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Two Quantitative Trait Loci for Prepulse Inhibition of Startle Identified on Mouse Chromosome 16 Using Chromosome Substitution Strains

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ABSTRACT

Prepulse inhibition (PPI) of acoustic startle is a genetically complex quantitative phenotype of considerable medical interest due to its impairment in psychiatric disorders such as schizophrenia. To identify quantitative trait loci (QTL) involved in mouse PPI, we studied mouse chromosome substitution strains (CSS) that each carry a homologous chromosome pair from the A/J inbred strain on a host C57BL/6J inbred strain background. We determined that the chromosome 16 substitution strain has elevated PPI compared to C57BL/6J ($P = 1.6 \times 10^{-11}$), indicating that chromosome 16 carries one or more PPI genes. QTL mapping using 87 F_2 intercross progeny identified two significant chromosome 16 loci with LODs of 3.9 and 4.7 (significance threshold LOD is 2.3). The QTL were each highly significant independently and do not appear to interact. Sequence variation between B6 and A/J was used to identify strong candidate genes in the QTL regions, some of which have known neuronal functions. In conclusion, we used mouse CSS to rapidly and efficiently identify two significant QTL for PPI on mouse chromosome 16. The regions contain a limited number of strong biological candidate genes that are potential risk genes for psychiatric disorders in which patients have PPI impairments.

SENSORIMOTOR gating is an inhibitory brain mechanism that filters extraneous stimuli to allow cognitive centers and motor output pathways to attend to relevant stimuli. Sensorimotor gating is thought to be governed by mechanisms that influence cognition; thus robust gating is an indication of integrity of higher cognitive processes (Perry and Braff 1994). Gating of the startle reflex (a primitive brainstem reflex circuit) can be measured by prepulse inhibition (PPI) (Geyer *et al.* 2001), a phenomenon in which a weak auditory or tactile prestimulus reduces the startle response to a subsequent startling stimulus. PPI is mediated by midbrain structures (Fendt *et al.* 2001) and is regulated by forebrain processes (Swerdlow *et al.* 2001a) that together extend through a cortico-striato-pallido-pontine (CSPP) neural circuit. This forebrain regulation occurs via dopaminergic, cholinergic, and glutamatergic processes (Swerdlow *et al.* 2001a), and pharmacological compounds that are known to influence these processes result in modulation of PPI (Geyer *et al.* 2001). In both rodents and humans, PPI of acoustic startle is not learned (it occurs on the first trial), is stable over time, and exhibits good test-retest reliability (Koch 1999; Ludewig *et al.* 2002); hence, PPI appears to be a reliable neurophysiological marker of sensorimotor gating.

Many studies have documented PPI impairments in schizophrenia patients (Braff *et al.* 1978, 1992, 1999, 2001; Bolino *et al.* 1994; Kumari *et al.* 1999, 2000; Weike *et al.* 2000; Ludewig *et al.* 2002). Nearly half of nonschizophrenic relatives of patients demonstrate reduced PPI, with only 20% of control individuals showing reduced PPI (Cadenhead *et al.* 2000), suggesting that the deficits are hereditary. PPI deficits correlate with clinical severity (Braff *et al.* 1999; Light and Braff 1999; Weike *et al.* 2000), cognitive symptoms such as thought disorder (Perry *et al.* 1999), and early age of onset of schizophrenia (Kumari *et al.* 2000), suggesting a strong relationship between disease symptoms and PPI impairment. PPI deficits have also been reported in other

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disorders that appear to have a common characteristic of loss of gating in sensory, motor or cognitive domains, notably obsessive-compulsive disorder (Swerdlow *et al.* 1993), attention deficit hyperactivity disorder (ADHD) (Hawk *et al.* 2003), Tourette syndrome (Swerdlow *et al.* 2001b), comorbid Tourette syndrome and ADHD (Castellanos *et al.* 1996), Huntington's disease (Swerdlow *et al.* 1995), bipolar disorder with acute psychotic mania (Perry *et al.* 2001), comorbid ADHD and nocturnal enuresis (Ornitz *et al.* 1999), blepharospasm (Gomez-Wong *et al.* 1998), nonepileptic seizures (Pouretemad *et al.* 1998), and post-traumatic stress disorder (Grillon *et al.* 1996).

The above findings provide evidence that PPI may be an endophenotype (intermediate trait) of these psychiatric disorders, particularly schizophrenia for which PPI deficits are the most consistently and extensively documented. While the term "endophenotype" has various meanings, in terms of genetic analysis, endophenotypes are heritable traits associated with a disorder that may have a less complex genetic basis (Gottesman and Gould 2003). As a result, they may be more amenable to genetic mapping studies than the disorder itself, for several possible reasons including reduced genetic complexity, more accurate and objective quantitative measurement, and reduced influence of environmental factors. Thus, identification of the genes involved in PPI regulation not only would shed light on brain circuits involved in sensorimotor gating and perhaps higher cognitive functioning, but also may identify genes that contribute to risk of the above disorders.

Inbred mouse strains demonstrate considerable strain variation in PPI of acoustic and tactile startle (Bullock *et al.* 1997; Logue *et al.* 1997; Paylor and Crawley 1997; Ralph *et al.* 2001; Varty *et al.* 2001; Willott *et al.* 2003), suggesting that the forebrain processes that regulate PPI vary across inbred strains and are genetically determined. The heritability (h^2) of PPI of acoustic startle has been estimated to range from 23 to 67% in inbred mouse strains (Joober *et al.* 2002; Willott *et al.* 2003), indicating that mapping genes underlying PPI regulation is feasible. Nearly 20 quantitative trait loci (QTL) for PPI have been implicated in mice (McCaughran *et al.* 1999; Hitzemann *et al.* 2001; Joober *et al.* 2002), and 2 PPI loci have been reported in rats (Palmer *et al.* 2003). However, only two murine QTL have been replicated in different studies, and no genes have yet been identified, no doubt due to the unmanageable size of the mapped regions (~30 cM).

Mouse chromosome substitution strains (CSSs), also known as consomic strains, have been developed to hasten genetic mapping of heritable traits. The mouse CSSs were created from a host C57BL/6J ("B6") strain and a donor A/J inbred strain, such that each CSS is homosomic for a particular donor A/J chromosome but otherwise has a B6 background (Nadeau *et al.* 2000). As detailed elsewhere (Nadeau *et al.* 2000; Belknap 2003; Singer *et al.* 2004), the CSS panel can be screened for phenotypic differences from the host B6 strain to rapidly focus on chromosomes containing candidate loci, after which a limited number of backcrosses or intercrosses between the relevant CSS and B6 can be performed to identify the locus on the corresponding chromosome. In these crosses, the ability to detect and resolve even modest QTL is greatly enhanced, since all other chromosomes are fixed B6 and, therefore, the variance due to segregating QTL on other chromosomes is no longer present. The CSS approach has been used to identify QTL for several complex traits, including anxiety (Singer *et al.* 2004, 2005), diet-induced obesity (Singer *et al.* 2004), serum levels of sterols and amino acids (Singer *et al.* 2004), testicular cancer (Matin *et al.* 1999; Youngren *et al.* 2003), pubertal timing (Krewson *et al.* 2004), and airway hyperresponsiveness (Ackerman *et al.* 2005).

In this study, we sought to identify a QTL for PPI using mouse chromosome substitution strains. In comparison to the host B6 strain, we detected significantly elevated PPI in the chromosome 16-substitution (CSS-16) strain. This observation indicates that mouse chromosome 16 harbors one or more PPI genes. We performed genetic intercross mapping and identified two significant PPI regions on chromosome 16, one of which overlaps a previously reported QTL region. These results were obtained using vastly fewer mice and chromosomal markers than traditional genetic mapping, thereby supporting the use of mouse CSSs as a powerful and rapid approach to map QTL for complex behavioral traits. We refined the list of most probable candidate genes in the QTL regions by identifying genes that have sequence variation between the B6 and CSS-16 strains.

MATERIALS AND METHODS

Mice: Generation of the mouse CSS panel has been described previously (Nadeau *et al.* 2000; Singer *et al.* 2004). Briefly, each strain in the panel has a chromosome pair substituted from the A/J strain onto a host C57BL/6J background. The panel nomenclature is C57BL/6J-#^{A}/Na, where # refers to the substituted chromosome. For simplicity, however, the strains are referred to as CSS-# in this report. CSS lines were obtained by embryo transfer from Case Western Reserve University and bred for approximately six generations in the Whitehead Institute for Biomedical Research (WIBR) vivarium prior to the start of experiments. C57BL/6J mating pairs were purchased from Jackson Laboratory and bred in the WIBR vivarium. Mice were weaned between 3 and 4 weeks of age and were housed a maximum of five per cage of the same sex. Mice were maintained on a 12 hr light:dark cycle (lights on at 0700 hr) with food and water *ad libitum*, except during behavioral testing. All experiments were performed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (NIH publication no. 86-23) and were approved by the Massachusetts Institute of Technology Committee on Animal Care.

Behavioral testing: Male 6- to 9-week-old mice were tested between 10 am and 6 pm using a startle monitor system

(Hamilton-Kinder, San Diego). Mice were habituated to the startle monitor on the 2 days prior to PPI testing by being placed in the monitor for 3 min with a constant 65-dB white noise background. PPI testing sessions began with a 3-min acclimatization period using a 65-dB background white noise that was maintained throughout the session. Since the startle reflex exhibits habituation and may alter PPI across a session (Blumenthal 1997), four pulse-alone trials of a 120-dB 40-msec white noise burst were presented at the beginning and end of the session to stabilize the startle magnitude. These trials were not used for any analyses. Mice were exposed to six replicate blocks of trials in pseudorandom order. Each block consisted of one pulse-alone trial, three trials in which the pulse was preceded by 100 msec by a nonstartling 20-msec prepulse of 70-, 75- or 80-dB intensities, and one null trial (no stimulus presented). The intertrial interval ranged from 6 to 8 sec in pseudorandom order. The maximum response within a 65-msec record window was used as the startle amplitude. The acoustic startle response (ASR) was defined as the mean startle amplitude of the six pulse-alone trials. For each prepulse intensity, the mean startle amplitude across the six replicate trials was calculated. We defined PPI as the percentage reduction in the ASR when preceded by the prepulse compared to the ASR alone using the equation $100 \times (1 - [\text{mean startle amplitude with prepulse/ASR}])$. Activity in the startle monitors was assessed by the null trials and was similar among all mice; therefore, there were no effects of activity level on PPI.

The 70- to 80-dB prepulse intensities used for our PPI test protocol were chosen to cover a range of intensities, including louder prepulses that have been found to elicit higher PPI levels (Swerdlow *et al.* 1993). However, louder prepulse intensities have been found by one study to elicit a startle response that may affect PPI measurements (Dahmen and Corr 2004). Therefore, we measured the startle threshold of B6 ($N = 12$) and CSS-16 ($N = 8$) male mice at increasing decibel levels to determine whether our prepulse intensities elicited a startle response. Startle threshold test sessions were similar to the PPI test protocol described above, except that each block of trials consisted of one trial each of 70- to 120-dB 20-msec white noise bursts at 5-dB increments, one trial of a 20 psi 40-msec air puff, and one null trial, with a 15-sec intertrial interval.

Identification of CSS lines with PPI variation: PPI measures of each strain were determined at three prepulse intensities (70, 75, and 80 dB). CSS mice and B6 mice were tested during the same sessions to allow for accurate comparison of their PPI measures. We tested 25 CSS-2 mice *vs.* 18 B6 mice, 12 CSS-16 mice *vs.* 22 B6 mice, and 18 CSS-18 mice *vs.* 11 B6 mice. For each strain, a main effect of strain on PPI was tested by a two-factor unbalanced analysis of variance (ANOVA) as implemented in R (Chambers *et al.* 1991). We applied a nominal significance level that corresponded to an overall 5% false-positive rate adjusted for the three strains tested, which was $P = 0.015$. For those strains that had a significant main effect, Student's *t*-tests were performed to test for significant differences in PPI levels at each prepulse intensity between the CSS and B6 strains. A nominal significance threshold of $P \leq 0.05$ was applied.

QTL mapping: F_2 intercross mice were generated by mating female CSS-16 mice to male B6 mice to generate F_1 progeny that were heterosomic B6 and A/J for chromosome 16, followed by brother-sister intercrossing. A total of 87 male F_2 intercross mice were generated, all of which were tested for PPI and genotyped for linkage analyses.

Genomic DNA from F_2 intercross mice and two each of B6 and CSS-16 mice was isolated from tail tips using established methods (Laird *et al.* 1991). DNA was genotyped for a total of 47 chromosome 16 SNPs (~1 cM density) selected from the Celera Discovery System database (SNPs are now available from the National Center for Biotechnology Information [NCBI] dbSNP database, http://www.ncbi.nlm.nih.gov/projects/SNP/MouseSNP.cgi/). Genotyping was performed using the Sequenom MassArray mass spectrometry system as described previously (Sklar *et al.* 2002). Genotype data from each SNP were included only if it met the following quality control criteria: (1) duplicate samples had identical genotypes, (2) SNPs were polymorphic in the F_2 intercross progeny, and (3) ~85% of genotypes were obtained. Of the 47 SNPs, 2 SNPs did not meet the >85% genotyping threshold, and 2 telomeric SNPS were monomorphic in that all intercross mice had only the B6 allele and not the A/J allele, presumably because the telomere of chromosome 16 in CSS-16 is of B6 origin (chromosomes were not genotyped completely to the telomeres during CSS panel construction; Singer *et al.* 2004).

PPI measures of the F_2 intercross mice were inspected prior to linkage analyses to select the optimal measure. The skewness and kurtosis of each measure was calculated using MAPMAKER/QTL (Lander *et al.* 1987) to determine whether the measures were normally distributed; hence, parametric linkage analyses were valid. The genetic variance of each PPI measure was estimated by determining the excess phenotypic variance in the F_2 intercross mice (due to genes) compared to the phenotypic variance in the B6 strain (due to noise), as described by Lander and Botstein (1989). Linkage analyses were performed using the 80-dB prepulse PPI measure due to its nearly normal distribution (skewness is 0.04, kurtosis is −0.22) and high estimated genetic variance (56%). Since only one measure was utilized for parametric linkage analyses, correction of significance levels to adjust for multiple testing was not required.

Parametric linkage analysis was performed using MAPMAKER/QTL (Lander *et al.* 1987). Genetic distances between SNPs were estimated from the intercross mouse genotype data. Additive, dominant, and recessive models are tested by MAPMAKER/QTL, where the phenotype of an individual F_2 intercross mouse i is given by $\text{Phenotype}_i = \text{mean} + (\text{Weight} \times \text{number of A/J alleles}) + (\text{Dominance} \times \text{Het}_i) + \text{Noise}$, where Weight is the additive component (the amount by which an A/J allele affects the phenotype), Dominance is the dominance component, and $\text{Het}_i = 1$ if individual i is a heterozygote; otherwise $\text{Het}_i = 0$. Since two-linked QTL were detected on chromosome 16, thereby violating the assumption in the linkage analysis of a single QTL, fixed QTL analysis was also performed to fit a two-locus model to the data. In this analysis, one putative QTL was fixed at the location of one of the detected linkage peaks, and a linkage scan was performed to search for a second QTL given the presence of the first QTL (Lander and Botstein 1989). A second QTL is likely to be present if the LOD score is substantially higher than the fixed QTL at the second QTL. The two-locus analyses tested all possible combinations of additive, dominant, and recessive effects at each QTL. To investigate whether epistatic interactions between the two loci exist, we used a general epistatic model as follows: for locus i, additive and dominance main effects were coded $A_i = [-1, 0, 1]$ and $D_i = [0, 1, 0]$, respectively, for genotypes [A/J, Het, B6]. In addition, four crossproduct terms were calculated: additive × additive epistatic effects, additive × dominance and dominance × additive effects, and dominance × dominance effects. Wald tests were performed to determine the significance of each epistatic term, and a likelihood ratio test was performed to jointly test all four epistatic terms. A nominal significance threshold of $P \leq 0.05$ was applied to the epistatic model tests.

We determined the chromosome-wide empirical significance threshold for chromosome 16 as follows. Single locus parametric linkage analysis of the 80-dB prepulse PPI measure was performed 10,000 times, each time randomly assigning

the observed PPI measures of the intercross mice. The maximum LOD score obtained from each of the 10,000 permuted analyses was noted, and the LOD score surpassed by 5% of the permuted analyses (LOD = 2.3) was taken as the chromosome-wide significance threshold, corresponding to a chromosome-wide $P \leq 0.05$. We utilized a 1-LOD confidence interval to define the boundaries of each QTL region detected by the fixed QTL analyses.

Candidate gene selection: Each chromosome 16 PPI QTL region was manually annotated using information from the University of California Santa Cruz Genome Bioinformatics website (http://www.genome.ucsc.edu). Specifically, we used evidence from the following tracks to annotate transcripts: RefSeq genes, MGC genes, mouse mRNAs, mouse ESTs, spliced ESTs, and CpG islands.

Transcripts in the QTL regions that were polymorphic between B6 and A/J were identified by investigating SNPs located within 10 kb of each transcript from the Celera Discovery System database that met the following criteria: (1) SNPs were genotyped in at least four inbred strains (A/J, B6, DBA/2J, and 129S1/SvImJ or 129X1/SvJ), (2) SNPs were homozygous in each strain, and (3) each allele was observed at least twice in the chromosomes analyzed. A transcript was considered to have sequence variation between B6 and A/J if ≥90% of SNPs had different alleles in the two strains. This criterion was applied to allow for the possibility of a small proportion of SNPs being artifactual (due to sequencing errors) and not polymorphic between the two strains. In this way, artifactual SNPs did not influence the selection of strong candidate genes with sequence diversity between B6 and A/J. Whole-genome shotgun reads from the Mouse Genome Sequencing Consortium (http://www.broad.mit.edu/mouse/) that were uniquely placed on the mouse genome, but that did not identify any polymorphisms, were used to confirm that transcripts in the QTL regions that did not have SNPs in the Celera database were not due to lack of sequence coverage, but indeed lacked sequence diversity between the strains. Genomic locations of SNPs from the Celera and WIBR databases were determined by aligning SNP flanking sequences to the NCBI build 33 mouse genome assembly (http://www.ncbi.nlm.nih.gov/genome/guide/mouse/) using the sequence search and alignment by hashing algorithm implementation for SNPs (ssahaSNP) (Ning *et al.* 2001).

To identify strong biological candidate genes in the PPI QTL regions, we queried the following public databases for biological and tissue expression information on the putative candidate genes: NCBI Entrez Gene (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene), NCBI PubMed (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed), and Mouse Genome Informatics (http://www.informatics.jax.org/).

RESULTS

PPI is elevated in CSS-16 compared to the B6 host strain: Due to the logistics of behavioral testing, we have initially focused our PPI studies on three substitution strains, CSS-2, CSS-16, and CSS-18. These strains carry donor A/J chromosomes (chromosomes 2, 16, and 18, respectively) that have previously been suggested to harbor PPI QTL (McCaughran *et al.* 1999; Hitzemann *et al.* 2001; Joober *et al.* 2002). PPI studies of the other substitution strains are being performed as time and resources permit. Individual male mice from each CSS and B6 were tested for PPI at 70- to 80-dB prepulse intensities at 5-dB increments with a 120-dB startle pulse, against a constant 65-dB background. A very significant main effect of strain on PPI level was detected for CSS-16 ($N = 12$ mice) compared to the B6 host strain ($N = 22$ mice) (ANOVA $P = 1.6 \times 10^{-11}$). This finding indicates that at least one QTL for PPI resides on chromosome 16. No significant effect of strain on PPI was detected for CSS-2 ($N = 25$) compared to B6 ($N = 18$) or for CSS-18 ($N = 18$) compared to B6 ($N = 11$) (ANOVA $P = 0.77$ and $P = 0.85$, respectively). As shown in Figure 1, the mean PPI levels at the 70- to 80-dB prepulse intensities of the CSS-16 strain were significantly elevated compared to the B6 strain (*t*-test $P = 6.0 \times 10^{-3}$–2.0×10^{-4}). The acoustic startle response to the pulse alone was not significantly different between CSS-16 and B6 (*t*-test $P = 0.26$). Assessment of the startle threshold in CSS-16 and B6 determined that the 70- to 80-dB intensities did not evoke a substantial startle response (<2% of the response elicited by the 120-dB pulse), indicating that the PPI differences between CSS-16 and B6 are associated with processing of the prepulse and not the brain stem startle reflex.

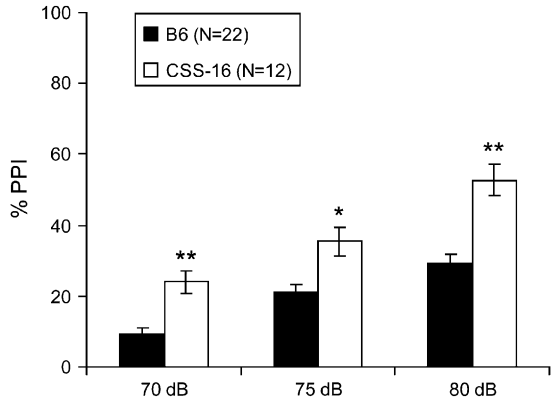

Figure 1.—Mean (±SEM) percentage PPI in the CSS-16 substitution strain compared to the B6 host strain. Significantly elevated PPI (*i.e.*, greater inhibition of the startle response) was observed in CSS-16 ($N = 12$) compared to B6 ($N = 22$) at 70- to 80-dB prepulse intensities. Asterisks indicate *t*-test significance levels in comparison to B6 at (*) $P < 0.01$ and (**) $P < 0.001$.

QTL mapping identifies two significant PPI loci on chromosome 16: We performed genetic intercross mapping to identify the PPI locus on chromosome 16. A total of 87 male F_2 intercross progeny were generated and tested for PPI at 70- to 80-dB prepulse intensities. In each test session, B6 mice and CSS-16 mice were also tested with the F_2 intercross progeny. This enabled us to confirm the significantly elevated PPI of CSS-16 compared to B6 observed in our original sample in an independent, larger group of mice (CSS-16, $N = 54$; B6, $N = 65$). A highly significant effect of strain on PPI was detected (ANOVA $P = 2.2 \times 10^{-16}$), and PPI levels at the 70- to 80-dB prepulse intensities were significantly

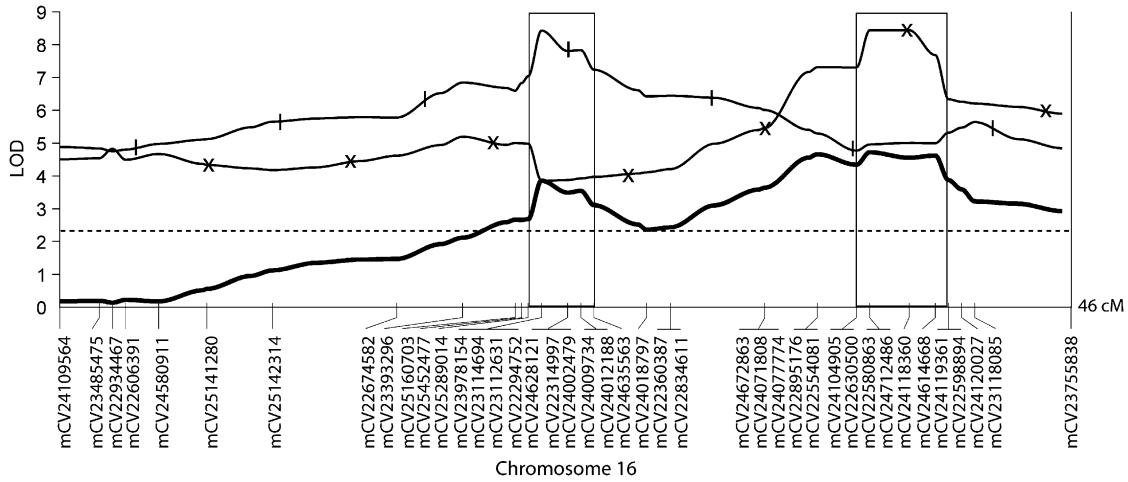

FIGURE 2.—Chromosome 16 F_2 intercross parametric linkage analysis results. Chromosome 16 SNPs ($N = 41$) were tested for linkage with PPI levels at an 80-dB prepulse intensity in 87 F_2 intercross mice. The single-locus model multipoint LOD curve is indicated by the solid line. Also shown are the results of two-locus fixed QTL analyses in which the proximal QTL peak (mCV23978154–mCV22294752; cross-hatched curve) or distal QTL peak (mCV24118360; ticked curve) was held constant. The 1-LOD confidence intervals of the proximal QTL and the distal QTL are indicated by the left and the right boxed regions, respectively. The chromosome-16 wide significance threshold (LOD 2.3) is indicated by the dotted line.

different between the two strains (*t*-test one-tailed $P = 9.3 \times 10^{-4}$–3.7×10^{-9}).

To maximize power to detect linkage, we selected the 80-dB prepulse PPI measure for analyses due to its high-estimated genetic variance (56%) and normal trait distribution. Single-locus parametric linkage analysis of the 87 F_2 intercross mice using 23 SNPs on chromosome 16 identified two PPI QTL. As shown in Figure 2 (solid line), fine mapping with 18 additional SNPs, for a total of 41 chromosome 16 SNPs, resulted in linkage peaks with LOD 3.9 at markers mCV23978154–mCV22294752 and with LOD 4.7 at markers mCV22580863–mCV24712486. Both QTL far exceeded the chromosome 16-wide empirical significance threshold of LOD 2.3. The QTL with LOD 3.9 had an empirical $P = 2.5 \times 10^{-3}$ (25 of 10,000 permutations of the linkage data resulted in maximum LOD scores >3.9) and the QTL with LOD 4.7 had an empirical $P = 2.0 \times 10^{-4}$ (2 of 10,000 permutations had maximum LOD scores >4.7).

To confirm that the two PPI QTL were separate loci, we further characterized each QTL by performing fixed QTL analyses using MAPMAKER/QTL to fit a two-locus model to the data. In these analyses, we fixed the existence and location of one QTL while parametric analyses were performed to scan for linkage to a second locus with an independent effect (LANDER and BOTSTEIN 1989). Fixed QTL analyses detected a two-locus maximum LOD 8.6 when either the QTL more proximal to the centromere was fixed (Figure 2, cross-hatched curve) or the QTL more distal to the centromere was fixed (Figure 2, ticked curve). The maximum-likelihood two-locus model was a significantly better fit (LOD >3.8) than either single-locus model, indicating that both QTL represent two independently statistically significant linkages. Using the 1-LOD confidence intervals to define approximate locus boundaries, the two-locus analysis refined the QTL more proximal to the centromere to a 3.0-cM (11.5 Mb) region from mCV25289014–mCV24635563 (Figure 2, left box) located from 40.0 to 51.5 Mb (NCBI mouse genome build 33) spanning chromosome 16qB4–qB5. The QTL more distal to the centromere was refined to a 4.2-cM (5.3 Mb) region from mCV22630500–mCV24119361 (Figure 2, right box), located from 78.6 to 83.9 Mb spanning chromosome 16qC3.1–qC3.3.

Since the two QTL were genetically linked, the appropriate mode of inheritance of each could be revealed only by the two-locus model. In contrast, in the single-locus scans, the perceived mode of inheritance at any location on the chromosome is influenced by both loci. The maximum-likelihood two-locus model determined that the proximal locus (at 40.0–51.5 Mb) fit an overdominance model, in that heterozygosity at the proximal QTL was associated with elevated PPI. The proximal QTL explained 19% of the variance in the F_2 intercross progeny (Table 1). The maximum-likelihood two-locus model determined that the distal QTL (at 78.6–83.9 Mb) fit a recessive model, in that homozygosity for the A/J (CSS-16) allele was related to elevated PPI and accounted for 23% of the variance (Table 1). The two PPI loci therefore together explained most of the estimated 56% genetic variance of the 80-db prepulse PPI measure utilized for these analyses.

The recessive effect of the distal QTL to increasing PPI, as indicated by the maximum-likelihood two-locus model, was supported by the mean PPI levels of the intercross mice grouped according to the nine genotype

TABLE 1

Maximum-likelihood two-locus model from parametric linkage analysis of the chromosome 16 F_2 intercross

	Position (cM)	Weight	Dominance	Variance explained (%)	Genetic effect of A/J allele
Proximal QTL	22.0	1.61	13.63	19	Overdominance; elevated PPI
Distal QTL	37.0	10.88	−9.82	23	Recessive; elevated PPI

Weight (additive component) and dominance (dominance component) of the QTL A/J allele (*i.e.*, CSS-16 allele) effect in the following genetic model: $\text{Phenotype}_i = \text{mean} + (\text{Weight} \times \text{Number of A/J alleles}) + (\text{Dominance} \times \text{Het}_i) + \text{Noise}$, where $\text{Het}_i = 1$ if individual i is a heterozygote; otherwise $\text{Het}_i = 0$.

combinations at the two QTL peaks (Table 2 and Figure 3). Mice that were homozygous for the A/J allele at the distal QTL peak had elevated PPI (51–58% PPI; Figure 3, right) compared to mice that were heterozygous (29–42% PPI; Figure 3, center) or homozygous B6 (24–43% PPI; Figure 3, left). Thus, the recessive effect of the distal QTL in increasing PPI was regardless of the genotype at the proximal QTL. Similarly, the overdominance inheritance model of the proximal QTL was supported by the mean PPI levels of the intercross genotype classes. When mice that were homozygous A/J at the distal QTL were excluded (to remove its unique contribution to increasing PPI), mice that were heterozygous at the proximal QTL had elevated PPI (44% PPI; Figure 3, short dashed line) compared to B6 or A/J homozygous mice (24–36% PPI; Figure 3, solid line or long dashed line, respectively), thereby supporting the "heterozygote only" effect of the proximal locus. However, caution is warranted when interpreting the data in Figure 3, since some genotype classes had only one or two mice due to few recombination events between the two linked QTL regions in the intercross progeny (Table 2).

No evidence for epistatic interactions between the PPI loci: We tested for interaction between the two chromosome 16 PPI QTL by performing linear regression analyses with a general epistatic model. The model included additive and dominance main effects at each locus, and the four possible epistatic effects (additive × additive, additive × dominance, dominance × additive, and dominance × dominance effects). None of the epistatic terms was significant (Wald tests $P = 0.31$, $P = 0.86$, $P = 0.48$, $P = 0.51$, respectively). Similarly, a joint likelihood ratio test of all four epistatic terms was not significant ($\chi^2 = 1.826$, d.f. $= 4$, $P = 0.77$).

Candidate genes in the chromosome 16 PPI loci: Manual annotation of the chromosome 16 PPI QTL regions identified 75 transcripts (including 35 known

TABLE 2

PPI levels of F_2 intercross mice classified by genotypes at the chromosome 16 proximal and distal PPI QTL peaks

Genotype			PPI (%)	
Proximal QTL	Distal QTL	*N*	Mean	Variance
	F_2 intercross progeny			
B6	B6	10	24.3	109.9
B6	Het	6	29.5	21.7
B6	A/J	1	58.6	NA
Het	B6	6	43.7	56.8
Het	Het	37	43.4	165.2
Het	A/J	2	59.9	16.6
A/J	B6	1	37.2	NA
A/J	Het	6	30.1	216.9
A/J	A/J	16	52.6	281.2
	Parental strains			
B6		65	34.3	105.1
CSS-16		54	48.9	202.1

Mean percentage PPI at an 80-dB prepulse intensity and variance of F_2 intercross mice utilized for linkage analyses. Intercross mice are grouped according to the nine genotype combinations of the markers at the peak of the proximal and distal chromosome 16 QTLs, where A/J indicates homozygosity for A/J (CSS-16) alleles, B6 indicates homozygosity for B6 alleles, and Het indicates heterozygosity. PPI data are omitted for two intercross mice that had missing genotypes for the marker at the distal QTL peak. For comparison, mean percentage PPI is shown for the B6 and CSS-16 parental mice tested during the same PPI test sessions at the intercross mice.

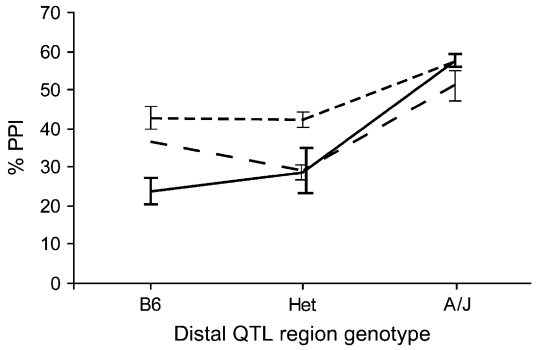

FIGURE 3.—Mean (±SEM) percentage PPI of F_2 intercross mice grouped according to genotypes at the chromosome 16 proximal and distal PPI QTL peaks. Genotypes at the distal QTL peak (mCV24118360) are plotted along the *x*-axis. Mean (± SEM) percentage PPI at an 80-dB prepulse intensity is plotted along the *y*-axis. Genotypes at the proximal QTL peak (mCV23978154–mCV22294752) are plotted and connected by lines corresponding to genotype. A/J (– – –) indicates homozygosity for A/J (CSS-16) alleles, B6 (—) indicates homozygosity for B6 alleles, and Het (- - -) indicates heterozygosity.

genes) in the proximal QTL region and 11 transcripts (including 6 known genes) in the distal QTL region. We sought to identify strong candidate genes in the QTL regions by investigating polymorphisms between the B6 and A/J (thus CSS-16) strains. Since most inbred mouse strains, including B6 and A/J, were derived from a single mixed but limited founder population (Silver 1995; Beck *et al.* 2000), comparison of their genomes results in a mosaic of segments of very low sequence diversity (*i.e.*, few SNPs) due to shared recent ancestry in the founder population or in a mosaic of segments of very high diversity (*i.e.*, many SNPs) due to ancestry from different subspecies in the founder population (Lindblad-Toh *et al.* 2000; Wade *et al.* 2002; Frazer *et al.* 2004). Therefore, the most probable location of the PPI gene underlying each of the chromosome 16 PPI QTL is in a segment of high sequence diversity between the B6 and A/J strains. Similar rationale has been used to identify candidate genes for several other complex traits (Grupe *et al.* 2001; Pletcher *et al.* 2004; Yalcin *et al.* 2004). A total of 33 transcripts (18 known genes) in the proximal QTL region and 9 transcripts (6 known genes) in the distal QTL region had high sequence diversity between the B6 and A/J strains, with a large proportion of SNPs (>90%) having different alleles in the two strains. These transcripts were consequently deemed strong PPI candidate genes.

DISCUSSION

We have identified two significant QTL for PPI of the startle response on mouse chromosome 16 using CSSs. The QTL were identified using only 87 F_2 intercross mice, 41 SNPs, and vastly fewer progeny and markers than utilized for traditional mapping methods using inbred, recombinant inbred, and outbred strains (Darvasi 1998; Mott and Flint 2002; Belknap 2003). Thus, as our study and others (Matin *et al.* 1999; Youngren *et al.* 2003; Krewson *et al.* 2004; Singer *et al.* 2004, 2005; Ackerman *et al.* 2005) have demonstrated, QTL mapping using CSS is an efficient approach to identifying loci for complex genetic traits.

Our two-locus linkage analyses indicated that the proximal PPI QTL on chromosome 16 had an overdominance effect, in that heterozygous mice had higher PPI levels compared to homozygotes of either parental genotype. In contrast, the distal PPI QTL on chromosome 16 appeared to have a recessive effect and, furthermore, homozygosity for the A/J (CSS-16) allele was sufficient for higher PPI regardless of the genotype at the proximal QTL. However, since the two QTL were linked, we could not conclusively determine the effect of each locus independent of the other in the intercross progeny. Therefore, we are currently performing backcrosses of informative recombinant intercross mice that segregate only one of the QTL. By comparing the PPI levels of recombinant and nonrecombinant backcross progeny, we will be able to resolve the mode of inheritance of each QTL, as well as more precisely refine the QTL region boundaries.

It is possible that the two PPI QTL interact epistatically, which will become apparent when analyzing the backcross PPI data, in that significant deviation from simple additive genetics effects would suggest interaction. Interactions are unexpected, however, given that we did not find significant evidence for epistasis under a general epistatic model, the inheritance models of each QTL indicated by the maximum-likelihood two-locus model were different, and the genetic variance remaining after accounting for both QTL was small (36 of 56% genetic variance accounted for).

To identify strong candidate genes in the chromosome 16 PPI QTL regions, we identified genes known to have sequence differences between the B6 and A/J inbred strains, presumably due to different ancestry from the founder population from which the strains were derived. There are several interesting biological candidate genes with sequence diversity in the proximal PPI QTL. Drd3 is the most intriguing candidate gene on the basis of numerous reports of PPI alterations in response to dopamine agonists and antagonists in mice, rats, and humans (Braff *et al.* 2001; Swerdlow *et al.* 2001a; Ralph-Williams *et al.* 2002, 2003) and pharmacological evidence for D3 receptor involvement in rat PPI (Caine *et al.* 1995; Bristow *et al.* 1996; Varty and Higgins 1998). Other strong biological candidate genes in the proximal QTL region include Lsamp, which mediates neuronal growth and axon targeting (Pimenta *et al.* 1995), Gap43, which is involved in axonal outgrowth and regeneration (Benowitz and Routtenberg 1997), Zbtb20, which is implicated in hippocampal neurogenesis (Mitchelmore *et al.* 2002), and Tagln3, which is selectively expressed in neuronal subpopulations (Ren *et al.* 1994) and is implicated in actin filament assembly (Mori *et al.* 2004). There is only one strong biological candidate gene with sequence differences between B6 and A/J in the distal PPI QTL region, Btg3, which is implicated in neurogenesis on the basis of high expression in the ventricular zone of the developing central nervous system (Yoshida *et al.* 1998). Of course, it is possible that other genes with sequence diversity in the QTL regions that have either known or unknown function may be responsible for the QTL. It is also feasible that the PPI gene in each QTL has low sequence diversity between B6 and A/J and thus did not meet our criteria for selection as a strong candidate gene. This latter scenario could occur if the PPI gene has different ancestry in the two strains despite the lack of sequence variation or if the causative sequence variant(s) in the gene arose since the divergence of the two strains from a common founder during their breeding history, for example.

We plan to pursue an integrated strategy to refine each of the chromosome 16 PPI regions and identify the

underlying PPI genes. Candidate genes in the QTL regions will be sequenced in A/J and B6 to identify potential functional sequence variants between the strains. Candidate genes will be prioritized for further study by performing mRNA expression profiling of brain regions in the CSPP circuit that regulates and mediates PPI (Fendt *et al.* 2001; Swerdlow *et al.* 2001a), on the basis that PPI genes must be expressed in at least one of these brain regions. Ultimately, to "prove" that a candidate gene is in fact the PPI gene, functional studies must be performed to characterize the role of sequence variants in PPI.

We observed a significant elevation of PPI in the CSS-16 line, which is homosomic A/J for chromosome 16, compared to B6. In contrast, previous studies have demonstrated that the A/J and B6 strains have similar PPI levels (Bullock *et al.* 1997; Logue *et al.* 1997; Paylor and Crawley 1997; Joober *et al.* 2002; Willott *et al.* 2003). This apparent discrepancy can be explained by the fact that, of the many genes that influence PPI, the CSS panel focuses on only one or a few PPI genes present on a particular chromosome. The chromosome 16 PPI genes appear to greatly enhance PPI, whereas the summation of the effects of all the PPI genes in A/J results in similar PPI levels compared to B6. Similarly, significantly elevated PPI compared to B6 has been reported in recombinant inbred strains derived from B6 and either A/J (Joober *et al.* 2002) or DBA/2J (Hitzemann *et al.* 2001). Although the latter two inbred strains had lower PPI than B6 in these studies, the strains apparently contributed one or more alleles that increased PPI relative to B6 in a proportion of the recombinant inbred strains.

Our distal PPI region is within a previously reported PPI region detected in a panel of recombinant congenic strains derived from the B6 and A/J strains (Joober *et al.* 2002). However, the effect of the QTL in our and the previous study appeared to differ. In our study, the QTL was associated with increased PPI in mice that were homozygous for the A/J (CSS-16) alleles across the QTL region. In contrast, in the previous study, the QTL mediated increased PPI in mice that were homozygous for the B6 alleles across the QTL region. It is possible that interactions with other PPI QTL that differed between the CSS-16 and B6 strains used in our study, and the recombinant congenic strains used in the previous study, could explain the disparate PPI effects.

The documented PPI deficits in patients with schizophrenia (Braff *et al.* 1978, 1992, 1999, 2001; Bolino *et al.* 1994; Kumari *et al.* 1999, 2000; Weike *et al.* 2000; Ludewig *et al.* 2002), obsessive-compulsive disorder (Swerdlow *et al.* 1993), ADHD (Castellanos *et al.* 1996; Hawk *et al.* 2003), acute manic bipolar disorder (Perry *et al.* 2001), and other neurological and psychiatric disorders (Swerdlow *et al.* 1995, 2001b; Grillon *et al.* 1996; Gomez-Wong *et al.* 1998; Pouretemad *et al.* 1998; Ornitz *et al.* 1999) provide impetus to investigate the human syntenic loci of rodent PPI QTL for risk genes for these illnesses. Our proximal chromosome 16 PPI region is syntenic with human 3q13.12–q13.31, where an ADHD linkage peak has been implicated (Bakker *et al.* 2003). Furthermore, the *DRD3* gene in this region was associated with impulsivity in one study (Retz *et al.* 2003), although association with ADHD *per se* has not been detected (Barr *et al.* 2000; Payton *et al.* 2001; Muglia *et al.* 2002). Our distal chromosome 16 PPI region is syntenic with human 21q21.1, where a locus for bipolar disorder has been reported (Detera-Wadleigh *et al.* 1996), although subsequent bipolar disorder studies have reported linkage peaks more telomeric (Detera-Wadleigh *et al.* 1997; Kelsoe *et al.* 2001). Patient association studies of the human orthologs of candidate genes from each of the mouse PPI loci will be the focus of future studies by our group.

In conclusion, we have rapidly identified two highly significant QTL for PPI of the startle response on mouse chromosome 16 using CSS. Furthermore, we have identified a small number of strong biological candidate genes in the loci that are feasible candidates for association studies in patients with PPI deficits.

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