp.SHRsr-(Klk-D1Mit3)], and [SHRsp.SHRsr-(D1Rat134-Mt1pa)].

We generated the lines by crossing SHRsp with SHRsr in a reciprocal design to form heterozygous F1 progenies. The F1 progenies were subsequently backcrossed to either SHRsp or SHRsr strain (BC1). The genotype of the BC1 progenies was determined by genome screening with chromosome-specific informative microsatellite markers at 10-cM intervals. Heterozygotes at loci of interest (within the targeted area) were selected and backcrossed again to SHRsp or SHRsr (BC2). We repeated the backcrossing 10 times, always selecting heterozygous breeders for the next backcross while performing a genome scan on the other chromosomes. During this process, >99.9% of loci not undergoing selection became homozygous for one strain, while the selected allele from the other strain remained heterozygous. After BC10, two heterozygotes were cross-bred, and the resulting offspring homozygous at loci of interest were bred to each other, thus fixing the alleles of interest in the homozygous state on the background of the other strain.

#### Genotyping

Genomic DNA was prepared from tail clipping of each animal by salt precipitation, as previously described (12). Microsatellite markers belonging to chromosome 1 ( $n = 21$ ) and to the remaining chromosomes ( $n = 120$ ) were genotyped in congenic animals to insure their inbreeding status outside of the selected congenic intervals. Genotyping was carried out by PCR amplification of 50 ng of DNA. The forward primer was labeled with [ $^{32}$ P]ATP (DuPont, NEN) using T4 polynucleotide kinase (Promega). The PCR reactions were processed on a thermal cycler (model PTC 100; MJ Research, Watertown, MA), and the products were loaded onto a 7% polyacrylamide gel, run using a Base Ace apparatus (Stratagene, La Jolla, CA) at 60 W (Feathervolt 3000, Stratagene) for 4 h and exposed to XAR-6 film (Kodak, Rochester, NY) for autoradiography.

#### Phenotyping

At the age of 6 wk, the JD was initiated and maintained throughout the remainder of the experiment (3 mo) in all rat lines. The following animals were used: SHRsr,  $n = 18$ ; SHRsp,  $n = 31$ ; [SHRsr.SHRsp-(Klk-Mt1pa)],  $n = 33$ ; [SHRsr.SHRsp-(Klk-D1Mit3)],  $n = 30$ ; [SHRsr.SHRsp-(D1Rat134-Mt1pa)],  $n = 31$ ; [SHRsp.SHRsr-(Klk-Mt1pa)],  $n = 33$ ; [SHRsp.SHRsr-(Klk-D1Mit3)],  $n = 31$ ; [SHRsp.SHRsr-(D1Rat134-Mt1pa)],  $n = 35$ .

The Japanese dietary regimen has been long used as the most suitable tool to induce stroke in the SHRsp strain (5, 10, 12, 21). The stroke phenotype was recorded, as previously described (12), as the latency to develop cerebrovascular events after the experimental protocol (stroke proneness) was initiated. Therefore, animals were monitored for signs of cerebrovascular events, as documented by presence of mono-, para-, hemi-, or quadriplegia, lethargy, akinesia, or sudden death. Moreover, animals were killed upon the appearance of symptoms of stroke. The whole brain was fixed in freshly prepared 4% paraformaldehyde. After fixation, each brain was sectioned in a coronal plane and embedded in paraffin. Histological sections (7- $\mu$ m thick) were stained with hematoxylin-eosin and observed under light microscopy.

#### BP, Body Weight, and Proteinuria Measurements

BP was measured throughout the experiment in conscious, restrained rats at 2-wk intervals by tail-cuff plethysmography (BP 2000, Apex). Multiple BP measurements were made by the same operator at each time point, as a rule between 10:00 and 14:00, and the mean value was taken as the representative systolic BP level.

Urine was collected from rats of parental and congenic lines by housing them in individual cages at each time point. Protein concentration was measured according to Bradford (2) with bovine albumin as standard.

Finally, rats were weighed regularly throughout the experiment.

#### Statistical Analysis

BP, body weight (BW), and proteinuria data are provided as means  $\pm$  SD. Between group analysis was performed by paired *t*-test or one-way analysis of variance, as applicable. Post hoc analysis was conducted using Scheffé's multiple-comparison test. Survivor function was estimated by the life-table method. Log-rank and Wilcoxon statistics were used for testing equality of survivor functions. Statistical significance was stated at the  $P < 0.05$  level. Analyses were carried out using Stata 8.2 (Texas Corp, 2005).

## RESULTS

### Congenic Lines

The chromosome 1 map in the congenic lines is shown in Fig. 1. We introgressed the entire region in the [SHRsr.SHRsp-(Klk-Mt1pa)] and in the [SHRsp.SHRsr-(Klk-Mt1pa)] lines (SHRsp and SHRsr configuration, respectively, into the opposite background); the *Klk-D1Mit3* area in the [SHRsr.SHRsp-(Klk-D1Mit3)] and in the [SHRsp.SHRsr-(Klk-D1Mit3)] lines (SHRsp and SHRsr configuration, respectively, into the opposite background); the *D1Rat134-Mt1pa* area in the [SHRsr.SHRsp-(D1Rat134-Mt1pa)] and in the [SHRsp.SHRsr-(D1Rat134-Mt1pa)] lines (SHRsp and SHRsr configuration, respectively, into the opposite background).

### Stroke Latency

Stroke occurrence in parental and congenic strains is shown in Fig. 2. The data obtained in parentals confirmed earlier findings (12). In fact, we observed 100% stroke survival in the SHRsr strain, whereas the SHRsp animals displayed unequivocal strokes starting at the 4th wk of JD that reached 100% stroke occurrence by the 6.5th wk. In the SHRsp-derived congenics, presence of the chromosomal segment introgressed from the SHRsr strain induced a significant delay of stroke occurrence. The phenotype was most evident in the [SHRsp.SHRsr-(Klk-Mt1pa)] and [SHRsp.SHRsr-(D1Rat134-Mt1pa)] lines (Fig. 2). In fact, they showed 61 and 63% stroke occurrence, respectively, at the 7th wk of JD and reached 100% mortality at the 9.5th and 13th wk of JD, respectively. On the other hand, among the SHRsr-derived lines we observed a significant occurrence of stroke events (20%) by the end of the 3-mo period in the [SHRsr.SHRsp-(Klk-Mt1pa)] and [SHRsr.SHRsp-(D1Rat134-Mt1pa)] lines {reciprocal of [SHRsp.SHRsr-(Klk-Mt1pa)] and [SHRsp.SHRsr-(D1Rat134-Mt1pa)] lines, respectively}.

### Histology

Consistency between the clinical phenotype and the histological evidence of stroke was 100%. In particular, on microscopic examination, the most frequent finding in both parental and congenic strains was the presence of acute and recent ischemic lesions (aged >6 h <2 days) affecting the gray and the white matter without a preferential distribution. Intravascular fibrin thrombi were often observed within the ischemic areas, as well as endothelial swelling with focal hemorrhagic diapedesis.

### BP, Proteinuria, and BW in Parental and Congenic Strains

**BP tail-cuff measurements.** Systolic BP levels are shown in Table 1. They were similar at baseline (6 wk of age) in all

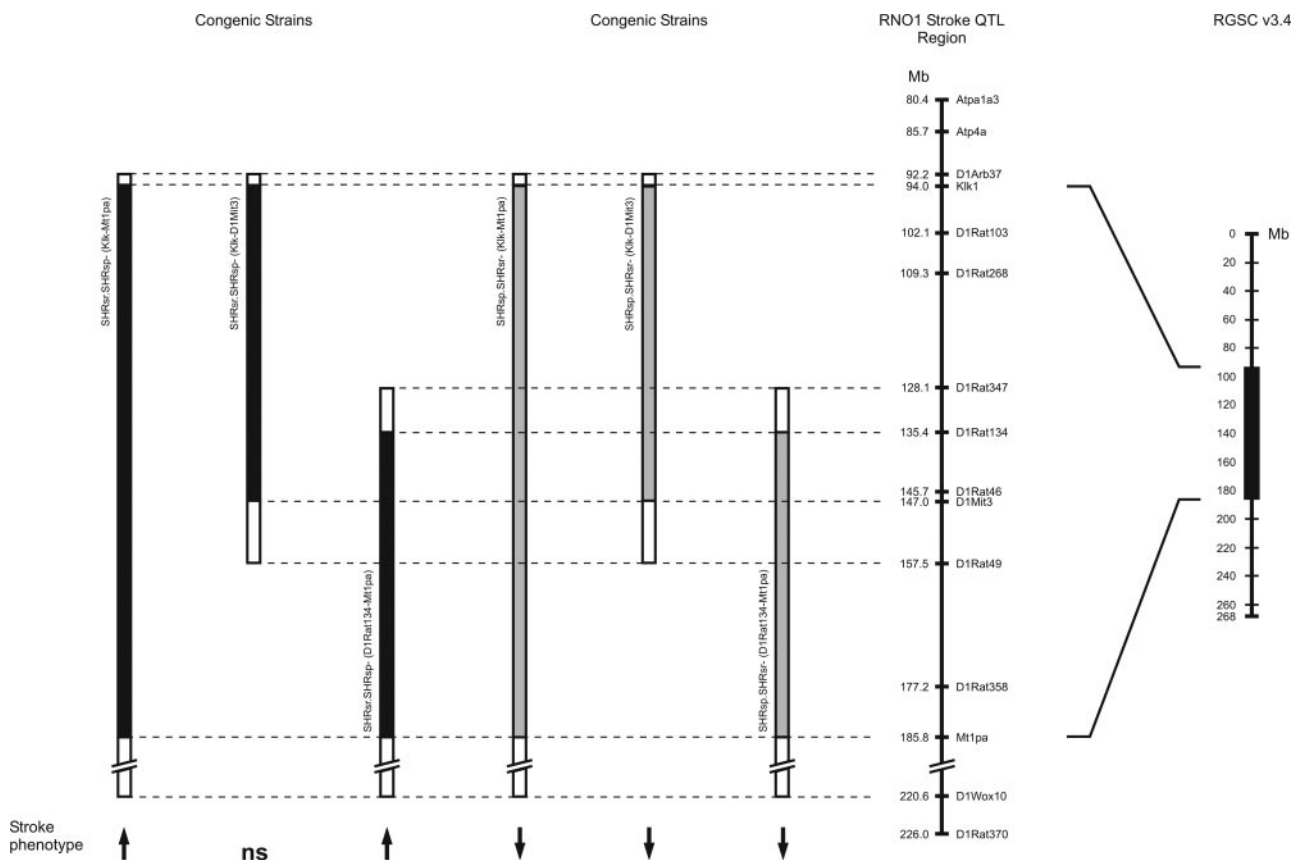


Fig. 1. Schematic representation of rat chromosome 1 and definition of congenic lines. The black and gray bars to the left of the map are the congenic segments. The open ends of these bars represent the intervals in which a crossover occurred. Each chromosomal segment was introgressed both in the stroke-prone spontaneously hypertensive rat (SHRsp) configuration within the stroke-resistant spontaneously hypertensive rat (SHRsr genome) {lines [SHRsr.SHRsp-(Klk-Mt1pa)], [SHRsr.SHRsp-(Klk-D1Mit3)], [SHRsr.SHRsp-(D1Rat134-Mt1pa)] from left to right, respectively} and in the SHRsr configuration within the SHRsp genome {lines [SHRsp.SHRsr-(Klk-Mt1pa)], [SHRsp.SHRsr-(Klk-D1Mit3)], [SHRsp.SHRsr-(D1Rat134-Mt1pa)] from left to right, respectively}. Arrows at bottom indicate a significant effect on stroke (as depicted in Fig. 2) compared with the parental line that was used as the recipient genome for establishment of a particular congenic line; ns, no significant effect observed; QTL, quantitative trait locus; RGSC v3.4, Rat Genome Sequencing Consortium, version 3.4.

lines. The BP increase under JD was similar in both parental and congenic lines.

**Proteinuria.** Table 2 shows the 24-h urinary protein excretion in all lines throughout the experiment. There was a rise of proteinuria after 2 wk of JD in the parental lines with a further marked increase during the following weeks in SHRsp. All SHRsp-derived lines significantly increased their proteinuria levels after 6 wk of diet (2 wk later than the original parental strain). Two of the SHRsr-derived lines, [SHRsr.SHRsp-(Klk-Mt1pa)] and [SHRsr.SHRsp-(D1rat134-Mt1pa)], showed a late marked increase of proteinuria starting at week 8 and 10, respectively.

**BW.** There was a significant difference in the growth of the parental strains, as previously reported (5, 12, 20). In fact, stroke-prone rats had a marked reduced growth under JD. Among the SHRsp-derived lines, the [SHRsp.SHRsr-(Klk-Mt1pa)] and [SHRsp.SHRsr-(D1Rat134-Mt1pa)] lines had a progressive increase of BW, whereas the [SHRsp.SHRsr-(Klk-D1Mit3)] line behaved as the SHRsp strain. Amongst the SHRsr-derived lines, we observed a reduction of growth in the [SHRsr.SHRsp-(Klk-Mt1pa)] and, particularly, in the [SHRsr.SHRsp-(D1Rat134-Mt1pa)] lines.

## DISCUSSION

Congenic strains are necessary to confirm the functional significance of previously identified QTLs (1, 3, 6, 7, 23) before more detailed molecular genetic approaches can be undertaken. We showed that chromosome 1 alleles significantly affect stroke susceptibility. In fact, this chromosome 1 segment led to a significant stroke delay when the allele was introduced from the stroke-resistant into the stroke-prone recipient genome and led to a significant degree of stroke susceptibility when the stroke-prone allele was introgressed into the stroke-resistant recipient genome. Therefore, these new data are consistent with our previous linkage data on the F2 population of SHRsr/SHRsp hybrid rats and with the 17% phenotype contribution estimated for the chromosome 1 QTL in the original linkage study (12).

Few potential candidate genes are known to map within the chromosome 1 area isolated in the congenic lines. Among them, the genes encoding adrenomedullin and insulin-like growth factor II receptor deserve particular attention. In fact, they have been shown to be highly expressed in the injured brain following middle cerebral artery occlusion in the rat model (20, 22) and to have a neuroprotective effect in ischemic

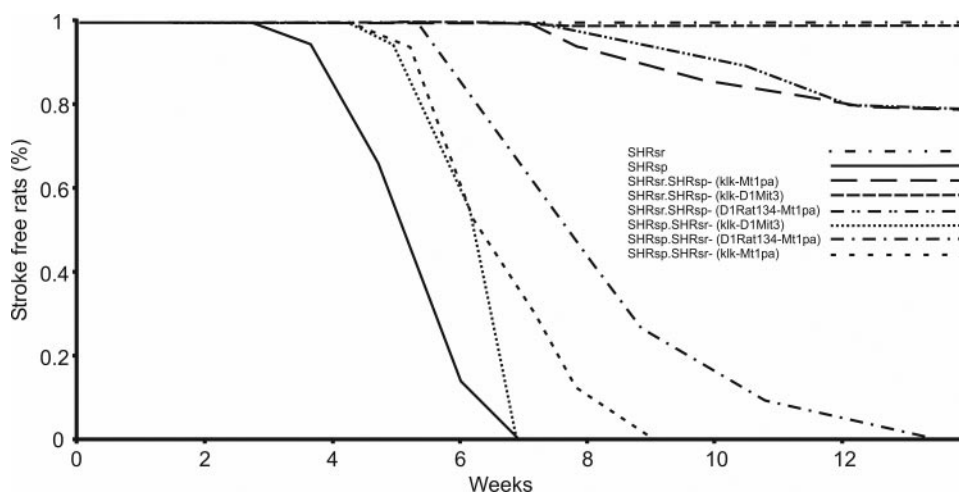


Fig. 2. Stroke survival of the parental and congenic lines. Survival of congenic lines was compared with that of their strain of origin. The overall comparison of the SHRsps-derived lines carrying the SHRsrs chromosomal area was significantly different vs. the SHRsps strain ( $P < 0.001$ ). Comparison of SHRsps vs. [SHRsps.SHRsrs-(Klk-Mt1pa)]:  $P < 0.001$ ; comparison of SHRsps vs. [SHRsps.SHRsrs-(D1Rat134-Mt1pa)]:  $P < 0.001$ ; comparison of SHRsps vs. [SHRsps.SHRsrs-(Klk-D1Mit3)]:  $P < 0.001$ ; comparison of [SHRsps.SHRsrs-(Klk-Mt1pa)] vs. [SHRsps.SHRsrs-(D1Rat134-Mt1pa)]:  $P = 0.312$ ; comparison of [SHRsps.SHRsrs-(Klk-Mt1pa)] vs. [SHRsps.SHRsrs-(Klk-D1Mit3)]:  $P = 0.110$ ; comparison of [SHRsps.SHRsrs-(D1Rat134-Mt1pa)] vs. [SHRsps.SHRsrs-(Klk-D1Mit3)]:  $P = 0.020$ . The overall comparison of the SHRsrs-derived lines, carrying the SHRsps chromosomal area, was significantly different vs. the SHRsrs strain ( $P = 0.003$ ). Comparison of SHRsrs vs. [SHRsrs.SHRsps-(Klk-Mt1pa)]:  $P = 0.002$ ; comparison of SHRsrs vs. [SHRsrs.SHRsps-(D1Rat134-Mt1pa)]:  $P = 0.004$ ; comparison of [SHRsrs.SHRsps-(Klk-Mt1pa)] vs. [SHRsrs.SHRsps-(D1Rat134-Mt1pa)]:  $P = 0.116$ .

brain (9). Although structural differences of the adrenomedullin gene do not exist between the original parental strains (13), it remains to be determined whether its gene expression is under allele specific regulation.

One of the main purpose of the congenic strategy is to narrow down the chromosomal span of the original QTL interval. The region of interest is quite large, and the present definition of the lower border of the reciprocal congenic lines that are presented is incomplete. It well may be that the borders in the reciprocal congenic lines may differ outside the QTL region. Despite considerable efforts, so far, we have been unable to identify additional polymorphic markers mainly due to the high degree of genetic homogeneity between the two closely related rat parental strains. However, with increasing single nucleotide polymorphism markers being generated for the rat, this may become feasible in the future. Interestingly,

even at the present stage, our findings suggest the relevance of a distinct chromosomal segment within the entire region that modulates stroke resistance and latency. A clear-cut stroke phenotype was related to the *D1Mit3-Mt1pa* area, which is specific only to the [SHRsps.SHRsrs-(Klk-Mt1pa)] and [SHRsps.SHRsrs-(D1Rat134-Mt1pa)] lines, showing a significant stroke resistance and, to their reciprocal strains, showing a significant stroke susceptibility. On the other hand, the observation that the [SHRsps.SHRsrs-(Klk-D1Mit3)] line had a certain degree of stroke resistance whereas its reciprocal line did not show increased susceptibility to stroke raises interesting perspectives about the possible relevance of the *D1Rat134-D1Mit3* area (which is shared by all lines).

In this regard, it is likely that increased susceptibility to stroke may require the interaction of multiple susceptibility variants in the target region of chromosome 1, and, therefore,

Table 1. Systolic blood pressure levels as determined by tail-cuff plethysmography

	Baseline	2 wk	4 wk	6 wk	8 wk	10 wk	12 wk
SHRsrs	111 ± 4.0	139 ± 7.0	185 ± 7.3	191 ± 12.8	191 ± 7.0	199 ± 1.0	188 ± 12
<i>n</i>	18	18	18	18	18	18	18
SHRsps	107 ± 7.0	151 ± 8.0	187 ± 13	201 ± 19.5			
<i>n</i>	31	31	26	5			
SHRsrs.SHRsps-(klk-Mt1pa)	110 ± 3.5	148 ± 18	174 ± 19	192 ± 7.0	199 ± 10	202 ± 9.0	197 ± 21
<i>n</i>	33	33	33	33	32	29	26
SHRsrs.SHRsps-(klk-D1Mit3)	108 ± 5.0	146 ± 7.0	176 ± 10.5	181 ± 9.2	197 ± 10	206 ± 8.0	208 ± 8.0
<i>n</i>	31	31	31	31	31	31	31
SHRsrs.SHRsps-(D1Rat134-Mt1pa)	112 ± 2.0	144 ± 11	166 ± 10.9	191 ± 4.0	195 ± 8.0	199 ± 10	201 ± 7.0
<i>n</i>	31	31	31	31	30	28	25
SHRsps.SHRsrs-(klk-Mt1pa)	114 ± 1.0	164 ± 15	186 ± 6.9	192 ± 7.0	195 ± 5.0		
<i>n</i>	33	33	33	20	3		
SHRsps.SHRsrs-(klk-D1Mit3)	106 ± 1.0	153 ± 12	190 ± 5.0	200 ± 4.0			
<i>n</i>	31	31	31	22			
SHRsps.SHRsrs-(D1Rat134-Mt1pa)	112 ± 4.0	153 ± 12	188 ± 12.5	192 ± 4.6	192 ± 6.0	198 ± 3.0	193
<i>n</i>	35	35	35	34	14	5	1

Systolic blood pressure (SBP) levels are presented as means ± SD. Baseline = 6 wk of age. Empty cells correspond to experimental time points when rats had already reached 100% stroke occurrence. SHRsrs, stroke-resistant spontaneously hypertensive rat; SHRsps, stroke-prone spontaneously hypertensive rat.

Table 2. Twenty four-hour urinary protein excretion during the dietary regimen

	Baseline	2 wk	4 wk	6 wk	8 wk	10 wk	12 wk
SHRsr	1.67 ± 1.1	31 ± 14	60 ± 18	56 ± 4.0	83 ± 4.4	80 ± 4.5	89 ± 9.0
<i>n</i>	18	18	18	18	18	18	18
SHRsp	1.57 ± 0.3	35 ± 3.4	215 ± 94*	194 ± 95*			
<i>n</i>	31	31	26	5			
SHRsr.SHRsp-(klk-Mt1pa)	2.9 ± 1.6	37 ± 14	46 ± 15†	75 ± 19†	270 ± 34*	241 ± 22*	294 ± 15*
<i>n</i>	33	33	33	33	32	29	26
SHRsr.SHRsp-(klk-D1Mit3)	1.7 ± 0.4	22 ± 10.6	74 ± 15†	58 ± 28†	81 ± 23‡	89 ± 71‡	58 ± 7.4‡
<i>n</i>	31	31	31	31	31	31	31
SHRsr.SHRsp-(D1Rat134-Mt1pa)	3.1 ± 2.8	11 ± 1.5	60 ± 14†	51 ± 22†	54 ± 27†	169 ± 86*	111 ± 86*
<i>n</i>	31	31	31	31	30	28	25
SHRsp.SHRsr-(klk-Mt1pa)	3.4 ± 1.2	38 ± 11	30 ± 5.3†	232 ± 91*	351 ± 50*		
<i>n</i>	33	33	33	20	3		
SHRsp.SHRsr-(klk-D1Mit3)	3.2 ± 2.3	39 ± 24	75 ± 36†	257 ± 89*			
<i>n</i>	31	31	31	22			
SHRsp.SHRsr-(D1Rat134-Mt1pa)	2.4 ± 1.1	24 ± 11	53 ± 7.5†	275 ± 84*	343 ± 60*	333 ± 77*	400
<i>n</i>	35	35	35	34	14	5	1

Protein excretion (mg) is presented as means ± SD. \* $P < 0.01$  vs. SHRsr, † $P < 0.01$  vs. SHRsp, ‡ $P < 0.01$  vs. the other congenic lines at the same experimental time point. Empty cells correspond to experimental time points when rats had already reached 100% stroke occurrence.

replacement of just one of these variants with a single resistance variant may be sufficient to protect against stroke. Conversely, if interaction of multiple variants is necessary to confer susceptibility to stroke, transfer of only one of these variants from SHRsp onto the SHRsr background will not be sufficient to increase susceptibility to stroke. Thus it is possible that the D1Rat134-D1Mit3 region still contains an important variant even though an effect was observed in only one of the reciprocal congenics for that area.

The relevance of the findings obtained from the lines carrying the Klk-D1Mit3 segment may also underscore the importance of the genetic background as a critical factor influencing the phenotypic expression in congenic strains.

Overall, the data we provide make detailed genetic approaches aimed at the identification of the underlying gene(s) possible. This includes the development of congenic sublines for the chromosomal region of interest, as well as the identification of differentially expressed genes within the area of interest. In particular, the generation of nonoverlapping congenic substrains may help us to formally test the hypothesis that increased susceptibility to stroke requires the interaction of multiple susceptibility variants in the target region of chromosome 1.

This information may be important to the human disease. In fact, we have already demonstrated the close resemblance between the SHRsp and human stroke with regard to the gene encoding atrial natriuretic peptide (14, 15, 17).

The SHRsp strain shows high susceptibility to develop renal damage before the onset of cerebrovascular lesions (5, 18, 21). It has been suggested that renal lesions might be involved in the pathogenesis of stroke as a potential intermediate phenotype. According to the proteinuria levels detected in our experimental context, an association between the renal and the cerebrovascular phenotypes can be clearly confirmed. In fact, the SHRsp-derived lines had a delayed occurrence of proteinuria compared with the original strain, whereas two out the SHRsr-derived lines showed a rise of proteinuria only at the time of stroke events (which occurred 2 or 3 mo after starting JD). Thus our new models may provide an interesting tool to clarify the role of proteinuria in the pathogenesis of stroke. Moreover, it will be interesting to determine whether the same

gene modulates both proteinuria and stroke occurrence in the SHRsp strain.

The issue of a stroke phenotype independence of BP, as previously reported in our experimental set-up (12), remains an interesting aspect to explore. We performed a noninvasive BP measurement throughout the experiment and obtained evidence in support of a lack of correlation between hypertension and stroke in our animal models. However, recently published American Heart Association recommendations caution against reliance on tail-cuff BP measurements in experimental animals to make claims about the BP independence of any phenotype (8). Thus we are aware that, in the absence of BP telemetry measurements, a clear definition regarding the relationship between stroke phenotype and BP under the effect of our stroke/QTL cannot be definitively assessed. By use of telemetry in future experiments aimed at the recording of all BP fluctuations in relation to stroke occurrence, our new animal models may provide important information relevant to this issue.

Finally, an interesting observation was obtained from monitoring the BW while the rats ingested the JD. We observed that the SHRsp-derived lines with a major stroke resistance also had increased BW and kept growing during JD compared with their strain of origin. On the other hand, the SHRsr-derived lines with a major stroke susceptibility had reduced growth compared with SHRsr. We suggest two possible explanations for these findings: there is an allelic influence on BW within the QTL on rat chromosome 1 or it is merely a secondary effect.

Earlier epidemiological studies suggested that coronary disease is a greater health-care burden than stroke. However, the recently reported Oxford Vascular Study demonstrated that this is not the case (11). The study showed high rates of acute vascular events outside the coronary arterial territory and a steep rise in event rates with age in all territories. As a matter of fact, stroke was no less common than coronary disease. The epidemiological findings have implications for prevention strategies, clinical trial design, and experimental research. Our experimental study opens new mechanistic perspectives. On the basis of our previous linkage data, we provided functional evidence for the role of a chromosome-1 QTL on stroke

susceptibility. This result represents a promising background for the final identification of the specific components that directly contribute to stroke susceptibility in this rat model of a complex human disease. We are currently pursuing more detailed molecular genetic approaches to identify specific stroke-susceptibility and stroke-protection genes.

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