Localization of two new X-linked quantitative trait loci controlling corpus callosum size in the mouse

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Abstract:
Corpus callosum (CC) size is a complex trait, characterized by a gradation of values within a normal range, as well as abnormalities that include a small or totally absent CC. Among inbred mouse strains with defects of the CC, BTBR T^+ tf/J(BTBR) mice have the most extreme phenotype; all animals show total absence of the CC and severe reduction of the hippocampal commissure (HC). In contrast, the BALB/cByJ (BALB) strain has a low frequency of small CC and consistently normal HC. Reciprocal F_1 crosses between BTBR and BALB suggest the presence of X-linked quantitative trait loci (QTLs) affecting CC size. Through linkage analysis of backcross male progeny, we have localized two regions on the X chromosome, having peaks at 68.5 Mb (~29.5 cM) and at 134.5 Mb (~60.5 cM) that are largely responsible for the reciprocal differences, with the BTBR allele showing X-linked dominant inheritance associated with CC defects.
Keywords: Axon guidance, BTBR, corpus callosum, hippocampal commissure, quantitative trait locus (QTL), X-linked.

Article:
The corpus callosum (CC), the largest fiber tract in the brain of placental mammals, connects the left and right cerebral hemispheres. In humans, CC agenesis occurs in 1% of the general population, with an incidence closer to 2.3% among developmentally disabled individuals (Jeret et al. 1985–86). Corpus callosum defects are often observed in syndromes characterized by mental retardation, hydrocephalus, interhemispheric cysts and epileptic seizures (Jeret et al. 1987; Serur et al. 1988) and are frequently associated with neuronal migration and axon guidance disorders such as lissencephaly, polymicrogyria and heterotopias (Wisniewski & Jeret 1994). Absent CC in humans is a complex genetic trait, showing autosomal and X-linked transmission (Richards et al. 2004).

The mouse is an excellent model to study the genetic factors influencing CC formation because the development of this commissure is similar in humans and in mice (Ren et al. 2006). BTBR mice show a total absence of the CC and a severely reduced hippocampal commissure (HC), while BALB and 129 substrains show varying degrees of CC deficiencies (Wahlsten et al. 2003). The difference between BALB/cWah1 and 129P1/ReJ mice arises from at least two autosomal loci (Livy & Wahlsten 1991; Wahlsten et al. 2006). Two or more loci also appear to be responsible for the difference between the anatomically normal B6 strain and either BALB/cWah1 or 129P1/ReJ (Wahlsten 1982) mice. In crosses of strains prone to absent CC with a robustly normal strain such as B6, no absent CC in F1 hybrids has been observed, and there is a very low frequency in backcrosses (Wahlsten 1982; Livy & Wahlsten 1991), with no evidence of a sex difference. Linkage analysis of crosses between NZB/ BlNJ and C57BL/6By, neither of which has abnormally small CC, has identified two autosomal quantitative trait loci (QTLs) that influence midsagittal CC size within a normal range (Le Roy et al. 1998), but no loci involved in total CC absence in common inbred strains have yet been identified. The identification of QTLs in these strains is a first step towards new insights into the regulation of commissure formation. As various X-linked CC defects occur in humans (Richards et al. 2004) and CC development is similar in humans and in mice, we examined the role of the X chromosome in CC formation in the mouse. We demonstrate this in reciprocal crosses between BALB and BTBR inbred mice, where many more afflicted F_1

male offspring with a BTBR-derived X chromosome show CC defects compared with reciprocal F_1 males with a BALB-derived X chromosome, and reciprocal F_1 female mice are not different from each other. We show that this difference arises from loci on the X chromosome where the BTBR allele shows X-linked dominant inheritance over the BALB allele.

Materials and methods

Animals

Inbred mouse strains BALB/cByJ (BALB) and BTBR T^+ tf/J (BTBR) were obtained from the Jackson Laboratory (Bar Harbor, ME, USA) and handled under protocols approved by the Institutional Animal Care and Use Committee. Mice were housed in temperature- and humidity-controlled (20–22°C) rooms as previously described (Bolivar & Flaherty 2003). Same-sex mice were housed four to five to a cage. F_1 and backcross populations were generated at the Wadsworth Center.

Corpus callosum size measurements

Corpus callosum staining and measurements were performed according to Wahlsten et al. (2003). Briefly, 8-week-old mice were euthanized by CO_2 gas asphyxiation. Brains were immediately removed and fixed in 10% phosphate-buffered formalin (Fisher Scientific, Hampton, NH, USA) for 48 h. Fixed brains were trimmed to a standard configuration by removing olfactory bulbs, paraflocculi, cranial nerves and any remaining spinal cord by cutting caudal to the medulla oblongata. Brains were then weighed before bisection down the midsagittal plane. Each hemisphere was stained in a 0.2% gold chloride solution (Sigma, St Louis, MO, USA) at 37°C. Staining was stopped after 45 min by immersion in 2.5% sodium thiosulfate solution (25 g of sodium thiosulfate anhydrous in 1000 ml of distilled water). The midsagittal plane of each hemisphere was imaged with a Leica MZ75 stereoscope using a Pixera Pro150ES camera. The areas of the CC and HC were measured using IMAGEJ version 1.33u software obtained from National Institutes of Health (http://rsb.info.nih.gov/ij/index.html). To correct commissure sizes for brain size, we calculated an abnormality index value (i) using the linear regression equation CC index (CCi) = (CC area)/(-0.1 + 2.2X) for CC, and HC index (HCi) = (HC area)/(0.1 + 0.40X) for HC, where X is brain weight in grams (Wahlsten et al. 2006). An index of 1.0 indicates that the area of the commissure is equal to the value expected for the corresponding brain size and is considered normal. Abnormal commissure size is defined by an index less than 0.5, while an index of 0 indicates that the structure is completely absent. Populations such as C57BL/6J and B6D2F2/J mice that never have CC defects always show CCi values above 0.5. The CCi and HCi values were used for all statistical and mapping analyses. Statistical analyses were performed using SYSTAT 11 (Systat Software Inc., Point Richmond, CA, USA) and JMP version 5.1 (SAS Institute, Cary, NC, USA) software.

Mapping analyses

Genomic DNA was purified from tails using the Gentra Puregene DNA isolation kit (Gentra, Minneapolis, MN, USA). Single nucleotide polymorphism (SNP) genotyping assays were designed with the Applied Biosystems Assaysby-design service (www.appliedbiosystems.com; Foster City, CA, USA), and SNP genotyping was carried out according to manufacturer's recommendations using an ABI7900HT sequence detection system (Applied Biosystems). The following X-chromosomal SNPs differing between BALB and BTBR mice were used for genotyping: rs1 3483752 (46.4 Mb, ~19.0 cM), rs13483824 (68.5 Mb, ~29.5 cM), rs13483933 (103.7 Mb, ~48.4 cM), rs13484035 (134.5 Mb, ~60.5 cM), rs13484074 (147.2 Mb, ~65.0 cM) and rs13484112 (162.4 Mb, ~73.0 cM). Information on SNP and Mb positions were obtained from the Web site http://www.ncbi.nlm.nih.gov/SNP/(NCBI build 34), and centimorgan (cM) positions of markers were approximated based on known cM value of nearest gene or marker according to JAX (http://www.informatics.jax.org). Data were compiled and analysed by MAP MANAGER version QTXb19 (Manly et al. 2001), software designed for detection and localization of QTLs through statistical association of trait values with marker loci in progeny of a cross. A permutation test (n = 1000) was used to determine the threshold value for detecting a QTL influencing the CC phenotype in our backcrosses. Multiple regression analysis of CCi was also carried out to confirm linkage.

Results and discussion

Among all the BTBR mice we examined (17 females, 17 males), none had a CC (Fig. 1). Reciprocal F_1 crosses between BTBR and BALB inbred mice were performed to determine whether there were genes on the X chromosome that influenced CC size. A reciprocal cross effectively isolates the X chromosome of one parental strain from the other in F_1 male offspring, while genetic background of the female reciprocals is heterozygous at all loci.

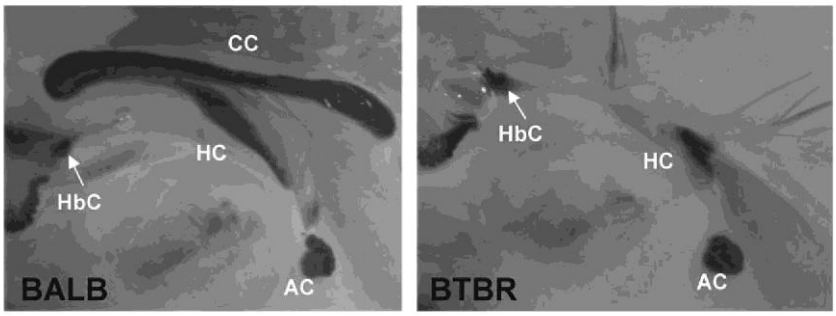

Figure 1: Midsagittal view of gold-chloride-stained hemisphere of brains from 8-week-old BALB and BTBR inbred mice. In BTBR mice, the CC is completely absent and the HC is abnormally small. These images are representative of CC size for all mice that we observed within each inbred strain (BALB: 24 females, 24 males; BTBR: 17 females, 17 males). There was no difference in CC size between males and females of the same strain. AC, anterior commissure; HbC, habenular commissure.

The distributions of CCi and HCi for all F_1 hybrid mice (n = 96) are shown in Fig. 2a, b. Corpus callosum size varied in the F_1 hybrid population, with 31% having CC abnormalities, whereas HC size was normal in the adult brains of all F_1 progeny, even for the mice showing CC defects; thus HC measurements were not included in the further analyses. All F_1 male progeny with a BALB X chromosome (Fig. 2f) (n = 24) had a normal CC, while all F_1 progeny inheriting an X chromosome from BTBR had similar frequencies of CC abnormalities (Fig. 2c–e) (n = 24 for each group). Specifically, there was a significant difference ($P < 0.0001$) in mean CCi between the two groups of reciprocal F_1 males (Fig. 2d, f) but not females (Fig. 2c, e), showing X-linked influence on CC size.

When analyzing a reciprocal cross, it is important to keep in mind that maternal effects, such as uterine environment, maternal care, or mitochondrial genome, can influence a phenotype in reciprocal offspring (Carlier et al. 1999). An interaction between mitochondrial DNA and the nuclear genome has been shown to modulate midsagittal CC size in mice (Roubertoux et al. 2003). However, here there was no evidence for maternal effects because the two groups of reciprocal F_1 female progeny did not differ in CC size.

We used a backcross breeding scheme to generate X chromosome recombinants. Analysis of backcross data is more straightforward than that of F_2 populations, and given that XX pairings for meioses can only occur in the female parents, we would expect the same number of meioses for linkage analysis when looking at only the X chromosome for either an intercross or a backcross. Female (BALB × BTBR) F_1 mice were crossed to both parental strains to generate N2 progeny. In the male N2 mice (n = 420) CC size varied from normal to absent. The N2 mice from the backcross to BTBR had a higher frequency of abnormal and absent CC than did N2 mice from either the BALB backcross or F_1 hybrids (Fig. 2g, h).

Analysis of variance for CCi indicated that there was no interaction (all $P > 0.2$) between genetic background [(BALB x BTBR) x BTBR] or [(BALB x BTBR) x BALB] and marker. Thus, genotyping and phenotyping results from both backcrosses were combined and were analysed by the MAP MANAGER program version QTXb19 (Manly et al. 2001) (Fig. 3). A permutation test was used to obtain a threshold value to establish significance of linkage (Churchill & Doerge 1994). Based on the permutation test, a highly significant linkage relationship ($P < 0.001$) was found on the distal X chromosome, with a peak likelihood ratio statistic (LRS) score of 12.4, at approximately 134.5 Mb (~60.5 cM). Another, more proximal peak located at 68.5 Mb (~29.5 cM) was also detected (LRS = 9.8, $P < 0.05$). The 95% confidence intervals for each QTL location, as determined by MAP MANAGER software, encompassed approximately 40 and 50 cM for the distal and

proximal QTLs, respectively. Confirmatory multiple regression analysis of backcross data with effect coding supported marker effects at SNP rs13483824 (P 1/4 0.006) and rs13484035 (P 1/4 0.0001). In the multiple regression analysis, about 16% of the phenotypic variance in the CCi was attributable to background genotype and 3% to a QTL near the rs13484035 marker. We have designated these two QTLs as Ccrs3 and Ccrs4, following the previously established nomenclature (http:// www.informatics.jax.org) for QTLs that influence midsagittal CC hemisphere surface size.

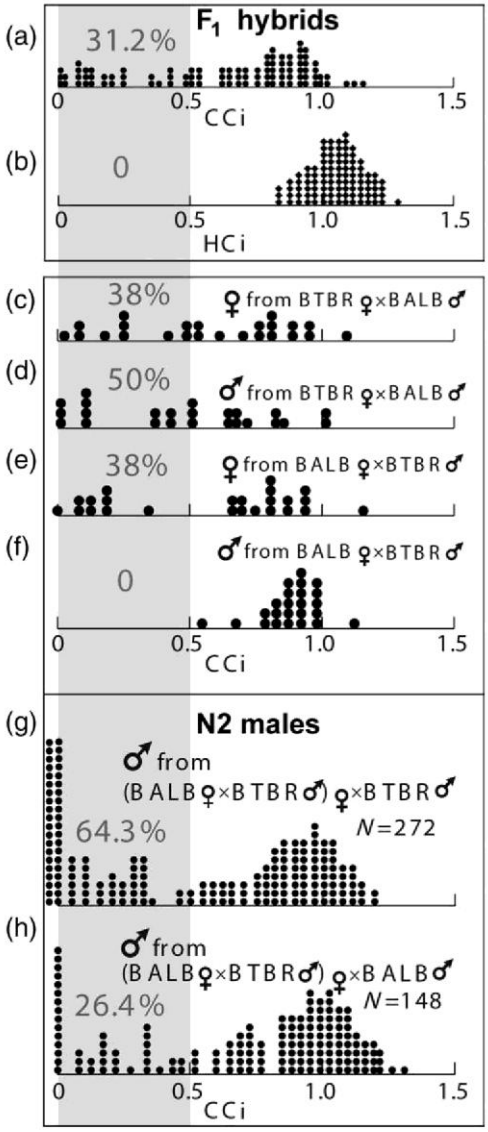

Figure 2: Index of abnormality for commissures in F_1 hybrid and backcross mice. Indices were calculated according to equations given by Wahlsten *et al.* (2006); CCi = (CC area)/(−0.1 + 2.2 × brain weight) and HCi = (HC area)/(0.1 + 0.4 × brain weight). A commissure with index value <0.5 is considered abnormal. (a–b) 31% of F_1 mice (reciprocal crosses, both male and female mice) had abnormally small CC, but none had abnormal HC (n = 96). (c–f) Male mice with a BALB X chromosome had normal CC, whereas the other three F_1 hybrid groups had similar frequencies of abnormal CC (n = 24 for each group). (g) Male mice from the backcross to a BTBR male (n = 272) had a much higher frequency of abnormal and totally absent CC than did either the F_1 hybrids or the backcross to BALB (n = 148), shown in (h).

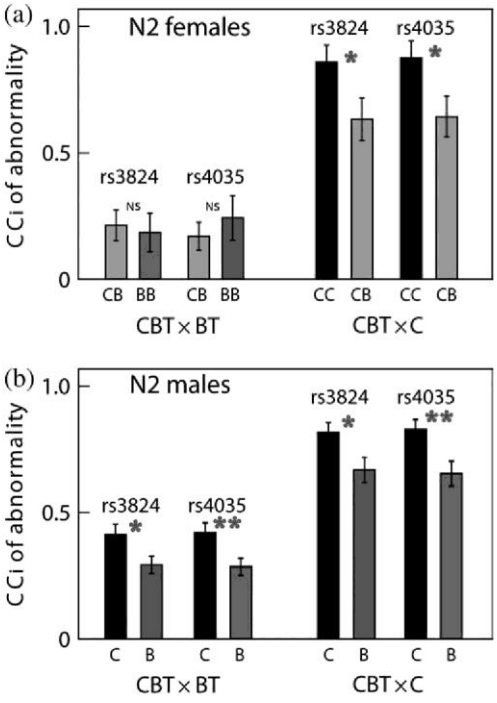

Figure 4: Allelic effects of QTLs for N2 female and male progeny of each backcross. (a) Allelic contributions determined in the N2 female population at each peak marker show that the BTBR or B allele is dominant over the BALB or C (C for BALB) allele at both loci. Mean CC index is significantly different (P < 0.02) between heterozygous mice (BTBR/BALB alleles) and those homozygous for the BALB allele, while it is not different between heterozygotes and those homozygous for the BTBR allele. (b) Having a BTBR allele at either marker reduces CC size in N2 male progeny of both backcrosses. C, BALB allele; B, BTBR allele; CBT × BT, backcross to BTBR (n = 50 females, n = 272 males); CBT × C, backcross to BALB (n = 50 females, n = 148 males). *P < 0.02; **P < 0.01; NS, not significant. Peak marker ID (SNP rs#) is indicated by the last four digits.

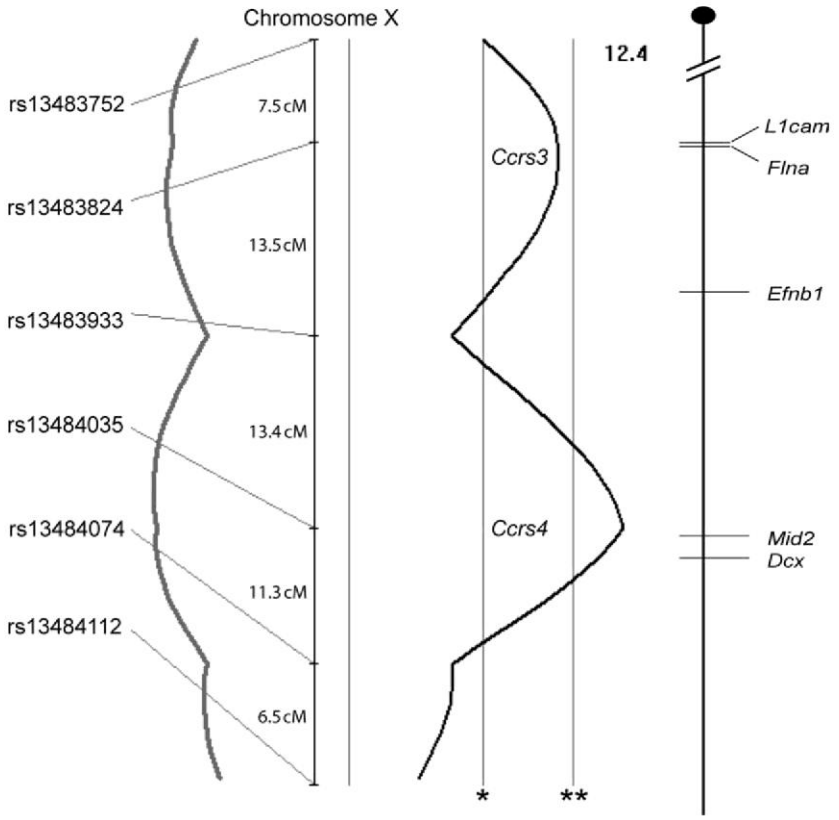

Figure 3: MAP MANAGER output for X chromosome interval mapping. By a permutation test, a LRS above 11 was shown to be highly significant for detecting a QTL. Peaks are at 68.5 Mb (~29.5 cM), LRS = 9.8* at marker rs13483824 and at 134.5 Mb (~60.5 cM), LRS = 12.4** at marker rs13484035 (*$P < 0.05$; **$P < 0.01$). Recombination frequency between SNP markers, as determined by mapping software, is indicated in cM. Relative locations of candidate genes (previously implicated in CC defects in humans) in the peak regions are indicated on the right.

BTBR alleles at SNP rs13484035 or rs13483824 significantly reduced CCi in N2 male brains from both backcrosses (Fig. 4b). However, the allelic effects for the X chromosome cannot be defined in males because they are hemizygous for the X chromosome, thus we examined inheritance in back-cross female offspring. At the 134.5 Mb marker rs13484035, heterozygous females differed significantly ($P < 0.05$) in CCi from females homozygous for the BALB allele, while females homozygous for the BTBR allele were not significantly different from heterozygotes (Fig. 4a). Comparisons were also done for the SNP rs13483824 at 68.5 Mb with similar results. These results confirm the X-linked dominant inheritance of the BTBR allele observed in F_1 progeny and further establish the dominant nature of the BTBR alleles at each QTL.

The critical regions for these QTLs are centered at 134.5 Mb (~60.5 cM) and at 68.5 Mb (~29.5 cM). Two interesting candidates present in these regions are Dcx (doublecortin) (137.3 Mb, ~62.5 cM) and L1cam (neural cell adhesion molecule L1) (68.5 Mb, ~29.5 cM). Because these genes have been associated with CC defects in humans (Gleeson et al. 1998; Kenwrick et al. 2000), we sequenced the coding regions of these genes in both BALB and BTBR mice. No sequencing polymorphisms were found in either of the coding regions of these genes. Publicly available SNP data (http://www.jax.org/phenome/snp.html) show two SNPs, reference SNP identifiers are rs29283011 and rs29285923, that differ between BALB and BTBR mice in the Dcx 3' untranslated region and could possibly be important for gene regulation. Additional candidate genes in the region are being identified.

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