Are IA-2 and RESP18 Involved in Trait of Blood Pressure?

To the Editor:

Congenic rat strains are important tools for the genetic dissection of essential hypertension.1 Garrett et al recently demonstrated that a blood pressure (BP) quantitative trait locus (QTL) exists within a newly defined 117-kb QTL region on rat chromosome 9.2 By using microarray technology, the authors first found that the mRNA of Resp18 (endocrine-specific protein 18) is ~7.27-fold lower in the kidney of S.R(9)x3A congenic rats (Dahl salt-resistant) than that of S rats (Dahl salt-sensitive). Furthermore, sequencing analysis revealed multiple mutations of Resp18, particularly the sequence variation (T/C) in exon 2. However, a fine-map analysis showed that Resp18 is located just outside of the 117-kb QTL region; thus, Resp18 was eliminated as a candidate gene.

The authors, however, did not address: (1) whether a homolog gene of Resp18 exists in genome, particularly in the 117-kb QTL region; (2) the significance of the Resp18 sequence variation (T/C) in exon 2; (3) the possibilities that RESP18 plays a role in BP.

We recently demonstrated that RESP18 not only shares significant sequence similarities with the N-terminal domain (amino acid 1–200) of IA-2, a dense-core vesicle (DCV)-transmembrane protein, but also shows a similar biological function to IA-2 in terms of exocytosis of neuronal transmitter and hormones.3,4 This means we have demonstrated that Resp18, as a DCV cargo protein, is also involved in DCV secretion (P. Yu et al, unpublished data, 2005). Furthermore, our bioinformatic analysis showed that Resp18 shares similar genomic structure and is tandemly arranged with IA-2 within a small genomic region in rat. Resp18 is located ~9 kb of the 5′ terminus of IA-2 (GenBank accession number AC121633), indicating that IA-2 is within the 117-kb QTL region.

We found that the sequence variation (T/C) in exon 2 (nt 272) of Resp18 actually results in a Ile/Pro (aa 62) change in the predicted protein product rather than a Ile/Val as reported.5 This variation (T/C) is not found in >100 expressed sequence tags of IA-2 (http://www.ncbi.nlm.nih.gov/BLAST/), suggesting it is a rare polymorphism or a mutation. Furthermore, our evolutionary analysis revealed that substitution of Ile/Pro is not present in all species we examined, whereas Ile and Val are present in these species (see below, bold in multiply sequences alignment). But the effects of the Ile/Pro change on DCV secretion of RESP18 need to be examined.

Mouse RESP18 57 FGYQLLIFHQIVPQEGMF, Rat RESP18 57 FQYQLLFLHHHIVPQGMF, Bovine RESP18 57 FKHLLQV-LQQIIMPDLF, Pig RESP18 57 FQHLQWLQIVPQGLF, Human RESP18a57 FQHLQVLLQIIPQGLF.

A recent in vivo evidence demonstrated that DCV formation and activity, which is regulated by chromogranin, is tightly coupled to the action of catecholamine and BP regulation.5 We speculated that IA-2 probably is the causative gene for the 117-kb BP QTL, and RESP18 might also possess an additive effect on the BP by regulating DCV cargo release or other mechanisms. To test this hypothesis, our experiments by using IA-2 deficient mice are underway.

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Response

The fundamental premise of substitution mapping is to be able to track the blood pressure (BP) effects of quantitative trait loci (QTL) that are introgressed within a congenic strain. Based on this paradigm, our report1 clearly excludes Resp18 as the QTL within the 117-kb region of interest. Cai contemplates the possibility that Resp18 plays a role in BP. In our opinion, a good experiment to test this is to study a minimal congenic strain around the Resp18 locus.

Cai brings out an interesting point of whether a homolog of Resp18 exists in the critical 117-kb region. IA-2, or ICA512, is one such gene, the protein product of which was originally reported to have 27% identity and 56% similarity with the 18-kDa Resp18 protein.2 We are glad that Cai could utilize the data presented in our manuscript and arrive at 2 speculations: (1) IA-2 is probably the causative gene for the 117-kb BP QTL. The least that this speculation could be based on is a single nucleotide polymorphism (SNP) of IA-2 between Dahl salt-sensitive (S) rats and Dahl salt-resistant (R) rats. We have identified numerous SNPs between the S and R rat within and around IA-2. Unpublished data demonstrate that, at least independently, none of these SNPs affect BP. Further, the microarray experiment reported in our article1 did not detect any renal differential expression of IA-2 between S and the congenic strain (GEO accession no. GSE 1775, probes D38222_s_at and rc_AI137484_at). Thus, so far we do not have any evidence, at the level of either the gene sequence or transcription, to suggest that IA-2 is the BP QTL. Cai indicates that experiments using mouse IA-2 are underway. We look forward to the results of this study. Nevertheless, phenotypic effects of knockout mice may not always reflect the properties of natural allelic variants of a gene in a congenic strain.3,4 (2) Resp18 and IA-2 may possess an additive effect on BP. This speculation is contrary to the data provided in our report,1 wherein we have demonstrated that the effects of the QTL gene within the 117-kb region and that of Resp18 are not additive.1

The amino acid substitution of Resp18 is correct in our report (ie, the sequence variation is from Isoleucine [Ile] to Valine [Val]). However, there is a discrepancy in the location of nucleotide coding for this amino acid substitution. The T/C variation reported in Figure 3 of our article1 is that of genomic
DNA. This variation corresponds to a SNP of A/G at position 286 (not 272 as reported) from the 5’ untranslated region of the mRNA transcript of Resp18. This SNP alters the amino acid 67 of Resp18 from Ile in S rats to Val in R rats.

Finally, please note that in Cai’s letter there is some confusion about amino acid 62. It is referred to as Ile (Isoleucine) in the text, but depicted as L (Leucine) within the sequence comparisons. We would like to clarify that amino acid 62 is L in the protein database (NP_062151 at http://www.ncbi.nlm.nih.gov) as well as in S and R rats.

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