Mamm Genome (2011) 22:209–234
DOI 10.1007/s00335-011-9315-9

# The genetic basis of adrenal gland weight and structure in BXD recombinant inbred mice

**Domenico L. Di Curzio · Daniel Goldowitz**

Received: 18 January 2011 / Accepted: 19 January 2011 / Published online: 24 February 2011


**Abstract** Adrenal gland function is mediated through secreted hormones, which play a vital role in the autonomic and hypothalamic-pituitary-adrenal (HPA)-axis-mediated stress response. The genetic underpinnings of the stress response can be approached using a quantitative trait locus (QTL) analysis. This method has been used to investigate genomic regions associated with variation in complex phenotypes, but it has not been used to explore the structure of the adrenal. We used QTL analyses to identify candidate genes underlying adrenal weight and adrenal cortical zone and medulla widths. We used 64 BXD recombinant inbred (RI) strains of mice ($n = 528$) and 2 parental strains (C57BL/6J and DBA/2J; $n = 20$) to measure adrenal weights and adrenal zone widths. For adrenal weight, we found significant QTLs on chromosome 3 for females (*Fawq1*) and Chr 4 for males (*Mawq1*) and suggestive QTLs on Chrs 1, 3, 10, and 14 for females and Chrs 2, 4, 10, 17, and X for males. We identified a significant QTL on Chr 10 (*Mawdq1*) and a suggestive QTL on Chr 13 for male adrenal total width. For male adrenal medulla width, we found a significant QTL on Chr 5 (*Mmwdq1*) and a suggestive QTL on Chr 1. We also identified significant QTLs on Chrs 10 (*Mxwdq1*) and 14 (*Mxwdq2*) for male X-zone width. There are 113 genes that mapped within the significant QTL intervals, and we identified 4 candidate genes associated with adrenal structure and/or function. In summary, this study is an important first step for detecting genetic factors influencing the structure of the adrenal component of the HPA axis using QTL analyses, which may relate to adrenal function and provide further insights into elucidating genes critical for stress-related phenotypes.

D. L. Di Curzio (✉)
John Buhler Research Centre, Department of Human Anatomy and Cell Science, University of Manitoba, Winnpeg R3L 3P4, MB, Canada
e-mail: ddicurzio@gmail.com

D. L. Di Curzio · D. Goldowitz (✉)
Centre for Molecular Medicine and Therapeutics, Child and Family Research Institute, Department Medical Genetics, Graduate Program in the Neurosciences, The University of British Columbia, Vancouver V5Z 4H4, BC, Canada
e-mail: dang@cmmt.ubc.ca

## Introduction

The adrenal gland is an endocrine organ and its function is mediated through the secretion of hormones (Ehrhart-Bornstein et al. 1998; Li et al. 2002; Shelton and Jones 1971). It plays a vital role in both the acute and the prolonged mammalian stress response. In all adult vertebrates, the adrenal gland comprises the adrenal medulla and three layers of the adrenal cortex that surround the medulla (Deschepper et al. 2004; Nussdorfer 1986). These cortical regions include the zona glomerulosa (ZG), zona fasciculata (ZF), and zona reticularis (ZR). In mammalian development, the medulla and cortex are derived from different germ layers that later fuse to form the adrenal gland during embryogenesis (Rüsse and Sinowatz 1998; Wurtman 2002). The medulla and each cortical region have many important functions associated with endocrine hormone synthesis, and the cortex and medulla are known to be activated distinctly during the stress response (Parker et al. 1993). Adrenal medullary chromaffin cells contain catecholamines and several neuropeptides (Li et al. 2002; Ulrich-Lai et al. 2006) that are released directly into the blood stream during "fight or flight" situations, while the ZF of the adrenal cortex secretes cortisol during the stress response and is regulated by neuroendocrine hormones

through the hypothalamic-pituitary-adrenal (HPA) axis (Ehrhart-Bornstein and Bornstein 2008; Fujieda and Tajima 2005; Keegan and Hammer 2002). However, both stress response systems are intimately linked (Ehrhart-Bornstein and Bornstein 2008; Goldstein and Kopin 2008; Wurtman 2002) and activate the adrenal cortex and medulla despite their divergent germ layer development, which suggests that the structure of the adrenal gland plays a role in its functions. Hence, an examination of adrenal structure may provide important findings to help narrow the search for understanding more about adrenal function and the stress response.

Although the adrenal gland has similar structural features and functions in all mammalian species, including the stress response, there are some structural characteristics and functions that are unique to the murine endocrine system and mice in particular. For instance, it was found that mice and some other rodents cannot secrete adrenal androgens from the ZR (Keegan and Hammer 2002; VanWeerden et al. 1992), and they secrete corticosterone from the ZF because they are unable to synthesize cortisol (Heikkilä et al. 2002). The inability of mice to secrete both adrenal androgens and cortisol has been linked to the lack of 17α-hydroxylase expression in the adrenal gland (Heikkilä et al. 2002; VanWeerden et al. 1992). In terms of structure, histological examination of the mouse cortex shows that in addition to the three primary cortical zones distinguished by varying cell morphology (Deschepper et al. 2004; Nussdorfer 1986; Shelton and Jones 1971; Zelander 1959), mice also display a thin layer referred to as the zona intermedia (Nussdorfer 1986; Shelton and Jones 1971) and a transient cortical layer referred to as the X-zone, which was first identified by Masui and Tamura (1924), Howard-Miller (1928), and Deanesly (1931).

There are also sex-dependent differences in the structure and function of the adrenal gland in mice. In particular, pubertal and adult female mice exhibit significantly higher adrenal cortex, adrenal medulla, and whole adrenal gland weights compared to males (Badr and Spickett 1971; Bielohuby 2007; Moog et al. 1954; Tanaka and Matsuzawa 1995) despite females having significantly lower body weights relative to male mice. Female mice also have larger adrenal glands by volume, show elevated serum corticosterone levels, and contain more adipose-stored lipids compared to males from approximately 5 weeks of age onward (Bielohuby 2007). In addition, although the function of the X-zone is debated and still uncertain (Gersh and Grollmann 1939; Hershkovitz et al. 2006; Keegan and Hammer 2002), this zone typically disappears in males at about 5–6 weeks of age (Deacon et al. 1986; Howard-Miller 1928; Keegan and Hammer 2002), yet it remains present in nulliparous females into adulthood until their first pregnancy when it degenerates as well (Deacon et al. 1986; Deanesly 1931; Holmes and Dickson 1971; Howard-Miller 1928; Masui and Tamura 1924; Sato 1968; Tanaka and Matsuzawa 1995).

Adrenal gland development, structure, and function have also been examined from the genetic perspective (Badr and Spickett 1971; Badr et al. 1968; Beuschlein et al. 2002; Janat and Shire 1987; Lemos et al. 2006; Lin et al. 1995; Moore et al. 1999; Pawlus 1983; Solberg et al. 2006; Tanaka et al. 1994, 1995; Vidal and Schedl 2000). Many studies have documented genes associated with adrenal structure and function in rodents and humans. In humans, one study examined the gene profiling data for fetal and adult adrenal glands, identifying significant gene expression differences for 25 transcripts that may help delineate mechanisms influencing adrenal development and growth (Rainey et al. 2002). In addition, a review of the many gene mutations related to human and/or rodent diseases or dysfunction of the adrenal gland and adrenogonadal development, as well as rodent gene knockout experiments, has been published by Keegan and Hammer (2002). Their review indicates that mutations in different genes can result in comparatively similar adrenal-related phenotypes. Mutations of the same genes, however, also result in multiple adrenal structural and functional alterations, indicating that the genetic underpinnings of adrenal gland structure and function are complex. When examining the genetic nature of complex phenotypes such as adrenal structure and function, quantitative trait locus (QTL) analyses are often effective for locating regions in the genome that are associated with variation in such phenotypes (Williams et al. 1998). As the adrenal gland is critical for the stress response, one useful strategy for analyzing the genetics underlying both adrenal structure and the stress response is the use of groups of inbred and recombinant inbred (RI) mice that express genetically mediated differences in stress response and anxiety. RI analyses are valuable for examining complex traits and performing QTL mapping when there is accessible genotypic data compiled and an abundance of animals available for investigation of each RI strain (Lad et al. 2007; Philip et al. 2010; Reiner et al. 2008).

In the current study, BXD RI strains were used. These mice are derived from the C57BL/6J (B6) and DBA/2J (D2) strains, which have been found to differ significantly for various phenotypes, including behavioural stress responses (Tarricone et al. 1995), stress responses to and consumption of alcohol (Belknap et al. 1993; Eleftheriou and Elias 1975; Gill et al. 1996; Kakihana et al. 1968; Roberts et al. 1992, 1995), and behavioural displays of anxiety (Trullas and Skolnick 1993). D2 mice show heightened anxiety-related behavioural responses during dark–light (DL) testing (Võikar et al. 2005), elevated plus maze (EPM) measures (Võikar et al. 2005; Yilmazer-Hanke et al. 2003), fear-sensitized acoustic startle response

(ASR) paradigms (Yilmazer-Hanke et al. 2003), open field (Ponder et al. 2007a; Tarricone et al. 1995) and elevated zero maze (EZM) testing, and contextual fear conditioning (Ponder et al. 2007a), as well as restraint stress (Tarricone et al. 1995) compared to B6 mice. Due to the observable phenotypic differences found between these inbred strains, a multitude of studies have endeavoured to identify "candidate" genes for QTLs that may cause or influence the phenotypic differences between B6 and D2 mice (Gill et al. 1996). The aim of this study is twofold. First, we directly analyzed the structure of the adrenal gland in the parental and BXD RI strains to determine if there are strain differences in adrenal gland weight and size that could be quantified and examined with a QTL approach. Second, by using inbred strains of mice that differ significantly in terms of anxiety and stress response, we wanted to indirectly examine whether there are genotypic and/or phenotypic connections between adrenal structure and function, particularly with respect to the adrenal stress response.

In the present study, we measured total adrenal weights and widths of adrenal zones in 64 BXD RI and both parental strains of mice. These measurements were analyzed for the detection of QTLs to implicate candidate genes that might be associated with strain- and sex-related adrenal weight and anatomical differences in adrenal gland size. We identified two significant QTLs for adrenal gland weight on Chrs 3 and 4 and suggestive QTLs on multiple chromosomes. For adrenal width measurements, we found significant QTLs on Chrs 5, 10, and 14 for the male adrenal measures but not for the females. We did find multiple suggestive QTLs for both male and female measures on various chromosomes. We also draw attention to a list of 113 genes located within these loci that are suggested to account for phenotypic differences amongst the parental and RI strains analyzed. From the QTL analyses and a bioinformatics-based investigation of the QTLs, we identified four candidate genes potentially linked to adrenal gland weight. Lastly, our results for adrenal weight and structure show some intriguing relationships with previous research performed on mice for stress and anxiety measures. To our knowledge, this article reports the first exploration of QTLs involved in two aspects of the adult mouse adrenal: weight and anatomical structure.

## Methods

### Animals

A total of 548 mice were used in this study, which included 64 BXD RI strains ($n = 528$, mean = 8.25 mice per strain) and 2 parental strains (C57BL/6J and DBA/2J, $n = 20$). There were approximately an equal number of males and females used ($n = 273$ and 275, respectively). The mice were examined between 48 and 86 days of age (mean ± standard error of the mean = 58.78 ± 0.33). The animals were produced at the Oak Ridge National Laboratory (ORNL) and then were transferred to the University of Tennessee Animal Facility in a temperature-controlled van. At the facility, the mice were housed in a specific pathogen-free environment, which was kept at approximately of 23.5°C on a 12-h light/dark cycle with 45–50% humidity. They were maintained on a diet of 5% fat Agway Prolab 3000 rat and mouse chow. All experimental procedures were conducted according to the Principles of Laboratory Animal Care protocols (NIH publication No. 86-23, revised 1985) and were approved by the Institutional Animal Care and Use Committee at the University of Tennessee Health Science Center.

### Adrenal removal and weight analysis

Cages of BXD mice were placed on a cart or rack and kept at one end of the room in which the mice were taken for analysis. Mice were then removed from each cage and weighed at the other end of the room, and placed in a separate cage. After 10 min, each mouse was then quickly cervically dislocated, and their ventra opened to provide access to the abdominal cavity. Whole kidneys were then extracted from the body one at a time, careful to include the adrenal glands located at the anterior tip of each organ. The adrenal glands were then carefully dissected from the kidneys and surrounding tissue under a Zeiss dissecting microscope. Lastly, the adrenal glands were weighed on a Mettler Toledo scale to a tenth of a milligram and placed in vials overnight in Bouin's fixative (75 ml picric acid, 25 ml 37% formalin, 5 ml glacial acetic acid).

### Tissue preparation and histology

After fixation, the adrenals were embedded in paraffin blocks and positioned in a horizontal plane for sectioning. There were two to five adrenals embedded in each block and were placed together according to histological date sacrificed and by sex. The embedded adrenal glands were sectioned at 8 μm using a Leitz rotating microtome (Leitz 1512; Leitz, Wetzlar, Germany). Two consecutive sections out of every 20 were mounted on Superfrost Plus slides (Fisher Scientific, Hampton, NH, USA) and stained with hematoxylin and eosin (H&E) for histological observation. After identifying the section that contained the most extensive region of each adrenal, additional sections were mounted ±10 from this section and stained with H&E. These latter sections were examined to select the section

with the largest adrenal area, and structural measures were performed on this adrenal section.

### Microscopy

All adrenal gland sections were analyzed using bright-field light microscopy. Morphometric examination was conducted using a Zeiss Axiovert 200 inverted light microscope with a Zeiss AxiocamHR colour camera, which was attached to a computerized Zeiss Axiovision 4.6 imaging system (Zeiss, Jena, Germany). The examination included quantitative measurements of the adrenal cortical zones and medulla widths, as well as qualitative assessment of the presence of the X-zone region and adipose tissue (lipoid zone). For the total width and some medulla measurements, images of the adrenal sections were taken using a 5× objective. To obtain more precise measures of the cortical regions and the medulla, the tissue typically was viewed with a 10× objective and optovar setting at 1.60.

### Adrenal cortex and medulla measurements

The adrenal width measurements were taken at the approximate "midline" where the width of the adrenals was the greatest across the horizontal sections analyzed. The total width and width of each region was measured along this midline, with the cortical zone widths being averaged for the measures taken on either side of the medulla ($n = 2$). The H&E staining provided visible morphological borders between each of the zones and the medulla (Fig. 1).

### Statistical analysis and QTL mapping

The adrenal weight and regional width measurements were acquired from 64 BXD lines and both parental strains in this study. A linear model was used to examine the effects of factors that may affect the adrenal measures being investigated. Sex, age, body weight, and the respective phenotypes of each BXD strain were taken into consideration in the analyses. Statistical analyses were conducted using the SPSS 16.0 software program. Both quantitative and qualitative assessments were analyzed separately for the different BXD strains and for male and female mice with MANOVA and GLM analyses. Two-tailed Student's *t* tests were conducted to compare the male and female data. In addition, separate Pearson's *R* correlations were performed to compare the adrenal width data for each region with the total adrenal weight data. For all analyses, *p* values less than or equal to 0.05 were deemed statistically significant. In addition, broad-sense heritability measures were calculated using a method derived from Hegmann and Possidente (1981) that compares between-strain and total differences with the calculation $h^2 = \mathrm{VA}/(\mathrm{VA} + 2\mathrm{VE})$, where VA is genetic variance and VE is environmental variance. QTL mapping analyses were performed for the adrenal gland weight and width measures. These analyses classified the BXD strains according to their genotypes using distinct chromosomal markers and compared them separately with the adrenal weight and width phenotypic variables. The significant and suggestive genome-wide loci were generated using 1000 permutation tests that randomly reassign trait values across the strains and compare the permuted and original data to examine the significance of the QTL(s). Both the likelihood ratio statistic (LRS) and logarithm of odds (LOD) values (LOD = LRS/4.61) were used to measure the linkage between quantitative phenotypic variations and genetic differences at a particular genetic locus (QTLs). The QTL maps were all generated using GeneNetwork (WebQTL) (www.genenetwork.org). Lastly, we surveyed the genes mapped within the significant QTLs using the National Center for Biotechnology Information (NCBI) Entrez Gene website (http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene) and the Jackson Laboratory's MGI Mouse Genome Database project (www.informatics.jax.org) to identify potential candidate genes.

## Results

### Adrenal weight measurements

For the parental strains, the main effects for both body weight and adrenal weight show that they do not differ significantly ($p = 0.281$ and $p = 0.237$, respectively), despite the B6 mice showing lower values for average body weight ($19.89 \pm 0.80$ g) and adrenal weight ($1.83 \pm 0.17$ mg) compared to D2 mice, which have an average body weight of $21.50 \pm 1.13$ g and average adrenal weight of $2.12 \pm 0.17$ mg. When accounting for sex differences within the parental strains, we found that body weight and adrenal weight still show no significant differences for both males and females; however, the D2 mice still exhibit slightly higher values for both males and females for body weight ($23.22 \pm 1.90$ and $20.07 \pm 1.19$ g) and adrenal weight measures ($1.91 \pm 0.23$ and $2.30 \pm 0.23$ mg) compared to the B6 male ($22.30 \pm 0.24$ g and $1.39 \pm 0.14$ mg) and female ($17.96 \pm 0.41$ g and $2.18 \pm 0.16$ mg) mice. When just considering sex, there is a significant difference between males and females ($p = 0.007$ and $p = 0.016$, respectively), with males having a higher body weight ($22.81 \pm 1.02$ g) and lower adrenal weight ($1.68 \pm 0.16$ mg) compared to females, which have an average body weight of $19.11 \pm 0.72$ g and average adrenal weight of $2.25 \pm 0.14$ mg.

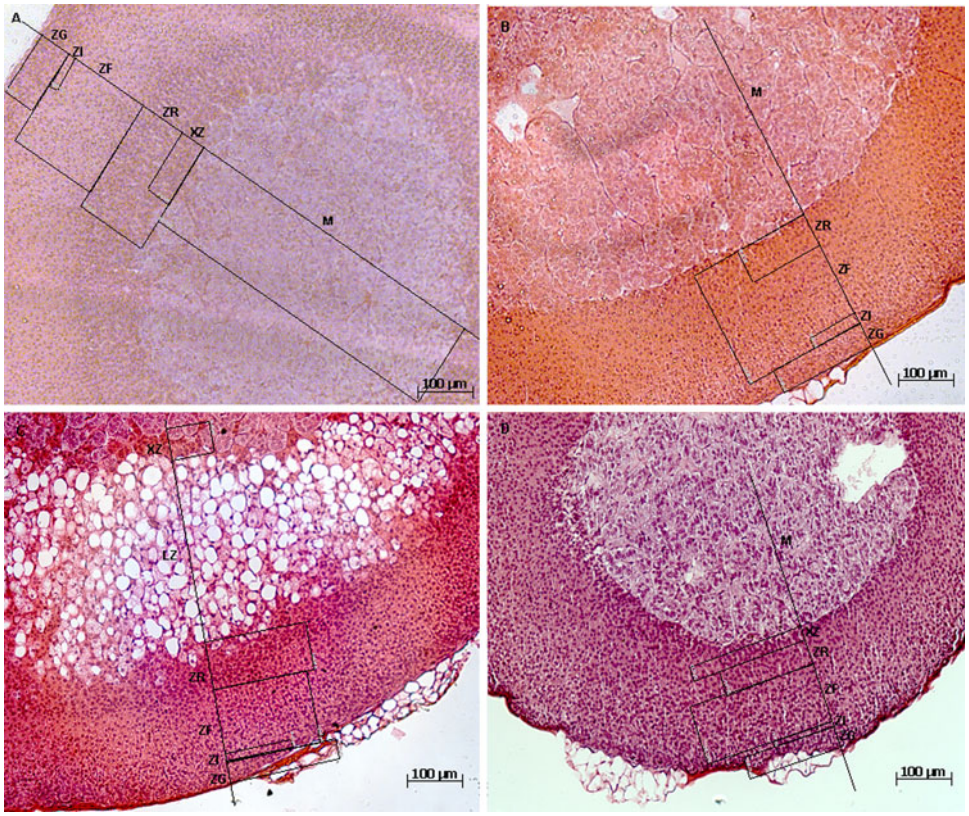


**Fig. 1** Sample width measurements from female (**a**, **c**) and male (**b, d**) adrenal glands sectioned horizontally. *ZG* zona glomerulosa, *ZI* zona intermedia, *ZF* zona fasciculata, *ZR* zona reticularis, *XZ* X-zone, *M* medulla, *LZ* lipoid zone

The overall measures of whole adrenal weights for both parental strains and all 64 BXD lines for the female and male mice show that females (2.45 ± 0.03 mg) have significantly heavier adrenal glands than the males (1.72 ± 0.02 mg; $F_{1,546} = 550.02$, $p < 0.0005$). As would be expected, the relative adrenal weight to body weight is also significantly higher for females compared to males ($F_{1,546} = 952.37$, $p < 0.0005$). In addition, there are significant strain differences in actual adrenal weight ($F_{65,482} = 2.54$, $p < 0.0005$) and relative adrenal weight ($F_{65,482} = 1.82$, $p < 0.0005$), and these strain differences are apparent for both measures in females ($F_{65,209} = 6.74$, $p < 0.0005$; $F_{65,209} = 8.48$, $p < 0.0005$) and males ($F_{65,207} = 5.79$, $p < 0.0005$; $F_{65,207} = 5.46$, $p < 0.0005$). The strain means for adrenal weight, age, body weight, and relative adrenal weight to body weight are shown separately for females and males in Tables 1 and 2, respectively. Age, sex, body weight, and left-right adrenal weight were also analyzed using Pearson product-moment correlations. The results show that both age and sex are positively correlated with adrenal weight, while body weight is negatively correlated with adrenal weight (Table 3). Sex shows a highly significant correlation with adrenal weight ($r = 0.71$, $p < 0.0005$) and accounts for 50% of the variance in adrenal weight. When controlling for sex, age is still significantly correlated with adrenal weight in both females ($r = 0.39$, $p < 0.0005$) and males ($r = -0.14$, $p = 0.024$). Meanwhile, the significant negative correlation between body weight and adrenal weight ($r = -0.26$, $p < 0.0005$) reflects the fact that males have lighter adrenal glands but weigh more (22.03 ± 0.19 g) than females (18.17 ± 0.15 g), who have heavier adrenal glands. Lastly, broad-sense heritabilities ($h^2$) confirm the robust link between sex and adrenal weight because the heritability values for adrenal weight for males and females are much higher than when heritability is calculated across both sexes (Table 6). It should be noted that Valdar et al. (2006) found other confounding environmental variables that influenced adrenal weight, the most significant being the seasonal effect within a family, but not between. We had insufficient numbers of mice to determine this point within the large number of BXD families that we sampled.

## QTL influencing adrenal weight

The QTL mapping of adrenal weight was performed for each sex using the 64 BXD RI and parental strains. An interval genome-wide QTL map of female adrenal weight showed a significant QTL located on Chr 3 and five suggestive QTLs were found on Chrs 1, 3, 4, 10, and 14 (Fig. 2a). The significant QTL was termed *Fawq1* for "female adrenal weight QTL" and mapped to a distal region of Chr 3 (Fig. 2b). After performing a marker regression analysis using WebQTL, *Fawq1* was found to

**Table 1** Female mice: age, body weight, and total adrenal weight measurements

| Strain | Total cases | Age (days) | Body weight (g) | Adrenal weight L (mg) | Adrenal weight R (mg) | Avg. adrenal weight (mg) | Adrenal weight / body weight (%) |
|---|---|---|---|---|---|---|---|
| BXD1 | 4 | 55.00 ± 2.31 | 16.73 ± 0.37 | 2.13 ± 0.08 | 1.90 ± 0.11 | 2.01 ± 0.08 | 0.012 |
| BXD2 | 4 | 56.25 ± 0.95 | 20.55 ± 0.64 | 2.48 ± 0.11 | 2.53 ± 0.11 | 2.50 ± 0.06 | 0.012 |
| BXD6[a] | 4 | 59.00 ± 3.46 | 18.73 ± 0.98 | 3.00 ± 0.18 | 2.58 ± 0.25 | 2.79 ± 0.21 | 0.015 |
| BXD8 | 3 | 60.00 ± 7.00 | 15.50 ± 1.01 | 2.60 ± 0.21 | 2.00 ± 0.10 | 2.30 ± 0.15 | 0.015 |
| BXD9 | 4 | 62.00 ± 3.46 | 18.70 ± 1.18 | 2.53 ± 0.26 | 2.25 ± 0.12 | 2.39 ± 0.19 | 0.013 |
| BXD11[a] | 3 | 55.67 ± 4.67 | 14.57 ± 1.33 | 3.10 ± 0.10 | 3.10 ± 0.21 | 3.10 ± 0.14 | 0.022 |
| BXD12 | 4 | 54.50 ± 0.96 | 15.38 ± 0.36 | 2.38 ± 0.14 | 2.15 ± 0.06 | 2.26 ± 0.10 | 0.015 |
| BXD13[a] | 4 | 61.00 ± 8.39 | 19.23 ± 1.66 | 2.50 ± 0.16 | 2.63 ± 0.33 | 2.56 ± 0.11 | 0.013 |
| BXD14 | 4 | 58.75 ± 2.25 | 19.53 ± 0.84 | 2.35 ± 0.17 | 2.08 ± 0.06 | 2.21 ± 0.10 | 0.011 |
| BXD15 | 4 | 48.50 ± 0.29 | 21.40 ± 0.66 | 2.58 ± 0.24 | 2.30 ± 0.19 | 2.44 ± 0.21 | 0.011 |
| BXD16[a] | 4 | 67.75 ± 5.01 | 23.88 ± 0.84 | 3.45 ± 0.10 | 2.68 ± 0.13 | 3.06 ± 0.10 | 0.013 |
| BXD18[a] | 7 | 61.00 ± 3.23 | 19.19 ± 0.85 | 3.17 ± 0.27 | 2.51 ± 0.14 | 2.84 ± 0.20 | 0.015 |
| BXD19 | 4 | 60.00 ± 4.00 | 15.33 ± 0.93 | 1.93 ± 0.08 | 1.80 ± 0.11 | 1.86 ± 0.04 | 0.012 |
| BXD20 | 4 | 54.75 ± 2.25 | 17.95 ± 1.85 | 2.63 ± 0.14 | 2.43 ± 0.10 | 2.53 ± 0.12 | 0.014 |
| BXD21 | 4 | 52.25 ± 2.32 | 17.55 ± 1.44 | 2.35 ± 0.18 | 2.13 ± 0.06 | 2.24 ± 0.09 | 0.013 |
| BXD24 | 4 | 61.25 ± 1.60 | 18.48 ± 0.92 | 2.25 ± 0.10 | 2.15 ± 0.10 | 2.20 ± 0.10 | 0.012 |
| BXD27 | 8 | 51.50 ± 0.50 | 14.53 ± 0.60 | 2.34 ± 0.08 | 2.26 ± 0.09 | 2.30 ± 0.08 | 0.016 |
| BXD28[a] | 3 | 53.00 ± 0.00 | 17.47 ± 0.78 | 3.43 ± 0.15 | 3.43 ± 0.26 | 3.43 ± 0.12 | 0.020 |
| BXD29 | 3 | 56.33 ± 0.33 | 14.57 ± 0.75 | 2.17 ± 0.09 | 2.13 ± 0.09 | 2.15 ± 0.06 | 0.015 |
| BXD31[b] | 1 | 55.00 | 14.80 | 2.50 | 2.10 | 2.30 | 0.016 |
| BXD32[a] | 4 | 82.00 ± 0.00 | 21.53 ± 0.43 | 3.40 ± 0.15 | 3.65 ± 0.16 | 3.53 ± 0.14 | 0.016 |
| BXD33[a] | 4 | 72.00 ± 7.00 | 17.70 ± 0.87 | 2.75 ± 0.23 | 2.55 ± 0.09 | 2.65 ± 0.16 | 0.015 |
| BXD34[a] | 4 | 53.25 ± 1.75 | 18.78 ± 0.46 | 2.88 ± 0.18 | 2.73 ± 0.10 | 2.80 ± 0.13 | 0.015 |
| BXD36 | 8 | 57.50 ± 2.12 | 17.13 ± 0.80 | 2.43 ± 0.14 | 2.23 ± 0.15 | 2.33 ± 0.15 | 0.014 |
| BXD38[a] | 4 | 58.75 ± 3.15 | 17.38 ± 0.57 | 2.90 ± 0.17 | 2.90 ± 0.24 | 2.90 ± 0.19 | 0.017 |
| BXD39[a] | 4 | 60.50 ± 5.50 | 18.55 ± 0.78 | 2.63 ± 0.28 | 2.53 ± 0.12 | 2.58 ± 0.20 | 0.014 |
| BXD40 | 2 | 50.00 ± 1.00 | 15.45 ± 0.55 | 2.00 ± 0.00 | 2.00 ± 0.00 | 2.00 ± 0.00 | 0.013 |
| BXD42 | 8 | 54.88 ± 1.13 | 16.39 ± 0.26 | 2.60 ± 0.15 | 2.31 ± 0.10 | 2.46 ± 0.12 | 0.015 |
| BXD43 | 4 | 66.00 ± 0.00 | 19.20 ± 0.40 | 2.35 ± 0.12 | 2.25 ± 0.06 | 2.30 ± 0.09 | 0.012 |
| BXD44 | 4 | 49.00 ± 0.00 | 16.75 ± 0.65 | 1.60 ± 0.04 | 1.53 ± 0.09 | 1.56 ± 0.05 | 0.009 |
| BXD45 | 4 | 54.00 ± 1.73 | 15.60 ± 0.73 | 2.48 ± 0.15 | 2.30 ± 0.12 | 2.39 ± 0.11 | 0.015 |
| BXD48[a] | 8 | 57.38 ± 2.95 | 18.88 ± 0.56 | 2.69 ± 0.15 | 2.64 ± 0.15 | 2.66 ± 0.15 | 0.014 |
| BXD50[a] | 4 | 71.25 ± 2.10 | 16.65 ± 0.56 | 2.75 ± 0.19 | 2.35 ± 0.21 | 2.55 ± 0.20 | 0.015 |
| BXD51[a] | 4 | 60.25 ± 2.36 | 18.10 ± 0.60 | 2.95 ± 0.06 | 2.55 ± 0.19 | 2.75 ± 0.07 | 0.015 |
| BXD55 | 4 | 65.25 ± 0.75 | 19.70 ± 0.36 | 2.13 ± 0.11 | 1.78 ± 0.14 | 1.95 ± 0.12 | 0.010 |
| BXD56[a] | 4 | 58.25 ± 0.25 | 16.68 ± 0.09 | 2.93 ± 0.22 | 2.55 ± 0.14 | 2.74 ± 0.16 | 0.016 |
| BXD60 | 6 | 57.00 ± 0.37 | 20.70 ± 1.37 | 2.60 ± 0.05 | 2.27 ± 0.08 | 2.43 ± 0.06 | 0.012 |
| BXD61 | 2 | 52.00 ± 0.00 | 19.60 ± 0.80 | 2.20 ± 0.20 | 2.30 ± 0.00 | 2.25 ± 0.10 | 0.011 |
| BXD62[a] | 4 | 73.00 ± 1.00 | 18.98 ± 0.65 | 3.33 ± 0.13 | 2.90 ± 0.10 | 3.11 ± 0.08 | 0.016 |
| BXD63 | 4 | 66.00 ± 5.20 | 18.55 ± 1.40 | 2.75 ± 0.16 | 2.33 ± 0.10 | 2.54 ± 0.13 | 0.014 |
| BXD65 | 4 | 56.75 ± 4.01 | 17.18 ± 1.29 | 2.65 ± 0.17 | 2.33 ± 0.10 | 2.49 ± 0.13 | 0.014 |
| BXD66[a] | 4 | 56.00 ± 1.00 | 16.03 ± 0.97 | 2.85 ± 0.12 | 2.55 ± 0.10 | 2.70 ± 0.08 | 0.017 |
| BXD67 | 4 | 59.00 ± 0.00 | 19.88 ± 0.59 | 2.45 ± 0.09 | 2.25 ± 0.16 | 2.35 ± 0.11 | 0.012 |
| BXD68[a] | 4 | 63.75 ± 0.75 | 18.10 ± 0.39 | 2.80 ± 0.07 | 2.73 ± 0.10 | 2.76 ± 0.09 | 0.015 |
| BXD69[a] | 4 | 57.00 ± 1.15 | 21.00 ± 0.64 | 3.28 ± 0.21 | 2.93 ± 0.11 | 3.10 ± 0.15 | 0.015 |
| BXD70 | 4 | 58.25 ± 0.25 | 16.78 ± 0.26 | 2.40 ± 0.08 | 2.28 ± 0.03 | 2.34 ± 0.04 | 0.014 |
| BXD71 | 4 | 56.50 ± 0.50 | 17.43 ± 1.40 | 1.75 ± 0.06 | 1.63 ± 0.08 | 1.69 ± 0.06 | 0.010 |

**Table 1** continued

| Strain | Total cases | Age (days) | Body weight (g) | Adrenal weight L (mg) | Adrenal weight R (mg) | Avg. adrenal weight (mg) | Adrenal weight / body weight (%) |
|---|---|---|---|---|---|---|---|
| BXD73 | 6 | 62.33 ± 0.62 | 17.82 ± 0.39 | 2.22 ± 0.07 | 2.00 ± 0.04 | 2.11 ± 0.04 | 0.012 |
| BXD75 | 4 | 56.75 ± 4.75 | 20.83 ± 0.77 | 2.45 ± 0.06 | 2.08 ± 0.06 | 2.26 ± 0.01 | 0.011 |
| BXD77 | 2 | 57.00 ± 0.00 | 24.05 ± 0.05 | 2.05 ± 0.15 | 2.00 ± 0.00 | 2.03 ± 0.08 | 0.008 |
| BXD80 | 4 | 57.50 ± 0.87 | 16.48 ± 1.17 | 1.95 ± 0.09 | 1.70 ± 0.04 | 1.83 ± 0.05 | 0.011 |
| BXD83 | 4 | 67.75 ± 1.31 | 16.55 ± 0.72 | 2.60 ± 0.07 | 2.20 ± 0.18 | 2.40 ± 0.12 | 0.015 |
| BXD84[a] | 4 | 62.25 ± 0.75 | 17.13 ± 0.77 | 2.78 ± 0.10 | 2.50 ± 0.08 | 2.64 ± 0.07 | 0.015 |
| BXD85 | 4 | 50.00 ± 0.58 | 21.83 ± 0.71 | 2.60 ± 0.14 | 2.40 ± 0.12 | 2.50 ± 0.10 | 0.011 |
| BXD86 | 4 | 53.75 ± 3.75 | 16.90 ± 1.34 | 1.95 ± 0.16 | 1.85 ± 0.13 | 1.90 ± 0.14 | 0.011 |
| BXD87[a] | 4 | 59.75 ± 0.85 | 15.60 ± 0.70 | 2.65 ± 0.16 | 2.73 ± 0.17 | 2.69 ± 0.16 | 0.017 |
| BXD89 | 4 | 61.50 ± 1.44 | 19.35 ± 0.75 | 2.33 ± 0.21 | 2.15 ± 0.20 | 2.24 ± 0.19 | 0.012 |
| BXD90 | 4 | 64.50 ± 1.44 | 20.78 ± 0.88 | 2.08 ± 0.19 | 2.18 ± 0.21 | 2.13 ± 0.20 | 0.010 |
| BXD92[a] | 4 | 58.25 ± 6.25 | 19.23 ± 0.62 | 3.05 ± 0.16 | 2.68 ± 0.19 | 2.86 ± 0.16 | 0.015 |
| BXD96 | 4 | 53.50 ± 3.18 | 18.35 ± 0.52 | 2.43 ± 0.13 | 2.20 ± 0.14 | 2.31 ± 0.13 | 0.013 |
| BXD97[a] | 4 | 56.75 ± 2.25 | 17.45 ± 0.36 | 2.70 ± 0.12 | 2.43 ± 0.09 | 2.56 ± 0.10 | 0.015 |
| BXD98 | 2 | 59.50 ± 1.50 | 18.25 ± 1.25 | 2.70 ± 0.20 | 2.40 ± 0.20 | 2.55 ± 0.20 | 0.014 |
| BXD99 | 4 | 54.50 ± 0.87 | 17.03 ± 0.45 | 2.43 ± 0.14 | 2.38 ± 0.11 | 2.40 ± 0.12 | 0.014 |
| BXD100 | 4 | 67.00 ± 0.00 | 20.85 ± 0.37 | 2.68 ± 0.06 | 2.30 ± 0.04 | 2.49 ± 0.05 | 0.012 |
| C57BL/6J | 5 | 61.00 ± 0.00 | 17.96 ± 0.41 | 2.48 ± 0.23 | 1.88 ± 0.20 | 2.18 ± 0.16 | 0.012 |
| DBA/2J | 6 | 59.33 ± 2.74 | 20.07 ± 1.19 | 2.32 ± 0.15 | 2.28 ± 0.35 | 2.30 ± 0.23 | 0.011 |

All data are shown as mean ± standard error of the mean

[a] Female BXD strains with significantly higher adrenal weight compared to all BXD strains examined

[b] Data available from only one female adrenal weighed from BXD31

**Table 2** Male mice: age, body weight, adrenal weight, and total adrenal weight measurements

| Strain | Total cases | Age (days) | Body weight (g) | Adrenal weight L (mg) | Adrenal weight R (mg) | Avg. adrenal weight (mg) | Adrenal weight / body weight (%) |
|---|---|---|---|---|---|---|---|
| BXD1 | 4 | 62.00 ± 4.62 | 24.28 ± 0.96 | 1.75 ± 0.10 | 1.70 ± 0.11 | 1.73 ± 0.05 | 0.007 |
| BXD2 | 4 | 56.50 ± 0.87 | 19.53 ± 2.83 | 1.60 ± 0.12 | 1.35 ± 0.18 | 1.48 ± 0.11 | 0.008 |
| BXD6 | 8 | 53.63 ± 2.07 | 21.18 ± 0.40 | 2.11 ± 0.10 | 2.14 ± 0.11 | 2.13 ± 0.08 | 0.010 |
| BXD8 | 4 | 67.75 ± 4.94 | 21.68 ± 1.38 | 1.78 ± 0.10 | 1.80 ± 0.18 | 1.79 ± 0.10 | 0.008 |
| BXD9 | 3 | 61.00 ± 1.00 | 22.73 ± 0.65 | 1.50 ± 0.06 | 1.50 ± 0.15 | 1.50 ± 0.10 | 0.007 |
| BXD11[a] | 1 | 52.00 | 20.70 | 2.00 | 1.90 | 1.95 | 0.009 |
| BXD12 | 4 | 54.00 ± 1.15 | 21.15 ± 0.47 | 2.03 ± 0.09 | 2.20 ± 0.04 | 2.11 ± 0.02 | 0.010 |
| BXD13 | 4 | 66.25 ± 7.43 | 23.98 ± 1.73 | 1.68 ± 0.06 | 1.55 ± 0.13 | 1.61 ± 0.08 | 0.007 |
| BXD14 | 4 | 62.50 ± 1.50 | 25.43 ± 1.20 | 2.10 ± 0.08 | 1.73 ± 0.05 | 1.91 ± 0.02 | 0.008 |
| BXD15 | 3 | 49.00 ± 0.00 | 24.20 ± 2.19 | 1.83 ± 0.03 | 1.87 ± 0.07 | 1.85 ± 0.05 | 0.008 |
| BXD16 | 4 | 55.00 ± 3.46 | 23.55 ± 1.06 | 2.48 ± 0.14 | 2.25 ± 0.06 | 2.36 ± 0.10 | 0.010 |
| BXD18 | 7 | 63.43 ± 3.99 | 25.70 ± 0.52 | 1.97 ± 0.09 | 1.66 ± 0.21 | 1.81 ± 0.13 | 0.007 |
| BXD19 | 4 | 65.75 ± 5.66 | 20.45 ± 0.90 | 1.53 ± 0.14 | 1.53 ± 0.09 | 1.53 ± 0.11 | 0.007 |
| BXD20 | 4 | 57.00 ± 0.00 | 22.23 ± 0.09 | 1.83 ± 0.05 | 1.93 ± 0.08 | 1.88 ± 0.06 | 0.008 |
| BXD21 | 2 | 48.50 ± 0.50 | 20.90 ± 1.60 | 1.90 ± 0.00 | 2.05 ± 0.35 | 1.98 ± 0.18 | 0.009 |
| BXD24 | 4 | 56.00 ± 1.78 | 16.75 ± 2.07 | 1.80 ± 0.13 | 1.65 ± 0.13 | 1.73 ± 0.13 | 0.010 |
| BXD27 | 7 | 57.71 ± 1.30 | 21.19 ± 0.95 | 1.74 ± 0.13 | 1.80 ± 0.13 | 1.77 ± 0.12 | 0.009 |
| BXD28 | 3 | 63.00 ± 11.50 | 21.40 ± 0.32 | 2.37 ± 0.07 | 2.27 ± 0.19 | 2.32 ± 0.12 | 0.011 |
| BXD29 | 4 | 54.25 ± 1.25 | 20.35 ± 0.86 | 1.70 ± 0.00 | 1.55 ± 0.05 | 1.63 ± 0.03 | 0.008 |
| BXD31 | 4 | 53.00 ± 1.73 | 22.68 ± 0.77 | 2.00 ± 0.09 | 1.90 ± 0.15 | 1.95 ± 0.04 | 0.009 |
| BXD32 | 4 | 77.75 ± 0.25 | 23.48 ± 1.66 | 1.90 ± 0.07 | 1.68 ± 0.05 | 1.79 ± 0.05 | 0.008 |

**Table 2** continued

| Strain | Total cases | Age (days) | Body weight (g) | Adrenal weight L (mg) | Adrenal weight R (mg) | Avg. adrenal weight (mg) | Adrenal weight / body weight (%) |
|---|---|---|---|---|---|---|---|
| BXD33 | 4 | 74.50 ± 1.85 | 23.48 ± 0.74 | 1.70 ± 0.20 | 1.68 ± 0.17 | 1.69 ± 0.08 | 0.007 |
| BXD34 | 4 | 57.00 ± 0.00 | 24.53 ± 0.31 | 2.13 ± 0.08 | 2.20 ± 0.15 | 2.16 ± 0.08 | 0.009 |
| BXD36 | 4 | 53.25 ± 0.25 | 19.63 ± 0.70 | 1.68 ± 0.03 | 1.75 ± 0.09 | 1.71 ± 0.05 | 0.009 |
| BXD38 | 4 | 53.50 ± 1.44 | 22.18 ± 0.06 | 1.78 ± 0.03 | 1.78 ± 0.05 | 1.78 ± 0.03 | 0.008 |
| BXD39 | 4 | 57.00 ± 0.00 | 15.48 ± 0.53 | 2.03 ± 0.11 | 2.10 ± 0.08 | 2.06 ± 0.09 | 0.013 |
| BXD40 | 4 | 54.00 ± 0.00 | 22.13 ± 0.50 | 1.50 ± 0.08 | 1.65 ± 0.10 | 1.58 ± 0.08 | 0.007 |
| BXD42 | 8 | 51.75 ± 1.10 | 21.23 ± 0.39 | 1.64 ± 0.09 | 1.40 ± 0.05 | 1.52 ± 0.07 | 0.007 |
| BXD43 | 4 | 50.00 ± 0.00 | 18.58 ± 0.92 | 1.65 ± 0.03 | 1.68 ± 0.08 | 1.66 ± 0.04 | 0.009 |
| BXD44 | 4 | 58.00 ± 0.00 | 21.98 ± 0.43 | 1.40 ± 0.04 | 1.50 ± 0.09 | 1.45 ± 0.05 | 0.007 |
| BXD45 | 4 | 60.50 ± 3.18 | 22.83 ± 2.44 | 1.65 ± 0.12 | 1.38 ± 0.11 | 1.51 ± 0.10 | 0.007 |
| BXD48 | 8 | 54.25 ± 1.66 | 21.81 ± 0.73 | 1.88 ± 0.09 | 1.86 ± 0.07 | 1.87 ± 0.07 | 0.009 |
| BXD50 | 4 | 66.75 ± 2.66 | 18.33 ± 0.48 | 1.45 ± 0.09 | 1.55 ± 0.03 | 1.50 ± 0.04 | 0.008 |
| BXD51 | 4 | 65.00 ± 0.58 | 21.33 ± 0.20 | 1.90 ± 0.11 | 1.70 ± 0.07 | 1.80 ± 0.09 | 0.008 |
| BXD55 | 4 | 68.50 ± 2.50 | 21.05 ± 1.67 | 1.28 ± 0.13 | 1.13 ± 0.06 | 1.20 ± 0.06 | 0.006 |
| BXD56 | 4 | 65.50 ± 0.29 | 24.00 ± 0.39 | 1.68 ± 0.03 | 1.50 ± 0.04 | 1.59 ± 0.03 | 0.007 |
| BXD60 | 5 | 57.00 ± 0.45 | 22.36 ± 2.14 | 1.86 ± 0.09 | 1.70 ± 0.11 | 1.78 ± 0.10 | 0.008 |
| BXD61 | 3 | 58.00 ± 0.00 | 22.77 ± 0.61 | 1.63 ± 0.03 | 1.60 ± 0.10 | 1.62 ± 0.06 | 0.007 |
| BXD62 | 3 | 52.67 ± 1.33 | 21.90 ± 1.20 | 2.17 ± 0.03 | 1.80 ± 0.06 | 1.98 ± 0.02 | 0.009 |
| BXD63 | 4 | 59.00 ± 3.72 | 22.33 ± 1.70 | 1.68 ± 0.08 | 1.58 ± 0.11 | 1.63 ± 0.09 | 0.007 |
| BXD65 | 4 | 62.00 ± 0.00 | 21.63 ± 0.60 | 1.73 ± 0.06 | 1.43 ± 0.06 | 1.58 ± 0.05 | 0.007 |
| BXD66 | 4 | 54.50 ± 0.29 | 20.38 ± 1.17 | 1.60 ± 0.09 | 1.55 ± 0.09 | 1.58 ± 0.08 | 0.008 |
| BXD67 | 4 | 59.00 ± 3.00 | 22.50 ± 0.45 | 1.58 ± 0.17 | 1.40 ± 0.04 | 1.49 ± 0.07 | 0.007 |
| BXD68 | 4 | 62.00 ± 0.00 | 24.65 ± 0.99 | 1.88 ± 0.03 | 1.75 ± 0.06 | 1.81 ± 0.03 | 0.007 |
| BXD69 | 3 | 50.00 ± 0.00 | 18.50 ± 0.45 | 1.73 ± 0.15 | 1.70 ± 0.00 | 1.72 ± 0.07 | 0.009 |
| BXD70 | 4 | 56.25 ± 2.25 | 19.18 ± 1.91 | 1.55 ± 0.10 | 1.38 ± 0.09 | 1.46 ± 0.09 | 0.008 |
| BXD71 | 4 | 57.00 ± 4.49 | 21.63 ± 0.52 | 1.50 ± 0.07 | 1.30 ± 0.07 | 1.40 ± 0.07 | 0.006 |
| BXD73 | 5 | 54.80 ± 0.80 | 18.90 ± 0.74 | 1.60 ± 0.14 | 1.50 ± 0.18 | 1.55 ± 0.15 | 0.008 |
| BXD75 | 4 | 54.25 ± 4.61 | 21.98 ± 1.40 | 1.65 ± 0.06 | 1.45 ± 0.05 | 1.55 ± 0.05 | 0.007 |
| BXD77 | 5 | 60.80 ± 1.72 | 30.36 ± 1.83 | 1.60 ± 0.07 | 1.58 ± 0.07 | 1.59 ± 0.05 | 0.005 |
| BXD80 | 4 | 59.00 ± 0.00 | 22.85 ± 0.39 | 1.63 ± 0.08 | 1.45 ± 0.10 | 1.54 ± 0.07 | 0.007 |
| BXD83 | 4 | 64.00 ± 4.53 | 22.28 ± 0.70 | 1.68 ± 0.05 | 1.68 ± 0.17 | 1.68 ± 0.10 | 0.008 |
| BXD84 | 4 | 65.00 ± 1.00 | 22.73 ± 0.60 | 1.70 ± 0.04 | 1.73 ± 0.05 | 1.71 ± 0.04 | 0.008 |
| BXD85 | 4 | 58.00 ± 5.20 | 27.20 ± 2.19 | 1.70 ± 0.04 | 1.63 ± 0.05 | 1.66 ± 0.04 | 0.006 |
| BXD86 | 4 | 52.00 ± 0.00 | 19.33 ± 0.25 | 1.48 ± 0.03 | 1.45 ± 0.09 | 1.46 ± 0.05 | 0.008 |
| BXD87 | 4 | 48.50 ± 0.29 | 17.10 ± 1.12 | 1.75 ± 0.03 | 1.63 ± 0.08 | 1.69 ± 0.05 | 0.010 |
| BXD89 | 2 | 66.00 ± 0.00 | 25.35 ± 0.35 | 1.65 ± 0.05 | 1.85 ± 0.05 | 1.75 ± 0.05 | 0.007 |
| BXD90 | 4 | 66.25 ± 0.75 | 24.30 ± 0.52 | 1.60 ± 0.11 | 1.48 ± 0.15 | 1.54 ± 0.13 | 0.006 |
| BXD92 | 4 | 58.50 ± 2.02 | 20.03 ± 1.20 | 1.73 ± 0.03 | 1.30 ± 0.11 | 1.51 ± 0.05 | 0.008 |
| BXD96 | 4 | 48.75 ± 0.75 | 22.28 ± 0.54 | 2.05 ± 0.09 | 2.03 ± 0.10 | 2.04 ± 0.07 | 0.009 |
| BXD97 | 4 | 49.00 ± 0.00 | 19.80 ± 0.41 | 1.65 ± 0.12 | 1.45 ± 0.10 | 1.55 ± 0.10 | 0.008 |
| BXD98 | 4 | 68.50 ± 2.50 | 23.05 ± 0.87 | 1.80 ± 0.16 | 1.53 ± 0.14 | 1.66 ± 0.05 | 0.007 |
| BXD99 | 4 | 55.50 ± 0.50 | 23.33 ± 0.56 | 1.63 ± 0.03 | 1.85 ± 0.06 | 1.74 ± 0.04 | 0.007 |
| BXD100 | 4 | 62.00 ± 0.58 | 25.88 ± 0.63 | 2.05 ± 0.10 | 1.85 ± 0.09 | 1.95 ± 0.09 | 0.008 |
| C57BL/6J | 4 | 61.00 ± 0.00 | 22.30 ± 0.24 | 1.55 ± 0.12 | 1.23 ± 0.20 | 1.39 ± 0.14 | 0.006 |
| DBA/2J | 5 | 61.40 ± 2.75 | 23.22 ± 1.90 | 1.90 ± 0.28 | 1.92 ± 0.22 | 1.91 ± 0.23 | 0.008 |

All data are shown as mean ± standard error of the mean

[a] Data available from only one male adrenal weighed from BXD11

**Table 3** Sex, age, body weight, and adrenal weight correlations

| | Sex | Age | Body weight | Adrenal weight L | Adrenal weight R | Avg. adrenal weight |
|---|---|---|---|---|---|---|
| Sex | 1 | | | | | |
| Age | 0.030 | 1 | | | | |
| Body weight | 0.558** | 0.246** | 1 | | | |
| Adrenal weight L | 0.712** | 0.158** | −0.267** | 1 | | |
| Adrenal weight R | 0.645** | 0.129** | −0.240** | 0.841** | 1 | |
| Avg. adrenal weight | 0.708** | 0.150** | −0.264** | 0.963** | 0.956** | 1 |

** Correlation is significant at the 0.01 level (2-tailed)

have a LRS value of 19.669 (LOD score = 4.267) (Table 9). This QTL spans a fairly small region of Chr 3, with the 1-LOD confidence interval between 127.0 and 129.5 Mb. Meanwhile, an interval whole-genome QTL map of male adrenal weight revealed a significant QTL mapped on Chr 4 along with several suggestive peaks on Chr 4, as well as five suggestive QTLs shown on Chrs 2, 10, 17, and X (Fig. 3a). The locus symbol for the male adrenal weight QTL is *Mawq1*, and a marker regression analysis using WebQTL calculated a LRS value of 18.363 (LOD score = 3.983) for *Mawq1* (Fig. 3b, Table 9). The QTL spans a fairly small region of Chr 4, with the 1-LOD confidence interval between 99.5 and 102.5 Mb.

## Adrenal width measurements

For the parental strains, the adrenal width measurements showed significant differences for the medulla ($p = 0.039$), the ZF ($p = 0.001$), and ZI ($p = 0.022$) measures. Although the average total width of the adrenal for the B6 mice (1127.80 ± 30.33 μm) was higher than the mean for the D2 mice (1058.51 ± 53.99 μm), they did not differ significantly ($p = 0.307$). Meanwhile, the ZG, ZR, and X-zone exhibited similar width measures between the parental strains. In terms of the significant differences, the D2 mice (483.10 ± 26.40 μm) had a larger medulla width compared to the B6 mice (412.94 ± 12.78 μm), whereas the B6 mice had larger ZF (169.85 ± 13.34 μm) and ZI (18.66 ± 1.47 μm) widths in comparison to those of the D2 mice (113.06 ± 7.25 and 14.03 ± 1.16 μm, respectively). Again, we accounted for sex differences within the strains and found that only the ZF width is significantly larger for the B6 mice for both males (143.66 ± 7.76 μm, $p = 0.029$) and females (190.80 ± 18.91 μm, $p = 0.006$) compared to that for the D2 mice (110.98 ± 8.63 and 114.78 ± 11.91 μm, respectively). Meanwhile, the medulla width was significantly larger only for D2 female mice (523.91 ± 40.02 μm, $p = 0.032$) and not the male mice ($p = 0.754$) compared to the B6 strain, while the ZI width was not significantly different among the strains for both the female ($p = 0.165$) and male ($p = 0.074$) mice. In addition, the total adrenal width was significantly larger for the B6 male mice (1065.04 ± 39.83 μm) in comparison to that for the D2 male mice (906.12 ± 38.56 μm; $p = 0.025$). Lastly, the ZR width displayed a unique pattern in which the male mice were significantly different ($p = 0.013$), with the B6 mice (65.39 ± 6.60 μm) exhibiting a wider ZR region than the D2 mice (44.88 ± 1.94 μm), whereas the female mice approached significance ($p = 0.059$), but the D2 mice showed a larger ZR (70.50 ± 4.41 μm) than the B6 mice (52.49 ± 7.49 μm) (Fig. 4).

The adrenal width data for the 64 BXD and parental strains for female mice analyzed (Table 4) show that the total width and the ZF and X-zone widths are typically larger than those obtained from the male mice for the 64 BXD and parental strains (Table 5), which often exhibit larger adrenal medulla and slightly larger ZR measures than females. Both tables also show that there are substantial strain differences in all regional adrenal measures, except for the female ZG ($p = 0.055$) width measures. In addition, broad-sense heritabilities ($h^2$) were calculated across both sexes and separately for males and females for adrenal width. The results showed that higher heritability values correspond to the adrenal phenotypes shown in Tables 4 and 5 that vary significantly between the strains and sexes (Table 6). For example, ZF width varies significantly for both males ($F_{65,139} = 2.69$, $p < 0.0005$) and females ($F_{65,137} = 5.33$, $p < 0.0005$) and shows high heritability values for males (34%) and females (57%).

Correlational analysis of the adrenal gland regional measures of width for the BXD and parental strains were compared with each other and with adrenal weight. As sex plays a prominent role in accounting for differences in adrenal weight, the adrenal width measures were controlled by sex and compared separately for males and females. The results showed that sex also has a significant main effect on most of the adrenal width measures, including the total, medulla, ZF, and X-zone widths, and it accounts for a significant amount of the total variance for these measures, ranging from 4.9 to 76%. For the female data, several correlations between the adrenal width and weight measures are significant, where the total adrenal width shows a significant positive correlation with all adrenal measures

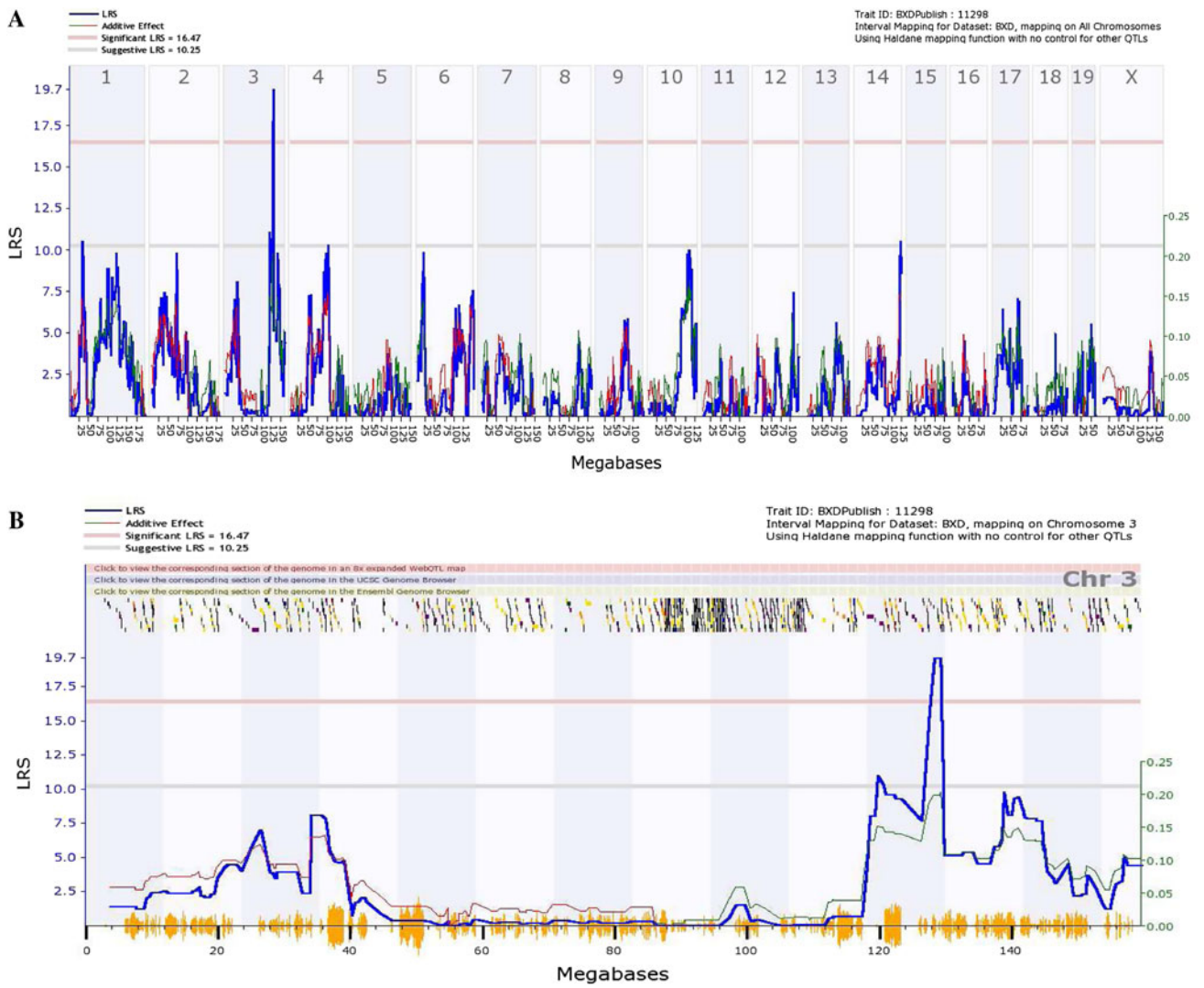


**Fig. 2** Genome-wide linkage map of female BXD adrenal weight. As shown in the legend, the *blue* trace shows the LRS for female BXD adrenal weight. **a** Interval genome-wide QTL map showing a significant QTL detected on chromosome 3 and five suggestive QTLs detected with one each on chromosomes 1, 3, 4, 10, and 14. **b** Interval QTL map with bootstrap analysis of chromosome 3 using female adrenal weight data. The *lower gray* horizontal line represents suggestive LRS genome-wide threshold at $p \leq 0.63$. The *upper pink* horizontal line represents significant LRS genome-wide threshold at $p \leq 0.05$. A positive additive coefficient (*green* line) indicates that DBA/2J alleles increase trait values. In contrast, a negative additive coefficient (*red* line) indicates that C57BL/6J alleles increase trait values. The *orange* seismograph marks on the *X* axis indicate SNP density

expect ZI width, and with age and body weight (Table 7). In addition, X-zone width shows a very robust positive correlation with both adrenal weight and ZR width. For the male data, there are also several significant correlations between the adrenal width and weight measurements, and the total adrenal width displays significant positive correlations with all adrenal measures and with body weight but not with age (Table 8). Moreover, adrenal medulla width shows a very robust correlation with total adrenal width ($r = 0.72$, $p < 0.0005$), accounting for 51% of the variance in total adrenal width ($F_{1,201} = 211.07$, $p < 0.0005$).

## QTL influencing adrenal width

The QTL mapping for all adrenal width measurements was calculated separately for male and female mice using the 64 BXD RI and parental strains. Significant QTLs were found for total adrenal, medulla, and X-zone width measures but only for the males. An interval genome-wide QTL map of male total width displayed a significant QTL located on distal Chr 10 and a suggestive QTL on Chr 13 (Fig. 5a). The locus symbol for male adrenal width QTL is *Mawdq1*. A marker regression analysis showed an LRS

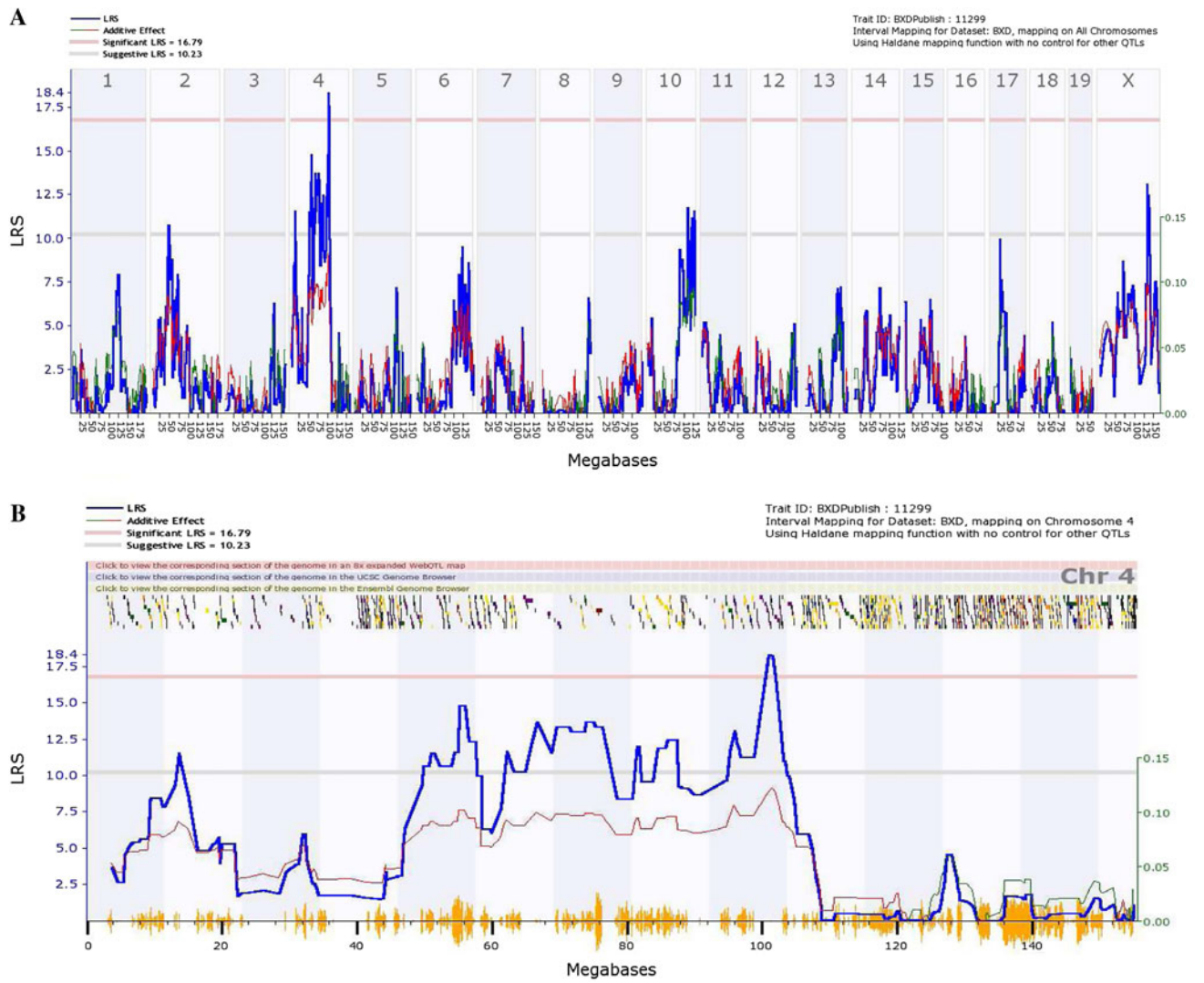


**Fig. 3** Genome-wide linkage map of male BXD adrenal weight. The *blue* trace shows the LRS for male BXD adrenal weight. **a** Interval genome-wide QTL map showing a significant QTL and several suggestive peaks detected on chromosome 4, as well as five suggestive QTLs with one each on chromosomes 2, 17, and X and two on chromosome 10. **b** Interval QTL map with bootstrap analysis of chromosome 4 using male adrenal weight data

value of 17.322 (LOD score = 3.757) for *Mawdq1*, and a 1-LOD confidence interval shows it spanning a small region between 119.0 and 120.0 Mb on Chr 10 (Fig. 5b, Table 9). Meanwhile, an interval genome-wide QTL map of male adrenal medulla width depicted a significant QTL mapped on Chr 5 and suggestive QTLs on Chrs 1 and 3 (Fig. 6a). *Mmwdq1*, the locus symbol for male medulla width QTL, mapped to a distal region on Chr 5 (Fig. 6b). A marker regression analysis yielded a LRS value of 16.876 (LOD score = 3.661) for *Mmwdq1* on Chr 5 (Table 9), and a 1-LOD confidence interval spans a region between 140.7 and 143.3 Mb. Lastly, an interval genome-wide QTL map of male X-zone width showed significant QTLs located on Chrs 10 and 14 (Fig. 7a). The significant QTLs are termed *Mxwdq1* for "male X-zone width QTL 1" on Chr 10 and *Mxwdq2* for "male X-zone width QTL 2" on Chr 14. Marker regression analysis showed an LRS value of 13.652 (LOD score = 2.961) for the peak of *Mxwdq1* on distal Chr 10 (Fig. 7b, Table 9), and a 1-LOD confidence interval depicts it spanning a region between 106.7 and 110.5 Mb. Lastly, a marker regression analysis showed an LRS value of 13.534 (LOD score = 2.936) for *Mxwdq2* on proximal Chr 14 (Fig. 7c, Table 9), and a 1-LOD confidence interval displays it spanning a region between 36.5 and 39.5 Mb.

In addition, there are some suggestive QTLs shown for most of the regional width measures for both males and females. For the female data, two suggestive QTLs are located on Chr 2 for total adrenal width; several suggestive QTLs are located on Chr 4 and one each on Chrs 2 and 17 for ZF width; three suggestive QTLs on Chr 7 for ZI width;

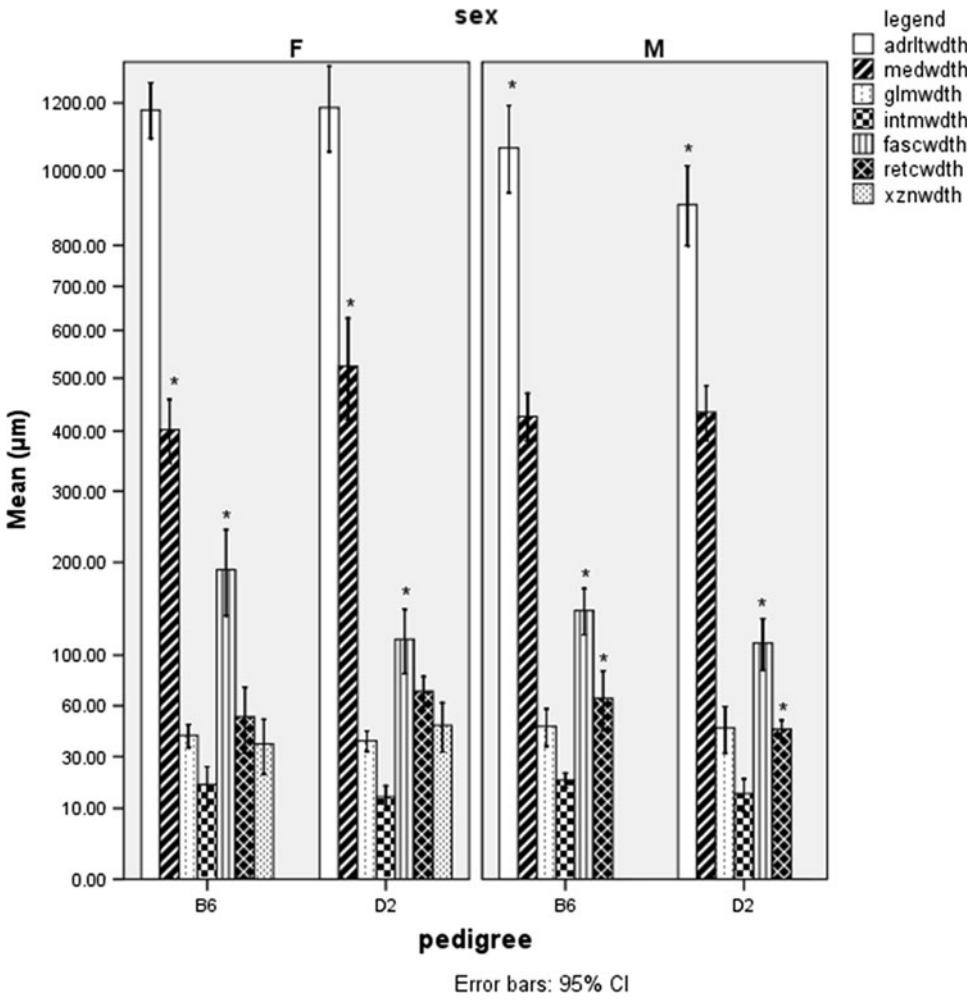


**Fig. 4** Bar graph depicting the parental strain means for the adrenal width measurements. The data are separated by sex for the B6 and D2 mice phenotypes. *Error bars* show the 95% confidence interval for the means. * indicates significance at the 0.05 level (2-tailed)

suggestive QTLs are found on Chrs 2, 3, 10, and 17 for ZR width; and one suggestive QTL on Chr 7 for medulla width (data not shown). For the male data, the ZF width shows several suggestive QTLs, which includes those on Chrs 4, 6, 7, 10, 13, and 19 (data not shown).

## X-zone and adipose analysis

As expected, the X-zone was present in all female mice analyzed since they are all nulliparous. Unexpectedly, the X-zone was also present in male mice of some BXD strains. For the females, the width of the X-zone varied significantly amongst the BXD strains from 27.42 µm for BXD 67 to 109.35 µm for BXD 11 ($F_{64,137} = 2.48$, $p < 0.0005$). This strain-related disparity seems to be associated largely with the presence of fatty adipose tissue. In the adrenal glands examined, adipose tissue was found primarily between the ZR and the X-zone, and it was present only in female mice. It typically appeared as a series of densely packed globules that developed and permeated the morphological border between these two inner cortical regions, forming a distinct lipoid zone. In 22 of the 64 female BXD lines examined, the X-zone was significantly larger compared to all strains for the width ($F_{1,273} = 33.71$, $p < 0.0005$) (Table 4). In 19 of these 22 strains, adipose tissue was densely dispersed throughout the inner cortical region, while four other strains showed some adipose tissue developing along this morphological border. Furthermore, the presence of this lipoid zone was associated with significantly higher adrenal weights in the 23 strains compared to all female strains examined ($F_{1,281} = 6.00$, $p = 0.015$) (Table 1).

Eight of the 64 male BXD strains (BXD 15, 16, 18, 33, 36, 38, 51, and 56) possessed a visible X-zone region (Table 2). Although the X-zone region was significantly smaller in these male strains in terms of width ($F_{1,225} = 73.56$, $p < 0.0005$) compared to that of the female mice examined, five of these eight strains showed the X-zone present in all animals tested within the strains. In addition, age was negatively correlated with X-zone width ($r = -0.61$, $p = 0.001$) in these eight strains. Moreover, in the three BXD lines in which only some of the animals in each strain displayed a visible X-zone, age was still negatively correlated with X-zone width

**Table 4** Female mice: adrenal total width and adrenal zone width measurements

| Strain | Total cases | Total width (μm) | Medulla width (μm) | Glomer width (μm) | Inter width (μm) | Fasc width (μm) | Retic width (μm) | X-zone width (μm) |
|---|---|---|---|---|---|---|---|---|
| BXD1 | 2 | 1135.51 ± 9.21 | 450.79 ± 29.61 | 44.03 ± 2.66 | 14.27 ± 0.12 | 136.56 ± 13.50 | 68.45 ± 26.22 | 37.89 ± 1.45 |
| BXD2[b] | 2 | 1253.38 ± 30.37 | 490.49 ± 22.78 | 46.98 ± 0.79 | 15.91 ± 0.41 | 182.12 ± 14.88 | 76.21 ± 5.40 | 66.47 ± 16.87 |
| BXD6 | 4 | 1461.83 ± 44.20 | 601.12 ± 43.23 | 46.56 ± 4.41 | 14.93 ± 0.47 | 228.63 ± 8.92 | 53.15 ± 5.47 | 52.88 ± 7.10 |
| BXD8 | 2 | 1064.81 ± 113.91 | 449.65 ± 64.51 | 38.61 ± 3.91 | 15.66 ± 0.84 | 142.96 ± 0.75 | 46.85 ± 12.16 | 50.10 ± 12.57 |
| BXD9 | 4 | 1271.12 ± 22.82 | 581.75 ± 29.97 | 41.95 ± 4.78 | 14.27 ± 1.42 | 153.21 ± 6.79 | 54.23 ± 4.42 | 42.92 ± 7.18 |
| BXD11[a, b] | 1 | 1306.77 | 414.77 | 38.55 | 14.63 | 192.10 | 69.13 | 109.35 |
| BXD12 | 2 | 1067.68 ± 47.25 | 404.61 ± 22.80 | 31.41 ± 4.51 | 10.52 ± 1.81 | 150.62 ± 6.05 | 68.40 ± 1.13 | 58.87 ± 4.63 |
| BXD13[b] | 2 | 1279.96 ± 22.03 | 445.34 ± 4.20 | 45.89 ± 1.60 | 14.97 ± 0.11 | 201.72 ± 14.95 | 66.92 ± 2.73 | 74.81 ± 1.04 |
| BXD14 | 2 | 1037.70 ± 5.80 | 412.07 ± 42.07 | 32.01 ± 2.73 | 11.94 ± 0.55 | 142.49 ± 6.50 | 67.89 ± 2.86 | 51.45 ± 5.01 |
| BXD15 | 4 | 1214.74 ± 52.26 | 568.50 ± 38.42 | 45.05 ± 2.09 | 13.82 ± 0.15 | 164.18 ± 11.97 | 42.96 ± 6.81 | 40.75 ± 3.05 |
| BXD16[b] | 2 | 1409.34 ± 251.39 | 428.64 ± 191.71 | 49.25 ± 0.71 | 17.50 ± 5.45 | 221.85 ± 16.17 | 86.39 ± 17.68 | 66.28 ± 3.12 |
| BXD18[b] | 7 | 1325.71 ± 28.72 | 461.64 ± 29.79 | 42.85 ± 5.51 | 15.37 ± 1.65 | 122.77 ± 9.87 | 92.87 ± 13.89 | 78.40 ± 10.84 |
| BXD19 | 3 | 1076.30 ± 23.60 | 435.34 ± 30.43 | 41.13 ± 1.01 | 13.68 ± 0.91 | 154.22 ± 15.60 | 53.86 ± 5.77 | 43.97 ± 6.37 |
| BXD20[b] | 3 | 1395.26 ± 72.98 | 622.83 ± 68.02 | 38.74 ± 3.41 | 15.34 ± 1.55 | 147.14 ± 8.46 | 73.84 ± 1.08 | 65.75 ± 3.25 |
| BXD21 | 2 | 1245.78 ± 94.33 | 528.37 ± 103.85 | 45.48 ± 4.10 | 16.81 ± 0.17 | 153.65 ± 18.82 | 64.73 ± 5.82 | 47.29 ± 6.91 |
| BXD24 | 2 | 1247.53 ± 54.42 | 521.17 ± 24.04 | 41.04 ± 5.69 | 12.51 ± 1.52 | 184.07 ± 21.53 | 63.84 ± 5.77 | 54.34 ± 5.92 |
| BXD27[b] | 4 | 1271.53 ± 33.43 | 541.24 ± 30.41 | 47.89 ± 5.42 | 15.00 ± 1.78 | 143.87 ± 8.40 | 63.86 ± 5.67 | 61.79 ± 4.12 |
| BXD28 | 3 | 1449.89 ± 106.68 | 461.57 ± 66.66 | 39.27 ± 3.62 | 12.16 ± 0.02 | 133.22 ± 7.97 | 75.79 ± 17.78 | 39.67 ± 9.29 |
| BXD31[a] | 1 | 1235.49 | 489.12 | 50.78 | 12.37 | 173.49 | 54.48 | 57.72 |
| BXD32 | 2 | 1411.23 ± 88.96 | 516.87 ± 20.83 | 33.42 ± 1.09 | 8.78 ± 0.35 | 102.86 ± 7.39 | 62.71 ± 24.37 | 40.90 ± 14.70 |
| BXD33 | 2 | 1217.19 ± 6.40 | 458.63 ± 41.58 | 33.59 ± 2.61 | 11.30 ± 0.61 | 195.99 ± 34.13 | 71.29 ± 14.59 | 57.45 ± 9.77 |
| BXD34 | 3 | 1203.64 ± 80.53 | 500.30 ± 46.41 | 46.02 ± 1.48 | 16.59 ± 1.09 | 179.93 ± 11.42 | 61.76 ± 7.84 | 58.91 ± 8.17 |
| BXD36[b] | 8 | 1289.41 ± 40.19 | 564.64 ± 25.09 | 40.33 ± 2.13 | 14.34 ± 0.82 | 127.68 ± 7.41 | 81.45 ± 8.40 | 63.56 ± 8.38 |
| BXD38 | 2 | 1330.00 ± 16.74 | 534.47 ± 115.48 | 44.51 ± 2.88 | 14.18 ± 3.04 | 171.70 ± 23.78 | 80.07 ± 21.85 | 57.62 ± 1.14 |
| BXD39[b] | 2 | 1303.38 ± 5.52 | 441.09 ± 16.85 | 50.29 ± 3.31 | 20.19 ± 1.01 | 224.44 ± 17.50 | 72.34 ± 4.09 | 64.72 ± 4.10 |
| BXD40 | 2 | 1137.89 ± 79.94 | 431.64 ± 58.88 | 39.31 ± 5.62 | 12.55 ± 0.56 | 202.11 ± 3.72 | 48.38 ± 4.06 | 47.01 ± 0.70 |
| BXD42 | 8 | 1116.28 ± 76.27 | 348.74 ± 45.20 | 46.39 ± 4.72 | 16.87 ± 1.17 | 155.73 ± 13.55 | 71.76 ± 8.38 | 57.07 ± 10.21 |
| BXD43 | 4 | 1287.82 ± 34.91 | 602.81 ± 37.47 | 40.29 ± 0.89 | 15.57 ± 0.19 | 163.20 ± 10.59 | 45.60 ± 6.05 | 48.40 ± 4.59 |
| BXD44 | 2 | 1037.93 ± 12.87 | 416.12 ± 3.24 | 46.44 ± 2.76 | 14.32 ± 1.31 | 155.08 ± 2.29 | 46.25 ± 0.78 | 36.67 ± 3.64 |
| BXD45 | 2 | 1396.88 ± 59.09 | 633.32 ± 31.23 | 51.90 ± 3.67 | 11.14 ± 1.50 | 189.82 ± 20.07 | 58.13 ± 1.24 | 57.95 ± 10.02 |
| BXD48 | 4 | 1344.69 ± 65.39 | 615.52 ± 67.46 | 41.49 ± 2.47 | 14.74 ± 2.16 | 184.47 ± 12.04 | 48.04 ± 2.57 | 44.26 ± 6.28 |
| BXD50[b] | 3 | 1406.93 ± 37.16 | 634.25 ± 38.47 | 46.91 ± 2.18 | 14.22 ± 0.28 | 156.53 ± 10.13 | 76.02 ± 11.59 | 60.23 ± 12.86 |
| BXD51[b] | 4 | 1514.22 ± 32.23 | 582.92 ± 37.60 | 55.37 ± 4.38 | 14.98 ± 0.67 | 232.24 ± 13.26 | 71.63 ± 10.85 | 66.82 ± 6.07 |
| BXD55 | 2 | 1193.36 ± 2.01 | 548.75 ± 0.27 | 43.65 ± 0.98 | 12.56 ± 0.76 | 145.49 ± 0.87 | 54.16 ± 1.45 | 53.93 ± 2.60 |
| BXD56 | 3 | 1348.00 ± 32.78 | 516.26 ± 20.33 | 50.00 ± 1.74 | 14.37 ± 0.79 | 214.72 ± 8.40 | 68.63 ± 6.83 | 51.74 ± 4.59 |
| BXD60 | 6 | 1337.82 ± 31.47 | 538.16 ± 16.02 | 48.66 ± 2.90 | 18.56 ± 1.46 | 181.47 ± 13.23 | 64.06 ± 6.17 | 49.25 ± 7.95 |
| BXD61 | 2 | 1262.57 ± 6.88 | 536.02 ± 25.19 | 43.03 ± 0.88 | 13.87 ± 0.63 | 174.33 ± 10.87 | 64.64 ± 3.80 | 47.96 ± 0.87 |
| BXD62[b] | 3 | 1436.28 ± 57.70 | 338.86 ± 96.00 | 54.27 ± 3.47 | 15.70 ± 1.55 | 259.65 ± 0.81 | 96.25 ± 11.81 | 74.49 ± 10.12 |
| BXD63 | 2 | 1408.99 ± 10.28 | 646.42 ± 4.59 | 47.18 ± 7.57 | 14.17 ± 0.42 | 212.68 ± 12.40 | 47.74 ± 0.32 | 43.61 ± 1.40 |
| BXD65[b] | 4 | 1209.61 ± 10.54 | 453.62 ± 20.17 | 42.04 ± 2.69 | 14.48 ± 0.76 | 175.09 ± 13.02 | 71.50 ± 4.74 | 71.18 ± 6.33 |
| BXD66[b] | 3 | 1305.66 ± 14.97 | 509.54 ± 24.25 | 39.36 ± 1.98 | 14.14 ± 1.23 | 195.73 ± 3.34 | 74.41 ± 5.70 | 69.27 ± 4.70 |
| BXD67 | 4 | 1118. 29 ± 51.13 | 413.49 ± 38.66 | 49.17 ± 5.62 | 13.94 ± 0.76 | 185.49 ± 11.99 | 44.24 ± 2.29 | 27.42 ± 2.01 |
| BXD68 | 2 | 1236.97 ± 108.46 | 471.13 ± 60.67 | 49.75 ± 2.20 | 15.58 ± 0.21 | 178.44 ± 29.53 | 51.09 ± 10.78 | 53.58 ± 8.03 |
| BXD69[b] | 3 | 1286.81 ± 44.48 | 475.64 ± 30.68 | 40.32 ± 2.61 | 14.21 ± 0.73 | 172.48 ± 11.54 | 86.12 ± 10.68 | 86.36 ± 11.17 |
| BXD70[b] | 3 | 1230.03 ± 23.78 | 522.08 ± 20.69 | 46.15 ± 0.86 | 13.05 ± 0.99 | 138.40 ± 4.28 | 69.70 ± 6.01 | 74.66 ± 9.31 |
| BXD71 | 3 | 1067.72 ± 58.14 | 500.74 ± 40.06 | 43.77 ± 1.15 | 15.30 ± 0.40 | 149.03 ± 10.08 | 38.43 ± 3.31 | 28.36 ± 2.60 |
| BXD73 | 4 | 1233.93 ± 29.37 | 527.33 ± 37.50 | 47.22 ± 4.46 | 16.04 ± 1.31 | 162.34 ± 16.09 | 43.23 ± 3.00 | 37.74 ± 7.29 |

**Table 4** continued

| Strain | Total cases | Total width (μm) | Medulla width (μm) | Glomer width (μm) | Inter width (μm) | Fasc width (μm) | Retic width (μm) | X-zone width (μm) |
|---|---|---|---|---|---|---|---|---|
| BXD75[b] | 4 | 1185.87 ± 24.32 | 483.71 ± 16.16 | 44.70 ± 0.83 | 15.64 ± .063 | 148.82 ± 3.15 | 67.22 ± 3.08 | 65.53 ± 10.00 |
| BXD77 | 2 | 1024.55 ± 83.02 | 390.20 ± 34.32 | 48.38 ± 4.18 | 13.06 ± 0.24 | 136.40 ± 12.73 | 54.76 ± 7.21 | 56.49 ± 16.92 |
| BXD80 | 3 | 1130.10 ± 31.54 | 530.51 ± 14.04 | 35.53 ± 1.64 | 12.02 ± 0.64 | 132.49 ± 6.98 | 51.59 ± 6.71 | 51.84 ± 1.20 |
| BXD83 | 3 | 1199.17 ± 11.42 | 501.56 ± 18.73 | 42.10 ± 3.15 | 13.58 ± 1.37 | 164.47 ± 9.03 | 58.17 ± 2.85 | 57.12 ± 8.30 |
| BXD84 | 3 | 1272.53 ± 56.52 | 517.29 ± 33.35 | 45.36 ± 0.96 | 16.41 ± 0.42 | 184.07 ± 12.86 | 59.09 ± 8.42 | 54.34 ± 6.70 |
| BXD85[b] | 2 | 1215.21 ± 16.10 | 517.88 ± 6.03 | 44.42 ± 0.53 | 15.01 ± 0.63 | 156.94 ± 22.64 | 59.19 ± 4.67 | 70.81 ± 18.28 |
| BXD86 | 3 | 1078.08 ± 93.66 | 534.32 ± 74.53 | 42.50 ± 4.51 | 13.10 ± 0.25 | 130.53 ± 5.85 | 36.69 ± 1.78 | 36.32 ± 2.48 |
| BXD87[b] | 2 | 1248.08 ± 27.68 | 486.16 ± 36.93 | 39.86 ± 3.16 | 14.88 ± 0.12 | 157.14 ± 22.24 | 72.74 ± 13.99 | 80.77 ± 4.35 |
| BXD89 | 2 | 1177.55 ± 67.22 | 457.99 ± 59.43 | 38.40 ± 8.04 | 11.05 ± 0.03 | 120.34 ± 32.53 | 51.99 ± 1.26 | 34.32 ± 2.55 |
| BXD90 | 4 | 1285.69 ± 57.49 | 551.22 ± 16.56 | 50.19 ± 6.30 | 14.77 ± 1.99 | 141.63 ± 12.05 | 61.53 ± 6.25 | 53.14 ± 1.98 |
| BXD92[b] | 4 | 1264.53 ± 53.36 | 485.91 ± 32.79 | 42.04 ± 1.35 | 14.92 ± 0.49 | 173.93 ± 1.61 | 75.92 ± 12.56 | 70.66 ± 8.70 |
| BXD96 | 3 | 1269.19 ± 44.37 | 556.90 ± 9.15 | 46.28 ± 1.02 | 15.23 ± 0.18 | 166.88 ± 14.65 | 55.37 ± 5.43 | 58.25 ± 1.85 |
| BXD97[b] | 3 | 1189.60 ± 12.90 | 429.61 ± 14.31 | 42.87 ± 1.65 | 14.62 ± 0.19 | 165.24 ± 6.82 | 66.60 ± 3.88 | 78.33 ± 4.39 |
| BXD98 | 2 | 1286.06 ± 20.77 | 605.43 ± 18.36 | 39.57 ± 9.12 | 13.02 ± 0.19 | 179.26 ± 23.68 | 54.37 ± 4.49 | 40.19 ± 1.55 |
| BXD99[b] | 4 | 1239.37 ± 68.59 | 533.79 ± 58.41 | 41.82 ± 1.22 | 16.05 ± 0.90 | 156.44 ± 5.18 | 66.48 ± 4.78 | 64.74 ± 6.26 |
| BXD100 | 3 | 1350.56 ± 20.40 | 601.36 ± 18.01 | 34.32 ± 1.43 | 13.91 ± 1.16 | 203.06 ± 3.21 | 61.52 ± 2.47 | 51.24 ± 4.17 |
| C57BL6 | 5 | 1178.02 ± 30.69 | 402.23 ± 20.25 | 41.18 ± 2.34 | 17.92 ± 2.64 | 190.80 ± 18.91 | 52.49 ± 7.49 | 36.52 ± 5.19 |
| DBA/2J | 6 | 1185.50 ± 50.99 | 523.91 ± 40.02 | 38.10 ± 2.18 | 13.54 ± 1.50 | 114.78 ± 11.91 | 70.50 ± 4.41 | 47.14 ± 5.80 |

All data are shown as mean ± standard error of the mean

*Glomer* glomerulosa, *Inter* intermedia, *Fasc* fasciculata, *Retic* reticularis

[a] Only 1 female adrenal weighed from BXD11 and BXD31 available for width measures

[b] Female BXD strains with significantly larger X-zone width compared to all BXD lines examined

($r = -0.73$, $p = 0.011$) such that those with a visible X-zone were typically younger than those that did not display an X-zone.

### Genes in QTL regions

In total, there are 113 genes mapped within the six significant QTL regions (Table 9). After locating these various genes in the QTL intervals, NCBI's Entrez Gene and the Jackson Laboratory's MGI Mouse Genome Database were used to investigate their known functions, which have been annotated from previous studies. The genes were surveyed to determine if there are interesting candidate genes with functions that are related to adrenal gland development, structure, and/or function. In our analysis, we identified four candidate genes using this approach, including *Pitx2*, *Hnl*, *Lepr*, and *Pde4b*.

## Discussion

### Overview

The adrenal gland is the efferent limb of the HPA axis and is critical for physiological responses to stress. The structure of the gland is such that different regions have distinct functional roles, including specific responses to stress. We obtained measures of adrenal gland weight and structure for a large series of the newly expanded BXD recombinant inbred lines derived from two sequenced strains of mice: C57BL/6J and DBA/2J. The variation in these measures is substantial among the multiple strains assessed, as well as between males and females. Statistically significant variation in these traits is highly correlated to gene variants that are mapped to Chr 3 (female adrenal weight), Chr 4 (male adrenal weight), Chr 5 (male adrenal medulla width), Chr 10 (male adrenal total width and X-zone width), and Chr 14 (male X-zone width). A total of 113 genes are identified in these genomic regions that are associated with the phenotypic differences observed between the sexes and strains of mice examined.

Our findings on adrenal gland structure and weight have interesting relationships with other animal work done on stress and anxiety. We found robust correlations between our BXD adrenal measures and behavioural and physiological measures of stress and anxiety that extend to genomic regions that subserve both the adrenal phenotypes reported in this article and previously reported QTLs found for stress, emotionality, and anxiety. We also highlight a view of adrenal function that emerges from significant

**Table 5** Male mice: adrenal total width and adrenal zone width measurements

| Strain | Total vcases | Total width (μm) | Medulla width (μm) | Glomer width (μm) | Inter width (μm) | Fasc width (μm) | Retic width (μm) | X-zone width (μm) |
|---|---|---|---|---|---|---|---|---|
| BXD1 | 2 | 1131.54 ± 76.02 | 579.68 ± 63.88 | 39.74 ± 3.47 | 14.46 ± 0.16 | 144.29 ± 3.03 | 70.66 ± 8.50 | 0.00 ± 0.00 |
| BXD2 | 3 | 1029.55 ± 41.70 | 497.75 ± 43.26 | 40.66 ± 2.86 | 12.30 ± 0.34 | 137.80 ± 3.46 | 61.04 ± 5.03 | 0.00 ± 0.00 |
| BXD6 | 6 | 1210.67 ± 44.36 | 511.88 ± 36.99 | 53.42 ± 3.18 | 20.75 ± 2.25 | 168.83 ± 12.71 | 67.73 ± 3.56 | 0.00 ± 0.00 |
| BXD8 | 3 | 1064.63 ± 28.29 | 526.28 ± 45.26 | 42.77 ± 0.81 | 13.25 ± 0.91 | 143.04 ± 14.90 | 59.86 ± 0.68 | 0.00 ± 0.00 |
| BXD9 | 3 | 1131.33 ± 48.23 | 580.49 ± 29.92 | 43.80 ± 0.90 | 15.41 ± 1.28 | 113.96 ± 6.38 | 69.41 ± 4.65 | 0.00 ± 0.00 |
| BXD12 | 2 | 962.84 ± 39.16 | 471.99 ± 20.71 | 32.11 ± 1.02 | 10.48 ± 0.91 | 117.24 ± 4.08 | 70.32 ± 7.78 | 0.00 ± 0.00 |
| BXD13 | 2 | 1019.48 ± 10.49 | 529.29 ± 46.07 | 36.99 ± 2.42 | 11.54 ± 0.43 | 116.84 ± 12.45 | 66.98 ± 8.81 | 0.00 ± 0.00 |
| BXD14 | 2 | 960.72 ± 54.01 | 457.25 ± 46.47 | 33.00 ± 0.90 | 13.17 ± 1.79 | 125.47 ± 13.80 | 70.29 ± 4.82 | 0.00 ± 0.00 |
| BXD15[a] | 2 | 1130.34 ± 27.53 | 657.67 ± 24.67 | 43.31 ± 2.93 | 12.88 ± 0.62 | 99.86 ± 0.78 | 44.54 ± 2.20 | 19.25 ± 1.58 |
| BXD16[a] | 3 | 1249.22 ± 49.27 | 514.58 ± 52.29 | 44.66 ± 4.33 | 14.51 ± 0.28 | 189.72 ± 5.62 | 69.57 ± 5.09 | 38.52 ± 4.40 |
| BXD18[a] | 6 | 1122.56 ± 25.77 | 593.27 ± 20.25 | 46.83 ± 3.74 | 16.77 ± 2.04 | 107.05 ± 8.58 | 49.49 ± 3.52 | 11.22 ± 5.06 |
| BXD19 | 2 | 996.24 ± 216.93 | 443.77 ± 113.11 | 39.26 ± 2.63 | 10.97 ± 3.13 | 150.65 ± 24.81 | 49.85 ± 3.07 | 0.00 ± 0.00 |
| BXD20 | 4 | 1204.90 ± 4.15 | 509.84 ± 21.76 | 48.11 ± 6.99 | 19.38 ± 2.48 | 178.80 ± 10.97 | 60.13 ± 15.29 | 0.00 ± 0.00 |
| BXD24 | 4 | 1170.92 ± 60.80 | 587.04 ± 65.55 | 50.04 ± 3.66 | 15.39 ± 1.38 | 142.65 ± 18.14 | 60.30 ± 2.30 | 0.00 ± 0.00 |
| BXD27 | 7 | 1157.20 ± 15.66 | 599.96 ± 20.74 | 39.44 ± 1.91 | 19.48 ± 1.48 | 140.07 ± 8.88 | 55.29 ± 3.22 | 0.00 ± 0.00 |
| BXD28 | 2 | 1157.93 ± 47.40 | 633.82 ± 11.42 | 35.48 ± 8.82 | 14.38 ± 0.47 | 132.65 ± 14.90 | 47.61 ± 5.88 | 0.00 ± 0.00 |
| BXD29 | 3 | 964.04 ± 18.38 | 483.57 ± 22.85 | 41.17 ± 0.48 | 13.36 ± 0.79 | 109.18 ± 0.93 | 64.72 ± 0.89 | 0.00 ± 0.00 |
| BXD31 | 2 | 1074.44 ± 41.15 | 597.37 ± 90.60 | 36.59 ± 3.69 | 12.61 ± 0.64 | 113.02 ± 26.61 | 51.20 ± 1.68 | 0.00 ± 0.00 |
| BXD32 | 2 | 1060.65 ± 125.58 | 565.12 ± 67.51 | 36.11 ± 4.29 | 12.58 ± 0.77 | 129.93 ± 12.04 | 64.68 ± 10.30 | 0.00 ± 0.00 |
| BXD33[a] | 3 | 1210.14 ± 52.47 | 650.39 ± 35.33 | 49.87 ± 1.36 | 16.86 ± 2.61 | 123.07 ± 12.61 | 59.30 ± 8.50 | 7.30 ± 7.30 |
| BXD34 | 3 | 1194.71 ± 21.01 | 596.10 ± 6.05 | 45.07 ± 2.40 | 14.67 ± 0.78 | 143.80 ± 9.10 | 85.43 ± 2.92 | 0.00 ± 0.00 |
| BXD36[a] | 3 | 1084.26 ± 38.21 | 552.73 ± 50.79 | 42.64 ± 1.16 | 12.04 ± 0.92 | 115.70 ± 4.56 | 54.65 ± 3.30 | 27.89 ± 1.36 |
| BXD38[a] | 4 | 1156.26 ± 32.94 | 542.62 ± 31.73 | 40.91 ± 3.77 | 13.72 ± 0.75 | 131.99 ± 19.88 | 57.87 ± 6.34 | 36.83 ± 9.87 |
| BXD39 | 3 | 1037.65 ± 41.89 | 485.41 ± 36.61 | 43.30 ± 0.57 | 13.10 ± 0.93 | 121.93 ± 6.18 | 75.22 ± 0.89 | 0.00 ± 0.00 |
| BXD40 | 3 | 958.60 ± 61.95 | 488.16 ± 50.58 | 39.11 ± 3.09 | 11.85 ± 0.88 | 108.82 ± 10.51 | 64.26 ± 2.15 | 0.00 ± 0.00 |
| BXD42 | 4 | 1101.15 ± 73.34 | 462.21 ± 72.20 | 51.12 ± 1.71 | 14.63 ± 0.83 | 136.71 ± 14.28 | 74.42 ± 7.85 | 0.00 ± 0.00 |
| BXD43 | 4 | 1059.56 ± 67.29 | 554.21 ± 38.19 | 39.25 ± 3.51 | 12.01 ± 0.34 | 122.65 ± 8.43 | 60.99 ± 3.13 | 0.00 ± 0.00 |
| BXD44 | 3 | 981.13 ± 16.99 | 458.59 ± 18.63 | 43.19 ± 1.24 | 14.85 ± 0.81 | 120.15 ± 6.52 | 72.89 ± 2.19 | 0.00 ± 0.00 |
| BXD45 | 2 | 1026.12 ± 81.35 | 383.76 ± 5.19 | 45.33 ± 3.18 | 17.25 ± 1.67 | 143.78 ± 19.76 | 66.94 ± 14.20 | 0.00 ± 0.00 |
| BXD48 | 8 | 1262.81 ± 37.31 | 664.31 ± 22.23 | 47.33 ± 1.92 | 13.99 ± 0.80 | 135.83 ± 9.72 | 76.55 ± 7.89 | 0.00 ± 0.00 |
| BXD50 | 4 | 1063.93 ± 23.90 | 605.43 ± 19.77 | 41.40 ± 1.78 | 12.73 ± 0.90 | 97.39 ± 6.16 | 59.44 ± 2.44 | 0.00 ± 0.00 |
| BXD51[a] | 2 | 1271.22 ± 39.42 | 576.78 ± 55.21 | 61.52 ± 6.78 | 12.98 ± 0.48 | 154.48 ± 17.55 | 48.08 ± 6.07 | 14.15 ± 14.15 |
| BXD55 | 4 | 1084.97 ± 62.89 | 560.38 ± 67.70 | 42.57 ± 3.64 | 12.51 ± 0.48 | 121.09 ± 12.70 | 57.44 ± 7.63 | 0.00 ± 0.00 |
| BXD56[a] | 2 | 1057.65 ± 128.58 | 490.60 ± 118.86 | 50.26 ± 9.32 | 11.15 ± 0.49 | 128.77 ± 9.67 | 49.48 ± 1.21 | 20.99 ± 1.30 |
| BXD60 | 4 | 1150.09 ± 24.61 | 570.08 ± 23.77 | 49.17 ± 5.24 | 17.71 ± 1.19 | 128.30 ± 5.74 | 65.48 ± 7.36 | 0.00 ± 0.00 |
| BXD61 | 3 | 1091.13 ± 32.46 | 567.20 ± 7.66 | 41.38 ± 2.60 | 12.88 ± 0.86 | 117.66 ± 7.98 | 78.82 ± 7.16 | 0.00 ± 0.00 |
| BXD62 | 3 | 1128.02 ± 136.67 | 531.38 ± 98.82 | 39.29 ± 1.30 | 13.23 ± 0.51 | 134.06 ± 7.75 | 101.20 ± 22.45 | 0.00 ± 0.00 |
| BXD63 | 2 | 1249.07 ± 72.74 | 712.67 ± 0.39 | 50.80 ± 2.55 | 14.69 ± 4.58 | 134.86 ± 23.00 | 45.67 ± 1.95 | 0.00 ± 0.00 |
| BXD65 | 3 | 1038.56 ± 19.94 | 476.24 ± 7.45 | 42.54 ± 4.45 | 13.18 ± 0.73 | 125.61 ± 12.88 | 82.21 ± 3.85 | 0.00 ± 0.00 |
| BXD66 | 3 | 1101.78 ± 33.07 | 534.08 ± 38.96 | 38.97 ± 2.35 | 14.83 ± 1.03 | 128.03 ± 4.87 | 83.26 ± 6.97 | 0.00 ± 0.00 |
| BXD67 | 4 | 1104.05 ± 51.58 | 516.67 ± 39.70 | 48.00 ± 3.43 | 17.00 ± 0.84 | 137.16 ± 10.04 | 55.98 ± 4.92 | 0.00 ± 0.00 |
| BXD68 | 3 | 1102.79 ± 37.20 | 547.20 ± 37.02 | 39.66 ± 1.90 | 13.69 ± 0.70 | 132.93 ± 2.03 | 81.12 ± 2.02 | 0.00 ± 0.00 |
| BXD69 | 3 | 1111.58 ± 60.22 | 469.35 ± 34.24 | 42.61 ± 1.15 | 13.70 ± 0.65 | 164.70 ± 8.14 | 83.48 ± 2.77 | 0.00 ± 0.00 |
| BXD70 | 4 | 969.45 ± 73.63 | 518.65 ± 68.50 | 35.78 ± 1.18 | 12.91 ± 1.13 | 108.38 ± 6.51 | 58.02 ± 4.72 | 0.00 ± 0.00 |
| BXD71 | 3 | 1149.26 ± 22.06 | 592.02 ± 13.35 | 46.09 ± 2.99 | 14.21 ± 0.38 | 109.33 ± 6.28 | 72.81 ± 5.86 | 0.00 ± 0.00 |
| BXD73 | 4 | 1083.49 ± 31.98 | 593.77 ± 44.92 | 46.94 ± 3.40 | 18.09 ± 1.65 | 109.58 ± 5.89 | 46.76 ± 2.58 | 0.00 ± 0.00 |
| BXD75 | 3 | 1022.65 ± 53.28 | 482.71 ± 36.98 | 44.04 ± 3.06 | 14.67 ± 0.03 | 119.45 ± 8.37 | 85.39 ± 9.23 | 0.00 ± 0.00 |

**Table 5** continued

| Strain | Total vcases | Total width (μm) | Medulla width (μm) | Glomer width (μm) | Inter width (μm) | Fasc width (μm) | Retic width (μm) | X-zone width (μm) |
|---|---|---|---|---|---|---|---|---|
| BXD77 | 3 | 1020.94 ± 26.47 | 510.18 ± 25.38 | 43.32 ± 4.21 | 14.30 ± 0.82 | 107.22 ± 6.44 | 78.95 ± 2.77 | 0.00 ± 0.00 |
| BXD80 | 2 | 1023.00 ± 75.94 | 538.11 ± 71.45 | 41.26 ± 3.33 | 15.30 ± 0.76 | 107.76 ± 1.19 | 71.19 ± 7.56 | 0.00 ± 0.00 |
| BXD83 | 2 | 1002.44 ± 12.94 | 565.91 ± 6.59 | 37.22 ± 1.30 | 10.05 ± 0.13 | 100.42 ± 2.47 | 59.85 ± 1.48 | 0.00 ± 0.00 |
| BXD84 | 3 | 1111.89 ± 61.54 | 533.75 ± 45.78 | 39.39 ± 1.36 | 12.69 ± 0.67 | 124.47 ± 4.79 | 98.78 ± 8.05 | 0.00 ± 0.00 |
| BXD85 | 4 | 1112.88 ± 27.83 | 539.44 ± 20.80 | 43.47 ± 1.58 | 14.57 ± 0.72 | 127.22 ± 13.12 | 80.00 ± 7.65 | 0.00 ± 0.00 |
| BXD86 | 2 | 1041.82 ± 12.61 | 548.81 ± 9.79 | 40.22 ± 1.82 | 14.67 ± 0.05 | 114.48 ± 6.92 | 67.24 ± 0.78 | 0.00 ± 0.00 |
| BXD87 | 2 | 1175.94 ± 27.03 | 599.59 ± 40.73 | 39.91 ± 2.59 | 14.41 ± 1.48 | 135.80 ± 14.16 | 90.00 ± 10.29 | 0.00 ± 0.00 |
| BXD89 | 2 | 1115.33 ± 65.14 | 667.32 ± 48.63 | 38.53 ± 0.46 | 15.29 ± 1.63 | 109.48 ± 3.83 | 38.13 ± 0.78 | 0.00 ± 0.00 |
| BXD90 | 4 | 1165.52 ± 37.21 | 632.02 ± 21.81 | 43.71 ± 3.06 | 14.06 ± 0.55 | 116.35 ± 7.89 | 60.43 ± 1.42 | 0.00 ± 0.00 |
| BXD92 | 2 | 1061.99 ± 23.94 | 528.20 ± 14.41 | 41.84 ± 1.46 | 14.21 ± 0.74 | 115.57 ± 1.30 | 81.59 ± 5.09 | 0.00 ± 0.00 |
| BXD96 | 2 | 1093.57 ± 136.90 | 518.86 ± 118.23 | 38.51 ± 4.73 | 13.66 ± 0.42 | 134.49 ± 1.92 | 81.17 ± 4.58 | 0.00 ± 0.00 |
| BXD97 | 3 | 1033.61 ± 47.86 | 490.34 ± 20.61 | 42.30 ± 2.89 | 14.37 ± 1.31 | 127.74 ± 11.85 | 76.52 ± 6.16 | 0.00 ± 0.00 |
| BXD98 | 3 | 1169.91 ± 30.65 | 634.35 ± 19.50 | 42.42 ± 3.41 | 13.73 ± 0.64 | 121.41 ± 1.91 | 72.79 ± 2.51 | 0.00 ± 0.00 |
| BXD99 | 4 | 1162.54 ± 28.54 | 640.21 ± 30.27 | 38.81 ± 1.41 | 14.14 ± 0.91 | 116.57 ± 4.24 | 69.60 ± 3.05 | 0.00 ± 0.00 |
| BXD100 | 2 | 1330.42 ± 85.43 | 580.97 ± 67.68 | 45.57 ± 0.67 | 13.87 ± 0.78 | 148.21 ± 39.43 | 108.24 ± 20.08 | 0.00 ± 0.00 |
| C57BL6 | 4 | 1065.04 ± 39.83 | 426.34 ± 13.75 | 46.56 ± 3.55 | 19.58 ± 0.92 | 143.66 ± 7.76 | 65.39 ± 6.60 | 0.00 ± 0.00 |
| DBA/2J | 5 | 906.12 ± 38.56 | 434.14 ± 18.25 | 45.55 ± 4.97 | 14.61 ± 1.96 | 110.98 ± 8.63 | 44.88 ± 1.94 | 0.00 ± 0.00 |

All data are shown as mean ± standard error of the mean

*Glomer* glomerulosa, *Inter* intermedia, *Fasc* fasciculata, *Retic* reticularis

[a] Male BXD strains with a visible X-zone region

**Table 6** Heritability of phenotypes

| Phenotype | Combined (across sexes) (%) | Males (%) | Females (%) |
|---|---|---|---|
| Adrenal weight | 16 | 54 | 59 |
| Total adrenal width | 23 | 34 | 45 |
| Adrenal medulla width | 25 | 29 | 33 |
| Zona glomerulosa width | 15 | 22 | 11 |
| Zona intermedia width | 19 | 33 | 14 |
| Zona fasciculata width | 22 | 34 | 57 |
| Zona reticularis width | 17 | 49 | 26 |
| X-zone width | 0 | 75 | 31 |

differences on measures of adrenal weight and ZF and adrenal medulla width that we found between B6 and D2 mice.

## Adrenal weight QTLs

Although QTL approaches have been used to examine adrenal weight while performing genetic analyses of the stress response in rats (Llamas et al. 2005; Solberg et al. 2006) and pigs (Muráni et al. 2010), this is the first report of QTLs associated with the mouse adrenal gland. Llamas et al. (2005) did not find a significant QTL for adrenal weight in the rat. Muráni et al. (2010), however, found four significant SNPs in the pig but none had orthology to the QTL regions of our present study. Solberg et al. (2006) found a significant QTL for adrenal weight, which they named stress-responsive adrenal 5 (*Sradr-5*), on Chr 7 in rats that is an orthologous region of the significant QTL that we found for male adrenal total width and the suggestive QTL for male adrenal weight, both on mouse distal Chr 10. *Sradr-5* also was orthologous to our significant Chr 3 QTL region for female adrenal weight. This same significant QTL region for female adrenal weight, along with a suggestive QTL for male medulla width on Chr 3, also shows some orthologous overlap with a significant QTL named stress-responsive corticosterone 1 (*Srcrt-1*) that they found on Chr 2 in rats. Lastly, the significant QTL *Srcrt-4*, on rat Chr 6, is orthologous to the mouse suggestive QTL for male adrenal weight on Chr 4. These studies (as well as others, e.g., Marissal-Arvy et al. 2004) reveal QTLs for adrenal-related phenotypes that may further suggest robust structure-function correlations.

The QTL analyses for male and female mice exhibited unique, significant QTLs; however, there were also overlapping suggestive QTLs located on the distal end of Chr 10 for the male and female weight data. Thus, although most of the QTLs detected are unique for males and females, the overlapping suggestive QTL region on Chr 10 indicates that some similar genes may account for strain

**Table 7** Age, body weight, adrenal weight, and adrenal width correlations for female mice

| | Age | Body weight | Adrenal weight | Adrenal width | Medulla width | Glomer width | Inter width | Fasc width | Retic width | X-zone width |
|---|---|---|---|---|---|---|---|---|---|---|
| Age | 1 | | | | | | | | | |
| Body weight | 0.277** | 1 | | | | | | | | |
| Adrenal weight | 0.402** | 0.296** | 1 | | | | | | | |
| Adrenal width | 0.293** | 0.184** | 0.530** | 1 | | | | | | |
| Medulla width | 0.033 | 0.062 | 0.011 | 0.595** | 1 | | | | | |
| Glomer width | −0.006 | −0.050 | 0.028 | 0.184** | −0.007 | 1 | | | | |
| Inter width | −0.083 | −0.007 | −0.010 | 0.025 | −0.050 | 0.358** | 1 | | | |
| Fasc width | 0.063 | 0.009 | 0.155* | 0.375** | −0.039 | 0.266** | 0.185** | 1 | | |
| Retic width | 0.216** | 0.179* | 0.406** | 0.378** | −0.106 | −0.017 | −0.004 | −0.024 | 1 | |
| X-zone width | 0.138* | 0.036 | 0.353** | 0.286** | −0.058 | −0.037 | −0.038 | −0.030 | 0.649** | 1 |

*Glomer* glomerulosa, *Inter* intermedia, *Fasc* fasciculata, *Retic* reticularis

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

**Table 8** Age, body weight, adrenal weight, and adrenal width correlations for male mice

| | Age | Body weight | Adrenal weight | Adrenal width | Medulla width | Glomer width | Inter width | Fasc width | Retic width | X-zone width |
|---|---|---|---|---|---|---|---|---|---|---|
| Age | 1 | | | | | | | | | |
| Body weight | 0.315** | 1 | | | | | | | | |
| Adrenal weight | −0.082 | 0.172* | 1 | | | | | | | |
| Adrenal width | −0.035 | 0.139* | 0.308** | 1 | | | | | | |
| Medulla width | 0.079 | 0.131 | 0.158* | 0.716** | 1 | | | | | |
| Glomer width | −0.008 | −0.051 | 0.012 | 0.330** | 0.050 | 1 | | | | |
| Inter width | −0.078 | 0.035 | 0.035 | 0.275** | 0.019 | 0.386** | 1 | | | |
| Fasc width | −0.129 | −0.016 | 0.294** | 0.482** | −0.115 | 0.115 | 0.286** | 1 | | |
| Retic width | −0.132 | 0.116 | 0.090 | 0.232** | −0.072 | −0.055 | −0.110 | 0.143* | 1 | |
| X-zone width | −0.076 | 0.065 | 0.191** | 0.168** | 0.005 | 0.013 | −0.121 | 0.176* | −0.141* | 1 |

*Glomer* glomerulosa, *Inter* intermedia, *Fasc* fasciculata, *Retic* reticularis

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

variation in adrenal weight. Such variation in adrenal weight and morphology could underlie the strain-specific differences in anxiety and stress responses observed for B6 and D2 mice (Ponder et al. 2007a; Shanks et al. 1990; Tarricone et al. 1995; Trullas and Skolnick 1993; Võikar et al. 2005; Yang et al. 2008; Yilmazer-Hanke et al. 2003). A direct examination of this possibility using these RI strains would provide further evidence of the potential genetic link between adrenal weight and adrenal function.

## Adrenal width QTL

This is the first study to identify QTLs for adrenal structure. Solberg et al. (2006) found a significant QTL on Chr 4 for adrenal weight in rats, which they termed *Sradr-3*, that is located in the orthologous, suggestive QTL for male adrenal medulla width on Chr 1. Another suggestive adrenal medulla QTL on mouse Chr 3 is orthologous to human genes *HSD3B1* (Chang et al. 1993) and *HSD3B2* (Rhéaume et al. 1992) located on human Chr 1 that have been found to be associated with adrenal hyperplasia and the significant QTL *Srcrt-1* in rat (Solberg et al. 2006). Thus, like the adrenal weight QTLs, the width QTLs have cross-species orthology that speak to a conservation of gene function.

For the measures that show significant QTLs, each phenotype is not only associated with a unique genomic region, it is also different between males and females. This

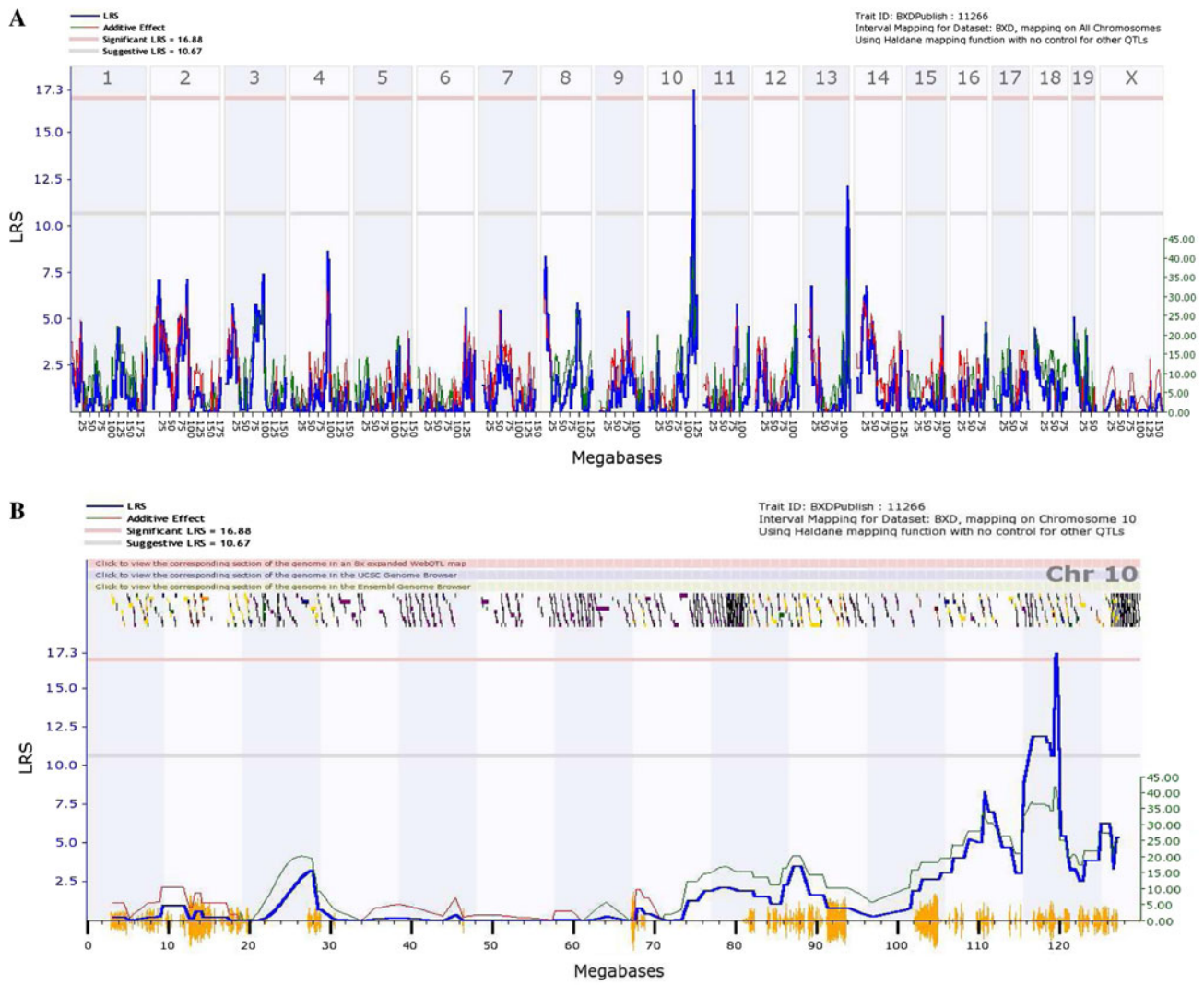


**Fig. 5** Genome-wide linkage map of male BXD adrenal total width. The *blue* trace shows the LRS for male BXD adrenal total width. **a** Interval genome-wide QTL map showing a significant QTL detected on chromosome 10 and a suggestive QTL detected on chromosome 13. **b** Interval QTL map with bootstrap analysis of chromosome 10 using male adrenal total width data

suggests that there are genes contributing to adrenal gland regional morphology that are specific to each cortical region and medulla that are also separate from those influencing adrenal weight.

The male X-zone QTL is derived from the eight BXD lines that have such a zone and really represents a qualitative trait. The same two genomic regions are identified when we treat this trait in an all-or-nothing fashion. This is presumably due to the confluence of alleles that underlie the appearance of an X-zone in males of some other strains of mouse (Deschepper et al. 2004).

Of final interest relative to our width measures is the QTL found for the male medulla. This QTL region is unique to the medulla measures because no other phenotypic measure performed in this study yields suggestive or significant QTLs on Chr 5. Since the adrenal medulla is specifically associated with the autonomic "fight or flight" stress response, this QTL may be related to both adrenal medulla width and the stress response, linking adrenal structure to function. Related to this, the stress-reactive D2 mice have a larger adrenal medulla region than the more stress-resistant B6 mice. Adrenal medullary hypertrophy occurs in rats subjected to a chronic stress paradigm, which coincides with the overall adrenal enlargement they experience (Ulrich-Lai et al. 2006). Although these results point toward a direct relationship between medulla width and function, it does not explain why our results do not show significant QTLs for the female medulla measures. Both males and females exhibit similar strain variability in the width measures, but the overall width of the male medulla is significantly larger than the female medulla. This could suggest that the

**Table 9** BXD phenotype-related loci and genes

| Adrenal measures phenotype | Chr | Mapping location | LRS | Gene list | | | | |
|---|---|---|---|---|---|---|---|---|
| Female adrenal weight (Fawq1) | 3 | 127.0–129.5 Mb | 19.669 | *Larp7* | *Neurog2* | *Alpk1* | 1500005C15Rik | 4930422G04Rik |
| | | | | *T2 bp* | BC002199 | 5730508B09Rik | 9830132P13 | D030025E07Rik |
| | | | | 4933424H11Rik | ***Pitx2***[a] | *Gm132* | *Enpep* | LOC435755 |
| | | | | *Elovl6* | *Egf* | 6330410L21Rik | ***Hnl***[a] | |
| Male adrenal weight (Mawq1) | 4 | 99.5–102.5 Mb | 18.363 | *Pgm2* | *Ror1* | *Ube2u* | *Cachd1* | *Raver2* |
| | | | | *Jak1* | E130102H24Rik | 0610043K17Rik | *Ak3l1* | *Dnajc6* |
| | | | | *Leprot* | C130073F10Rik | ***Lepr***[a] | RP23-149D11.4 | B020004J07Rik |
| | | | | RP23-149D11.5 | RP23-149D11.7 | ***Pde4b***[a] | *Sgip1* | *Ifna* |
| Male total width (Mawdq1) | 10 | 119.0–120.0 Mb | 17.322 | 4930564G21Rik | *Grip1* | 4930483C13Rik | *Helb* | *Irak3* |
| | | | | *Tmbim4* | 1190005P17Rik | *Hmga2* | 1700006J14Rik | 9230105E05Rik |
| | | | | 4930471E19Rik | | | | |
| Male medulla width (Mmwdq1) | 5 | 140.7–143.3 Mb | 16.876 | EG231836 | *Ftsj2* | *Nudt1* | *Snx8* | *Eif3b* |
| | | | | *Chst12* | *Grifin* | *Lfng* | *Ttyh3* | *Iqce* |
| | | | | AA881470 | *Amz1* | *Gna12* | *Card11* | *Sdk1* |
| | | | | 3200001G23Rik | *Kdelr2* | D330005C11Rik | *Foxk1* | C330006K01Rik |
| | | | | *Slc29a4* | D930005D10Rik | 4921504P13Rik | *Papolb* | *Mmd2* |
| | | | | *Wipi2* | B130019G13Rik | *Tnrc18* | | |
| Male X-zone width (Mxwdq1) | 10 | 106.7–1105 Mb | 13.652 | *Lin7a* | 3110033O22Rik | *Myf5* | *Myf6* | *Ptprq* |
| | | | | LOC628877 | Ppp1r12a | C430003N24Rik | *Pawr* | *Syt1* |
| | | | | A830054O04Rik | 5330428N10Rik | C030018G05Rik | EG368203 | C030044C18Rik |
| | | | | 2900074G08Rik | A130086K04Rik | 6430709C05Rik | 3110043J17Rik | *Nav3* |
| | | | | A830061P03Rik | 9230102K24Rik | E2f7 | 1700020G17Rik | *Csrp2* |
| | | | | *Zdhhc17* | | | | |
| Male X-zone width (Mxwdq2) | 14 | 36.5–39.5 Mb | 13.534 | 4930596D02Rik | 4930474N05Rik | *Gcap14* | *Rgr* | *Lrit1* |
| | | | | *Lrit2* | *Pcdh21* | *Ghitm* | *Nrg3* | |

[a] Candidate gene

males have a more highly sensitized autonomic stress response than female mice if a link does exist between adrenal width and the stress response.

## Interaction between adrenal width and weight

We found that adrenal weight and total width show a robust correlation, yet the QTL analyses for these phenotypes reveal only two overlapping suggestive QTLs on Chrs 2 and 10 out of a total of 3 significant and 17 suggestive regions between them. These results indicate that these factors have little effect on one another, which is interesting considering that the regression analysis shows total width accounting for 40% of the variance in adrenal weight. It makes sense that the larger the adrenal gland (determined by width), the heavier it would weigh. Since total cell number, cell density, and cell size measures were not performed in this study, these factors may account for why only a few overlapping QTL regions exist between weight and width phenotypes. It is likely that a combination of adrenal size and cellular architecture that influence adrenal weight, and an analysis of this possibility may identify more suggestive and significant QTLs in similar genomic regions as those found for adrenal weight.

## Candidate genes

The majority of the 113 genes identified in the QTL regions have gene functions yet to be elucidated. This is particularly the case with the QTL region on Chr 10, where a majority of the candidate loci are unannotated. Many other genes in these intervals are annotated relative to multiple biological functions, but they have not been determined to be associated with the adrenal gland or the endocrine stress response. Given what is known, the number of candidate genes is considerably reduced by focusing only on those that have a biological relevance to the adrenal gland structure, development, and/or function. Of the six significant QTLs, only those for adrenal weight have annotated genes that would be suggestive for follow-up. These four alleles include pituitary homeobox 2 (*Pitx2*), which has

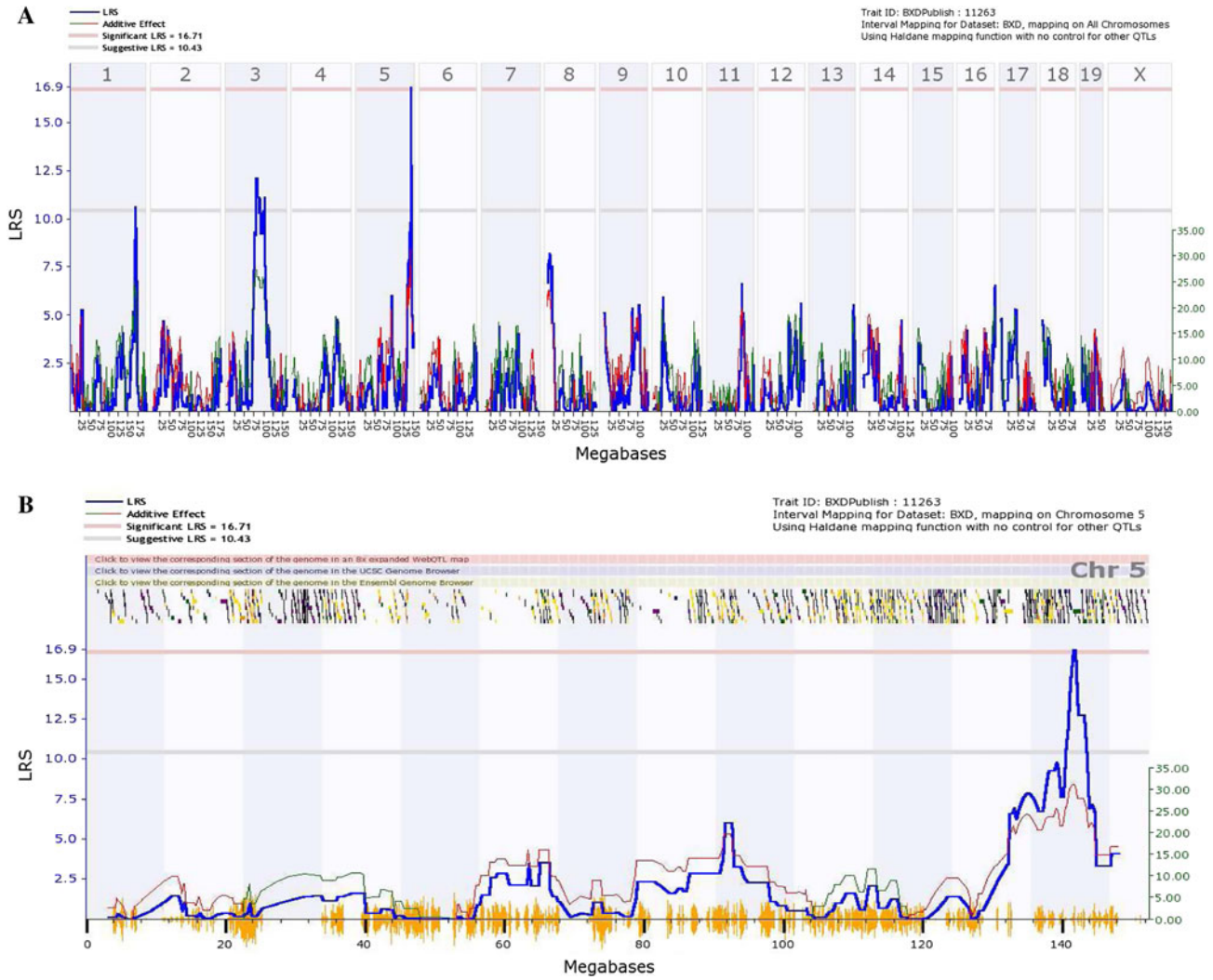


**Fig. 6** Genome-wide linkage map of male BXD adrenal medulla width. The *blue* trace shows the LRS for male BXD adrenal medulla width. **a** Interval genome-wide QTL map showing a significant QTL detected on chromosome 5 and suggestive QTLs detected on chromosomes 1 and 3. **b** Interval QTL map with bootstrap analysis of chromosome 5 using male adrenal medulla width data

been implicated in regulating gene transcription in the pituitary gonadotrope of adrenal 4 binding protein/steroidogenic factor-1 (*Ad4BP/Sf-1*), which is essential for animal reproduction and endocrine regulation (Shima et al. 2008); hypothalamic norepinephrine level (*Hnl*), which has been mapped on distal Chr 3 (131.843 Mb; Eleftheriou 1974) and is located in the QTL *Nsila3* on Chr 3 from an analysis of AXB/BXA RI strains, which involves B6 progenitors that exhibit more activation of locomotor activity in an open field compared to the A/J progenitor strain (Gill and Boyle 2005); phosphodiesterase 4B, cAMP-specific (*Pde4b*), which influences anxiogenic-like effects on behaviour as *Pde4b*−/− mice exhibited increased anxiogenic-like behaviour and heightened plasma corticosterone levels (Zhang et al. 2008); and the leptin receptor (*Lepr*), which influences the effects of leptin action of brown adipose tissue (Cannon and Nedergaard 2004; Oldfield et al. 2002), sympathetic nervous system tone (Bates et al. 2004), and hypothalamic regulation of feeding (Oldfield et al. 2002). It has also been suggested that *Lepr* provides mechanistic pathways for understanding how stress-related traits may be associated with risk factors for cardiovascular disease (Liu et al. 2007).

## Differences between parental strains

There are three major results that emerge from a comparison of the B6 and D2 parental strains for the adrenal gland weight and structure. First, the D2 and B6 mice, when considering both sexes, do not differ significantly in either adrenal weight or total adrenal width, but the relative adrenal weight to body weight for D2 mice is heavier

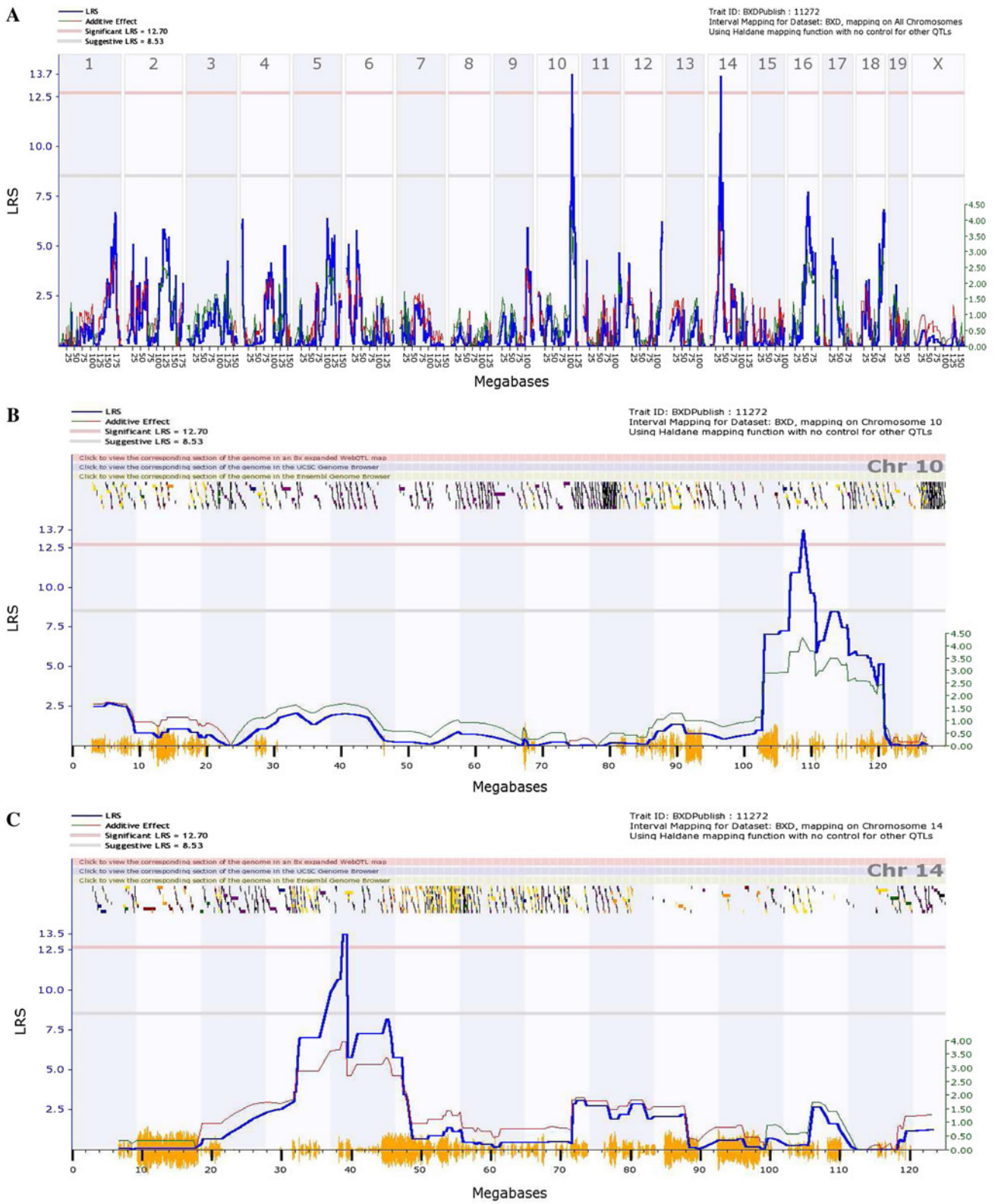


**Fig. 7** Genome-wide linkage map of male BXD X-zone width. The *blue* trace shows the LRS for male BXD X-zone width. **a** Interval genome-wide QTL map showing significant QTLs detected on chromosomes 10 and 14. **b** Interval QTL map with bootstrap analysis of chromosome 10 using male X-zone width data. **c** Interval QTL map with bootstrap analysis of chromosome 14 using male X-zone width data

compared to that for B6 mice for both males and females in our study. Second, the width of the ZF is significantly larger in B6 mice compared to that in D2 mice regardless of sex. Third, D2 mice have a significantly larger adrenal medulla, in terms of width, compared to the B6 mice (a difference that is most pronounced in females). It is of interest to consider the intersection between the genetic underpinnings of adrenal gland structure and its role in the stress response. These three quantitative findings on adrenal weight and structure and the marked strain differences in response to stressful situations (Anisman et al. 1998; Ponder et al. 2007a; Shanks et al. 1990; Tarricone et al. 1995; Trullas and Skolnick 1993; Võikar et al. 2005; Yang et al. 2008; Yilmazer-Hanke et al. 2003) provide some new insights about the relationship between adrenal gland structure and function.

Our results for adrenal weight provide support for the notion that lower relative adrenal weight to body weight is found for more stress-resistant strains. This was also found by Deschepper et al. (2004) in their mouse strain survey. Previous research with male rats has shown that the most robust determinant of adrenal weight differences is the trophic effect of ACTH on the adrenal ZF (Akana et al. 1983). This would suggest that adrenal gland weight is associated with function, where adrenal weight is related to hormone and steroid secretion for the stress response. Evidence for this connection is found in the early work of Selye (1936), which showed that rats experiencing extended periods of "physical and mental challenges" have enlarged adrenals. It is also known that chronic variable stress in rats induces both adrenal weight increase and adrenal hypertrophy (Aguilera et al. 1996; Herman et al. 1995; Prewitt and Herman 1997; Ulrich-Lai et al. 2006; Zelena et al. 2003). This increase in adrenal weight is also seen in chronically stressed depressed patients (Amsterdam et al. 1987; Nemeroff et al. 1992). Moreover, various human pathologies have been shown to affect adrenal gland weights, and most of these pathologies are stress-related and some also have known genetic loci associated with the stress-related differences in adrenal weight (Redei 2008).

Our findings also speak to the roles of the medulla and ZF in the stress response in mice. A direct association between structure and function might lead one to assume that D2 mice exhibit larger medulla and ZF regions, as they are more stress-reactive and have a more sensitive stress response than B6 mice. While D2 mice exhibit a larger medulla region compared to B6 mice, B6 mice have an unexpectedly larger ZF width than D2 mice. If a larger ZF can be translated to an increase in function, then B6 mice have a more responsive HPA axis than D2 mice. The B6 mouse ZF subsequently releases more corticosterone during stressful situations that binds to additional glucocorticoid receptors activating the negative feedback loop of the HPA axis and leading to a more efficient stress response than the more anxious D2 mice. The hypothesized inefficiency of the D2 stress response may be illustrated by the results from Roberts et al. (1995) who found that compared to B6 mice D2 mice show elevated corticosterone response to EtOH and saline following withdrawal from initial EtOH administration. This work (Roberts et al. 1995) coincides with previous work indicating that D2 mice show an adverse or greater stress response to EtOH compared to B6 mice (Belknap et al. 1993; Eleftheriou and Elias 1975; Gill et al. 1996; Kakihana et al. 1968; Roberts et al. 1992).

Relative to our results with the medulla, in two measures of autonomic responsivity [the tail suspension test (TST) and handling-induced convulsions (HIC)], D2 mice are far more reactive than B6 mice (Lad et al. 2007; Roberts et al. 1995). D2 mice also have significantly more HIC compared to B6 mice during withdrawal from chronic (Crabbe 1998; Crabbe et al. 1983; Goldstein and Kakihana 1974; Griffiths and Littleton 1977) and acute (Gililland and Finn 2007; Roberts et al. 1992, 1995) exposure to EtOH. While studies with adrenalectomized mice have been contradictory [e.g., compare the findings of Sze et al. (1974) and Lamblin et al. (1996) with those of Gililland and Finn (2007) and Strong et al. (2009)], no studies have directly measured medullary epinephrine and/or norepinephrine levels during TST or withdrawal HIC from acute or chronic EtOH exposure comparing D2 and B6 mice. An analysis of these measures would help to elucidate the relationship between the TST and HIC with the autonomic "fight or flight" stress response. It would also determine whether D2 mice show greater adrenal medullary catecholamine response compared to B6 mice on these measures, thus providing support for adrenal structure-function interpretations.

The hypotheses presented here suggest that adrenal weight and structure are causally linked to the adrenal's role in the stress response. However, the negative relationship between weight and total width of the male adrenals for the parental strains and the similarity in adrenal width and weight for the females in our study suggest that other variables play important roles not only in linking adrenal structure to adrenal weight but also in connecting structure to function. The connection between these adrenal phenotypes likely involves multiple factors that need further examination to elucidate the complex interrelationship between adrenal structure and function.

## Stress and anxiety phenotypes

This study has focused on investigating adrenal weight and structure in BXD RI strains that have been shown to vary in behavioural and physiological responses to stress and

anxiety (e.g., Ponder et al. 2007a; Tarricone et al. 1995; Võikar et al. 2005; Yang et al. 2008; Yilmazer-Hanke et al. 2003). In comparing our adrenal measures to these stress and anxiety phenotypes, we found robust correlations between ZF width and different measures for stress and anxiety. For example, from the work of Yang et al. (2008), we find that the number of arm entries for the EPM paradigm is negatively correlated with ZF width ($r = -0.534$, $p = 0.0393$), while female ZF width is also negatively correlated with the number of open arm entries ($\rho = -0.532$, $p = 0.0399$). The percent of freezing associated with conditioned stimulus (CS) and unconditioned stimulus (UCS) pairings in a fear-conditioning paradigm (Yang et al. 2008) is also positively correlated with ZF width ($r = 0.582$, $p = 0.0212$). In the study by Roberts et al. (1995), plasma corticosterone levels 1 h after saline administration in various BXD strains shows a significant positive correlation with male ZF width ($\rho = 0.566$, $p = 0.0163$). These significant correlations suggest that a larger ZF region is associated with heightened anxiety and elevated corticosterone levels; this provides evidence for a direct link between adrenal structure and stress-related function.

It is difficult to determine the genetic underpinning of stress and anxiety, including adrenocortical basal and stress-induced glucocorticoid levels, because such complex phenomena are strongly contingent on the environment (Redei 2008). Despite this, numerous studies have performed QTL analyses in rats (Cui et al. 2003; Jirout et al. 2010; Llamas et al. 2005; Marissal-Arvy et al. 2004; Potenza et al. 2004; Solberg et al. 2006), chickens (Buitenhuis et al. 2003; Bureau et al. 2009), and pigs (Désautés et al. 2002; Jouffe et al. 2009; Muráni et al. 2010) to investigate genomic regions that are associated with stress and anxiety phenotypes. As indicated above, some of these studies have shown orthologous genomic overlap with our adrenal phenotype QTLs. Meanwhile, QTL analyses in the mouse have identified significant genomic regions on all 19 autosomal chromosomes and the X chromosome that are associated with stress and anxiety-related phenotypes. After more extensive examination of these QTL results with different strains of mice and larger sample sizes, some of the significant QTL results have not been replicated or have been attributed to other behavioural responses rather than stress and anxiety (Flint 2002; Henderson et al. 2004; Turri et al. 2004; Willis-Owen and Flint 2006). The consistent QTL results are briefly discussed here, with a focus on those that overlap with the significant and suggestive QTLs that are found in our study.

The male medulla measures show a suggestive QTL in a distal region of Chr 1 that maps within the significant QTL identified by Flint et al. (1995) and close to the genomic regions repeatedly identified by Turri et al. (2001) that are associated with behavioural responses to various tests of anxiety, including open field, EPM, elevated square maze, DL box, and the mirror chamber. The significant and suggestive QTLs on Chr 4 for male adrenal weight, as well as the suggestive QTLs for both male and female ZF width, overlap with some of the QTLs influencing both locomotor activity and stress-related phenotypes (Flint 2001, 2002; Henderson et al. 2004; Liu et al. 2007; Roberts et al. 1995; Thifault et al. 2008; Turri et al. 2001). Additional evidence for this association is found on Chrs 3 (female adrenal medulla width and adrenal weight) and 17 (female ZR and male adrenal weight), where significant and suggestive QTLs identified in our study overlap with several of the stress and anxiety-related QTLs from the aforementioned studies (Roberts et al. 1995; Thifault et al. 2008; Turri et al. 2004; Williams et al. 2009). These results suggest that genomic regions associated with stress and anxiety and locomotor activity also influence adrenal gland morphology and provide genetic links between these complex phenotypes.

More compelling evidence for genetic links between our adrenal phenotypes and stress and anxiety measures are found on Chr 10. The distal end of Chr 10 shows several overlapping QTLs for our adrenal data. Several studies also identify significant QTLs in a distal region of Chr 10 that are associated with behavioural measures of emotionality (Flint 2002), anxiety-like behaviours related to learned and innate fear (Ponder et al. 2007b), seizure susceptibility (Gershenfeld et al. 1999), and exploratory and fear-like behaviours in mice (Gershenfeld and Paul 1997; Gershenfeld et al. 1999; Zhang et al. 2005). The significant QTL for male total adrenal width and the suggestive QTLs for male and female adrenal weight map in a region of Chr 10 that encompasses a QTL termed *Exq1* (exploratory and excitability QTL) that is associated with exploratory and fear-like behaviours in mice involving open field ambulation and DL box paradigms (Gershenfeld et al. 1999; Zhang et al. 2005). These results indicate that the pleiotropic locus *Exq1*, which has been found to affect exploration, fear-like behaviours, and seizure susceptibility, also shows a robust influence on adrenal measures of width and weight.

## Conclusion

This analysis is the first step toward identifying genes controlling adrenal gland morphology that may be associated with adrenal gland function. We have effectively identified numerous significant and suggestive genomic regions that are associated with adrenal gland weight and structure. Many of these adrenal-related QTLs are located in genomic regions similar to those shown to influence a

variety of stress and anxiety-related behavioural and physiological phenotypes, which strengthens the genetic link between adrenal structure and the stress response. The significant differences displayed between B6 and D2 mice for measures of adrenal weight and ZF and adrenal medulla width also provide a strain-related link between adrenal structure and function. In addition, some interesting candidate genes are located in these loci that potentially have a substantial influence on adrenal structure and function. Our investigation has enabled us to identify specific candidate genes that can be examined to clarify their role(s) in adrenal gland weight and structure and the stress response.

**Acknowledgments** Animals used in this study were provided primarily by Oak Ridge National Laboratory. Generous funding from the National Institutes of Health (NIH) enabled this project to be performed (DA020677 and AA016666). We thank Dr. Elissa Chesler, Richard Cushing, Meifen Lu, Barbara Jackson, Leslie Galloway, Darla Miller, Dr. Robert Williams, Heena Lad, and Dr. Douglas Matthews for their expert assistance, as well as Dr. Nicole Gallo-Payet, Dr. James P. Herman, and Dr. Yvonne Ulrich-Lai for their expertise and assistance with adrenal gland endocrinology. We also thank those in the Goldowitz Lab who assisted with this project, including Suvina To, Gurjit Rai, Mussawar Ahmed, Christopher Yeh, Derek Rains, and Ann Lu.

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