Quantitative Trait Loci Modulating Corpus Callosum Size in the Mouse Brain

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Abstract

The corpus callosum is the brain's primary pathway for interhemispheric communication between the left and right hemispheres, and abnormal function and anatomy of the corpus callosum have been associated with a variety of disorders in humans and animals. In this study, we mapped quantitative trait loci (QTLs) modulating corpus callosum size in mice. By using mouse brain sections and stereological techniques, we estimated the midsagittal corpus callosum size in 191 BXD recombinant inbred mice and their parent strains (C57BL/6J and DBA/2J). Analysis showed midsagittal corpus callosum size to be a heritable trait ($h^2 = .42$). We detected a suggestive QTL modulating corpus callosum size near the marker S10Gnf071.990 on chromosome 10 at approximately 73 Mb, in close proximity to a previously defined QTL modulating striatal volume. We also found correlations between corpus callosum size and other behavioral and anatomical phenotypes. Overall, our results implicate a genetic role in modulation of corpus callosum size.

1 Introduction

The corpus callosum is comprised of bundles of nerve fibers that connect the neurons of the left and right hemispheres of the brain, thereby enabling interhemispheric communication. This commissure has been noted as being the most important pathway for the transfer of sensory, motor, and higher-order information between the two cerebral cortices of the brain, thereby coordinating interhemispheric integration of perceptual, mnemonic, learned, and volitional information [43]. Split-brain patients, who have their corpora callosa severed in an attempt to alleviate intractible epilepsy, provide a direct illustration of this. After their surgery, the two hemispheres of the brain cannot communicate directly with each other, and therefore response to stimuli directed to one hemisphere might differ from that directed at the other. For example, split-brain patients can name objects shown in the right visual field, which projects to the verbal left hemisphere. In contrast, they are unable to name objects shown in the left visual field (which projects to the nonverbal right hemisphere) but can identify them with their left hand [43]. It is interesting to note, however, that in patients with damaged, partially removed, or completely removed corpora callosa, behavior and everyday functions remain largely intact, and such patients show few outward signs of their condition [43]. Furthermore, it should be noted that lateralization, or specialization of brain functions by the two hemispheres, varies widely among individual brains. A function normally associated with one hemisphere may be controlled by the other hemisphere in any particular individual [1].

In humans, the corpus callosum consists of over 200 million nerve fibers aligned in a broad, thick band running from rostral to caudal and medial to lateral [1]. Nerve fibers mostly project to corresponding cortical areas (homotopic callosal connections) while some project to noncorresponding areas in the contralateral hemisphere (heterotopic callosal connections) [43]. Typically, the morphology of the corpus callosum is examined in the mid-

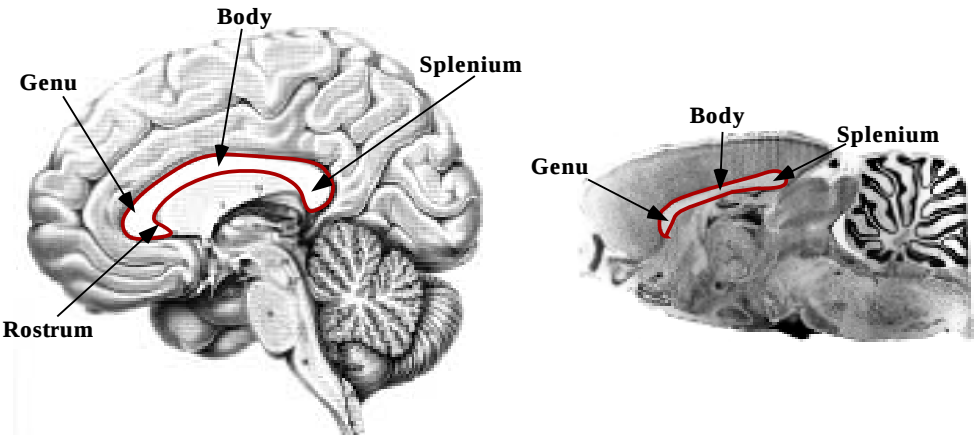

Figure 1: The corpus callosum in a human brain (left) and a mouse brain (right). Rostral is to the left and caudal is to the right.

sagittal plane, and is divided into four parts: the rostrum, body, genu, and splemium (see Figure 1). Investigators have measured the area [8] [13], length [8] [13], and shape [5] [44] of the midsagittal corpus callosum, and have found that these parameters are associated with a variety of disorders. Studies have found that patients with Attention Deficit Hyperactivity Disorder have significantly smaller rostra than normal individuals [19], and callosal development and connectivity occurs abnormally in schizophrenics, as the corpus callosum develops bidirectionally [30] and causes confusion over interhemispheric transmissions [11]. Dyslexics have a smaller callosal genu [22] and larger splenium and total callosal area when compared to nondylexics [16] [35]. Finally, patients suffering from Alzheimer's disease have significantly reduced callosal bodies [44].

The midsagittal area of the corpus callosum has been shown to be a reliable predictor of a number of biological traits. It is clear that certain factors, including sex and handedness, seem to account for some variation in corpus callosum size. Women appear to have larger corpus callosum to forebrain size ratios than men [25], and increased strength of handedness seems to suggest increased lateralization [25], although much debate remains about the role of these two factors and others in accounting for variations in corpus callosum size [7].

Within animal species, the morphology and function of the corpus callosum is very similar to that of the human. The primary role of the corpus callosum in mammals appears to be interhemispheric communication and coordination of responses, and much variation lies in the structure of the corpus callosum. Surgical interruption of corpus callosum function in animals has established the role of the corpus callosum in interhemispheric communication. For example, severing parts of the corpus callosum inhibits transfer of visual information in cats [34] and monkeys [4], of depth perception in cats [38], and of other sensory and cognitive functions [32]. Furthermore, as in the human, animal corpora callosa contain unevenly distributed nerve fibers in different callosal regions [23] [29], and most connections are homotopic while a smaller number are heterotopic [24] [42].

Investigators have reported that strains of mice differ in midsagittal corpus callosum area, suggesting a genetic role, and that genetic factors could underly pathological and normal variation in corpus callosum size [2] [26]. Wahlsten et al. looked at 21 strains and found a twofold difference in corpus callosum size between strains of BTBR crossed with BALB/cWah1, 129P1/Rej, and 9XCA/Wah mice [47]. Rosen et al. found a difference in corpus callosum size of a similar dimension with NZB/BINJ mice having larger corpora callosa than DBA/2J mice [41]. Le Roy et al. identified quantitative trait loci (QTL) linked to midsagittal corpus callosum area in an F2 cross between NZB/BINJ and C57BL/6By strains, and discovered QTLs on chromosomes 1 and 4 and a significant interaction between these two loci [31].

Recent research has found that changes in the size of brain structures in mouse such as the striatum [40] and cerebral ventricles [51] appear to be genetically modulated. In the latter study, recombiant inbred (RI) strains were used, which provide significant advantages in mapping precision when compared to the study of F2 mice [49]. Recombinant inbred strains are isogenic strains of mice derived from two parents through many generations of inbreeding. After sufficient matings, a set of strains are created such that they have different

homozygous alleles from the two parents at different locations in the genome. This allows for many possible phenotypic variations to be examined. In this experiment, we wished to confirm and extend the QTL findings of Le Roy [31] using alternate genetic and bioinformatic techiques. The objective of this study, therefore, is to map quantitative trait loci responsible for variation in the size of the corpus callosum in the mouse brain and to identify possible genes that could modulate corpus callosum size. Specifically we measured the midsagittal area of the corpus callosum in 34 BXD RI lines and their parent strains and mapped this phenotype against genome-wide markers.

2 Materials and Methods

2.1 Protocol

The midsaggital area of the corpus callosum was estimated from coronally cut sections of mouse brains from 191 mice representing 34 strains of recombinant inbred (RI) mice and their parent strains (C57BL/6J and DBA/2J). These traits were then mapped to 720 genome-wide polymorphic markers. Midsagittal corpus callosum area was also correlated against approximately 430 known behavioral, anatomic, and electrophysiologic phenotypes as well as the expression levels of over 12,000 RNA transcripts using WebQTL (`http://webqtl.org`).

2.2 Subjects

We used 191 mice from 34 BXD RI strains as well as the two parent strains. There were, on average, about five mice per RI strain. All data for this study were obtained from the histological brain sections of the Mouse Brain Library (`http://mbl.org`). Mice were obtained from the Jackson Laboratory (Bar Harbor, ME) and prepared at BIDMC, Boston, MA as detailed previously [40]. All procedures were approved by that institution's animal

care and use committee and conform to NIH guidelines for the humane treatment of animals. Briefly, subjects were deeply anesthetized with Avertin (0.8 mL i.p.) and transcardially perfused with saline, followed by fixitive (glutaraldehyde/paraformaldehyde), and their brains removed and weighed. After variable postfixation times, the brains were embedded in 12% celloidin and sliced in the coronal plane at 30 μm. Two series of every tenth section, representing a one-in-five sample of the brain, were stained with cresyl violet and mounted onto glass slides. These series were offset by five sections wih the result being that we sampled every fifth section throught the length of the corpus callosum.

2.3 Midsagittal area of the corpus callosum

The midsagittal area of the corpus callosum in each coronally sectioned mouse brain was estimated by measuring the dorsal-ventral length of the structure at the midsagittal region using StereoInvestigator (Microbrightfield Inc., Colchester, VT; see Figure 2). All measurements were made under 10X magnification with a Nikon Eclipse E800 microscope (Nikon Inc.). Wherever the corpus callosum appeared on a section, a reference line was drawn along the dorsal-ventral axis of the brain section by beginning a contour line at a point superior to the corpus callosum and ending the line at a point inferior to its ventral border. The length of the corpus callosum lying on that line was measured to within $\pm$ 2.5 μm for each section on which the corpus callosum appeared (5–11 sections per slide) and all measurements were recorded. The midsagittal area of the corpus callosum was then estimated for each slide using Cavalieri's rule, a method of estimating area and volume from serial section measurements (see Equation 1) [39]. In the cases where two reliable estimates of midsagittal area were available for each mouse brain (N = 160), they were averaged. In cases where there were missing sections from one of the slides (N = 31), the missing slide was removed from the analysis.

$$A = d(\sum_{i=1}^{n}(y_i)) - (t)y_{MAX} \quad (1)$$

where A is the estimated area, d is the distance between sections, t is the section thickness, y_i is the midsagittal corpus callosum measurement, and y_{MAX} is the maximum measurement.

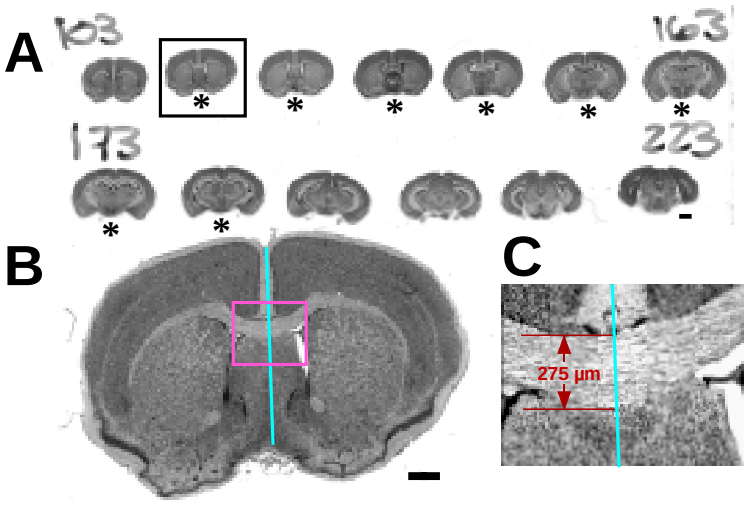

Figure 2: Panel A. Sections of coronally cut mouse brains on a microscope slide. Sections 113 through 183 (asterisked) contain visible corpora callosa. Scale = 1 mm. Panel B. One mouse brain section from Panel A. The corpus callosum is visible and runs from medial to lateral. The blue line is a reference line drawn along the dorsal-ventral axis. Scale = 500 μm. Panel C. The corpus callosum from rectangular area of Panel B. The midsagittal corpus callosal length along the reference line is measured in μm.

2.4 Analysis and genetic mapping

Intraobserver reliability was calculated by remeasuring ten randomly selected slides while blinded to their identity and calculating a Pearson product–moment correlation coefficient. The morphometric data were analyzed using ANOVA and multiple regression techniques (JMP, SAS Institute, Cary, NC). QTL analysis was carried out with WebQTL using a set of 720 markers distributed across all chromosomes in the BXD RI set. A likelihood ratio

statistic (LRS) for linkage was computed, and significance was determined by permutation tests and bootstrap analysis [6] [46]. Using WebQTL, we correlated our anatomic phenotypes to a database of 430 published phenotypes of BXD RI sets (Published Phenotypes Database, WebQTL). Trait data was also compared using WebQTL to a gene expression database of over 12,000 forebrain RNA transcripts (UTHSC Brain mRNA U74Av2 Database).

2.5 Determining candidate genes

Genes or QTLs within three cM of any suggestive or significant QTL found in this study were examined using a bioinformatics database (Mouse Genome Informatics, `http://www.jax.org`). Any gene or locus having been connected to activity in the brain is examined, the literature on that gene reviewed, and its significance analyzed subjectively.

3 Results

3.1 Summary and reliability of measurements

To assess the reliabiliy of the data, the midsaggital corpus callosum areas of 10 randomly selected slides were blindly remeasured by the same observer. The test-retest reliability coefficient for midsagittal corpus callosum area was 0.99. The average percentage difference between original and remeasured midsagittal areas was $\pm$ 0.6% μm^2.

The measured midsagittal corpus callosum areas of 191 subjects representing 34 strains and the two parent strains were included in the data analysis. 103 subjects were male and 88 were female. Ages ranged from 31 days to 493 days with an average of 93.4 $\pm$ 6.3 days and a median of 60 days. The midsagittal areas of the corpora callosa ranged from $2.26 * 10^5$ μm^2 to $7.57 * 10^5$ μm^2. Strain averages (mean $\pm$ SEM) ranged from 2.66 $\pm$ $0.30 * 10^5$ μm^2 (BXD38) to 5.68 $\pm$ $0.44 * 10^5$ μm^2 (BXD15; see Figure 3). The grand mean of midsaggittal

area of all 34 strains was $4.16 \pm 0.07 * 10^5$ μm^2 and the median was $4.08 * 10^5$ μm^2.

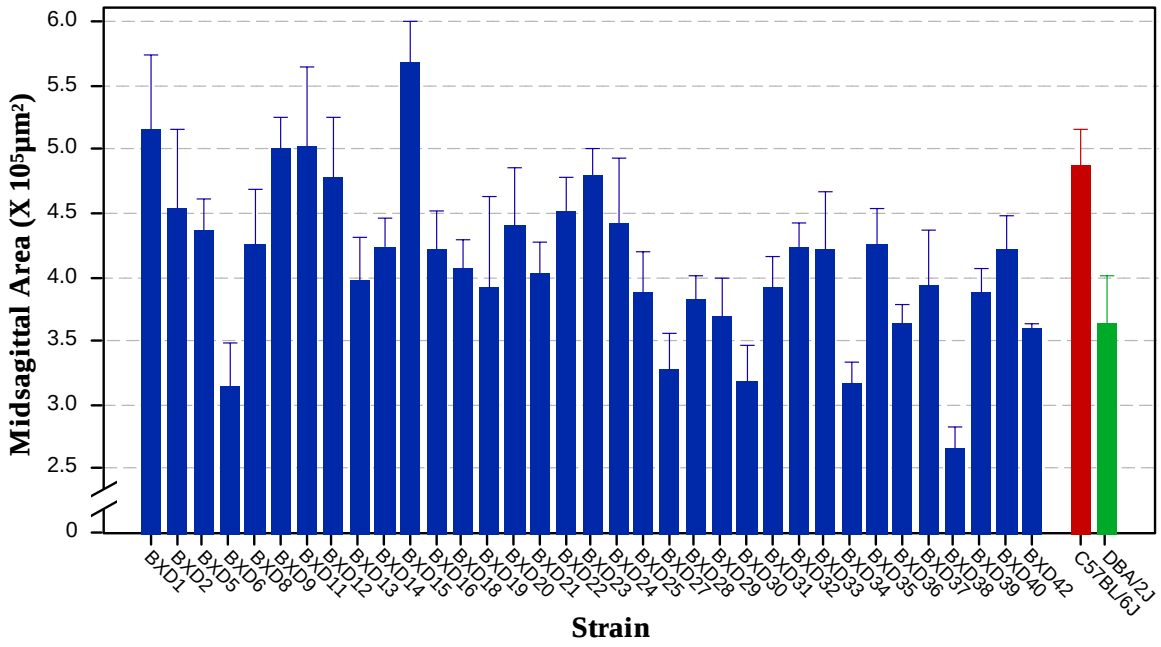

Figure 3: Histogram of mean midsagittal corpus callosum area of BXD RI strains. Parent strains are on the right.

3.2 Heritability of midsagittal corpus callosum size

ANOVA with Strain as the independent variable and the midsagittal corpus callosum area as the dependent variable indicated a significant effect of Strain ($F_{33,138} = 2.75$, P $< .001$). Regression analysis using Age, Sex, and Brain Weight as predictors of midsagittal area of the corpus callosum was significant ($F_{3,168} = 23.6$, P $< .001$), with both Age (t $= 3.44$, P $< .001$) and Brain Weight (t=5.92, P $< .001$) contributing significantly to the regression. Because these variables significantly predict midsagittal area of the corpus callosum, we extracted the residuals from this equation to begin our genetic mapping. This serves to remove the effects of Age, Brain Weight, and Sex from the phenotype. Strain remained a significant predictor of midsagittal corpus callosum area ($F_{1,32} = 8.69$, P $< .01$) when using residuals

as the dependent measure. The average within-strain and between-strain variances were calculated, yielding a heritability factor (h^2) of .42 [21].

3.3 Interval mapping shows a suggestive QTL

Linkage was computed with the residual midsagittal area of the corpus callosum (RMSACC) as the phenotype using WebQTL, which has over 720 polymorphic markers throughout the genome. WebQTL computes a Likelihood Ratio Statistic (LRS) score, which reflects the chance that the phenotype is related to specific genetic marker. This LRS score is mathematically identical to the more common Logarithmic Odd (LOD) score, with the LRS equal to LOD/4.6.

Using WebQTL to compute linkage with RMSACC as the phenotype, we found a suggestive QTL near marker S10Gnf071.990 (LRS = 12.4) on chromosome 10 at 73.28 Mb. Composite interval mapping (controlling for this QTL interval) revealed no additional significant QTL. ANOVA using Genotypes ("B" or "D") of the strains at the chromosome 10 marker as the independent variable and midsagittal corpus callosum size as the dependent variable showed a significant effect of Genotype ($F_{1,32} = 8.70$, $P < .01$). Strains with a "B" allele in this interval had larger residuals and thus larger midsaggital corpus callosum sizes than those with a "D" allele (mean RMSACC $\pm$ SEM = $2.48 \pm 1.04 * 10^5$ μm^2 vs. -2.11 $\pm$ $1.17 * 10^5$ μm^2, respectively).

3.4 Analysis of correlations with published phenotypes and gene expression levels

RMSACC values were compared to all values in the Published Phenotypes database using WebQTL and correlations were computed. Several interesting correlations were found (see Table 1). In particular, RMSACC seems to be negatively correlated with total hippocampal

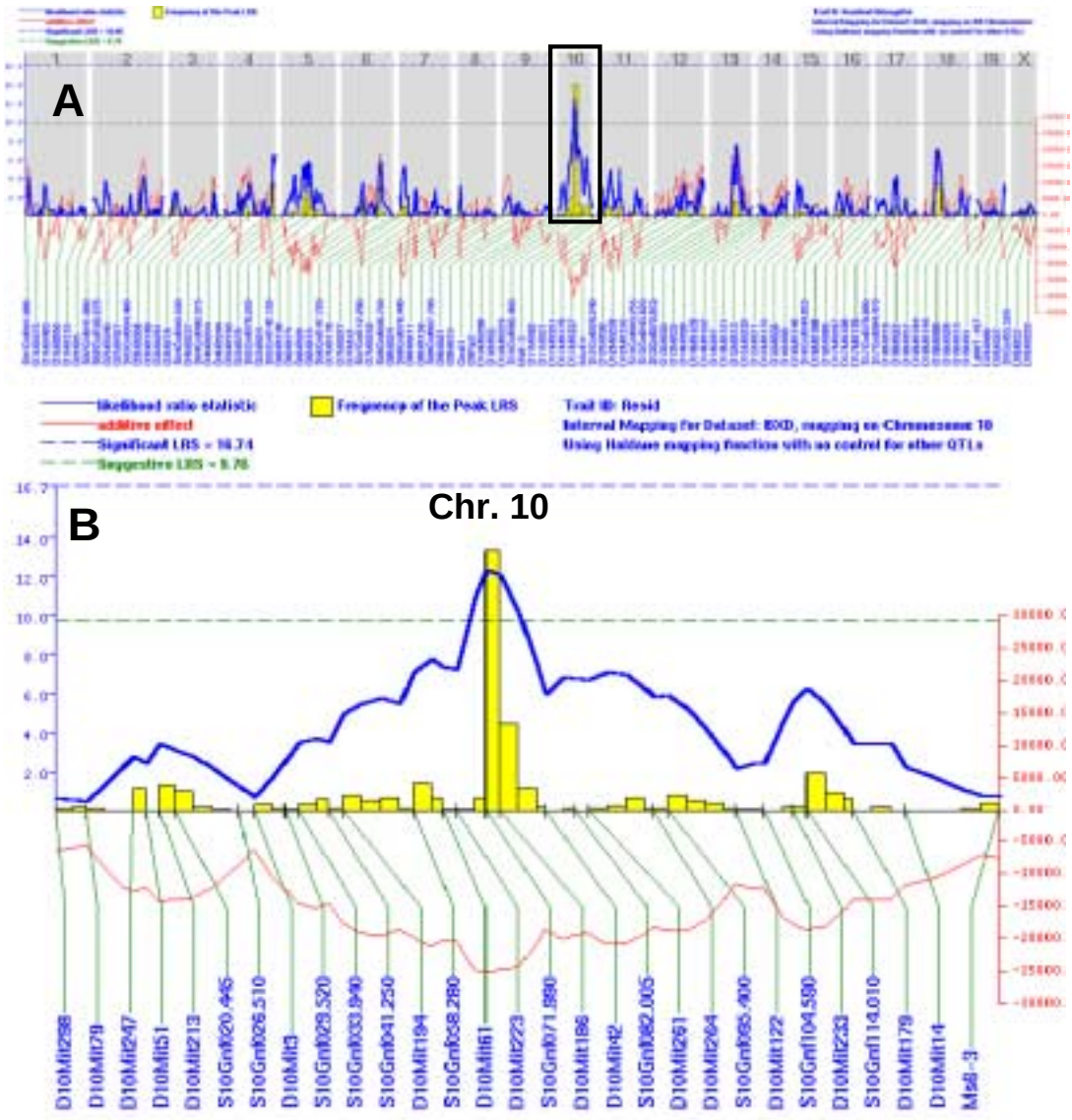

Figure 4: Panel A. LRS scores and bootstrap analysis for RMSACC across the entire genome. The solid blue line represents the LRS scores as determined by marker regression at each of the markers along the x-axis. The yellow histogram represents the results of the bootstrap analysis. The red line is the additive effect. Panel B. An expanded view of chromosome 10 from Panel A containing the suggestive QTL. The dotted line at LRS = 9.8 demonstrates level for suggestive QTL.

granule cell growth in a study involving introducing adult BXD RI mice to a maze. (r = -.68, P < .02; see Figure 5) [28]. There were no significant correlations between RMSACC and RNA transcript levels.

#	Phenotype	Ref	Correlation	# Strains	P Value
1	Total Hippocampus Granule Cell Number	[28]	-.6924	11	.01840
2	% Freezing in Response to Auditory Stimulus US in Contextualized Fear Conditioning Paradigm	[36]	.4825	21	.0226
3	Saline Open Field Activity	[9]	-.4935	29	.0258
4	Saccharin Consumption	[3]	-.4831	20	.0298
5	Change in Body Temperature due to 4 mg/kg Methamphetamine	[20]	.4227	25	.0344
6	Ethanol Induced Conditioned Place Preference – Percent Time on Drug paired Floor During First 30 Minutes of Test	[10]	.4670	20	.0369
7	Maximal Threshold to Ethanol Induced Ataxia	[18]	.4171	25	.0372
8	Mean Life Span, Longevity in days	[12]	.4396	22	.0398
9	Open-Field Activity Following Saline Injection	[37]	-.4526	20	.0442

Table 1: Correlations and P Values of RMSACC trait data versus values in the Published Phenotypes Database. The nine significant results are shown.

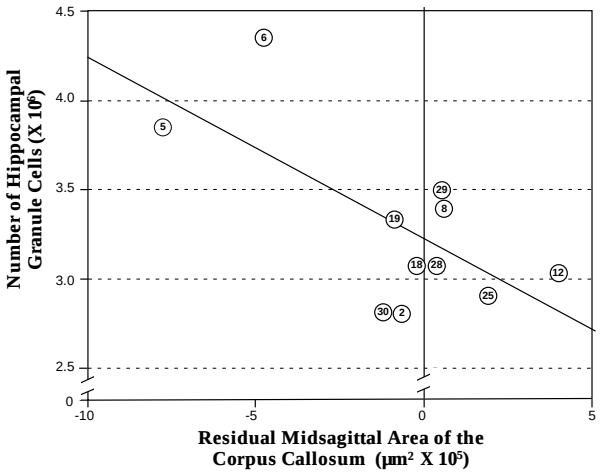

Figure 5: Scatter plot of RMSACC trait data versus hippocampal granule cell number in BXD mice. There is a high negative correlation between the two variables.

3.5 Possible candidate genes

One candidate QTL, Bsc10a (brain size control 10a) was found on chromosome 10 at 40 cM from the centromere, the exact area of the suggestive QTL we found. A previous study implicated this gene in modulation of mouse striatum volume and brain weight [40].

4 Discussion

Using measurements of midsagittal corpus callosum area from 34 BXD RI strains of mice and their parent strains, we found that corpus callosum size is a heritable trait, and mapped a suggestive QTL on chromosome 10 for corpus callosum size. Further examination showed a strong negative correlation between corpus callosum size and growth of adult hippocampal granule cells in BXD RI strains, as well as the presence of a QTL, Bsc10a, previously shown to modulate striatal volume and brain size independently, at the locus we found.

4.1 Variation in corpus callosum size

There is much variation in corpus callosum size in both humans and animals. The size of the corpus callosum is dependent on a variety of factors, such as the size of surrounding brain structures and diameters of nerve fibers, that are in part modulated genetically. Furthermore, we found age and brain weight to account for a significant portion of normal variation in corpus callosum area. Variation can also be caused by environmental factors or genetic heritability or an interaction between both genes and environment. It should be kept in mind that genetic factors account for only a portion of the variability in corpus callosum size, and that the determination of heritability of corpus callosum size and the mapping of QTLs assume that we are mapping a trait that could be indirectly influenced by other nongenetic traits.

4.2 Reliability of estimation of midsagittal corpus callosum area

Measuring midsagittal corpus callosum area has mostly been accomplished by examination of brain sections cut in the sagittal plane. In our experiment we did not have access to brains cut in this plane and were therefore forced to estimate midsagittal corpus callosum area from coronal sections, which could lead to some possible sources of error. Nevertheless, reliability was very high (.99), and is unlikely to contribute much variation to the data. Similarly, Cavalieri's Rule, the method used to approximate midsaggital area, is an accurate method of estimation for our purposes [39]. Although judgement of the experimenter in measurements under microscopy and use of Cavalieri's Rule may result in some discrepancies from the actual midsagittal corpus callosum area, the consistency of the measurements provides reliability in intrastrain and interstrain comparisons. Furthermore, the ratio of midsagittial corpus callosum areas of the parent strains in this study is very similar to the ratio of the same parent strains computed from another study [48]. We are therefore confident that our data represent a reliable and valid estimate of midsagittal corpus callosum area.

4.3 Heritability of Corpus Callosum Size

We found that corpus callosum size is a heritable trait in mice. This is consistent with other studies that have demonstrated interstrain differences in corpus callosum size [31] [41]. Corpus callosum size differs markedly in the BXD strains studied, including twofold differences (see strain BXD 15vs. BXD38) between strains. ANOVA confirmed that strain is a major determinant of corpus callosum size in the BXD RI set and contributes significantly to variation in this structure. We estimated that narrow-sense heritability is approximatly .42. This is relatively average for a morphometric trait and is similar to calculated heritabilities for ventricle size [51] and olfactory bulb size [50] in BXD RI strains.

4.4 Mapping showed suggestive QTL on chromomsome 10

Linkage analysis showed a suggestive corpus callosum size QTL on chromosome 10. Examination of the additive effect at the vicinity of the locus on chromosome 10 shows that the presence of a "B" allele from the C57BL/6J parents appeared to increase midsaggital corpus callosum area. Examination of the parent strains for the BXD RI set confirm that DBA/2J mice have small corpora callosa, whereas C57BL/6J mice have appreciably larger corpora callosa. These results strongly suggest that the C57BL/6J parent contributes to an increase in corpus callosum size. The fact that the "B" allele accounts for most of the additive effect to the trait value at chromosome 10 and the LRS score of 12.4 indicates that it can be likely that there is a gene at or near the QTL on chromosome 10 that modulates corpus callosum size.

In 1998, Le Roy et al. also studied QTLs implicated in midsagittal corpus callosum area and found in F2 generation mice from C57BL/By and NZB/BINJ parent strains two loci on chromosomes 1 and 4 that interacted significantly but no QTL on chromosome 10 [31]. Our study did not find any suggestive or significant QTLs on chromosomes 1 and 4 and thus we cannot confirm the results of the prior study. This may be due to the fact that we used different parent strains, studied RI, not F2, mice, performed analysis with different sets of genetic markers, and used different methods of analysis, or purely due to experimental variation.

4.5 Correlation with adult hippocampal granule cell growth

We considered the RMSACC as a phenotype and compared it to all values in the Published Phenotypes database to see whether there was a linkage or correlation with any other phenotype(s). The only notable correlation between between corpus callosum size and a phenotype was with total hippocampal granule cell number from a study that measured the number

of granule cell counts in BXD RI mice after they completed several trials of a water maze [28]. It has been suggested that hippocampal granule cells are generated in adulthood for the purpose of memory of and adaptation to new knowledge and new levels of complexity [27] [17]. Interestingly, the corpus callosum has been implicated in studies involving memory, specifically interhemispheric transfer of memory information [15] [14]. The high negative correlation between corpus callosum size and growth of hippocampal granule cells could indicate that that increased numbers of fibers in the corpus callosum may offset some of the need to grow new adult granule cells in order to enhance memory of and adaptation to new knowledge or skill. However, one must always be careful of overinterpreting correlational data. A more direct test of this correlation could be further examined by monitoring hippocampal granule cell growth versus original corpus callosum size after adult mice of BXD RI strains are introduced to an intellectual task that requires memory and use of new knowledge.

4.6 Bsc10a as a candidate QTL

The only previously reported locus implicated with brain anatomy or function between 37 and 43 cM on chromosome 10 was Bsc10a (brain size control 10a) at 40.0 cM from the centromere. In a previous study, Bsc10a was found to have a significant independent effect on brain size as well as a significant effect on striatal brain volume [40]. In our study, marker regression analysis was performed using only RMSACC scores after brain weight had been factored out, but the QTL on chromosome 10, which lies in close proximity to Bsc10a, still registered up as a suggestive loci. Thus Bsc10a may have an independent effect on corpus callosum size, especially since Bsc10a is the only QTL in proximity to our QTL. It is possible that Bsc10a modulates corpus callosum size in addition to brain volume, since Bsc10a has already been implicated in the size of another brain structure, the striatum.

5 Conclusion

The corpus callosum is the most important and largest commisure connecting the two brain hemispheres, and is crucial in organizing interhemispheric communication involving sensory, motor, and higher-order information. While changes in the corpus callosum structure and function have been recognized to be associated with several clinical manifestations and behavioral abnormalities, etiologies for the changes in corpus callosum structure and function are not known. In this study, we examined the genetic modulation of normal variation in the midsagittal area of the corpus callosum. Our findings confirmed that corpus callosum size is a highly heritable trait in mice. Furthermore, our QTL analysis identified a suggestive QTL on chromosome 10 where Bsc10a, a gene implicated with modulation of brain size and striatal volume, lies. Moreover, we found a correlation between corpus callosum size and growth of adult hippocampal granule cells. These findings support our hypothesis that variation in midsagittal area of the corpus callosum may be modulated by genes associated with brain size and function.

QTL analysis of small sets of RI strains is somewhat conditional. To expand on this study, statistical confidence could be improved by increasing precision of phenotypes, using larger RI sets, or generating RIX progeny [45], a set of RI F1 hybrids whose genetics allow further refinement of position and effects of QTLs.

This study has contributed significant new knowledge to the search for genetic modulation of corpus callosum size. Hopefully, through new research building on the methods and results of this study, scientists will be able to isolate the primary genetic contributors to variation in mouse corpus callosum size and extrapolate that knowledge to humans. Thus, the significance of this study is to eventually promote genetic measures to prevent diseases of or related to the corpus callosum through medical technologies such as gene therapy and stem cell transplantation.

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