Use of contiguous congenic strains in analyzing compound QTLs

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Rapp JP, Joe B. Use of contiguous congenic strains in analyzing compound QTLs. Physiol Genomics 44: 117–120, 2012. First published November 22, 2011; doi:10.1152/physiolgenomics.00136.2011.—Genetic analysis of polygenic traits in rats and mice has been very useful for finding the approximate chromosomal locations of the genes causing quantitative phenotypic variation, so-called quantitative trait loci (QTL). Further localization of the causative genes and their ultimate identification has, however, proven to be slow and frustrating. A major technique for gene identification in such models utilizes series of congenic strains with progressively smaller chromosomal segments introgressed from one inbred strain into another inbred strain. Under the assumption that a single causative locus underlies a QTL, nested series of congenic strains were earlier suggested as an appropriate configuration for the congenic strains. It is now known that most QTL are compound, that is, the QTL signal is caused by clusters of loci where alleles exert positive, negative, and interactive effects on the trait in a given strain comparison. It is argued that in this situation an initial series of nonoverlapping contiguous congenic strains over a relatively large chromosomal region will lead to a better appreciation of the underlying complexity of the QTL and therefore more rapid gene identification. Examples from the literature where this strategy would be helpful, as well as a case where it would be potentially counterproductive, are given.

Keywords: quantitative trait loci; polygenic inheritance; Dahl rats; hypertension

Discovery of the multiple loci causing variation in quantitative traits is difficult because the phenotype of a quantitative trait does not uniquely predict the underlying causative genotypes. Considerable progress has been made in the theory and techniques for locating quantitative trait loci (QTLs) to regions of chromosomes in model organisms especially those using inbred strains with different quantitative phenotypes (2–4, 15, 16, 18, 22, 26, 30, 33). Much less effort has been given to the development of techniques to systematically identify the underlying genes (3, 29). Considering the analysis of QTLs in rats and mice, the follow-up technique most often applied after locating a QTL in segregating populations (or by other techniques) is the construction of congenic strains. This involves the marker-assisted introgression of a large region of chromosome containing a QTL from one inbred (donor) strain into another inbred (recipient) strain by well-established techniques (14, 18, 31, 32). The phenotype of the initial congenic strain is then compared with the recipient strain to establish that the congenic chromosomal segment in fact has an effect on the phenotype.

The obvious follow-up technique to identify the gene(s) underlying the QTL congenic interval is to reduce the originally introgressed chromosomal segment by subsequent construction of congenic substrains derived from the original congenic interval, which may be 25–50 cm (or Mb) in size. It is this early stage of reducing the target region that has not received much theoretical consideration. In our view this accounts for the slow progress made in going from QTL to quantitative locus identification.

Originally we suggested the (admittedly obvious) method shown diagrammatically in Fig. 1 (24). The figure uses blood pressure in rats, specifically inbred hypertensive rats, compared with an inbred normotensive strain, as an example. The example of blood pressure is used since our experience with it illustrates the problems in QTL analysis discussed below. In Fig. 1 the original congenic strain (strain 1) was produced by introgressing a segment of chromosome from a normotensive strain into the hypertensive strain. This resulted in a reduced blood pressure in strain 1 compared with the hypertensive strain. A series of hypothetical nested congenic substrains constructed from the original congenic strain are shown (Fig. 1, strains 2–7). These are produced by crossing congenic strain 1 to the recipient (hypertensive) strain and then intercrossing the resultant F1 rats to make an F2 population. Animals carrying recombinant chromosomes in the F2 population are identified by genotyping, backcrossed to the recipient strain to duplicate the desired chromosome, which is then fixed in the homozygous state by further selective marker-assisted breeding. The strains thus produced are phenotyped, and as the introgressed region from the normotensive strain crosses the location of the QTL between strains 4 and 5 there is a step change in blood pressure. Thus the QTL is between markers D and E shown at the top of the Fig. 1.

Conceptually this is easy, but in practice strain differences are not always so clear because of high phenotypic variability, especially with blood pressure (9). Also the location of the QTL as the difference between two strains is not sufficient but has to be confirmed by constructing a shorter congenic strain just encompassing the interval D to E and comparing this to the hypertensive strain. If a phenotypic effect is still present then another iteration of congenic substrains in the interval D to E is invariably required to narrow the QTL interval enough for gene identification. More importantly it is now widely recognized that QTL regions identified in logarithm of the odds (LOD) plots of segregating populations (or by other means) are usually compound. Such regions can contain alleles with positive or negative effects at more than one locus in the QTL interval, and there may be interactions between loci in the QTL interval. Examples of these problems in our work with blood pressure were observed on chromosomes 1 (27), 2 (6), 3 (17), 5 (7), 9 (11), and 10 (28). Moreover, the QTL may not be anywhere near the LOD peak because of ghost peaks between QTLs (19, 20). Such effects can certainly be dissected using...
the scheme in Fig. 1, but it can be quite confusing, and consideration of a different approach is also warranted.

Given that the ultimate goal is to localize each causative locus within a large QTL region into a small congenic interval and that more than one such locus is likely in a large (or even a small) QTL region, it makes sense to start out making small congenic intervals earlier in the process. Figure 2 shows a hypothetical set of contiguous congenic substrains in a large QTL region that contains two causative loci acting additively where the donor allele at QTLa reduces blood pressure and the donor allele at QTlb increases blood pressure. In this situation it is immediately obvious that substrate 5 in Fig. 2 shows an augmented phenotypic effect due to QTLa because of the removal of the effect of QTlb; this is a significant advantage. There are examples in the literature of linked QTL with opposite effects on blood pressure (1, 23, 28). In the case where QTLs a and b are additive in the same direction the effect of each will be smaller than the original congenic strain. In the case where QTLs a and b are nonadditive (they interact) it is likely that each would show up with weak phenotypic effects the sum of which is not the same as the original congenic strain. In this case construction of a double congenic from small nonoverlapping substrains could be done to prove an interaction as has been done for interacting loci that were on different chromosomes (25). The point is that the strains for construction of a double congenic are likely to be immediately available.

One situation where the use of contiguous congenic strains is likely to fail is where there are two linked causative loci in a QTL region such that interacting alleles at both loci are required for an effect but that neither allele alone causes an effect. We observed this exact situation for blood pressure in Dahl S rats on chromosome 5 (7), and here the nested congenic strains worked eminently well. A counterexample is, however, also instructive. Initially we found a blood pressure QTL on chromosome 13 in Dahl rats using nested congenic strains (34). Further analysis using nested strains proved futile (unpublished). Moreno et al. (21) made 23 (highly redundant overlapping) congenic strains covering all of rat chromosome 13, which revealed a complex genetic architecture consisting of four regions containing interacting alleles that influence blood pressure. In this case contiguous strains were successful at defining a complex architecture where nested strains failed. Other examples in Dahl S rats where the contiguous congenic strain construction would probably have been the better approach to unravel a complex genetic architecture (two or more underlying causative loci) are on chromosomes 1 (27), 2 (6), and 10 (8). For didactic purposes Fig. 2 has only two theoretical causative loci, but the usefulness of the contiguous approach is enhanced where there are three or more causative loci underlying the QTL. Our experience is that there are always at least two.

It is rational to conclude that the best method to apply to an initial round of congenic strains depends on the underlying complex architecture of the QTL. Since this is unknown a priori, there is risk with either method. The example on chromosome 5 above is rare in our experience; thus the use of contiguous congenic strains in a large QTL region early in an analysis merits consideration.

The barrier to the analysis in Fig. 2 is of course the construction of the contiguous congenic substrains. The idealized strains in Fig. 2 in reality would be overlapping in some places and might have gaps between strains in other places. Overlaps and gaps should be minimized. As long as the gaps were not too extensive the chance of missing a QTL would not be inordinate. In any case the substrains in Fig. 2 can be constructed from the recombinant chromosomes that are generated in the initial phase of producing nested recombinant chromosomal segments, i.e., those shown in Fig. 1. Typically these are produced in an F2 population of ~200 rats derived from crossing the initial congenic strain with the recipient strain. It is emphasized that it is not necessary to construct (or phenotype) the congenic strains in Fig. 1 to make the strains in Fig. 2; it is only necessary to obtain the recombinant chromosomes and to work with them in a backcross to the recipient strain (see below). In Fig. 1 only nested fragments starting at

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**Fig. 1. Illustration of an idealized set of nested congenic substrains constructed from an initial congenic strain.** The initial congenic strain (strain 1) was made by introgressing a segment of chromosome from a donor normotensive strain into a recipient hypertensive strain. It is assumed that the segment contains a single quantitative trait locus (QTL) where the donor allele lowers blood pressure by 20 mmHg. The diagram is modified from Rapp and Deng (24).

**Fig. 2. Illustration of an idealized set of contiguous congenic substrains constructed from an initial congenic strain.** The initial congenic strain (strain 1) was made by introgressing a segment of chromosome from a donor normotensive strain into a recipient hypertensive strain. It is assumed that the segment contains two additive QTLs where the donor allele at QTLa lowers blood pressure by 20 mmHg and the donor allele at QTlb increases blood pressure by 10 mmHg. Note that in both Figs. 1 and 2 the initial congenic strain lowers blood pressure by 20 mmHg.
the left of the diagram are shown, but of course similar 
fragments starting from the right of the figure will also be 
generated. Thus in the initial screen the end fragments for 
generating congenic strains 2 and 8 in Fig. 2 should easily be 
obtained in the F2 population and/or in the following steps. It 
is, therefore, only necessary to consider further how to generate 
recombinant fragments for the construction of the internal 
substrains 3–7 in Fig. 2. For example an F2 animal carrying the 
recombinant chromosome 2 in Fig. 1 is backcrossed to the 
recipient strain, and crossovers at markers E, F, and G are 
evaluated to generate the recombinant fragment used in con-
structing substrate 3 in Fig. 2. If the region spanned by 
markers E, F, and G were (for example) 4 cM, then four 
crossovers per 100 meioses would be expected in that region. 
Thus screening 100 backcross animals should yield appropriate 
recombinants for constructing substrate 3 in Fig. 2. Similarly 
recombinant chromosome 3 in Fig. 1 is used to generate 
substrate 4 of Fig. 2, etc.

Consideration should also be given as to how the statistical 
analysis of the phenotypes (blood pressure) of the congenic 
strains generated in Fig. 2 should proceed. All nine strains in 
Fig. 2 can be compared by a one-way analysis of variance 
(ANOVA) with blocks. For example, if one wanted 20 rats to 
be phenotyped for each strain it would be difficult to raise and 
take blood pressure on all 180 rats concomitantly. Ten statist-
tical blocks of 18 rats (2 rats per strain for 9 strains) could be 
taken blood pressure on all 180 rats concomitantly. Ten statis-
tical comparisons are also appropriate. The latter can place the strains into subsets with 
same blood pressure, and of course pair-wise strain com-
parisons. The former can place the strains into subsets with 
comparable blood pressure QTL on chromosome 7 in Dahl rats by a 177kB congenic segment containing Cyp11b1. 

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Garrett MR, Rapp JP. Two closely linked interactive blood pressure 
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DISCLOSURES

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AUTHOR CONTRIBUTIONS

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