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A QTL on chromosome 1 modulates inter-male aggression in mice

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Abstract

Aggression between male conspecifics is a complex social behavior that is likely modulated by multiple gene variants. In this study the BXD recombinant inbred mouse strains (RIS) were used to map quantitative trait loci (QTLs) underlying behaviors associated with intermale aggression. Four hundred and fifty-seven males from 55 strains (including the parentals) were observed at an age of 13 $\pm$ 1 week in a resident-intruder test following 10 days of isolation. Attack latency was measured directly within a 10 minute time period and the test was repeated 24 hours later. The variables we analyzed were the proportion of attacking males in a given strain as well as the attack latency (on days 1 and 2, and both days combined). On day 1, 29% of males attacked, and this increased to 37% on day 2. Large strain differences were obtained for all measures of aggression, indicating substantial heritability (intraclass correlations 0.10–0.18). We identified a significant QTL on chromosome (Chr) 1 and suggestive QTLs on mouse Chrs 1 and 12 for both attack and latency variables. The significant Chr 1 locus maps to a gene-sparse region between 82 and 88.5 Mb with the C57BL/6J allele increasing aggression and explaining about 18% of the variance. The most likely candidate gene modulating this trait is *Htr2b* which encodes the serotonin 2B receptor and has been implicated in aggressive and impulsive behavior in mice, humans, and other species.

Keywords

Attack latency; resident-intruder; anxiety; *Htr2b*; serotonin; social behavior; impulsive behavior; aggression; recombinant inbred strains; mouse

Introduction

Aggression is a behavior present in virtually every animal species, including humans. The fact that this behavior is so highly conserved suggests that the neurobiological underpinnings are likely to be similar across species. In every species investigated, significant individual variation is present for aggression-related traits, and there is overwhelming evidence that much of this variation is heritable (Maxson, 1981, Maxson *et al.*, 2013). The reason that this genetic variation has not become fixed during evolution is most probably because this trait is subject to a form of balancing selection conserving such genetic variation across a wide range of environments and stages (Broadhurst & Jinks, 1974). Van Zegeren showed that, at different stages of the development of a mouse population, either very high or very low levels of aggression would be advantageous (Van Zegeren, 1980) and it can easily be seen that such a situation would prevent the fixation of alleles associated with extreme levels of aggression and be conducive to maintaining genetic variation for a trait.

Since the early beginnings of the field of behavioral genetics, mice have been used extensively to investigate the genetics of aggressive behavior (Ginsburg & Allee, 1942, Scott, 1958). Mouse aggression is a complex phenotype with different types, such as offensive and defensive aggression (Brain, 1981, Brain *et al.*, 1991, Miczek *et al.*, 2001) or maternal aggression (Ferrari *et al.*, 2000, Palanza *et al.*, 1993), that can be tested in different ways (e.g., using standard opponents vs. same-strain opponents, isolated vs. non-isolated males, resident-intruder testing vs. a neutral cage test). Results from these different tests often do not correlate well across inbred strains (Roubertoux *et al.*, 1999), suggesting that different genetic and neurobiological architectures underlie distinct forms of aggression.

In the past few decades, some progress has been made in identifying gene variants important for aggression (Maxson *et al.*, 2013). Most of these were identified through mutation studies, the most notable probably being genes involved in the serotonergic (5-HT) system, such as MAOA (Brunner, 1996, Brunner *et al.*, 1993, Cases *et al.*, 1995) and several 5-HT receptors (Bevilacqua *et al.*, 2010, Saudou *et al.*, 1994). However it should be noted that, in humans, variants in these genes are mostly involved with abnormal impulsive aggression, and comorbid with depression, substance abuse, and borderline mental retardation (Bevilacqua & Goldman, 2013, Brunner, 1996).

Relatively few loci or gene variants have yet been identified using QTL mapping approaches. Brodtkin and colleagues (2002) identified significant QTLs on chromosomes (Chrs) X and 10 in a cross between the inbred strains A/J and NZB/BINJ. Using the same highly-aggressive NZB strain, Roubertoux and colleagues (2005) studied a cross with C57BL/6J and identified several QTLs on Chrs 8 and 12. Finally, Nehrenberg *et al.* (2010) identified QTLs on Chrs 7 and 19 in a cross between C57BL/6J and the NC900 line selected for high aggression.

Given the paucity of data on natural-occurring variation in aggression, we performed a large-scale study using an extensive set of recombinant inbred strains (RIS) derived from C57BL/6J and DBA/2J (Peirce *et al.*, 2004). We tested males belonging to 55 strains with the aim of identifying QTLs in this genetically diverse population. Both parental strains

show only low levels of aggression (Guillot *et al.*, 1994, Sluyter *et al.*, 1999) possibly leading to a situation where many strains would show no or very little aggression. To avoid this, we used isolated males and a resident-intruder test on two successive days, known to increase aggression (Hoffmann *et al.*, 1993, Roubertoux *et al.*, 1999).

Materials and Methods

Subjects

Fifty-three BXD mouse strains were used in this study along with both parental strains, C57BL/6J and DBA/2J (see supplementary worksheet S1 for exact numbers of animals tested per strain). A total of 457 males was tested when 3–4 months old. All animals used were housed and bred in the SPF mouse facility of the University of Bordeaux (Pessac) in a climate-controlled breeding room (temperature: 21+/-1 °C, humidity: 55+/-10%, 12 hour light-dark cycle with lights on at 7 am). Food (Safe, type 113, sterilized) and water (softened, sterilized) were available *ad libitum*. Animals were housed 2–4 in clear plastic cages (162x406x176 mm, Tecniplast) filled with poplar wood shavings (Souralit).

Resident intruder test

Prior to the aggression tests, animals were observed once in an open field (Delprato *et al.*, 2017) and, 4 days later in a radial maze test (1 session/day for 6 days; Delprato *et al.*, 2015). At the beginning of the radial-maze testing, animals were housed individually. Starting on the 5th day after the end of the radial-maze test, the resident-intruder tests took place on two subsequent days, that is, after a total of 10 days of isolation housing. As standard opponents, we used males of the pacific A/JOLaHsd strain, which never initiate attacks. Standard opponents were used in up to 10 different tests. The observation started when the intruder was introduced into the home cage of the test mouse and lasted until an attack occurred or a maximum of 10 minutes. Attacks were defined as an animal lunging towards its opponent and attempting to bite it. Observations were immediately stopped at this point to avoid wounding. Attack latency in seconds was measured directly using a stopwatch, assigning a score of 600 seconds to animals that didn't attack, and the test was repeated the next day, using a different A/J male intruder. From these data an "attack" variable was derived: individuals that attacked were given a score of 1 (attack latency < 600 sec) and those that didn't were given a score of 0 (attack latency = 600). A third variable ("combined attacks") was derived by combining the data from both days, animals that attacked at least once on either testing day obtaining a score of 1 and those that never attacked a score of 0. Hence, the strain mean for these attack variables reflects the proportion of attacking males in that strain.

Raw data

All data have been deposited in the GeneNetwork database and are publicly available. GeneNetwork trait IDs: Latency day 1: 18432, Latency day 2: 18463, Latency mean of days 1 and 2: 19507, Attack day 1: 18464, Attack day 2: 18465, Attack total: 18466.

Statistical analysis

All statistical analyses were performed with SAS University Edition 2.5 (SAS Institute Inc., Cary, NC, USA). Attack latencies are reported as means with SEMs. Attacks are expressed as proportion of attacking males per strain. To determine the significance of strain effects, we used a parametric one-way ANOVA for repeated measures for the attack latencies and the Kruskal-Wallis ANOVA (Siegel, 1956) for the attack variables. Although there is no direct repeated-measures Kruskal-Wallis test, this can still be done by analyzing the summed scores of days 1 and 2 for the overall effect and the difference between days 1 and 2 for the strains*day interaction. As a measure of the strength of strain differences, we estimated the intraclass correlation as follows. The between-strain and within-strain variance components were derived from the expected mean squares from one-way ANOVAs that were run using the SAS procedure GLM. The estimate of the intraclass correlation then equals $r_i = V_{\text{between strains}} / (V_{\text{between strains}} + V_{\text{within strains}})$.

Bioinformatics

The genetic and correlation analyses were performed in GeneNetwork (www.genenetwork.org; Delprato *et al.*, 2015, Delprato & Crusio, 2017, Parker *et al.*, 2017). Other traits mapping to the Chr 1 QTL interval were identified with the Mouse Genome Informatics/Strains, SNPs and Polymorphisms database (www.informatics.jax.org/strains_SNPs.shtml) and GeneWeaver (www.geneweaver.org). Potential candidate genes located within the interval were prioritized based on functional association using Gene (www.ncbi.nlm.nih.gov/gene), DAVID (version 6.8; david.abcc.ncifcrf.gov), GeneWeaver (www.geneweaver.org), and PubMed (www.ncbi.nlm.nih.gov/pubmed).

QTL mapping

The QTL mapping module of GeneNetwork was used to identify QTLs for the aggression trait data. QTL significance was assessed using the likelihood ratio statistic (LRS) obtained after 1000 permutations and 2000 bootstrap tests. QTLs were deemed significant if $P_{\text{genome-wide}} < 0.05$ and suggestive if $P_{\text{genome-wide}} < 0.63$, which yields, on average, one false positive per genome scan (Lander & Kruglyak, 1995). The analyses presented were run on raw data. Winsorizing did not affect the results (not shown).

Correlation analyses

A correlation analysis was done in the GeneNetwork BXD brain tissue gene-expression databases using the trait data for the proportion of males attacking at least once on either of the testing days. We used Spearman's rank correlation and the top 200 correlates for 10 brain regions. The following brain tissue datasets were considered in the analysis: basolateral amygdala [INIA Amygdala cohort Affy MoGene 1.0 ST Mar 2011 RMA, Males], cerebellum [SJUT Cerebellum mRNA M430 (March 2005) RMA], hippocampus [Hippocampus Consortium M430v2 (June 2006) RMA], hypothalamus [INIA Hypothalamus Affy MoGene 1.0 ST (Nov10) RMA, Males], prefrontal cortex [VCU BXD PFC SAL M430 2.0 (Dec 2006) RMA], striatum [HBP Rosen Striatum M430V2 (April 2005) RMA], adrenal gland [INIA Adrenal Affy MoGene 1.0ST (Jun12) RMA Males], ventral tegmental area [VCU BXD VTA Sal M430 2.0 (June09) RMA], nucleus accumbens

[VCU BXD NAc Sal M430 2.0 (Oct07) RMA], pituitary gland [INIA Pituitary Affy MoGene 1.0ST (Jun12) RMA Males].

Gene correlates were screened in DAVID (version 6.8; david.abcc.ncifcrf.gov) based on gene ontology classification in order to identify genes with relevance to aggression. Gene ids were updated in Gene (www.ncbi.nlm.nih.gov/gene), when needed.

Results

Strain differences

We found significant strain differences for latencies and proportions of attacking males on days 1 and/or 2. For the attack latencies (overall: $F_{50,370}=2.74$, $P<0.0001$; day 1: $F_{54,392}=1.93$, $P<0.0001$; day 2: $F_{50,370}=2.86$, $P<0.0001$) there was a significant decrease of overall latencies from day 1 to day 2 ($F_{1,370}=1.37$, $P<0.0001$) that differed among strains (days*strain: $F_{50,370}=1.40$, $P=0.044$). In contrast, for the attack variables (day 1: $\chi^2=111.3$, $df=54$, $P<0.0001$; day 2: $\chi^2=113.7$, $df=50$, $P<0.0001$), the change from day 1 to day 2 was not different among strains ($\chi^2=53.7$, $df=50$, $P>0.33$). Combined attacks also differed strongly among strains ($\chi^2=112.7$, $df=50$, $P<0.0001$). From the ANOVAs we derived the intraclass correlations, which were moderate to low (latency day 1: 0.103, latency day 2: 0.184, attacks day 1: 0.148, attacks day 2: 0.175, combined attacks: 0.180).

QTL mapping

In our initial analyses, no significant loci were found, although two QTLs on Chr 1 were suggestive or borderline suggestive for all variables (Figs. 1–6). Another suggestive QTL was found for attack latency on day 2 on chromosome 12 (Fig. 2). However, there were 5 BXD strains (BXD61, BXD65, BXD66, BXD89, and BXD90) in which not a single animal attacked on either day. Obviously, negative scores are not possible, so this constitutes a floor effect. Including strains with identical phenotypical scores, even though their underlying genetic propensity for aggression might vary widely, could constitute a potential confound and obscure possible QTL effects. We therefore ran the analyses again, but excluded these 5 strains. This resulted in one of the suggestive QTLs on Chr 1 reaching significance for the mean latency (days 1 and 2 combined), the proportion of attacking males on day 2, and for total attacks (see Figs. 3, 5, and 6, respectively). For the other variables, this QTL was suggestive, and in one case (attack latency on day 2) close to significant. The Chr 1 QTL peak has LRS values of 17.1 (mean latency), 21.1 (total attacks), and 19.1 (attacks day 2), and for all three variables occurs at 83.9 Mb near rs13475957. The B6 allele increases aggression scores and explains about 18% of the variance. The QTL interval extends from 78.0 to 88.0 for mean attack latency, 82.0 to 88.5 Mb for total attacks, and 79.0 to 91.0 Mb for attacks day 2 and contains ~95 genes and 175 genes, respectively. The suggestive QTL on Chr 12 remained for attack latency on day 2 (LRS=12.6, interval 72.0–76.0 Mb) and for the mean attack latencies (LRS=12.7, interval 73.0–76.0 Mb). However, if we control for the effects of the Chr 1 locus using composite interval mapping and the rs13475957 locus, the Chr 12 peak disappears. Similarly, controlling for the Chr 12 QTL locus (SNP rs6346026), reduces the LRS of the Chr 1 peak (Fig. 7).

Positional candidates

Of the 96 genes within the Chr 1 interval (see supplementary worksheet S2) for which gene ontological and functional annotation is available, one gene, *Htr2b*, 5-hydroxytryptamine (serotonin) receptor 2B, is the only one known to be associated with behavior, specifically, with impulsive aggression. Besides *Htr2b*, 10 genes are linked to neural related processes (*Efhdl*: EF-hand domain family member, *Akp3*: alkaline phosphatase 3, intestine, not Mn requiring, *Chmd*: chondromodulin, *Dner*: delta/notch-like EGF repeat containing, *Ecell*: endothelin converting enzyme-like 1, *Irs1*: insulin receptor substrate 1, *Itm2c*: integral membrane protein 2C, *Nppc*: natriuretic peptide type C, *Neu2*: neuraminidase 2, *Nmur1*: neuromedin U receptor 1).

Brain tissue correlates

The aggression phenotype for which a significant QTL was obtained, total attacks, correlated most strongly with the striatum mRNA ($|0.55-0.81|$) dataset. The other tissue datasets correlated as follows: prefrontal cortex ($|0.55-0.77|$), hypothalamus ($|0.56-0.76|$), cerebellum ($|0.51-0.74|$), ventral tegmental area ($|0.53-0.73|$), nucleus accumbens ($|0.52-0.69|$), pituitary gland ($|0.44-0.66|$), adrenal gland ($|0.46-0.64|$), basolateral amygdala ($|0.44-0.63|$), and hippocampus ($|0.43-0.63|$). Gene correlates deemed relevant to aggression are associated with behavioral traits such as fear response, locomotion, social behavior, vocalization, learning, and addiction. Other relevant gene correlates are related to neurodegenerative disease, olfactory receptor signaling and steroid processes. (See supplementary worksheet S3 for a list of the genes with relevance to the trait.)

Discussion

In this study the BXD family of strains was used to identify loci and candidate genes involved in isolation-induced intermale aggressive behavior as measured using the resident-intruder test. Overall, the BXDs are not very aggressive; which is not surprising considering that compared to other mouse strains neither are the parental strains (Guillot *et al.*, 1994), especially the C57BL/6J (both in a neutral cage and a resident-intruder test, cf. Sluyter *et al.*, 1999). In spite of this, there were significant differences among strains that enabled us to identify a QTL on Chr 1 for mean attack latency and for total attacks after eliminating a floor effect by removing the data for five strains for which not a single animal attacked on either test day. It should be noted that some BXD strains score either higher or lower than the parental strains, indicating that both parents harbor alleles that increase and that decrease the propensity to attack.

A total of 21 QTLs are listed in the MGI database for the 82.0 to 88.5 Mb QTL interval on Chr 1, such as loss of righting induced by ethanol (Bennett *et al.*, 2006)<http://jpet.aspetjournals.org/content/319/1/299>, cocaine related behavior (Jones *et al.*, 1999), and emotionality (De Ledesma *et al.*, 2006). Three of these have possible relevance for aggression: *Emo4* (ID MGI:3582653): emotionality in an open field test (De Ledesma *et al.*, 2006), *Cocrb1*: cocaine related behavior in an open field test (Jones *et al.*, 1999), and *W10Q7* associated with growth and body size (Rocha *et al.*, 2004). Another QTL that may be relevant to the trait is associated with blood pressure (Woo & Kurtz, 2003).

Based on functional relevance to the phenotype, the strongest candidate within the narrow QTL interval is *Htr2b*, a gene that encodes one of several serotonin receptor subtypes. *Htr2b*^{-/-} mice are hyperactive and have deficits in pre-pulse inhibition of the startle response, social interaction, memory, and attention, and increases in wakefulness as well as impulsivity (Pitychoutis *et al.*, 2015).

HTR2B mutations are also associated with aggression in other species, such as pigs (Chu *et al.*, 2017) and zebrafish (Theodoridi *et al.*, 2017). In humans, a specific 5-HT_{2B} receptor stop codon (HTR2B Q20*) observed in a Finnish male population segregates with severe impulsivity, psychosis, and early-onset schizophrenia (Bevilacqua *et al.*, 2010). These individuals exhibit increased aggressive behavior towards themselves and others, as well as criminal activity, substance abuse, suicide, and depression (Bevilacqua & Goldman, 2013), especially after alcohol use (Tikkanen *et al.*, 2015). The biological basis for impulsive aggression is based on the hypothesis that normal levels of serotonin have an inhibitory effect on brain regions that are involved in aggressive behavior so that reduced serotonin activity increases aggression (Goodman & New, 2000). Serotonin modulates and in some instances inhibits aggressive impulses via 5-HT receptors of the prefrontal cortex which in turn modulates serotonin signaling in subcortical regions (Siever, 2008). In agreement with this, it has been shown that excessive aggressiveness and impulsivity of male *nNOS*^{-/-} mice is caused by decreasing 5-HT turnover and deficient 5-HT_{1A} and 5-HT_{1B} receptor function in brain regions regulating emotion: hypothalamus, cerebellum, midbrain, hippocampus, amygdala, and cerebral cortex, indicating that neuronal NO signaling is upstream of serotonin regulation of aggression (Chiavegatto *et al.*, 2001).

A possible interaction was detected between Chrs 1 and 12. Within the Chr 12 interval is the *Hif1a* gene, which regulates the adaptive response to hypoxia and activates the transcription of many genes involved in this process (The Uniprot Consortium, 2017). There is some evidence for a relationship between hypoxia and increased aggressive behavior (Nakazawa & Tang, 2006). This may be relevant to the NO role in serotonin regulation because NO is a major signaling molecule in the response to hypoxia. It increases blood flow and oxygen supply while reducing oxygen consumption. This is speculative but worthy of further investigation.

Aggressive behavior is associated with several human psychiatric and neurodegenerative disorders such as PTSD, borderline personality disorder, ADHD, Autism, Huntington's Disease, Parkinson's Disease, and others. The identification of the QTL region in mice for aggressive behavior is relevant to aggression in humans given the conservation of aggressive behavior across species. The QTL interval on mouse Chr 1 is syntenic with human Chr 2. The physiological basis of aggressive behavior has a long history of investigation but there is little information concerning the underlying genetic factors due to the complexity of the phenotype and the small genetic contribution from many genes. However with finer mapping methods, enlarged RIS sets, and more species under study, it may be possible to identify additional genes associated with aggressive behavior and understand its relationship to brain disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Attack latencies on day 1

(A) BXD strain differences for attack latency day 1 (ordered by strain). (B) Whole genome QTL scan including all BXD strains. The x-axis represents chromosome number and megabase (Mb) position and the y-axis represents the likelihood ratio statistic (LRS) of linkage. Blue lines represent LRS across the genome. The pink and gray horizontal lines are approximate threshold values which are used to assess whether a peak is significant ($P < 0.05$) or suggestive ($P < 0.63$) respectively. Red lines indicate negative values (C57BL/6J alleles increasing trait values) and green lines indicate positive values (DBA/2J alleles increasing trait values). Gray lines are shown when the parental strain is unknown. The yellow bars represent the relative frequency of peak LRS at a given location from 2000 bootstrap resamples. (C) As B, but with five BXD strains (61, 65, 66, 89, 90) excluded.

Figure 2. Attack latencies on day 2
(A) BXD strain differences for attack latency on day 2 (ordered by strain). (B) Whole genome QTL scan including all BXD strains (see Figure 1 legend for description). (C) As B, but with five BXD strains (61, 65, 66, 89, 90) excluded.

Figure 3. Mean attack latencies on days 1 and 2

(A) BXD strain differences for mean attack latencies on days 1 and 2 (ordered by strain). (B) Whole genome QTL scan including all BXD strains (see Figure 1 legend for description). (C) As B, but with five BXD strains (61, 65, 66, 89, 90) excluded.

Figure 4. Proportion of males attacking on day 1

(A) BXD strain differences for proportion of males attacking on day 1 (ordered by strain).

(B) Whole genome QTL scan including all BXD strains (see Figure 1 legend for description).

(C) Whole genome QTL scan with five BXD strains (61, 65, 66, 89, 90) excluded.

Figure 5. Proportion of males attacking on day 2

(A) BXD strain differences for proportion of males attacking on day 2 (ordered by strain).

(B) Whole genome QTL scan including all BXD strains (see Figure 1 legend for description).

(C) As B, but with five BXD strains (61, 65, 66, 89, 90) excluded.

Figure 6. Proportion of males attacking at least once on days 1 and 2

(A) BXD strain differences for proportion of males attacking at least once on days 1 and 2 (ordered by strain). (B) Whole genome QTL scan including all BXD strains (see Figure 1 legend for description). (C) As B, but with five BXD strains (61, 65, 66, 89, 90) excluded.

Figure 7. Chromosome 1 and chromosome 12 interaction

(A) Whole genome QTL scan for attack latency day 2 excluding 6 BXD strains (B) Composite interval QTL scan suppressing the chromosome 1 QTL locus, rs13475957 (C) Composite interval QTL scan suppressing the chromosome 12 QTL locus, rs6346026.