Minireview

Genetic effects on an animal model of anxiety

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Abstract Genetic effects on behavioural measures thought to model anxiety have been reported on 15 mouse chromosomes. In general the individual effect from each locus is small, contributing to 10% or less of the total variation, but through use of crosses between inbred rodents the power to detect such effects is high: 39 loci have been reported at stringent levels of significance. Novel multivariate analyses of these data go some way to characterizing the genetic architecture of anxiety and also to validating the tests that are used for its measurement. However, we are still some way from finding the molecular variants that explain the heritability of the trait. © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Quantitative trait locus; Anxiety; Rodent

1. Introduction

Over half a century of quantitative genetic research, using both animal and human subjects, leaves no room for doubt that genetic differences are an important factor in driving individual variation in anxiety [1]. However, finding the genetic variants that are responsible has proved to be far more difficult than most people expected, primarily because the genetic effects have turned out to be smaller than anticipated, and also, it is surmised, because they act in a more complicated fashion than was appreciated. In the majority of cases, since the phenotype is measured quantitatively, the genetic loci are referred to as quantitative trait loci or QTL, a convention I will adhere to in this review.

The problem of identifying small genetic effects has come to plague the analysis of almost all complex traits in humans, including those of medical importance such as depression and anxiety, but finding the location of such effects has not hampered progress in animal studies, where the use of crosses between inbred strains of mice simplifies the genetic analysis considerably. Considering only studies that have mapped genetic loci that influence behaviour, over the last seven years 45 publications have reported at least one statistically significant result [1]. In total, well over 200 QTL have been reported. Of these, at least 80 QTL are believed to influence one or more animal measures of anxiety (Table 1). The problem for those working with animals is not the detection of QTL, of which there is an excess, but to progress from gene location to gene [2].

My interest here is the genetic effects that influence variation in behaviour in animal models of human susceptibility to anxiety. Such models are not perfect. Indeed, some may be criticized for having little or nothing to do with anxiety on the grounds that the behavioural variation can be explained by variation in levels of activity rather than emotion [3]. Most of the anxiety models that can be applied to mice, the rodent geneticists prefer, depend on measuring a change in an animal's activity. For example, the commonly used elevated plus maze looks for differences in the number of entries an animal makes into exposed, presumably threatening, sections of the apparatus; a fast moving animal will tend to make more entries than a slow moving one. Of course, it is possible to apply corrections, such as the activity level in non-threatening environments, but nevertheless problems of interpretation still surround the use of the apparatus and others like it, which include the light–dark box and open-field arena. Tests of anxiety developed for work with rats, such as fear-potentiated startle, are more robust to such criticism of validity [4,5], but are more time consuming to carry out and may not be adaptable for use with mice. It should be noted that, because the genetic effects are small, a genetic mapping experiment requires hundreds of mice [6]. A conditioned test, such as the fear-potentiated startle, requires up to a week or more of training. Therefore, achieving adequate sample sizes to detect QTL for the more reliable measures of anxiety is demanding.

2. Genetic mapping of anxiety in rodents

Table 1 shows the published mapping results for measures of anxiety in rodents and it is dominated by the simple, activity-based tests. Only one report gives a result for using a conditioned test, two-way active avoidance, in the rat. Nevertheless, whatever one's view of the relevance of the phenotype, it is clear that the genetic effects discovered to date are many and small. Fifteen of the mouse's 19 chromosomes are implicated in influencing behaviour in at least one test and some chromosomal regions appear to influence almost a dozen different (but correlated) measures of anxiety. In the majority of cases the probability that the investigators have found a QTL is beyond reasonable doubt. A simple criterion for establishing significance for studies of the kind reported here (an F2 intercross) is whether the LOD score exceeds 4.3: Table 1 shows 39 results that exceed this stringent threshold. And in one case the results have been replicated [7].

The effect sizes, expressed as a percentage of the variation

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Table 1
QTL that influence anxiety in rodents

Measure	Species	Strains	Chr.	Pos.	LOD	Effect	Reference
Acoustic startle response	Mouse	NZB B/NJ	4	66	4.8	7.3	[26]
	Mouse	NZB B/NJ	4	51	3.9	10	[26]
	Mouse	NZB B/NJ	7	3	2.3	3.9	[26]
	Rat	RHA RLA	10	34	3.5	5.5	[13]
	Rat	RHA RLA	15	44	4.8	2.8	[13]
EPM % open arm entries	Mouse	DeFries High and Low	1	80	15.4	5.6	[12]
	Mouse	DeFries High and Low	4	46	3.2	1.2	[12]
	Mouse	A/J CBA/J	5	60	25	42	[8]
	Mouse	A/J CBA/J	5	60	12	51	[8]
	Mouse	DeFries High and Low	15	22	12.9	4.7	[12]
	Mouse	DeFries High and Low	18	22	7.9	2.9	[12]
	Rat	RHA RLA	5	80	4.1	2.6	[13]
	Rat	LEW SHR	6		2.8	6.5	[27]
EPM no. closed entries	Mouse	A/J CBA/J	7	Tyr	5		[8]
	Mouse	A/J CBA/J	7	Tyr	2.3		[8]
	Rat	LEW SHR	7		2.9	18.4	[27]
EPM no. open arm entries	Mouse	A/J CBA/J	5	60	28	41	[8]
	Mouse	A/J CBA/J	5	60	26	35	[8]
LD latency	Mouse	DeFries High and Low	1	80	6.8	2.5	[12]
	Mouse	DeFries High and Low	11	20	5.3	1.9	[12]
	Mouse	DeFries High and Low	14	20	8.7	3.1	[12]
	Mouse	DeFries High and Low	15	24	17.9	6.5	[12]
	Mouse	DeFries High and Low	18	34	6.5	2.3	[12]
LD transitions (3 days)	Mouse	A/J C57BL/6	1	67	2.5	2.5	[28]
	Mouse	A/J C57BL/6	6	10	2.9	3.5	[28]
	Mouse	A/J C57BL/6	10	74	9.5	8.4	[28]
	Mouse	A/J C57BL/6	15	42	2.5	2.5	[28]
	Mouse	A/J C57BL/6	19	21	4	3.9	[28]
LD transitions, Day 1	Mouse	A/J C57BL/6	10	74	8.9	7.9	[28]
LD transitions, Day 2	Mouse	A/J C57BL/6	19	21	2.5	2.5	[28]
Mirror chamber – latency	Mouse	DeFries High and Low	5	18	3.5	1.3	[12]
	Mouse	DeFries High and Low	12	60	3.2	1.2	[12]
	Mouse	DeFries High and Low	15	22	17.7	6.4	[12]
OF activity (5 min)	Mouse	DeFries High and Low	1	74	27.4	9.9	[12]
	Mouse	DeFries High and Low	4	36	3.5	1.3	[12]
	Mouse	DeFries High and Low	7	52	13	4.7	[12]
	Mouse	DeFries High and Low	12	40	3.6	1.3	[12]
	Mouse	DeFries High and Low	15	20	12.5	4.5	[12]
	Mouse	DeFries High and Low	18	32	5.4	2	[12]
	Mouse	DeFries High and Low	X	14	5.3	1.9	[12]
	Mouse	A/J C57BL/6	1	100	7.1	6.3	[29]
	Mouse	A/J C57BL/6	10	74	8.8	8.3	[29]
	Mouse	A/J C57BL/6	15	42	3.6	3.6	[29]
	Mouse	A/J C57BL/6	19	24	3.2	3.2	[29]
	Rat	RHA RLA	3	0	5	2.6	[13]
	Rat	RHA RLA	5	88	3.1	1.9	[13]
OF activity (last five min of the second 15-min trial)	Mouse	A/J C57BL/6	1	69	3.1	4	[29]
	Mouse	A/J C57BL/6	3	44	4.1	8	[29]
	Mouse	A/J C57BL/6	10	74	14.7	12.7	[29]
	Mouse	A/J C57BL/6	19	24	3.6	4.1	[29]
OF activity in centre	Rat	LEW SHR	4	30	10.4	61.3	[27]
	Rat	LEW SHR	5	30	3.7	3.7	[27]
OF centre time (day 1, first 5 min)	Mouse	A/J C57BL/6	1	73	7.7	6.8	[28]
	Mouse	A/J C57BL/6	19	19	2.5	2.8	[28]
OF defecation	Mouse	DeFries High and Low	1	74	14.5	5.2	[12]
	Mouse	DeFries High and Low	14	20	9.4	3.4	[12]
	Mouse	DeFries High and Low	X	50	5.4	1.9	[12]
	Rat	RHA RLA	3	8	3.2	2	[13]
	Rat	RHA RLA	6	50	3.4	8	[13]
	Rat	RHA RLA	19	56	3.3	3.2	[13]
	Rat	RHA RLA	X	64	6.2	3.7	[13]
OF vertical movement, T1	Mouse	A/J C57BL/6	1	79	4.5	5.9	[29]
	Mouse	A/J C57BL/6	10	74	8.5	7.6	[29]
	Mouse	A/J C57BL/6	19	24	3.8	4.2	[29]
OF vertical movement, T2	Mouse	A/J C57BL/6	1	102	5.8	5.4	[29]
	Mouse	A/J C57BL/6	8	26	3.1	3	[29]
	Mouse	A/J C57BL/6	10	74	6.1	5.4	[29]
	Mouse	A/J C57BL/6	11	54	3.3	5	[29]
	Mouse	A/J C57BL/6	19	24	4.7	5	[29]
Two-way active avoidance	Rat	RHA RLA	5	76	9.5	5.7	[13]
	Rat	RHA RLA	10	22	4.1	3	[13]

in the phenotype, are small; almost all are less than 10% of the total. The egregious exceptions are the studies by Ramos et al. and the study by Cohen et al. The latter studied elevated plus maze behaviour in a cross between CBA/J and A/J strains [8]. The authors find a QTL that explains about half the phenotypic variance on chromosome 5. However, this study has probably identified a known Mendelian mutation, which explains the large effect size. It is reasonable to assume that an animal's visual acuity is likely to influence its behaviour in the elevated plus maze. The cross Cohen and colleagues used for QTL mapping included one strain, CBA/J, that has a retinal degeneration mutation. The mutation is recessive and lies on chromosome 5, within the QTL peak reported by Cohen et al. [8]. While the data do not prove that the mutation underlies the QTL, the evidence points in that direction.

A QTL with an unusually large effect size is also reported by Ramos and colleagues [9]. In a cross between two rat strains (LEW and SHR) they find a QTL on chromosome 4 that accounts for 61.3% of the variance of inner locomotion in an open-field arena. Two features of this finding are odd. First, the QTL analysis detects an allele in the LEW strain that increases activity, yet the LEW strain actually has a lower activity than the SHR strain. If the direction of effect and effect size are correct, then there must be QTL alleles operating in the opposite direction whose total effects exceed that of the detected QTL. This is impossible if the single QTL effect size already explains more than half the total variance, unless, in the inbred strain, there happens to be a particular conformation of alleles that obscures the effect. Second, they find that the effect is not just sex specific but also depends on the strain origin: LEW grandmother. The LOD score for the 48 female animals with a LEW grandmother is 7.2, but no other strain combination exceeds a LOD of 2. This is incompatible with the LOD score of 10.4 for all females (there must be an effect from the other animals to increase the LOD). These two observations suggest we should treat this result with caution. Overall, therefore, effect sizes are small.

3. Genetic validation of emotionality

We can conclude that there is substantial evidence for the presence of multiple QTL that influence behaviour in animal models of anxiety. But it is possible to use genetic approaches to go one step further and examine the validity of the animal models, asking whether the genetic evidence supports the predictions of psychological theory [10]. We have tackled this question in two separate experiments, using mice and rats, to see if variation in susceptibility to anxiety, or emotionality as it is often called in the rodent literature [11], is a unitary construct.

We have mapped phenotypes believed to reflect, in both mice [7,12] and rats [13], different aspects of susceptibility to anxiety, and asked to what extent the action of the QTL matches the expectation that a single psychological process underlies emotionality. In the mouse study we used five tests of emotionality to phenotype over 1600 F2 animals. We found that QTL on chromosomes 1, 4, 15 and 18 influenced at least one measure obtained in all five tests, as would be expected if they represented the genetic basis of emotionality [12].

We also mapped QTL influencing fearful behaviour in an F2 cross of over 800 Roman High and Low avoidance rats (RHA and RLA respectively). These rats are the product of bi-directional selection for two-way active avoidance acquisition in a shuttle box [14], and the behavioural differences of the two strains are consistent with an inter-strain variation in responses to fear stimuli. Again we used a large battery of behavioural measures: measures of conditioned fear (both contextual and cued), open-field and elevated plus maze behaviours, acoustic startle response and spontaneous activity as well as two-way active avoidance acquisition. We detected three QTL chromosomes, 5, 10 and 15, that influenced more than one behavioural measure of emotionality [13].

We had to decide whether any of the QTL acted on emotionality in a manner consistent with what is known about the psychological processes underlying anxiety. There were two predictions that could be made: 1) the QTL should act consistently across different tests of anxiety; 2) and an allele of the QTL that has a supposedly anxiolytic effect in one test (for example, by increasing entries into the open arms of an elevated plus maze) should act consistently in other tests (for example, by increasing avoidance responses in the shuttle box).

By categorizing QTL on the basis of the direction that each allele works and their pattern of action across tests, we can define how a QTL operates. In the mouse study a QTL on chromosome 4 influences locomotor activity, not emotionality: it acts on activity in the home cage (a non-threatening environment) and has no effect on the time spent in anxiogenic regions of the elevated plus maze (the open arms). By contrast, QTL on mouse chromosomes 1 and 15 did not influence spontaneous activity but did have effects on time spent in the open arms of the maze, in the light compartment of the light–dark box and in the centre of the open field, all of which are anxiogenic regions [15–18]. These QTL, on chromosomes 1 and 15, are thus candidates for loci that influence emotionality.

In the rat mapping experiment multivariate analysis indicated that three loci (on chromosomes 5, 10 and 15) had broad effects across different test measures. The QTL on chromosome 5 matched the pattern of activity of a gene influencing an animal's reaction to a fear stimulus, and parallels the effects of drugs used to treat anxiety disorders in humans [19,20]. At this locus, on rat chromosome 5, the allele that increased avoidances in the shuttle box also decreased cue and contextual fear conditioning, while increasing time in the open arms of the elevated plus maze and activity in the open field.

As I have explained above, QTL analysis has the power to detect remarkably small effects. I have now argued that it can determine whether different phenotypes are under the control

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Table 1. Measure: behavioural tests used to measure anxiety. OF, open-field arena; EPM, elevated plus maze; LD, light–dark box. Strains: the inbred strains used in the QTL mapping experiment. Chr. and Pos.: gives the position in centiMorgans for the QTL. Tyr: refers to the tyrosinase gene (*c* locus) on mouse chromosome seven. LOD: the likelihood measure of the presence of a QTL on the chromosome. Effect: percentage of the phenotypic variances explained by the QTL.

of the same QTL and, at least at one level, establish that susceptibility to anxiety (emotionality) is a unitary construct as predicted by some theories of anxiety.

4. Gene identification

Successful genetic mapping is powerful because it is carried out in inbred strains where genetic variation has been considerably reduced: the effects of any one locus are relatively increased compared to their effect in a more complex population of animals where many other loci may be contributing to the phenotype and there may even be multiple alleles at each QTL. But the power to detect a small genetic effect is bought at the price of poor mapping resolution. For a QTL explaining 4% of the phenotypic variance, the 95% confidence interval will be about 40 centiMorgans when mapped in an F2 with 400 animals [21]. In other words, most of the studies reported in Table 1 locate a QTL to within half a chromosome. We might reasonably ask if this is much of an advance on earlier quantitative genetic studies that gave estimates of the heritability of each phenotype. Genetic mapping has so far done nothing to uncover the molecular basis of individual variation in tests of anxiety.

There are two strategies which may accelerate fine-mapping and gene identification. The first is to start using outbred animals for mapping. We have shown that the use of genetically heterogeneous mice, derived from known inbred progenitor strains and randomly intercrossed for more than 30 generations, can be used to map small effect QTL to under a centiMorgan [22]. We have also shown theoretically that a cross between an inbred strain and heterogeneous mice can be used both to screen the genome and to fine-map QTL, so that the burden of genotyping is reduced to a point where most laboratories should be able to localize a QTL to within a couple of centiMorgans [23].

The second strategy is to use quantitative complementation, as pioneered in Drosophila [24]. The idea behind the quantitative complementation test is that crosses between a strain carrying a recessive mutation in the gene influenced by the QTL and different inbred strains will uncover alleles that have different quantitative effects on the trait. A cross is set up between a strain bearing a QTL that increases the phenotype (I) and a mutant. The phenotypes of the F1 progeny are compared with the F1 offspring of I and the wildtype. An analogous pair of crosses is established, replacing the I strain with a strain bearing a different QTL allele, one that decreases the phenotype. The QTL is detected by a test for an interaction between mutant and strain, using an analysis of variance. No one has yet carried out quantitative complementation in mice, but John Schimenti has reported the use of deletions to refine the localization of QTL influencing sterility and segregation distortion associated with mouse t haplotypes [25].

Progress in mapping and sequencing the mouse and human genomes, the development of better methods of high resolution mapping, together with better functional tests of a QTL (such as gene expression analysis) are opening the doors to finding the molecular variants that govern variation in complex traits such as anxiety. Fine-mapping experiments can now uncover a series of candidate genes that might influence a phenotype. The challenge over the next few years is to confirm those candidates and move into the next phase of the molecular dissection of anxiety: functional characterization of the genes involved.

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