Author's response to reviews

Title: Genetic mapping of a new heart rate QTL on chromosome 8 of spontaneously hypertensive rats

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Author’s response to reviews: see over
Reviewer: Nengjun Yi

We thank the reviewer for his suggestions and the answers for specific commentaries follow below:

Reviewer’s report:

1) They did not adjust heart rate values for systolic or diastolic blood pressure traits. From Table 2, heart rate significantly correlated with these variables. Therefore, a better analysis should include these traits as covariates in the analysis.

Answer: Although heart rate significantly correlated with blood pressure levels (p=0.0069 for systolic blood pressure) (Table 2), the R squared value suggested that these variables are poorly correlated (0.0335 for systolic blood pressure). Thus, we did not adjust heart rate values for systolic or diastolic blood pressure traits before QTL mapping analysis.

2) Table 3 shows the results from AVONA for three markers, APOA02, R1106 and R830, which are located in the significant region. The AVONA analysis showed insignificant difference among the genotypic values for these three markers. This contradicts the finding. Is your interval mapping wrong or your AVONA wrong?

Answer: Please note in Table 3 that, by ANOVA analysis, there is a significant allelic effect for the R1106 marker (377±6 vs. 370±3 vs. 354±8* bpm, for BN/BN, BN/SHR, and SHR/SHR respectively; *p=0.0306). In contrast, there is no significant allelic effects on heart rate levels associated to APOA02 and R830.
markers, which are flanking the 6.78 cM heart rate QTL mapped on rat chromosome 8 (Figure 2). Therefore, there are no contradicting data or mistakes in our interval mapping or in the statistical analysis conducted.
Reviewer: Michal Pravenec

We thank the reviewer for his suggestions and criticism and the specific queries are answered below.

Reviewer’s report:

1) As already mentioned, the authors used data from their original paper that was published more than 10 years ago (Schork et al., Genome Research 5:164-172, 1995). Accordingly the markers look archaic. Unfortunately, DNA from the F2 rats is not available for additional genotyping.

Answer: The potential limitation of the genetic markers density was acknowledged and extensively discussed in the manuscript and we have no evidence that this would invalidate the results described in this paper.

2) The authors claim that QTL associated with heart rate variability is independent upon blood pressure but they have mapped another QTL associated with blood pressure in the close vicinity. It is therefore possible that a single locus affects both blood pressure and heart rate. The authors should also cite Kren et al. (V. Kren, M. Pravenec, S. Lu, D. Krenova, J.-M. Wang, N. Wang, T. Merriouns, A. Wong, E. St. Lezin, D. Lau, C. Szpirer, J. Szpirer, T. W. Kurtz: Genetic isolation of a region of chromosome 8 that exerts major effects on blood pressure and cardiac mass in the spontaneously hypertensive rat, J Clin Invest 99:577-581, 1997) where genetic isolation of a QTL associated with blood pressure and potentially also with heart rate is described: “…The heart rate of the SHR-Lx congenic strain tended to be greater than that of the SHR progenitor strain; however, differences in heart rate
were not consistently observed throughout the experiment… “It is interesting that the authors also observed increased heart rate associated with the BN alleles so their data seem to be consistent with results of Kren et al. observed in the SHR.BN congenic strain.

Answer:

Although it is possible that a single locus affects both blood pressure and heart rate phenotypes we believe that the mapped QTL associated with basal values of heart rate is independent from blood pressure. The previously blood pressure-related QTL mapped (Shork et al., 1995) is relatively far away (4.7MB) from the present heart rate QTL (see Figure 1). In addition, the segment transferred from the BN-Lx strain into the SHR, during the development of the congenic SHR-Lx strain described by Kren et al. (1997), is in fact very large (Figure 1). This segment contains 477 out of 1452 genes present in chromosome 8. Curiously, the congenic SHR-Lx segment includes both the blood pressure (Shork et al., 1995) and the heart rate QTLs mapped by our group (Figure 1), and it is therefore feasible that different loci are affecting blood pressure and heart rate in that sub-strain. We do not believe that the heart rate QTL identified in the present study is sharing candidate genes with the previously blood pressure QTL mapped in our study (Schork et al., 1995).
Figure 1. Chromosome 8 illustration and the blood pressure- and heart rate-related QTLs mapped by both groups. Gray bar represents the blood pressure QTL mapped by Kren et al. (1997), and delimited by D8Mit5 and D8Mgh6 markers; Black bar represents the blood pressure QTL mapped by Schork et al. (1995), and delimited by R19 (D8Mit5) and R850 (D8Mgh6) markers; Diagonally stripped bar represents the blood pressure QTL for us mapped in present manuscript (Silva et al.), and delimited by APOA02 (~D8Arb8) and R830 (D8Mit12) markers.

Nevertheless, the suggested reference (Kren et al., J Clin Invest 99:577-581, 1997) is really interesting and we have included it in the Discussion section (page 10, line 15). However, even though these two QTLs are contained within chromosome 8, it is important to emphasize that these authors did not observe statistically significant differences in heart rate levels between congeneric SHR-Lx
strain and SHR. Indeed, transfer of a segment of chromosome 8 from the BN-Lx strain onto the SHR background resulted only in substantial reductions in blood pressure and cardiac mass. Moreover, in our study we did not observe an allelic effect on blood pressure values for the genetic markers located within the heart rate QTL, suggesting that this may be, indeed, a heart rate specific QTL.

Altogether, we do not believe that the same gene(s) are pleiotropically having the effects in both phenotypes. To clarify some of these issues we added the following paragraph in the discussion (page 10, line 15):

“Interestingly, Kren et al. reported a blood pressure QTL on chromosome 8 which maps nearby the heart rate-related QTL reported in the present study. However, a congenic SHR-Lx strain harbouring this particular region showed only changes in basal blood pressure and cardiac mass with no heart rate effects. Therefore, it is less likely that the same gene(s) are influencing the effects on heart rate here described.”
Reviewer: Morton Printz

Reviewer's report:

General

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

None

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

None

Discretionary Revisions (which the author can choose to ignore)

None