

# Quantitative Trait Loci Modulate Ventricular Size in the Mouse Brain

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## ABSTRACT

Cerebral ventricular size in humans varies significantly. Abnormal enlargement of the ventricles has been associated with schizophrenia, and hydrocephalus can lead to serious cognitive and motor deficiencies in humans and animals. In this study, we mapped quantitative trait loci (QTLs) modulating cerebroventricular size in mice. We hypothesized that genes underlying hydrocephalus might also modulate normal variation in ventricular size. By using digital images of mouse brain sections and stereological techniques, we estimated the volume of the combined lateral and third ventricles, as well as the volume of the entire brain, in 228 AXB and BXA recombinant inbred mice and their parent strains (A/J and C57BL/6J). Ventricle size, expressed as percentage of brain volume, is a heritable trait ( $h^2 = 0.32$ ). We detected a major QTL controlling variance in volume on chromosome (Chr) 8 near the markers *D8Mit94* and *D8Mit189*. We also detected a strong epistatic interaction affecting ventricular volume between loci on Chr 4 (near *D4Mit237* and *D4Mit214*) and on Chr 7 (*D7Mit178* and *D7Mit191*). These three QTLs, labeled *Vent8a*, *Vent4b*, and *Vent7c*, are close to genes that have been previously implicated in hydrocephalus. *J. Comp. Neurol.* 461: 362–369, 2003. © 2003 Wiley-Liss, Inc.

**Indexing terms:** QTL; ventricle; recombinant inbred; stereology

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There is a large amount of variability in cerebral ventricular size in humans, a significant fraction of which can be accounted for by age and other environmental variables (Giedd et al., 1996; Lange et al., 1997; Baaré et al., 2001). In a study of mono- and dizygotic twins, Baaré and colleagues (2001) suggest, in fact, that there is no heritability of cerebral ventricular volume, and that most variance of this trait can be accounted for by common and unique environmental factors. This is in contrast to the report of Reveley and colleagues (1984), who found that a significant portion of the variance is accounted for by common gene polymorphisms. In their population, the heritability of variation in ventricular volume was close to 85%.

Enlargement of the cerebral ventricles has been associated with schizophrenia (Jeste et al., 1982; Suddath et al., 1990; Marsh et al., 1994), and there is evidence to suggest a genetic linkage between these two phenotypes (Shihabuddin et al., 1996). When ventricles become pathologically enlarged (hydrocephalus), severe cognitive and motor difficulties can result. Hydrocephalus is associated with numerous negative effects in both children and adults, including intellectual disabilities, urinary incontinence, sexual impairment, epilepsy, visual impairment, and deafness (Simpson and Hemmer, 1993). There is a

wide variety of inherited disorders associated with hydrocephalus, including ciliary dyskinesia (al-Shroof et al., 2001), Dandy-Walker malformation (Cavalcanti and Salomao, 1999; McKee et al., 2001), and a number of X-linked disorders (Brooks et al., 1994; Czarnecki et al., 1996; Kenwick et al., 1996; Katsuragi et al., 2000).

In mice, the hydrocephalus phenotype has been linked to no fewer than 14 genes, of which nine have been mapped. Overexpression of transforming growth factor- $\beta$ 1

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(*Tgfb1*) in transgenic mice, the gene for which is located on chromosome (Chr) 4, results in enlarged ventricles (Galbreath et al., 1995; Wyss-Coray et al., 1995). Disruption of the nuclear factor I-A (*Nfia*) gene on Chr 4 leads to hydrocephalus, perinatal lethality, and agenesis of the corpus callosum (das Neves et al., 1999). These symptoms resemble the human CRASH syndrome (corpus callosum hypoplasia, mental retardation, adducted thumbs, spastic paraplegia, and hydrocephalus), caused by a mutation of the *L1* gene on Chr X. The gene responsible for the hydrocephalus 3 mutation (*hy3*) has been mapped to Chr 8 (Berry, 1961; McLone et al., 1971; Raimondi et al., 1973; Sakuragawa and Yokoyama, 1994). The forkhead box c1 (*Foxc1*) gene that causes congenital hydrocephalus and many other abnormalities has been mapped to Chr 13 (Kume et al., 1998; Hong et al., 1999). Finally, the hydrocephaly with hop gait (*hyh*) gene (Bronson and Lane, 1990; Perez-Figares et al., 1998) and the nearby brain hernia (*bh*) gene on Chr 7 are also associated with hydrocephalus (Bennett, 1959; Wallace, 1963).

Although these genes have potentially important ramifications for understanding the etiology of hydrocephalus, a systematic genetic mapping of the normal variation of ventricular size phenotype has not previously been attempted. At the outset of the present study, we made the assumption that cerebroventricular volume in mice is a variable and heritable trait and, furthermore, that genes that modulate normal variation in ventricle size in normal subjects may also contribute to pathological hydrocephaly in mice and humans. By using stereologic techniques, we have estimated ventricle size in 26 AXB and BXA recombinant inbred (RI) strains that are part of the Mouse Brain Library (<http://mbl.org>). We find a significant quantitative trait locus (QTL) on Chr 8 and a significant epistatic interaction between loci on Chrs 4 and 7.

## MATERIALS AND METHODS

### Subjects

We used 228 mice (119 male and 109 female) from 26 AXB and BXA RI strains as well as the two parent strains (A/J and C57BL/6J; Marshall et al., 1992). This set is particularly appropriate because A/J have small ventricles, whereas C57BL/6J have large ventricles. All data for this study were obtained from digital images of mouse brain sections available at the Mouse Brain Library. Mice were obtained from the Jackson Laboratory (Bar Harbor, ME) and prepared at the University of Tennessee Health Science Center as detailed previously (Rosen and Williams, 2001). 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 fixative (glutaraldehyde/paraformaldehyde), and their brains removed and weighed. After variable postfixation times, the brains were embedded in 12% celloidin and sliced in either the coronal or horizontal plane at 30  $\mu\text{m}$ . In total, four series of every tenth section were stained with cresyl violet and mounted on glass slides using Permount. Two of these series, representing a one-in-five sample of the brain, were photographed at a resolution of 25  $\mu\text{m}/\text{pixel}$ . Because initial brain weight and final brain volume are known for all cases, we were able to

estimate shrinkage on a case-by-case basis. Differential shrinkage was not a confound in this study.

### Measurement of brain and ventricle volume

We calculated mouse brain and ventricle volumes from two series of sections using point counting and Cavalieri's rule and averaged the results (Gundersen and Jensen, 1987). For coronal sections, brain volume was estimated by measuring every fortieth section (eight to ten sections measured per slide), whereas every twentieth section was used for horizontal sections (five to eight sections measured per slide). In cases in which there were missing or damaged sections, a piece-wise parabolic estimation was used (Rosen and Harry, 1990).

Ventricular volume (lateral and third ventricles only) measurement was performed in the same fashion, with the exception that every section in which the ventricles appeared was sampled. In addition, a finer point grid was used for the measures of the ventricle than for those of brain volumes (Fig. 1). Brain and ventricular volumes of 10 randomly selected mouse slides were blindly remeasured to assess experimental reliability. All measurements, as well as the subsequent statistical analysis and QTL mapping, were carried out on personal computers.

### Analysis and genetic mapping

The morphometric data were analyzed using ANOVA and multiple regression techniques [StatView (SAS Institute, Cary, NC) and DataDesk (Data Description, Ithaca, NY)]. QTL analysis was carried out with QTX (<http://mapmgr.roswellpark.org>; Manly et al., 2001), by using a set of 669 microsatellite markers typed by Williams and colleagues distributed across all chromosomes in the AXB and BXA RI set (Williams et al., 2001b). QTX computed a likelihood ratio statistic (LRS) for linkage testing. Significance was determined by permutation tests, and bootstrap analysis was used to determine support intervals of QTLs (Churchill and Doerge, 1994; Visscher et al., 1996).

### Photomicrograph production details

The photomicrograph (Fig. 1) is a composite photograph of four brightfield montages, which were acquired using Virtual Slice (Microbrightfield, Inc., Colchester, VT). The contrast, levels, and sharpness of the images were manipulated in Adobe Photoshop (Adobe Systems, Inc., San Jose, CA), and the images were composited in Canvas (Deneba Software, Miami, FL). All figures were created on Macintosh computers (Apple Computer, Cupertino, CA).

## RESULTS

### Measurements are highly reliable

The measured brain volumes ranged from 104.7 to 226.5  $\text{mm}^3$  (mean  $\pm$  SEM =  $158.1 \pm 1.5 \text{ mm}^3$ ) on these celloidin-embedded brains, which represent a 50–60% reduction from the in vivo values. The size of the ventricles ranged from merely 0.33% up to 6.31% of the total brain volume. The average of all 26 strains was  $1.66\% \pm 0.13\%$ , and the median was 1.41%. One subject's ventricle size differed by greater than 2 SD from the average of that strain (AXB10) and was excluded from the analysis, leaving 227 subjects (Fig. 2).

To assess the reliability of the data, the brain and ventricular volumes of 10 randomly selected slides were

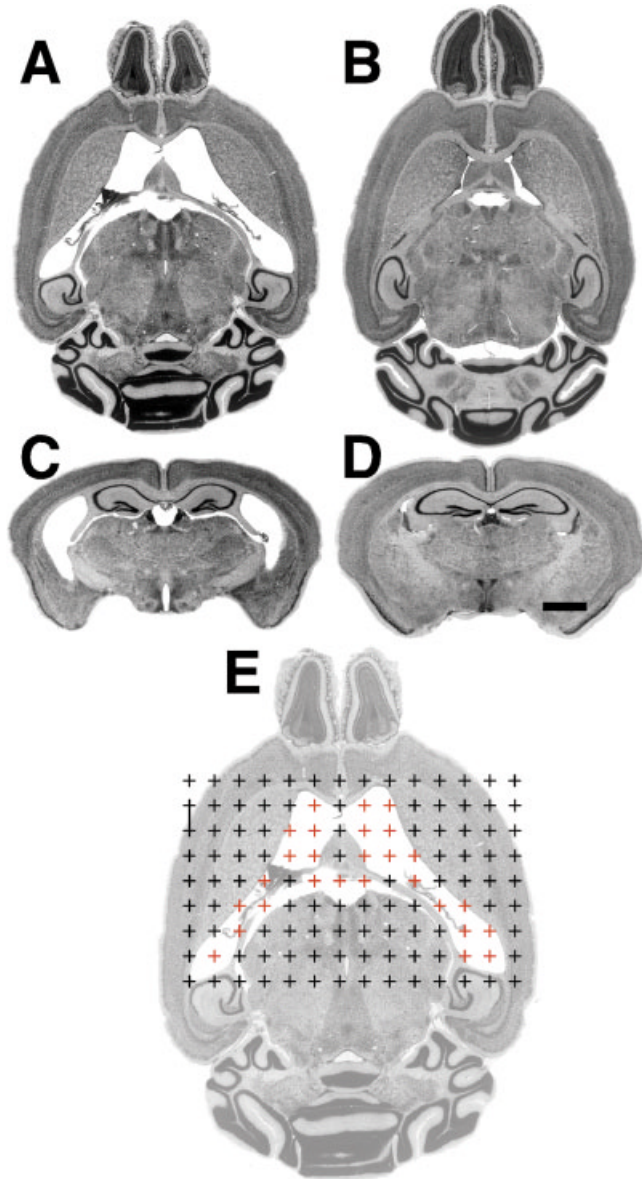


Fig. 1. Horizontal and coronal sections from subjects with large and small ventricles and illustration of the point-counting procedure. **A:** Horizontal section from subject 376 (AXB24) demonstrating large cerebral ventricles. **B:** Horizontal section from subject 619 (BXA16) illustrating relatively small ventricles. **C:** Coronal section from subject 667 (AXB24) showing large ventricles. **D:** Coronal section from subject 515 (AXB04) demonstrating relatively small cerebral ventricles. **E:** Illustration of the principles of the point-counting procedure for estimating ventricular volume. A grid (0.5 mm × 0.5 mm in this example) is overlaid on top of the image, and those points of intersection that fall within the ventricles are counted (red crosses). In this subject (376 from A), there are 27 points within the ventricles, yielding a surface area measurement of 13.5 mm<sup>2</sup>. Estimates of ventricular volume are computed using Cavalieri's rule from measures of the surface area from systematically sampled serial sections. Scale bar = 1 mm.

blindly remeasured. The test-retest reliability coefficient for brain and ventricular volume was 0.99. The average percentage difference between the original and the remea-

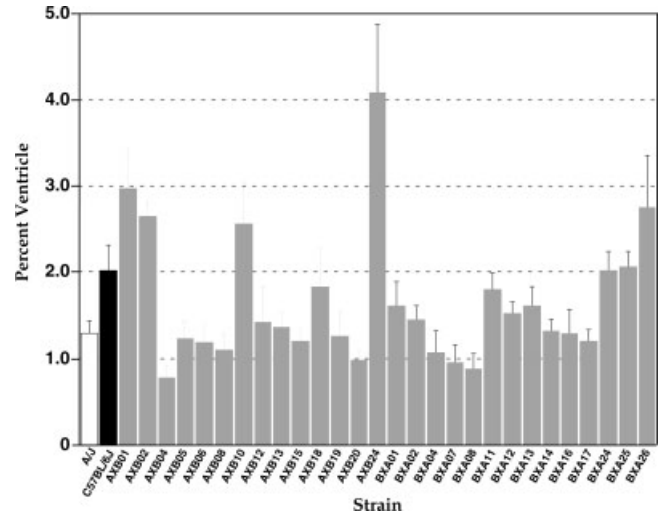


TABLE 1. Linkage Statistics for Cerebral Ventricle Volume Phenotypes

Trait	Chr	Locus	LRS	%Var	P	Add
Residual Ventricle Volume	3	D3Mit154	11.5	36	0.0007	-0.59
	4	D4Mit71	10.8	34	0.00101	0.58
	8	D8Mit94	12.6	38	0.00038	-0.63
	8	D8Mit63	11.2	35	0.00082	-0.59
	8	D8Mit189	12.6	38	0.00038	-0.63
	8	D8Mit131	10.2	32	0.00143	-0.57
Rank order of Residual Ventricle Volume	3	D3Mit154	10.5	33	0.0012	-4.33
	4	D4Mit71	14.2	42	0.00016	4.92
	7	D7Mit191	12.2	37	0.00049	-4.71
	8	D8Mit289	11.5	36	0.00071	-4.53
	8	<b>D8Mit94</b>	<b>19.6</b>	<b>53</b>	<b>0.00001</b>	<b>-5.61</b>
	8	D8Mit63	14.8	43	0.00012	-5.00
	8	<b>D8Mit189</b>	<b>19.6</b>	<b>53</b>	<b>0.00001</b>	<b>-5.61</b>
	8	D8Mit25	11.4	36	0.00072	-4.49
	8	D8Mit131	14.2	42	0.00016	-4.92
	12	D12Mit1	13.0	39	0.00031	-4.77

Abbreviations: Chr—the chromosome containing the locus; Locus—the microsatellite marker used to genotype the mice; LRS—the likelihood ratio statistic; %Var—percentage of the total phenotypic variance apparently accounted for by differences in genotype in the interval defined by the marker; P—the point-wise probability that the linkage is a false positive; Add—estimate of the additive effect of genetic variation. A negative number indicates contribution of the C57Bl/6J allele. Bolded loci are significantly linked to the phenotype (genome-wide  $P < .05$ )

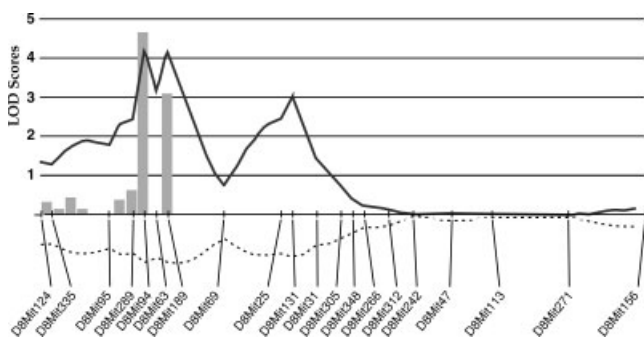


Fig. 3. LOD scores and bootstrap analysis for rank order of ventricular volume. The solid line represents the LOD scores as determined by marker regression at each of the microsatellite markers along the x-axis. The gray histogram represents the results of the bootstrap analysis and shows that the overwhelming majority of the 1,000 permutations in this analysis aligned within the QTL interval defined by the marker regression. LRS scores are equal to 4.6 times the LOD score. Dotted line is the additive effect.

Chr 8 markers (LRS = 19.6, genome-wide  $P = .028$ ). Suggestive QTLs were identified on Chrs 3, 4, 7, and 12 as well as on the more distal regions of Chr 8 (Table 1). Interval mapping of the QTL on Chr 8 with bootstrap analysis showed that the overwhelming majority of permutations aligned with the significant QTL, and we have named this QTL *Vent8a* (Fig. 3). Composite interval mapping (controlling for this QTL interval) did not reveal additional QTLs.

ANOVA using the genotype of each of the strains at both Chr 8 markers (they are identical) as independent measures and percentage ventricle as the dependent measure revealed a significant effect of genotype ( $F_{1,24} = 8.61, P < .01$ ). Strains with a “B” allele in this interval had larger ventricles than those with an “A” allele (mean  $\pm$  SEM =  $1.99\% \pm 0.208\%$  vs.  $1.20\% \pm 0.072\%$ , respectively).

**Significant interactions between markers on Chr 4 and Chr 7**

Analysis of potential interactions for both traits was performed with QTX, and the results are reported in the Table 2. There were significant interactions between

markers on Chrs 4 and 7 for rank-ordered residual ventricular volume. Specifically, the interaction between *D4Mit237* and *D7Mit178* was highly significant (LRS = 45.1, genome-wide  $P < .001$ ). There were suggestive interactions between the *Vent8a* and loci on Chrs 13 and 19. We further explored the Chr 4 and Chr 7 interaction using ANOVA, with the genotypes for these two markers as the independent measures and percentage ventricle as the dependent measure. As expected, there were no main effects, but there was a significant interaction between the two markers ( $F_{1,22} = 21.31, P < .001$ ). Examination of the means illustrated that those strains with “B” alleles on both markers ( $n = 7$ ) had larger ventricles than those with a “B” allele only on Chr 4 ( $n = 10$ ) or Chr 7 ( $n = 4$ ;  $D4B$  and  $D7B = 2.51 \pm 0.33$ ;  $D4A$  and  $D7A = 1.16 \pm 0.07$ ;  $D4A$  and  $D7B = 1.24 \pm 0.09$ ). We have labeled these QTLs *Vent4b* and *Vent7c*.

Finally, we computed the mean percentage ventricle size for those strains that had “B” alleles at *D8Mit94*, *D4Mit237*, and *D7Mit178* ( $n = 7$ ) and compared that with ventricle size for all the other strains. Those strains with “B” alleles at all three markers had significantly larger ventricles than those that did not ( $t = 4.38, df = 24, P < .001$ ; mean  $\pm$  SEM =  $2.51 \pm 0.33$  vs.  $1.38 \pm 0.10$ , respectively).

**Polymorphisms and mapping the QTL region**

After mapping QTLs to relatively large portions of three chromosomes, it is important to reduce the length of these intervals before considering candidate genes. One method involves scanning the haplotypes of each candidate interval using SNPs and microsatellites. The purpose of this analysis is to find relatively small pockets of polymorphisms within the QTL interval that are more likely to harbor the key gene responsible for the strain differences in ventricular volume. We plotted all known markers along each of the target regions of Chrs 8, 4, and 7 and paid particular attention to intervals in which haplotypes of the A/J and C57Bl/6J parental strains differ (our thanks to Dr. Robert W. Williams and members of the Complex Trait Consortium for providing this unpublished data set).

As can be seen in Figure 4, haplotypes of the A/J and C57Bl/6J differ across the entire QTL interval on Chr 8, thereby offering little assistance in refining QTL position.

TABLE 2. Linkage Interactions for Cerebral Ventricle Volume Phenotypes

Train	Chr1	Locus1	Chr2	Locus2	LRS	IX	Main1	Main2
Residual Ventricle Volume	3	D3Mit154	14	D14Mit265	32.1	10.1	11.5	4.6
	4	D4Mit237	7	D7Mit178	35.4	28.5	0	6.9
	4	D4Mit237	7	D7Mit191	31.9	22.6	0	9.3
	4	D4Mit71	19	D14Mit168	35.3	10.5	10.8	2.2
	4	D4Mit251	8	D8Mit312	31.2	23.2	6.1	0
	4	D4Mit68	8	D8Mit312	30.9	23.6	6.2	0
	7	D7Mit114	13	D13Mit122	31.1	25.8	4.7	0.8
	8	D8Mit95	8	D8Mit312	40.1	31.8	5.2	0
	8	D8Mit94	13	D13Mit248	34.5	10.8	12.6	0.3
	8	D8Mit94	13	D13Mit13	34.9	9.2	12.6	0.1
	8	D8Mit63	8	D8Mit242	31.5	8.8	11.2	1
	8	D8Mit63	13	D13Mit248	32.9	15.4	11.2	0.3
	8	D8Mit63	13	D13Mit13	34.3	16.6	11.2	0.1
	8	D8Mit189	13	D13Mit248	34.5	10.8	12.6	0.3
Rank Order Residual Ventricle Volume	8	D8Mit189	13	D13Mit13	34.9	9.2	12.6	0.1
	8	D8Mit25	13	D13Mit248	32.7	15.2	9.8	0.3
	8	D8Mit25	13	D13Mit13	33.6	16	9.8	0.1
	8	D8Mit131	13	D13Mit248	33.8	11.3	10.2	0.3
	8	D8Mit131	13	D13Mit13	34	9.8	10.2	0.1
	8	D8Mit242	15	D15Mit180	31	20.6	1	8.6
	14	D14Mit71	17	D17Mit175	33.3	28	0.9	2.7
	4	D4Mit172	7	D7Mit191	33.7	20.2	0.6	12.2
	4	<b>D4Mit237</b>	<b>7</b>	<b>D7Mit178</b>	<b>45.1</b>	<b>35.4</b>	<b>1.3</b>	<b>8.8</b>
	4	<b>D4Mit237</b>	<b>7</b>	<b>D7Mit191</b>	<b>39.1</b>	<b>25.2</b>	<b>1.3</b>	<b>12.2</b>
	4	<b>D4Mit214</b>	<b>7</b>	<b>D7Mit178</b>	<b>42.7</b>	<b>32.7</b>	<b>1.1</b>	<b>8.8</b>
	4	D4Mit214	7	D7Mit191	36.6	21.7	1.1	12.2
	4	D4Mit6	7	D7Mit178	35.8	26.2	0.5	8.8
	4	D4Mit6	7	D7Mit191	32.7	18.4	0.5	12.2
7	D7Mit294	12	D12Mit1	31.2	9.5	6.7	13	
8	D8Mit94	13	D13Mit13	32.9	8	19.6	0.8	
8	D8Mit94	19	D14Mit168	32.6	9.6	19.6	0.5	
8	D8Mit63	13	D13Mit13	32.4	16.3	14.8	0.8	
8	D8Mit189	13	D13Mit13	32.9	8	19.6	0.8	
8	D8Mit189	19	D14Mit168	32.6	9.6	19.6	0.5	
8	D8Mit25	19	D14Mit168	31.1	19.2	11.4	0.5	

Abbreviations: Chr1 and Chr2—the chromosome of the first and second interacting locus, respectively; Locus1 and Locus2—the markers for the first and second interacting locus, respectively; LRS—the likelihood ratio statistic for the interaction as a whole; IX—the LRS for the interaction effect; Main1 and Main2—the LRS for the main effects of the Chr1 and Chr2 loci, respectively.

Bolded loci interact significantly ( $P < .05$ )

In contrast, for Chrs 4 and 7, there are a number of short subintervals that have different haplotypes. These hot spots are highly divergent between the parental strains and are, therefore, much more likely to harbor the QTL responsible for differences in ventricular volume. Use of higher density haplotype maps (see Discussion) should provide some improvement in QTL localization.

## DISCUSSION

The size of cerebral ventricles is dependent on a variety of factors, including cerebrospinal fluid dynamics and, to a lesser extent, size of surrounding brain structures. Cerebral ventricle size is, therefore, likely to reflect changes in these brain factors, which are in turn modulated, at least in part, by genetic factors. Our determinations of heritability and mapping of QTLs assumes that we are mapping a trait that is an indirect measure of what may be a variety of other traits.

### Ventricular size is a heritable phenotype

Ventricular size is definitely a heritable trait in mice. Inbred strains differ markedly, and ANOVA confirmed that strain is a major determinant. We estimated that narrow-sense heritability is approximately 0.32. This is comparatively low for a morphometric trait but similar to the calculated heritabilities of olfactory bulb size in BXD recombinant inbred strains (Williams et al., 2001a). Note that narrow-sense heritability excludes epistatic interactions, and, because we have shown significant epistasis, broad-sense heritability is likely to be appreciably higher

(perhaps in the neighborhood of 0.5). Although heritability estimates are highly labile and are strongly modulated by the environmental background, our heritability index indicates appreciable genetic control. Moreover, our mapped QTLs account for at least 53% of the variance of this trait (Table 1), which is a measure of our *known* heritability.

These results contrast with those of Baaré et al. (2001) in humans, who found in their population of mono- and dizygotic twins that there was essentially no heritability of lateral ventricle size. It could be that the fundamental differences between mouse and human cerebroventricular systems underlie this difference. Along these lines, our measures included the lateral and third ventricles, whereas only the lateral ventricles were measured by Baaré et al. In addition, previous work had demonstrated a strong genetic component to ventricular size in humans (Reveley et al., 1984). It should be pointed out, however, that the degree of heritability demonstrated here suggests that epigenetic factors account for a large portion of the variance for this trait.

### Ventricular size linked to a QTL on Chr 8 and significant interactions between Chrs 4 and 7

The linkage analysis shows a significant ventricular size QTL on Chr 8 and additional suggestive QTLs on Chrs 3, 4, 7, and 12. Moreover, a significant epistatic QTL pair was found between Chrs 4 and 7. In the case of the significant additive effect on Chr 8 and interactive QTLs on Chrs 4 and 7, the presence of a “B” allele appeared to

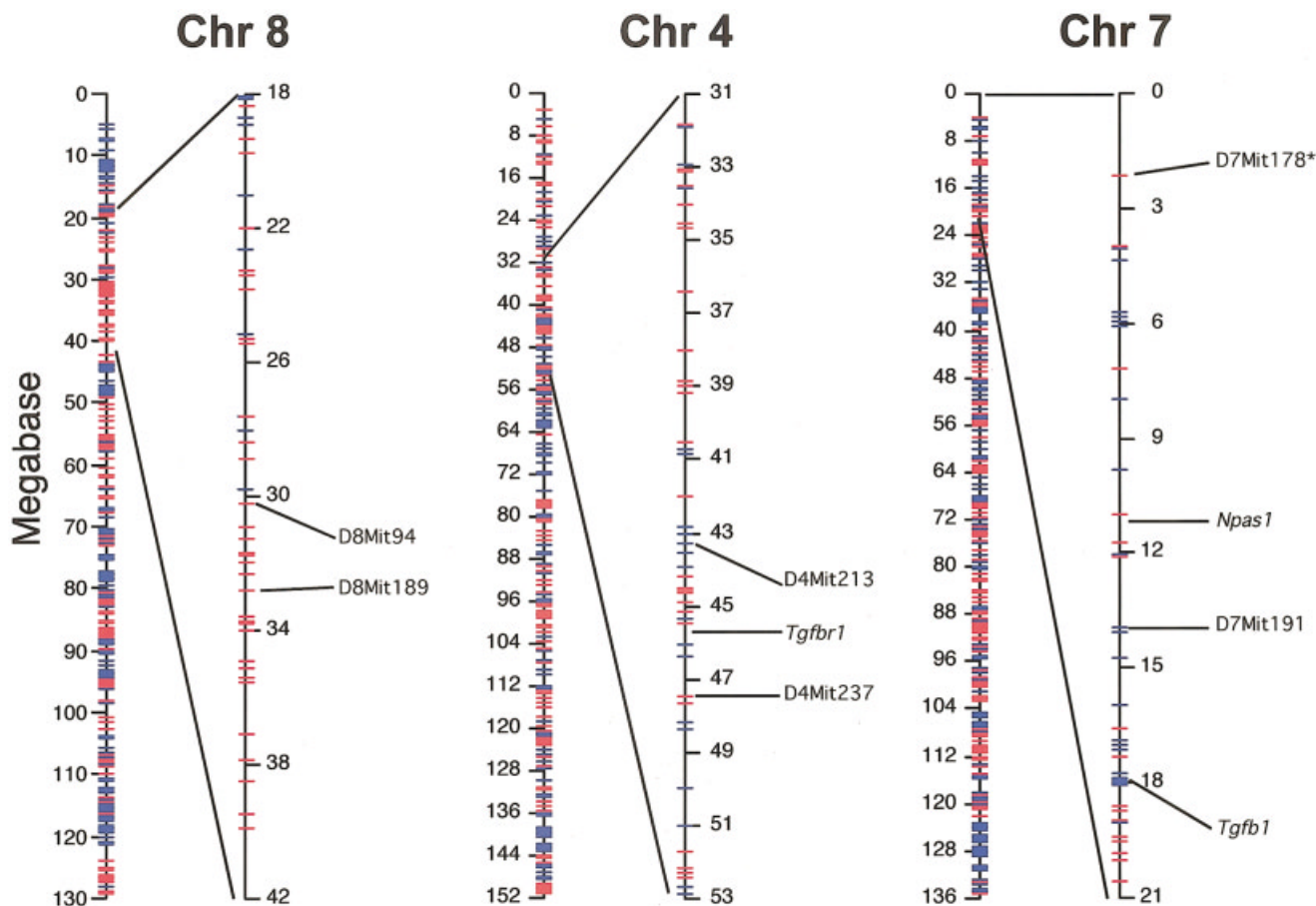


Fig. 4. Diagram illustrating distribution of polymorphic markers along Chrs 8, 4, and 7. Red markers are polymorphic; blue markers are homologous. For each chromosome, an expanded region containing those markers with the highest LOD scores appears to the right. The position of the markers and any candidate genes (*italicized*) are noted on this expanded section. Asterisk indicates estimated location.

increase the size of the cerebral ventricles. Specifically, strains having “B” alleles at each of the three loci had, on average, larger ventricles than those strains with “B” alleles at only one loci. Examination of the parent strains for this RI set confirm that A/J have small ventricles, whereas C57BL/6J have large ventricles and are known to be susceptible to hydrocephalus (Marshall et al., 1992). These results strongly suggest that alleles inherited from the C57BL/6J parent directly contribute to an increase in ventricle size. Ventricular size, therefore, appears to be a polygenic trait modulated by the additive effect of several genes. This assertion is further supported by the wide and continuous phenotypic variation for the RI mouse strains.

### Proximity of QTLs to genes underlying hydrocephalus

Our original hypothesis was that the genes related to hydrocephalus may also modulate the normal variation of cerebral ventricle size. We therefore examined the regions in proximity to our QTLs for genes that might be related to hydrocephalus. The region on Chr 8 that contains *Vent8a* (Fig. 4) has relatively few genes that are expressed during brain development. Cortixin is a protein localized to neocortical neurons perinatally (Coulter et al., 1993),

and its gene (*ctxn*) is located at 30.25 megabases (Mb) on Chr 8 (Watson et al., 1994). There is no evidence to suggest, however, that this protein relates to hydrocephalus. The gene responsible for the hydrocephalus 3 mutation (*hy3*) maps to a telomerically more distant region on Chr 8 (Sakuragawa and Yokoyama, 1994). Among the suggestive QTLs, the region of Chr 4 is located in close proximity to the *Nfia* gene, whose disruption has been shown to induce hydrocephalus and other abnormalities in mice (das Neves et al., 1999).

Examination of the chromosomal regions associated with *Vent4b* and *Vent7c* shows a number of potentially interesting genes that may relate to hydrocephalus (Fig. 4). Transgenic mice overexpressing transforming growth factor- $\beta$ 1 (*Tgfb1*) exhibit hydrocephalus (Galbreath et al., 1995; Wyss-Coray et al., 1995). More recent reports have confirmed that this model of congenital hydrocephalus reasonably represents what is known about its etiology in humans (Cohen et al., 1999; Hayashi et al., 2000; Moinuddin and Tada, 2000). Interestingly, *Tgfb1* is located in close proximity to the region of Chr 7 (17.92 Mb) that interacts with *Vent4b* in our model. Moreover, the gene for the receptor of *Tgfb1* (*Tgfb1*) is located at 46.25 Mb on Chr 4, which is within the region defined by marker re-

gression analysis in the current experiment. The centromeric area of Chr 7 is a gene-rich region and contains other potentially interesting genes related to our ventricular phenotype. *Npas1*, a member of the bHLH-PAS family of transcription factors, is located at 11.59 Mb and is expressed in brain during development (Zhou et al., 1997). In addition, two other genes related to hydrocephalus have been mapped to Chr 7 around 25 Mb (*hyh*) and 33 Mb (*bh*; Wallace, 1963; Bronson and Lane, 1990; Perez-Figares et al., 1998; Chae et al., 2002). Inspection of the other suggestive interactions shows an interaction between the *Vent8a* and a region on Chr 13 that is in close proximity to the *Foxc1* gene, which has been shown to induce hydrocephalus (Kume et al., 1998; Hong et al., 1999).

## SUMMARY AND CONCLUSIONS

Hydrocephalus in humans has many different etiologies, and it is likely to be a polygenic trait (Laurence, 1993). Several genes (*Nfia*, *hy3*, *Foxc1*), as well as overexpression of *Tgfb1* in transgenics, had been linked to hydrocephalus in mice. However, no close examination of the genetic basis of the normal variation in ventricular size phenotype had been conducted.

Our results suggest that ventricular size is a highly heritable trait. Moreover, we demonstrate that ventricular size is a polygenic trait with epistatic gene interactions. Single-locus association and interval mapping tests point to a significant QTL on Chr 8 (*Vent8a*) and suggestive QTLs on Chrs 3, 4, 7, and 12. A highly significant interactive QTL has been found between Chrs 4 and 7 (*Vent4b* and *Vent7c*, respectively), and suggestive epistatic QTLs were identified between *Vent8a* and Chr 13, Chrs 8 and 19, and Chrs 7 and 12. The location of hydrocephalus-related genes in close proximity to the QTLs on Chrs 4, 7, 8, and 13 lends support to our hypothesis that normal variation in cerebral ventricular size may be modulated by genes associated with hydrocephalus.

QTLs derived from analysis of small sets of RI strains are often considered conditional (Belknap et al., 1996). Increasing the precision of phenotypes or using larger RI sets could both increase statistical confidence. In addition, recent investigations have tested a powerful method of refining QTLs mapping in RI lines simply by generating a set of RI F1 hybrids, so-called RIX progeny (Threadgill et al., 2002). These "RIX" represent an elegant means by which to refine the position and effects of the QTLs mapped in this study.

Finally, the two parental strains that we have used in our mapping study of ventricular volume have both been sequenced, one as part of the public effort (<http://www.ensembl.org>; C57BL/6J) and one by Celera Genomics (<http://www.celera-radiscoversystem.com>; A/J). This makes it possible to compare haplotypes of these two strains on any interval thought to harbor a QTL. Subintervals within the QTL region that have haplotypes that are identical by descent are unlikely to be of interest, whereas those subintervals within the QTL region that have contrasting haplotypes and high concentrations of SNPs are likely to be of intense interest. Thus it will soon be possible to refine further all of the QTL intervals reported in this study by essentially convolving the LRS plot of a QTL by a haplotype contrast function. It will be possible to do this

systematically for the hundreds of traits that have already been mapped using the AXB,BXA RI set.

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