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Quantitative trait loci for energy balance traits in an advanced intercross line derived from mice divergently selected for heat loss

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Running title: Quantitative trait loci for energy balance traits in mice

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ABSTRACT

Obesity in human populations, currently a serious health concern, is considered to be the consequence of an energy imbalance in which more energy in calories is consumed than is expended. We used interval mapping techniques to investigate the genetic basis of a number of energy balance traits in an F_{11} advanced intercross population of mice created from an original intercross of lines selected for increased and decreased heat loss. We uncovered a total of 137 quantitative trait loci (QTLs) for these traits at 41 unique sites on 18 of the 20 chromosomes in the mouse genome, with X-linked QTLs being most prevalent. Two QTLs were found for the selection target of heat loss, one on distal chromosome 1 and another on proximal chromosome 2. The number of QTLs affecting the various traits generally was consistent with previous estimates of heritabilities in the same population, with the most found for two bone mineral traits and the least for feed intake and several body composition traits. QTLs were generally additive in their effects, and some, especially those affecting the body weight traits, were sex-specific. Pleiotropy was extensive within trait groups (body weights, adiposity and organ weight traits, bone traits) and especially between body composition traits adjusted and not adjusted for body weight at sacrifice. Nine QTLs were found for one or more of the adiposity traits, five of which appeared to be unique. The confidence intervals among all QTLs averaged 13.3 Mb, much smaller than usually observed in an F_2 cross, and in some cases this allowed us to make reasonable inferences about candidate genes underlying these QTLs. This study combined QTL mapping with genetic parameter analysis in a large segregating population, and has advanced our understanding of the genetic architecture of complex traits related to obesity.

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INTRODUCTION

Energy balance in biological organisms is achieved when the amount of energy consumed equals that expended. While energy consumption consists simply of the number of calories eaten, energy is expended both internally in the production of heat and externally during physical exercise (Schoeller, 2009). The maintenance of an appropriate energy balance clearly is critical since increased weight gain leading to obesity can occur if more energy is consumed than expended.

Much of our knowledge of the genetics of obesity has come from discovery of many quantitative trait loci (QTLs) located throughout the genome in mice that affect traits such as body weight, weight gain, and especially various measures of fat (Cheverud et al., 2001; Rocha et al., 2004; Leamy, Pomp & Lightfoot, 2009b; 2012; Kelly et al., 2011; Cheverud et al., 2011). While fewer studies in mice have been conducted for energy consumption and expenditure, the basic components of energy balance, several QTLs have been found for these traits as well. For food intake measures, it is interesting that many of the QTLs found thus far map to different sites than those affecting body weight and adiposity measures (Allan, Eisen & Pomp, 2005; Kelly et al., 2010; Leamy et al., 2012). This also appears to be the case for QTLs affecting energy expenditure as assessed from voluntary exercise (primarily wheel-running) traits in mice (Lightfoot et al., 2007; 2008; 2010; Leamy, Pomp & Lightfoot, 2008; 2009a; 2009b; Nehrenberg et al., 2010; Kelly et al., 2010; Mathes et al., 2011).

Measures of energy expenditure related to metabolic rate have rarely been subjected to QTL analyses in rodent models. A notable exception is heat loss measured by indirect calorimetry. This trait was analyzed by Moody et al. (1999) who made use of two F_2 mouse populations derived from lines which had undergone divergent selection for high and low heat

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loss. Mice from the high line had increased heat loss but also tended to be more active with less body fat than mice from the low line (Nielsen et al., 1997b), suggesting a genetic link of heat loss with body fat. And in fact Moody et al. (1999) discovered five significant and four suggestive QTLs for heat loss, several of which mapped in the confidence intervals of QTLs found for different measures of fat in these mice, especially the percentage of brown adipose tissue.

Leamy et al. (2005) estimated genetic parameters for heat loss, food intake, and body weight and composition traits in an F_{11} advanced intercross population (AIL; Darvasi & Soller, 1995) derived from crosses of mice from inbred versions of the high and low heat loss selection lines used by Moody et al. (1999). Heritability estimates for these traits varied, but suggested that a reasonable amount of genetic variability had been preserved in the development of this population from the selection lines. There also were some interesting patterns among the genetic correlations; for example, heat loss was positively associated with food intake but negatively associated with adiposity (Leamy et al., 2005). This population therefore appeared to be an ideal one for a comprehensive QTL study aimed at identifying genes for adiposity and associated energy balance traits. We report here the results of such a study conducted to search for QTLs affecting all of these traits, and to discover their patterns of effects. We were particularly interested in differentiating QTLs acting on these traits independently of overall body size, and therefore analyzed body composition traits both adjusted and not adjusted for body weight at sacrifice.

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MATERIALS AND METHODS

The population and traits

We used an advanced intercross (AIL-F11) population of mice originally developed from lines selected for low (ML) and high (MH) heat loss during a 16 generation period (Nielsen et al., 1997a; 1997b). This selection was successful in achieving a divergence of ~50% in heat loss, 20.6% for feed intake per unit metabolic size, and 40% for body fat percentage (Nielsen et al., 1997a; 1997b). Mice were randomly sampled from each of the two selection lines and full-sib matings were done for seven generations to establish mostly inbred high (MHI) and low heat loss (MLI) lines. An intercross of these two inbred lines then was made and continued for 8 generations at which time the population was divided into two replicates and at generation 10 into four replicates. Single-pair matings in generation 10 were replicated, producing F_{11} mice in 8 different groups (4 replicates each with 2 parities). Litter sizes were standardized to 8 at birth, and all pups were weaned at 3 weeks of age. Altogether, a total of 18 traits were measured: 8 whole body traits (body weights at 4 different ages, 2 weight gain traits, heat loss and feed intake), and 10 body composition traits (4 measures of fat, 3 organ weights, and 3 bone traits). Table 1 gives a list of these traits and their abbreviations and more detailed descriptions of the measurements may be found in Leamy et al. (2005). All procedures involving the rearing and husbandry of the mice were approved by the Institutional Animal Care and Use Committee at the University of Nebraska – Lincoln (Protocol 02-02-010).

Genotyping and molecular markers

SNPs were selected from the Wellcome-CTC Mouse Strain SNP Genotype Set (http://mus.well.ox.ac.uk/mouse/INBREDS). DNA samples from five MHI and five MLI mice

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representing a subset of the parents used to make this AIL were included in the original Wellcome-CTC genotyping of 13370 SNPs, and from these data we selected 768 evenly spaced SNPs that were predicted to be fully informative within the AIL population based on fixed alternative genotypes between these MHI and MLI mice. These SNPs were genotyped using Illumina Goldengate technology by the Illumina FastTrack service lab (San Diego, CA). Of the 768 SNPs designed for the array, 658 provided data representing high quality and full informativity between the MHI and MLI parental lines.

Appendix 1 provides a listing of all 658 markers with their positions (in Mb) on each of the 20 chromosomes. The mapping resolution in the F_{11} population was enhanced because of a 4.4-fold expansion of the genome. The frequencies of the three genotypes at each of the SNPs on each chromosome are illustrated in Appendix 2. This figure shows that heterozygote frequencies across most chromosomes consistently track around the expected frequency of 50%, as do both homozygotes around their expected frequency of 25%, with some variability as expected.

Preliminary analyses

We created 10 additional traits by adjusting each of the 10 body composition traits for body weight at sacrifice (WTFINAL). For the 7 non-bone traits, this was accomplished by dividing each value by WTFINAL and then multiplying by 100 to express these values as percentages (traits were designated PFAT, PLIVER, etc.). This was particularly useful in allowing us to directly compare our QTL results for these traits to those from other mouse QTL studies that also used percentages (Cheverud et al., 2001; Gordon et al., 2008; Kelly et al., 2011; Leamy et al., 2012). We adjusted each of the bone traits (designated BMD[a], BMC[a], and

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BAREA[a]) by using WTFINAL as a covariate in the QTL analysis (see below). This allowed us to compare our QTL results for BMD[a] to those found by Leamy et al. (2013) who also adjusted BMD in their mouse population in this same fashion. Beyond these comparisons with other studies, use of the adjusted body composition traits allowed us to discover QTLs affecting these traits that were independent of overall body weight.

Prior to the QTL analyses, we tested each of the 28 total traits for potential effects of several variables. Multivariate analyses of variance showed significant effects of sex, replication, and parity for all the traits, as did the 20 body composition traits for cohort and the age at sacrifice as well. For food intake (INTAKE) and heat loss (HL), body weight at the time the mouse entered the calorimeter also was significant. Additional significant covariates for HL included the percentage of body weight lost in calorimeter and the amount of food (g) remaining in the calorimeter, and a random factor, the calorimeter unit in which each mouse was placed. After appropriate adjustment by these covariates and factors, we calculated basic statistics (means and standard deviations) for each of these traits (Table 1). Because of some original technical problems with the PIXImus, occasional mortality among the mice, a few measurement difficulties and recording errors, as well as the number of mice available for genotyping, total sample sizes varied from 1456 to 1525 among the traits.

QTL mapping

We used the QTLRel program implemented in R (Cheng et al., 2010; Cheng et al., 2011) to map QTLs for each of the 28 traits. The QTL program accounted for the structural relatedness among individuals in our advanced intercross population by calculating identity coefficients (Lynch and Walsh, 1988) from the pedigree information supplied. We used information only

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from generations 7 to 11 since the relative contribution of earlier generations to the overall amount of inbreeding achieved was considered marginal. As in our previous studies (Leamy et al., 2012; 2013), we used the Haley-Knott interval mapping (Haley & Knott, 1992) option in QTLRel to impute genotypic values between SNPs separated by more than 1 cM. At all actual and imputed markers, QTLRel evaluated the phenotypic values of each trait with a model that included additive and dominance genetic effects as well as all appropriate covariates (sex, replication, etc.) and factors outlined above to adjust for their potential effects. For all markers on each of the 20 chromosomes, the program calculated likelihood ratio values that were converted into LOD (likelihood of odds) scores.

We evaluated all of the LOD scores generated for each trait by estimating both 5% (significant) and 10% (suggestive) experimentwise thresholds in QTLRel with the permutation method of Churchill & Doerge (1994). Genotypic (rather than phenotypic) values were shuffled in QTLRel program so that the family structures were maintained. We ran this permutation procedure with 1000 iterations and recorded the 95th and 90th percentile LOD values in each of these runs. The 95th percentile values were used as the 5% experimentwise (significant) thresholds and the 90th percentile values were used as the suggestive threshold values.

We considered the highest LOD score on each chromosome that reached the suggestive threshold value as representing the site of a putative QTL. Multiple LOD score peaks exceeding this value on the same chromosome also were regarded as potential QTL sites if the peaks were separated by a drop of at least 1.5 LOD units. We also used QTLRel to estimate confidence intervals for each of the QTLs that were defined by 1.5 LOD drops on either side of the peak position (Manichaikul et al., 2006).

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193 At the site of each putative QTL, QTLRel computed additive (*a*) and dominance
194 genotypic values (*d*) and tested these values for significance ($P < 0.05$). These values were
195 computed from probabilities so were subject to possible inflation. The additive genotypic value
196 estimates one-half of the difference between the phenotypic values for the two homozygotes and
197 thus is useful in describing the magnitude of effect of each QTL. The dominance genotypic
198 values estimate the difference between the mid-homozygous and the heterozygous values, and
199 where significant, suggest that those QTLs exhibit dominance (Falconer & Mackay, 2006). If *d*
200 values approximately equal *a* values, this suggests complete dominance whereas *d* values greater
201 than +*a* values (or less than –*a* values) indicate overdominance (Falconer & Mackay, 2006).
202 QTLRel also estimated the percentage of the total phenotypic variation of the trait explained by
203 each QTL.
204 Once the locations of all putative QTLs were determined, we used QTLRel to test for
205 their potential interactions with sex. This was done by the calculation of a probability associated
206 with the difference between likelihood values produced in models run with and without a sex by
207 QTL interaction. Any of these probabilities less than the conventional 0.05 level were
208 considered to be statistically significant (Kenney-Hunt et al., 2008; Leamy et al., 2012). Where
209 these interactions occurred, we tested the effect of the QTL in the separate sexes and used the
210 suggestive threshold value to assess significance.
211

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RESULTS

Energy balance traits

Table 2 shows results of the QTL analysis of the eight whole body traits measured in the live F_{11} mice, including those for heat loss (HL) and feed intake (INTAKE), two key energy balance traits. For HL, two QTLs were discovered, one on distal chromosome 1 and another on proximal chromosome 2. Both exhibit additive genetic effects and account for 2.5% and 2%, respectively, of the total variation. A single QTL on chromosome 5 with significant additive effects was found affecting INTAKE.

Whole body traits

For the whole body traits (all weights, HL, and INTAKE), 37 QTLs were identified, with 27 reaching the 5% experimentwise level of significance (Table 2). LOD scores vary considerably, with the highest values (> 20) found for QTLs on the X chromosomes affecting the body weight at 3 (WT3), 6 (WT6), and 12 (WT12) weeks of age and at sacrifice (WTFINAL). Figure 1 illustrates the trends in LOD scores throughout the genome for each of the four body weight traits. The QTLs for the whole body traits are found on 12 of the 20 chromosomes, with chromosome X being most represented (10 occurrences). Confidence intervals for the QTLs range from 3.0 to 24.0 Mb, averaging 12.2 Mb with a standard deviation of 5.97 Mb.

Many of the positions for the QTLs affecting the weight traits are similar. Two QTLs, one on chromosome 12 at 77.0-80.8 Mb, and another on chromosome 13 at 57.4-58.9 Mb, may well represent the same underlying gene with pleiotropic effects on the weight of the mice at each of the four ages. Some QTLs show a more restricted pleiotropy; for example, a QTL on chromosome X at 66.3 Mb affects weight only at the later ages (WT6, WTK12, and WTFINAL),

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and a potentially common QTL on chromosome 8 (77.9-82.5 Mb) affects WT3, WT12, and WTFINAL (Figure 1). Additional instances of potential pleiotropy are seen for the four QTLs affecting GAIN3-6, all of which map in similar locations to QTLs for WT6 or WT12. Other QTLs such as the three on chromosome X for WT3 do not exhibit pleiotropy and affect only single traits. This is also the case for the single QTL on chromosome 3 affecting GAIN6-12, the QTL on chromosome 5 affecting INTAKE, and the two QTLs on chromosomes 1 and 2 affecting HL, none of which appear to colocalize with QTLs for any of the other whole body traits.

As evidenced by the significant additive genotypic values for all 37 QTLs affecting the whole body traits (Table 2), they exhibit a predominantly additive mode of action. Significant dominance effects occur for only four QTLs, and the average (absolute) mean of the *d* values (0.21) is well less than that of 0.80 for the *a* values (*d*/*a* ratio = 27%; $P < 0.01$ in a *t*-test for paired data). Dominance is partial or complete for three QTLs although a QTL on chromosome 5 affecting WT12 exhibits overdominance. The percent of the total phenotypic variation in the whole body traits contributed by the QTLs ranges from less than 1% (0.62%) to 5.77%, averaging 1.72%.

A total of 18 of the 37 QTLs exhibited significant interactions with sex, suggesting that their effects differed in male versus female mice. In 8 of these instances, the QTL effects were significant only in the male mice. An example of this is illustrated in Figure 2A for a QTL on chromosome 3 affecting GAIN6-12. Note that that the means of the three genotypes at this locus in females are quite similar whereas in males, MLI/MLI and MLI/MHI individuals show a greater weight gain than do MHI/MHI individuals. The remaining 10 QTLs show significant effects in both sexes, and in most (8) instances the effect is greater for males. An example of this

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257 is illustrated in Figure 2B for a QTL on chromosome 6 affecting WT6 where trends across the
258 genotypes are similar in both sexes, but are more pronounced in males.
259
260 **Body composition traits**
261 Table 3 shows results of the QTL analysis of the seven body composition traits adjusted
262 and not adjusted for weight at sacrifice. Figure 3 also llustrates genome trends in LOD scores for
263 two measures of fat, FAT and SUBQ, and their percentages of the final weight (PFAT and
264 PSUBQ). A total of 52 QTLs were found for the body composition traits, 41 of which had LOD
265 scores that exceeded the 5% experimentwise level of significance. These QTLs are found on
266 precisely the same 12 chromosomes as those for the whole body traits already presented in Table
267 2. Again, chromosome X is the most represented (8 occurrences), although 6 QTLs are found on
268 chromosome 3. Confidence intervals for the QTLs range from 2.5 to 31.6 Mb, averaging 15.3
269 Mb, somewhat higher than those for the whole body traits. The number of QTLs affecting the
270 body composition traits varies from 0 for PHEART to 7 for PSPLEEN.
271 In general, there is considerable commonality in the QTLs among the traits and especially
272 between trait pairs. For example, four of the 6 QTLs for FAT and PFAT share similar locations
273 and may well represent the same underlying gene or genes. Similar trends occur for the other
274 trait pairs except for BAT/PBAT, each of which is affected by only one QTL. There also is
275 apparent pleiotropy among the QTLs for different traits such as those on chromosomes 2
276 (19.4-21.0 Mb) and 3 affecting the FAT/PFAT and SUBQ/PSUBQ traits. Beyond the trait pairs,
277 one or more of QTLs on chromosomes 8, (82.5 Mb), 12 (80.8 Mb), 17 (43.1 Mb) and X (118.9
278 Mb) affecting WTFINAL (Table 2) also map near those affecting FAT, PFAT, SUBQ, PGON,
279 PBAT, LIVER, PLIV, SPLEEN, and PSPLEEN.

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Additive genotypic values are significant for all 52 body composition QTLs, whereas 14 QTLs showed significant dominance genotypic values. Dominance is somewhat more prevalent in these QTLs compared to those for the whole body traits, although the mode of action for the majority of the body composition QTLs is primarily additive. Where significant, dominance is mostly partial or complete, although there are several instances of overdominance (for example, a QTL on chromosome 2 affecting FAT). The percent of the total phenotypic variation in the whole body traits contributed by the QTLs ranges from less than 1% (0.95%) to 7.82%, averaging 2.49%, somewhat higher than the comparable value for the whole body traits.

A total of 11 of the 52 body composition QTLs exhibited significant interactions with sex, a proportion considerably less than that for the QTLs affecting the whole body traits. Two of these QTLs affect females only, an example of which is illustrated in Figure 2C. This figure shows that a chromosome 2 QTL significantly decreases the mean liver weight of MHI/MHI compared with MLI/MLI female mice, but there is no significant difference in genotype means in male mice. For six other QTLs, five of which affect PSPLEEN, the effect is greater in females than in males. An example of this is illustrated in Figure 2D where it can be seen that both males and females show the same significant trend in genotypic means for a chromosome 19 QTL affecting PSPLEEN, but this trend is more pronounced in females. Only three QTLs, all affecting GON or PGON, were significant for males only or had greater effects in males, although this trait is different in the two sexes, being a measure of the right epididymal fat pat in males and the perimetrial pad in females.

Bone traits

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Table 4 shows results of the QTL analysis of the three bone traits not adjusted and adjusted for weight at sacrifice, and figure 4 illustrates trends in LOD scores across the genome for the BMD and BMC trait pairs. A total of 48 QTLs were found for these six traits, 42 exceeding the 5% experimentwise level of significance. The QTLs are found on 16 of the 20 chromosomes, with chromosome 9 (8 occurrences) being most represented. Confidence intervals for the QTLs range from 2.7 to 24.0 Mb, their average of 12.1 being nearly identical to the comparable value for the QTLs affecting the live body traits (Table 2). There is a large number of QTLs affecting the unadjusted BMD (13) and BMC (14) traits whereas QTLs affecting BMD[a] (8) and BMC[a] (7) and especially both BAREA and BAREA[a] (3 each) are fewer in number. A QTL on chromosome 4 has the greatest effect on both BMD[a] and BMC[a], although it was not detected for either of the unadjusted bone mineral traits.

Some pleiotropy again is apparent among the QTLs, especially those affecting each pair of traits. For example, QTLs on chromosome 1 (at 199.8 Mb), 9 (at 40.2 and 82.6-84 Mb) and 17 (at 31.6 Mb) affect both BMD and BMD[a]. Some QTLs also appear to be common across traits, an example being one on chromosome 1 at 187-188.8 Mb affecting both the adjusted and unadjusted BMD and BMC traits. Again, at least four of the QTLs affecting WTFINAL (those on chromosomes 8, 11, 12, 13) map to similar or identical positions as those affecting one or more of the bone traits.

All except 2 of the 48 QTLs affecting the bone traits show significant additive genotypic effects, with the mean of their absolute values = 0.028. Significant dominance effects occur for 13 QTLs, with the mean of the absolute *d* values = 0.013 (mean *d*/a ratio = 0.49). Most of the dominance tends to be partial or complete, with only two clear instances of overdominance (one QTL on chromosome 9 at 59.6 Mb affecting BMD[a], and another QTL on chromosome 5 at 108.8

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affecting BAREA[a]). The percentage of the total phenotypic variation in the bone traits contributed by the QTLs ranges from 0.91% to 10.7%, and averages 2.78%.

Only five of the QTLs for the bone traits exhibited sex interactions, so the effects of the majority of these QTLs were consistent in both sexes. Further, all five QTLs showing interactions occurred for BMD or BMC, not the adjusted values for these traits (BMD[a] and BMC[a]) or for either the raw or adjusted BAREA traits.

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DISCUSSION

We undertook this study to search for QTLs affecting energy balance traits in a unique F_{11} mouse population derived from an intercross of lines that had undergone long-term divergent selection for heat loss measured by indirect calorimetry. An important goal was to uncover QTLs for heat loss itself, and to discover whether they might be commonly affecting other traits, especially measures of fat. We did find two QTLs for HL, although expected more given the history of the F_{11} population.

Among the 28 traits, however, we were successful in uncovering a total of 137 QTLs that were located at various sites on all chromosomes except 14 and 16. QTLs were found for all traits except PHEART, and the number affecting these traits varied from only 1 (GAIN6-12, INTAKE, and BAT) to as many as 14 (BMC). A major finding was that the X chromosome harbored the greatest number of these QTLs as well as the QTLs with the greatest effects on many phenotypes.

Leamy et al. (2005) previously showed that there were a number of significant genetic correlations among the traits in this F_{11} population, so it was not surprising that many of the QTLs we found exhibited apparent pleiotropy. As a consequence, a number of the 137 QTLs presumably represent common underlying genetic variation. In fact a tally of all non-overlapping confidence intervals for these QTLs suggests that they may reside in as few as 43 unique genomic locations. This number is conservative since more sites presumably would emerge with an increase in mapping precision, but in general the precision of the QTLs as assessed by the mean of their confidence intervals, 13.3 Mb, was quite good. This value is comparable to that of 12.5 Mb estimated for QTL confidence intervals affecting similar traits in an F_{10} advanced intercross mouse population analyzed by Leamy et al. (2012; 2013), and well

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below that of 23 Mb calculated by Kelly et al. (2011) for comparable traits in an F_4 advanced intercross mouse population.

Energy consumption and expenditure QTLs

The two QTLs we discovered for HL were far fewer than the nine QTLs found for this same trait by Moody et al. (1999) in their HB (high heat loss selection line crossed with C57BL/6J) mouse population, and also mapped to different positions. Moody et al. (1999) tested whether the three QTLs (one on chromosome 1 and two on chromosome 3) exhibiting the greatest effect on heat loss in the HB population would replicate in an F_2 population (LH) created from crossing mice from the outbred lines they had selected for increased (MH) and decreased heat loss (ML). The single QTL on chromosome 1 was confirmed, although with a considerably reduced effect, and neither QTL on chromosome 3 replicated (Moody et al., 1999). The HB and LH populations both share an LH progenitor, so this disparity in results presumably reflected differences in the alleles segregating in the low heat loss lines (BL and ML) and/or in the interactions of alleles on the two separate genetic backgrounds (Moody et al., 1999). These differences also help to explain why the F_{11} advanced intercross population produced from inbreeding and crossing of mice in the selection lines has yielded QTLs for HL that differ in number and location from those originally found in the HB population. It is also possible the alleles at heat loss QTL that were still segregating in the selection lines were lost during the inbreeding process, or that they were not well represented in the specific mating pairs leading to these inbred lines.

It is interesting that the HL QTL we discovered on chromosome 1 (128.7 Mb) maps near a QTL on this same chromosome (at 127.2) that affects spleen weight (both SPLEEN and

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PSPLEEN). Although we cannot know whether there is a single gene underlying these QTLs that in fact is pleiotropically affecting both HL and SPLEEN, it is certainly possible given that the genetic correlation of these two traits estimated by Leamy et al. (2005) is a moderately high +0.48. This also seems reasonable because mice in the MH selection group tended to have larger spleens than those in the ML line, this presumably being a reflection of their greater energy consumption and expenditure (Moody, Pomp & Nielsen, 1997). In addition, the spleen is a high metabolic rate organ that has been shown to make important contributions to resting energy expenditure in humans (Javed et al., 2010). The greatest number of QTLs among the body composition traits were found for the spleen traits (SPLEEN and PSPLEEN), and perhaps some of these other QTLs may be involved with energy balance as well. If so, selection for spleen weight may be an efficient alternative to produce lines divergent for energy balance.

The other heat loss QTL that we discovered on chromosome 2 (at 25.6 Mb) maps within the confidence intervals of QTLs for two adiposity traits: FAT and SUBQ (also PFAT and PSUBQ). Moody et al. (1999) found a similar result for 4 of 9 HL QTLs in their HB mouse population. Thus it seems possible that our chromosome 2 HL QTL may have pleiotropic effects on both heat loss and adiposity. Genetic correlations of HL with the four adjusted and unadjusted adiposity traits in the F_{11} mouse population all are less than |0.2| and are non-significant (Leamy et al., 2005), however, so it is not surprising that we did not find more evidence for this sort of pleiotropy. While the nature of this chromosome 2 QTL is unknown, *Pax8*, *paired box gene 8* at 24.4 Mb (Planchov et al., 1990) is an interesting possibility for a candidate gene that could affect both heat loss and adiposity. Mutations in *Pax8* cause hypothyroidism with its consequent effects on metabolism and growth that have been documented in mice (Planchov et al., 1990) and in humans (Trueba et al., 2005).

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We found just one QTL on chromosome 5 affecting feed intake (INTAKE), the primary measure of energy consumption. This seems surprising given that by generation 15, Nielsen et al. (1997b) achieved a nearly 21% divergence in feed intake between the high and low selection lines from which the F_{11} population was generated. And Leamy et al. (2005) found a fairly low, but significant heritability of 0.27 for this trait in the F_{11} population. On the other hand, Moody et al. (1999) found no QTLs for feed intake in the HB F_2 mouse population . So perhaps it is understandable that we found little detectable genetic (QTL) variation for this trait in our F_{11} mouse population. However, studies in other mouse populations have yielded QTLs for feed intake on several different autosomes (Allan, Eisen & Pomp, 2005; Leamy et al., 2012) and on the X chromosome (Liu et al., 2001b).

Body weight QTLs

The pleiotropic patterns exhibited by the QTLs affecting body weight at each of the four ages were consistent with those reported in previous mouse QTL studies (Cheverud et al., 1996; Vaughn et al., 1999; Rocha et al., 2004; Gordon et al., 2008). For example, Cheverud et al. (1996) found QTLs affecting early growth in mice (body weight from weeks 1 to 3) that were distinct from those affecting later growth (body weight from weeks 6 to 10), but some QTLs that affected both early and late growth. We also found QTLs (on chromosomes 8, 12, and 13) affecting both early (WT3) and late growth (WT6, WT12, WTFINAL), as well as other QTLs affecting early growth only, late growth only, or body weight at a single age. In all cases the additive genetic effects of those QTLs exhibiting pleiotropy were consistent in sign but tended to increase in magnitude from early to late growth, as also is typical (Cheverud et al., 1996; Vaughn et al., 1999).

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It was somewhat surprising to find so many X-linked QTLs that tended to exhibit the highest LOD scores and contributions to the total variation of the body weight traits. This sort of result has not usually been found (for example, Leamy, Pomp & Lightfoot, 2009b; Leamy et al., 2012), but mapping information for the X chromosome is somewhat more limited because many previous mouse studies have analyzed only the 19 autosomes (Cheverud et al. 1996; 2011; Vaughn et al., 1999; Rocha et al., 2004; Gordon et al., 2008; Kelly et al., 2011; Leamy et al., 2012). Some body weight and adiposity QTLs have been found on this chromosome (Dragani et al., 1995; Rance, Hill & Keightley, 1997), many of which are listed in the Mouse Genome Informatics Database (2013). In addition, some of the previously mapped X-linked QTL have very strong effects. However, none of these appear to map in similar positions to those we have found, and this may be a consequence of the imprecision of mapping or instead suggest that the X-linked body weight QTLs we have uncovered may be novel. Since no QTLs for heat loss were mapped to the X chromosome, it seems unlikely that these QTLs played a role in the selection response observed in the MH and ML lines, but rather that they represent variability segregating in the base population from which selection originated.

Nearly one-half (14) of the 29 body weight QTLs showed significant interactions with sex, with all except two affecting males only or showing greater effects in males. Although a few previous studies in mice have failed to detect QTL by sex interactions for body weight (Rocha et al., 2004; Leamy et al., 2012), these kinds of interactions are quite common in other studies (Vaughn et al., 1999; Cheverud et al., 2001; 2011; Gordon et al., 2008). Some studies also have shown that a preponderance of body weight QTLs affect male rather than female mice (Dragani et al., 1995; Vaughn et al., 1999). Significant interactions of sex with epistatic (two-locus) QTL effects also have been found (Leamy, Gordon & Pomp, 2011), and it is possible that

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these may have modified or even masked the effect of some of the body weight QTLs in the female F_{11} mice. Whatever the physiological mechanism involved in these differential QTL effects, they are an important component of the genetic architecture of body weight.

We can only speculate about the identity of the body weight QTLs because their confidence intervals usually include a number of potential candidate genes, even with the finer resolution afforded by an AIL. For example, the Mouse Genome Database (2013) lists 38 protein coding genes even in the smallest (3 Mb) confidence interval found for any body weight QTL (one on the X chromosome at 101.4 Mb affecting WT3). This kind of result is typical in QTL mapping experiments and has made the transition from QTL to gene difficult, although some progress is being made. For example, Oliver et al. (2005) fine-mapped an X-linked growth QTL to a small region containing *Gpc3* (*glypican 3*), and provided strong evidence from expression data for this being the gene underlying the QTL. *Gpc3* actually is within the confidence interval of a QTL we discovered on chromosome X at 50.4 Mb affecting WT3, and thus represents a potential candidate gene for this QTL as well. If we have mapped this same gene, however, its effect on body weight in the F_{11} mice is much smaller than was previously found in other mouse populations (Liu, Bunger & Keightley, 2001a).

Adiposity QTLs

We found a total of 28 QTLs affecting the adiposity traits that are located in nine non-overlapping regions on seven different chromosomes, including one QTL on each of chromosomes 1, 2, 3, 6, and 17, and two on each on chromosomes 10 and X (see Table 5). Only the QTL on chromosome 17 matches any of the QTLs found by Moody et al. (1999) for these same adiposity traits in their MB mouse population. Other QTLs for various measures of

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adiposity previously have been mapped near those we have discovered on chromosomes 2 and 10 (Mouse Genome Database, 2013) as well, but not those on chromosomes 1, 3, 6, and X. These 5 obesity QTL sites therefore may be unique, and add to the current total of 170 obesity QTL locations listed in the Mouse Genome Database (2013).

Among the nine adiposity QTLs we discovered, seven affected two or more adiposity traits whereas only two were trait-specific. We expected this high level of pleiotropy because genetic correlations previously calculated among the four traits all were positive and quite high, varying from +0.71 to +0.95 (Leamy et al., 2005). We also found considerable commonality among the QTLs affecting the adjusted/unadjusted trait pairs. Of the six QTLs affecting FAT for example, four replicated with PFAT and two (on chromosomes 2 and X) did not. Further, both non-replicating QTLs affected at least one other adiposity trait, so they were not unique to FAT. Only two QTLs on chromosomes 1 and 10 affected one trait, and it is noteworthy that both had LOD scores reaching the suggestive, but not significant, experimentwise threshold. Nonetheless, the differences among the QTLs affecting the trait pairs are sufficient to suggest caution in comparing QTL results for unadjusted versus adjusted trait values.

Four adiposity QTLs (on each on chromosomes 6 and 17, and two on chromosome X) also mapped in the same general locations as QTLs affecting one or more of the body weight traits. This apparent pleiotropy for QTLs affecting both body weight and adiposity traits is not uncommon, even when adiposity measures have been adjusted for overall body size (Cheverud et al., 2001; Kelly et al., 2011; Leamy et al., 2012). The adiposity QTL on distal chromosome X mapped to a similar location for QTLs affecting GAIN3-6, WT6, WT12 and WTFINAL. If common, this gene appears to influence body weight in mice from six weeks of age until the time of sacrifice, as well as adiposity measured at that time. The additive genotypic values of this

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potentially common QTL, however, is consistently positive for the adiposity traits but negative for the body weight traits, suggesting that it is exhibiting antagonistic pleiotropy.

Although many protein coding genes fall within the confidence intervals of the obesity QTLs we discovered, we used the Mouse Genome Database (2013) and found some potential candidates for each of the QTLs (Table 5). For example, all three genes listed as candidates for the obesity QTL on chromosome 6 have well documented effects on adiposity, metabolism, and homeostasis in mice (Bera et al., 2008; Bjursell et al., 2007; Shen et al., 2009). Similarly, *Brs3, bombesin-like receptor 3,* at 57 Mb on chromosome X, is a possible candidate for our proximal X obesity QTL since mice with mutations at this locus exhibit obesity, an impaired glucose metabolism, and a reduced metabolic rate (Ladenheim et al., 2008). Interestingly, Xu et al. (2012) have shown that *Brs3* shows a sexually dimorphic expression in the hypothalamus that apparently is a reflection of its role in the regulation of sex typical behaviors in mice. For the other (more distal) X-linked obesity QTL, we found only one potential candidate: *Cited1* (at 102.2 Mb). Alterations in this gene are associated with an increased incidence of diabetes and obesity in mice (Novitskaya, Baserga & de Caestecker, 2011).

Organ weight QTLs

We found several QTLs affecting liver, heart, and especially spleen weights in the F_{11} mice. This was as expected since liver and spleen weights significantly differed between the heat loss selection lines (Moody, Pomp & Nielsen, 1997) from which the F_{11} population was derived. Moody et al. (1999) uncovered 5 QTLs for the percentage of liver weight in their MB mouse population, but only one of these on chromosome 7 maps within the confidence interval

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of a QTL we discovered for LIVER. They also found two QTLs on chromosomes 1 and 7 affecting the heart weight percentage but we found none for PHEART.

The QTLs for the organ weights mostly were independent from those we found for the adiposity QTLs. The only exceptions were QTLs on chromosome 17 affecting LIVER, SPLEEN, and PSPLEEN and an X-linked QTL affecting PLIV and PSPLEEN, both of which mapped within the confidence intervals of adiposity QTLs. It is interesting that the QTL on chromosome 17 affected LIVER but not PLIVER, suggesting it may have pleiotropic effects on overall body size, and in fact it also maps in a similar location to a QTL for WTFINAL. Of the candidate genes listed for this chromosome 17 adiposity QTL (Table 5), *Rcan2* is attractive because alterations in this gene reduce diet-induced obesity and liver weight (Sun et al., 2011). The QTL on chromosome X affects PLIVER and not LIVER, and may be the same as the QTL previously described possibly exhibiting antagonistic pleiotropic effects on the body weights versus the adiposity traits.

We found four QTLs on chromosomes 1, 8, 12, and 17 for SPLEEN, all of which were replicated for PSPLEEN. Of these QTLs, only that on chromosome 17 mapped close to one for LIVER, and may well represent the same QTL described above. A QTL on chromosome 12 (at 81.9 Mb) had the greatest effect on spleen weight. *Psen1*, *presenilin 1* (at 83.6 Mb), is a potential candidate gene for this QTL since when altered it can cause many effects, including enlargement of the spleen (De Strooper et al., 1998). Another possibility is *Ucp1*, *uncoupling protein 1* (at 83.3 Mb), that affects thermoregulation and brown fat development (Jacobsson et al., 1985), but when knocked out also causes a reduction in spleen cell numbers (Adams, Kelly & Porter, 2010).

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It was interesting that five of the seven PSPLEEN QTLs, but no SPLEEN QTLs, exhibited significant interactions with sex. This disparity may be a simple consequence of scaling a small organ weight by overall body weight, however, and in fact preliminary analyses of variance showed a much greater sexual dimorphism for PSPLEEN than for SPLEEN. Further, the differential effects of the QTLs in males versus females, one example of which was previously illustrated in Figure 1, were rather subtle. Nonetheless, this interaction occurred even for two X-linked QTLs, including one at 136.6 Mb that appears to be unique. A possible candidate for this QTL is *Sty14* (at 133.9), a gene that exhibits sexually dimorphic expression in the brain (Xu et al., 2012). Inactivation of this gene causes an increase in adrenocorticotropin secretion that in turn is known to alter spleen weights (Veenema et al., 2003).

Bone QTLs

Heritability estimates among the traits analyzed by Leamy et al. (2005) in the F_{11} mouse population were highest for the unadjusted BMD (0.65) and especially BMC traits (0.85), and thus it was not surprising that we also found the greatest number of QTLs for these two traits (13 for BMD, 14 for BMC) as well. BAREA had a much lower heritability (0.26), and we found only 3 QTLs affecting this trait. In fact across all traits, there is a significant positive association (Spearman correlation = +0.74, $P < 0.01$) between the number of QTLs affecting the traits and their heritabilities. Adjusting BMD and BMC traits for WTFINAL reduced the number of QTLs by about one-half, however, suggesting that at least some of these QTLs were influencing overall growth. And in fact 7 of the QTLs for both BMD and BMC mapped close to those affecting the body weights, especially WTFINAL. Two QTLs (both X-linked) for BMD and three QTLs for BMC also mapped near QTLs for one or more adiposity traits.

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QTLs on chromosome 9 were important contributors to the adjusted bone mineral traits, three affecting BMD[a] and two affecting BMC[a]. A possible candidate for the most proximal QTL at 40.2 Mb on this chromosome affecting both of these traits is *Zfp202*, *zinc finger protein*, *202*. This gene also is located at 40.2 Mb, and when altered causes abnormal bone mineralization (Mouse Genome Database, 2013). The most distal chromosome 9 QTL (84.0 and 81.2 Mb) affecting both BMD[a] and BMC[a] may be *Col12a1*, *collagen, type XII*, *alpha 1* at 79.6 Mb. Mutations in this gene produce lower mineral apposition rates and a reduced mineralized surface/total bone surface (Izu et al., 2011). Leamy et al. (2013) also found two QTLs at similar locations on chromosome 9 (35.0 and 82.9 Mb) affecting total bone mineral density in their F_{10} advanced intercross population. Another QTL with an intermediate location (59.6 Mb) on chromosome 9 affected BMD[a] in the F_{11} mice, and a possible candidate is *Glce*, *glucuronyl C5-epimerase* (at 62.1 Mb), mutations in which lead to excessive bone mineralization (Li et al., 2003).

Except for an additional QTL on chromosome 9 affecting BMD[a], only one QTL was found for the adjusted bone mineral traits that was not detected for the unadjusted bone mineral traits. This QTL on chromosome 4 (at 140.3 and 137.8) also had the greatest effect on these traits, accounting for nearly 11% of the total variance for BMD[a]. This value may be inflated, however, because this QTL occurs in a region where markers are very sparse. Others also have mapped QTLs for bone mineral density in this region (Klein et al., 2001; Masinde et al., 2002; Koller et al., 2003).

SUMMARY

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In summary, we conducted an extensive genome-wide scan for a wide variety of metabolism traits within an advanced intercross line derived from lines divergently selected for heat loss. While only two QTLs for heat loss were detected, we uncovered a total of 137 QTLs at 41 unique sites on 18 of the 20 chromosomes in the mouse genome, with X-linked QTLs being most prevalent and having the strongest effects. The number of QTLs affecting the various traits generally was consistent with previous estimates of heritabilities in the same population, with the most found for two bone mineral traits and the least for feed intake and several body composition traits. QTLs were generally additive in their effects, and some, especially those affecting the body weight traits, were sex-specific. Pleiotropy was extensive within trait groups (body weights, adiposity and organ weight traits, bone traits) and especially between body composition traits adjusted and not adjusted for body weight at sacrifice. This study, combining QTL mapping with genetic parameter analysis in a large segregating population, advances our understanding of the genetic architecture of complex traits related to obesity.

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FIGURE LEGENDES

Figure 1. Quantitative trait locus maps of body weight at each of the four ages, Shown are distributions of LOD scores on each of the 20 chromosomes (LOD scores on the X chromosome are truncated to a maximum of 10). The horizontal line represents the 95% experimentwise threshold level used in determining statistical significance

Figure 2. Mean genotypic values of QTLs vary depending on the sex of the mice. Shown are differential effects of QTLs for weight gain from 6 to 12 weeks (GAIN6-12), weight at six weeks (WT6), unadjusted liver weight (LIVER) and spleen weight percentage (PSPLEEN) in male and female mice.

Figure 3. Quantitative trait locus maps of four fat traits. Shown are distributions of LOD scores on each of the 20 chromosomes for FAT, PFAT, SUBQ, and PFAT. The horizontal line represents the 95% experimentwise threshold level used in determining statistical significance.

Figure 4. Quantitative trait locus maps of four bone traits. Shown are distributions of LOD scores on each of the 20 chromosomes for BMD, BMD[a], BMC, and BMC[a]. For BMC and BMC[a], LOD scores on the X chromosome are truncated to a maximum of 8). The horizontal line represents the 95% experimentwise threshold level used in determining statistical significance.

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807 Table 1. Basic statistics for the traits used in the QTL analysis.
808

Trait (units)	Abbreviation	N	Mean	Std Dev
3-week body weight (g)	WT3	1513	14.41	1.940
6-week body weight (g)	WT6	1518	28.71	2.539
12-week body weight (g)	WT12	1511	33.61	2.984
Weight when sacrificed	WTFINAL	1525	32.28	2.869
Weight gain from 3 to 6 weeks (g)	GAIN3-6	1506	14.31	1.866
Weight gain from 6 to 12 weeks (g)	GAIN6-12	1504	4.87	1.863
Feed intake (g/kg$^{0.75}$/day)	INTAKE	1521	84.23	14.391
Heat Loss (kcal/kg$^{0.75}$/day)	HL	1525	146.77	15.836
Total body fat (g)	FAT	1520	4.37	0.838
Subcutaneous fat pad (g)	SUBQ	1523	0.127	0.038
Gonadal fat pad (g)	GON	1523	0.157	0.074
Brown adipose tissue (g)	BAT	1517	0.045	0.012
Liver weight (g)	LIVER	1520	1.69	0.224
Heart weight (g)	HEART	1519	0.187	0.032
Spleen weight (g)	SPLEEN	1519	0.112	0.028
Total body fat as % of kill weight	PFAT	1520	13.52	2.169
Subcutaneous fat pad as % of kill weight	PSUBQ	1523	0.394	0.111
Gonadal fat pad as % of kill weight	PGON	1523	0.472	0.208
Brown adipose tissue as % of kill weight	PBAT	1517	0.140	0.036
Liver weight as % of kill weight	PLIVER	1520	5.24	0.463
Heart weight as % of kill weight	PHEART	1519	0.581	0.097
Spleen weight as % of kill weight	PSPLEEN	1519	0.350	0.078
Bone mineral density (g/cm^2)	BMD	1456	0.062	0.004
Bone mineral content (g)	BMC	1456	0.735	0.070
Bone area (cm^2)	BAREA	1456	11.78	0.747
Bone mineral density (g/cm^2)-WTFINAL adjusted	BMD[a]	1456	0.062	0.004
Bone mineral content (g)-WTFINAL adjusted	BMC[a]	1456	0.735	0.054
Bone area (cm^2)-WTFINAL adjusted	BAREA[a]	1456	11.78	0.668

809
810 Shown are the sample size (N), mean, and standard deviation (Std Dev) for each of the 28 traits
811 (with their units and abbreviations) measured in the F_{11} mice. Standard deviations reflect
812 adjustments made for various classification factors and covariates.
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816 Table 2. QTL results for the whole body traits measured in the F_{11} mice.

Trait	Ch	Location	Conf. Interval	LOD	*a*	*d*	%	Sex
HL	1	128.7	111.0—133.5	6.87†	**3.8402**	-0.2316	2.50	
	2	25.6	12.8—29.4	5.09†	**3.4861**	-0.3666	1.95	
INTAKE	5	108.0	106.2—111.8	4.55†	**-2.6615**	-0.7727	1.71	
WT3	8	77.9	68.5—85.8	3.81	**0.2604**	**0.2858**	0.89	**M**,F
	12	80.8	78.1—82.9	4.88†	**0.3361**	-0.0772	1.47	
	13	57.4	54.7—60.5	4.03	**0.3366**	0.0138	1.25	
	X	50.4	48.3—54.8	4.57†	**-0.2413**	0.0828	1.11	
	X	78.8	68.8—88.9	6.43†	**-0.3716**	-0.0631	2.41	
	X	101.4	100.0—103.0	19.49†	**-0.6090**	0.0961	5.77	
WT6	2	104.5	98.0—108.7	4.54†	**0.4045**	0.226	0.73	
	6	125.7	116.8—128.9	4.01	**0.4737**	0.0835	0.85	**M**,F
	7	124.3	116.8—132.2	4.13†	**-0.6451**	-0.5557	1.93	
	12	80.8	78.1—82.9	10.19†	**0.7008**	-0.2101	1.92	**M**,F
	13	57.4	54.7—59.5	5.21†	**0.5295**	0.1007	1.03	
	X	66.3	58.1—67.2	9.93†	**-0.6627**	-0.2258	2.38	M
	X	107.7	107.7—124.7	35.24†	**-1.1317**	**0.4093**	5.37	**M**,F
WT12	2	101.4	101.2—114.5	3.86	**0.4607**	-0.2314	0.62	
	2	147.5	146.5—152.2	4.05	**0.5261**	-0.2354	0.97	M,**F**
	5	139.8	134.4—142.3	4.02	**0.4095**	**0.8124**	1.06	
	8	77.9	66.6—83.3	4.76†	**0.6204**	0.2274	0.79	
	12	77.0	74.8—81.9	6.19†	**0.6702**	-0.0032	1.10	M
	13	57.4	56.6—65.0	5.77†	**0.6759**	0.1217	1.10	
	X	66.3	58.1—68.5	8.67†	**-0.7503**	-0.312	2.06	M
	X	118.9	104.2—125.8	20.43†	**-1.1371**	0.1121	3.57	**M**,F
WTFINAL	2	21.4	5.8—25.6	4.05	**0.6495**	0.0971	1.08	M,**F**
	7	139.1	136.4—141.9	4.88†	**-0.6036**	-0.1701	1.09	
	8	82.5	67.8—86.8	3.83	**0.4983**	-0.0121	0.65	**M**,F
	12	80.8	77.1—82.9	8.92†	**0.7630**	-0.1584	1.59	M
	13	58.9	41.0—65.0	4.04	**0.5537**	0.0895	0.79	
	17	43.1	29.7—50.7	4.19†	**0.5164**	-0.0162	0.74	
	X	66.3	58.1—67.2	11.67†	**-0.8473**	0.1347	2.50	M
	X	118.9	108.6—125.8	22.60†	**-1.1271**	0.2942	3.66	**M**,F

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GAIN3-6	6	118.4	113.5—123.4	4.96†	**0.3491**	**0.1819**	0.83	**M**,F
	7	124.5	118.9—132.6	4.40†	**-0.5311**	-0.3709	1.94	
	12	76.9	71.3—86.9	4.14†	**0.3508**	-0.105	0.77	M
	X	107.7	107.7—126.4	12.59†	**-0.5524**	0.2551	2.02	M
GAIN6-12	3	31.4	27.2—41.1	3.88	**0.3248**	0.1302	1.30	M

Shown are all QTLs affecting the traits measured on the live F_{11} mice that had LOD scores reaching the 10% (suggestive) or 5% (†) experimentwise level of significance. Locations on each chromosome (Ch) and confidence intervals of the QTLs are given in Mb (from NCBI Build 37). Also shown is the percentage contribution (%) of each QTL to the total variance of each trait, and its additive (*a*), dominance (*d*) genotypic effects (bolded values indicate significance at $P < 0.05$). Interactions of QTLs with sex are indicated as M (significant in males only), F (significant in females only) or both M and F (significant in both sexes where bolded values indicate the sex for which the QTL had the greater effect).

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828 Table 3. QTL results for the body composition traits measured in the F_{11} mice.

Trait	Ch	Location	Conf. Interval	LOD	*a*	*d*	%	Sex
FAT	2	19.7	11.6—26.2	5.45†	**0.2148**	0.0548	2.49	F
	3	99.8	83.1—114.7	4.64†	**-0.1248**	**0.2227**	2.83	
	6	124.0	118.8—126.4	5.44†	**0.1691**	-0.0325	1.75	
	10	75.3	65.0—91.5	4.18†	**-0.1435**	-0.0349	1.24	
	17	48.6	34.9—57.1	4.49†	**0.2135**	0.0262	2.45	
	X	54.2	48.3—58.1	5.45†	**-0.1337**	0.0583	1.49	
PFAT	1	25.5	21.4—35.8	3.75	**-0.3262**	0.0436	1.13	
	2	21.0	12.8—29.4	3.98	**0.4411**	0.1667	1.93	
	3	95.6	80.6—102.1	8.98†	**-0.5424**	**0.5023**	5.22	
	6	124.0	117.4—126.6	4.50†	**0.3774**	-0.0858	1.61	
	10	72.7	67.4—92.7	4.42†	**-0.4117**	-0.0143	1.76	
	X	107.7	101.5—126.4	4.45†	**0.3491**	-0.1171	1.35	
SUBQ	2	19.4	12.7—29.0	7.42†	**0.0120**	0.0017	4.78	
	3	81.6	79.7—106.9	4.40†	**-0.0062**	0.0030	1.72	
	6	124.0	119.0—126.6	4.27†	**0.0070**	-0.0019	1.91	
	10	92.0	82.7—100.0	4.56†	**-0.0091**	**-0.0091**	4.32	
	17	48.6	34.9—62.7	3.77	**0.0093**	0.0014	2.88	
PSUBQ	2	19.4	11.5—29.4	5.92†	**0.0314**	0.0067	3.56	
	3	81.6	79.7—100.9	6.57†	**-0.0229**	0.0091	2.39	
	10	92.0	84.0—98.2	4.76†	**-0.0270**	**-0.0271**	4.17	
	10	127.2	114.0—130.3	3.81	**-0.0227**	-0.0035	2.16	
GON	6	124.0	118.0—126.3	6.48†	**0.0175**	-0.0011	2.02	M
	X	58.4	54.1—66.5	4.01	**-0.0265**	-0.0002	1.06	M
PGON	3	95.6	79.5—104.5	4.46†	**-0.0280**	**0.0627**	3.01	
	6	124.0	117.4—126.3	6.11†	**0.0477**	0.0090	2.18	**M**,F
	X	109.9	101.5—126.4	6.38†	**0.0467**	-0.0147	2.10	
BAT	6	125.7	122.6—128.9	4.13†	**0.0024**	0.0001	1.77	
PBAT	X	107.7	104.1—125.8	3.85	**0.0056**	0.0010	1.52	
LIVER	2	74.1	69.2—78.9	4.40†	**0.0443**	-0.0045	1.32	F
	2	104.5	98.0—106.7	4.05†	**0.0344**	**0.0222**	0.95	

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	2	140.1	135.9—146.2	4.47†	**0.0458**	-0.0077	1.74	
	7	122.7	114.4—129.8	4.88†	**-0.0689**	-0.0335	3.14	
	8	83.6	81.6—85.8	3.80	**0.0390**	-0.0110	1.06	
	17	44.2	31.6—49.8	3.85	**0.0410**	0.0080	1.11	
PLIV	1	96.7	89.4—117.1	3.82	**0.0663**	**0.1151**	2.79	
	6	99.5	91.7—112.4	4.58†	**-0.1243**	**-0.0939**	3.33	
	11	102.1	98.8—107.8	4.55†	**-0.0889**	-0.0333	2.46	
	19	7.5	5.3—10.2	4.01	**0.0760**	-0.0205	1.53	M,**F**
	X	107.7	108.6—125.8	6.70†	**0.0963**	0.0292	2.79	
HEART	3	77.1	70.0—77.1	4.08†	**0.0041**	**0.0056**	0.99	
	8	127.6	126.4—130.1	4.09†	**0.0065**	-0.0009	1.67	
SPLEEN	1	127.2	124.4—133.5	4.11†	**0.0047**	-0.0026	1.76	
	8	74.8	51.2—80.8	6.91†	**0.0072**	0.0002	2.88	
	12	81.9	78.3—82.9	19.26†	**0.0098**	**-0.0087**	7.82	
	17	38.0	31.8—49.4	10.47†	**0.0085**	-0.0017	4.79	
PSPLEEN	1	127.2	123.3—131.6	4.57†	**0.0149**	-0.0056	1.63	
	8	67.4	58.5—82.5	5.40†	**0.0170**	-0.0018	1.74	M,**F**
	12	81.9	80.8—84.7	16.23†	**0.0234**	**-0.0254**	5.03	
	17	38.0	31.8—49.4	8.49†	**0.0207**	-0.0046	2.84	M,**F**
	19	4.0	4.0—6.5	3.81	**0.0090**	**-0.0114**	1.10	M,**F**
	X	124.7	106.8—126.4	9.50†	**0.0185**	**0.0169**	3.46	M,**F**
	X	136.6	133.1—137.8	9.41†	**0.0167**	**0.0177**	3.11	M,**F**

Shown are all QTLs affecting the body composition traits measured in the F_{11} mice that had LOD scores reaching the 10% (suggestive) or 5% (†) experimentwise level of significance. Locations on each chromosome (Ch) and confidence intervals of the QTLs are given in Mb (from NCBI Build 37). Also shown is the percentage contribution (%) of each QTL to the total variance of each trait, and its additive (*a*), dominance (*d*) genotypic effects (bolded values indicate significance at $P < 0.05$). Interactions of QTLs with sex are indicated as M (significant in males only), F (significant in females only) or both M and F (significant in both sexes where bolded values indicate the sex for which the QTL had the greater effect).

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839 Table 4. QTL results for the bone traits measured in the F_{11} mice.
840

Trait	Ch	Location	Conf. Interval	LOD	*a*	*d*	%	Sex
BMD	1	188.8	185.6—189.3	9.01†	**-0.001183**	0.000237	3.73	
	7	124.5	104.1—134.8	4.41†	**-0.001229**	-0.000470	3.87	
	8	74.8	67.4—84.5	6.80†	**0.001088**	0.000253	2.78	
	9	40.2	34.8—43.4	4.89†	**-0.000905**	0.000267	2.26	
	9	82.6	75.9—94.6	6.22†	**-0.001032**	-0.000260	2.41	
	10	12.6	9.5—15.8	4.02	**0.000771**	0.000462	1.95	
	12	80.8	78.3—82.9	4.29†	**0.000677**	**-0.000620**	1.65	
	13	52.4	46.6—57.8	5.13†	**0.000921**	0.000166	2.33	
	15	98.8	96.9—102.0	4.12†	**-0.000794**	-0.000220	1.84	M,**F**
	17	31.6	27.7—32.9	8.79†	**0.001072**	**-0.000760**	4.23	
	18	41.4	37.6—63.3	3.84	**-0.000616**	**0.000486**	1.53	**M**,F
	X	66.3	58.1—71.5	5.58†	**-0.000908**	-0.000038	2.88	
	X	103.5	100.0—124.6	8.50†	**-0.001060**	0.000064	3.59	
BMD[a]	1	188.8	185.5—189.3	7.56†	**-0.000988**	0.000199	2.60	
	4	140.3	134.2—142.9	4.80†	**0.001580**	**0.001921**	10.74	
	8	89.3	72.4—89.3	4.33†	**0.000711**	0.000002	1.32	
	9	40.2	34.8—42.1	7.08†	**-0.001110**	0.000137	3.03	
	9	59.6	51.4—69.7	4.80†	-0.000640	**0.001315**	4.47	
	9	84.0	74.4—93.1	6.29†	**-0.000951**	-0.000330	2.00	
	17	31.6	27.3—32.9	6.80†	**0.000805**	**-0.000720**	2.69	
	18	24.1	17.0—30.4	3.81	**-0.000689**	0.000405	1.66	
BMC	1	30.8	22.1—35.8	4.59†	**0.0118**	0.0025	1.36	
	1	187.0	185.6—189.3	7.71†	**-0.0164**	0.0011	2.34	
	2	140.1	132.3—146.2	3.77	**0.0116**	-0.0057	1.77	F
	3	81.6	76.8—90.7	8.33†	**0.0131**	**-0.0100**	2.52	
	7	118.6	100.7—134.5	4.21†	**-0.0145**	0.0128	2.99	
	8	77.9	70.4—84.5	7.23†	**0.0173**	**0.0081**	2.28	
	9	82.6	74.6—96.3	4.56†	**-0.0132**	0.0004	1.54	
	10	3.1	3.1—10.8	4.11†	**0.0105**	**0.0068**	1.39	
	11	90.3	88.3—92.3	5.17†	**0.0124**	**-0.0079**	1.89	M
	12	80.8	78.1—82.9	7.25†	**0.0164**	-0.0061	2.65	**M**,F
	12	104.0	91.0—106.6	3.72	**0.0115**	0.0003	1.23	
	13	55.2	54.5—58.1	5.43†	**0.0159**	-0.0011	2.01	
	17	26.7	17.9—29.7	5.27†	**0.0139**	-0.0066	2.22	
	X	107.7	107.7—118.8	32.16†	**-0.0306**	0.0108	9.48	

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BMC[a]	1	25.5	21.5—37.7	4.06†	**0.0098**	-0.0019	0.91
	1	187.0	185.5—189.3	6.25†	**-0.0122**	-0.0002	1.26
	3	81.6	76.8—94.3	7.35†	**0.0078**	**-0.0105**	1.39
	4	137.8	126.8—141.6	5.38†	**0.0220**	0.0211	6.25
	9	40.2	34.8—42.1	7.70†	**-0.0169**	0.0007	2.27
	9	81.2	74.6—96.3	4.45†	**-0.0114**	-0.0021	1.03
	X	107.7	107.7—118.8	13.96†	**-0.0172**	0.0053	3.02
BAREA	3	81.6	79.7—82.4	9.89†	**0.1946**	-0.0497	3.96
	12	80.8	75.1—81.9	4.41†	**0.1416**	0.0186	1.54
	X	107.7	107.7—124.7	23.28†	**-0.2911**	**0.1118**	6.84
BAREA[a]	3	81.6	79.7—82.4	7.93†	**0.1436**	-0.0666	1.93
	5	108.8	108.4—113.3	3.93	-0.0558	**0.1681**	1.14
	X	107.7	107.7—124.7	11.52†	**-0.1915**	0.0601	2.98

Shown are all QTLs affecting the unadjusted and adjusted ([a]) bone traits measured on the live F_{11} mice that had LOD scores reaching the 10% (suggestive) or 5% (†) experimentwise level of significance. Locations on each chromosome (Ch) and confidence intervals of the QTLs are given in Mb (from NCBI Build 37). Also shown is the percentage contribution (%) of each QTL to the total variance of each trait, and its additive (*a*), dominance (*d*) genotypic effects (bolded values indicate significance at $P < 0.05$). Interactions of QTLs with sex are indicated as M (significant in males only), F (significant in females only) or both M and F (significant in both sexes where bolded values indicate the sex for which the QTL had the greater effect).

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Table 5. Adiposity QTLs and their potential candidate genes.

Chrom	Location (Mb)	Adiposity Traits	No. of Genes	Candidate Genes
1	21.4-35.8	PFAT	47	*Rims1,* Arhgef4
2	11.5-29.4	FAT, PFAT, SUBQ, PSUBQ	339	*Cacna1b*, *Ehnt1, Cel*
3	79.5-114.7	FAT, PFAT, SUBQ, PSUBQ, PGON	511	Prkab2, Nhlh2, *Kcna3*
6	117.4-126.6	FAT, PFAT, SUBQ, GON, PGON, BAT	134	Ankrd26, Adipor2, *Gdf3,*
10	65.0-100.0	FAT, PFAT, SUBQ, PSUBQ	395	*Arid5b, Igf1*
10	114.0-130.3	PSUBQ	225	*Hmga2, Lrp1, Mmp19*
17	34.9-62.7	FAT, SUBQ	397	*Ehmt2, Lta, Tnf, Rcan2*
X	48.3-66.5	FAT, GON	86	*Gpc3, Hprt, Brs3*
X	101.5-130.5	PFAT, PGON, PBAT	113	*Cited1*

Shown are the chromosome (Chrom) and location of the QTLs affecting the various adiposity traits, as well as the number of protein-coding genes located within their confidence intervals, and potential candidate genes for the QTLs.

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Figure 1

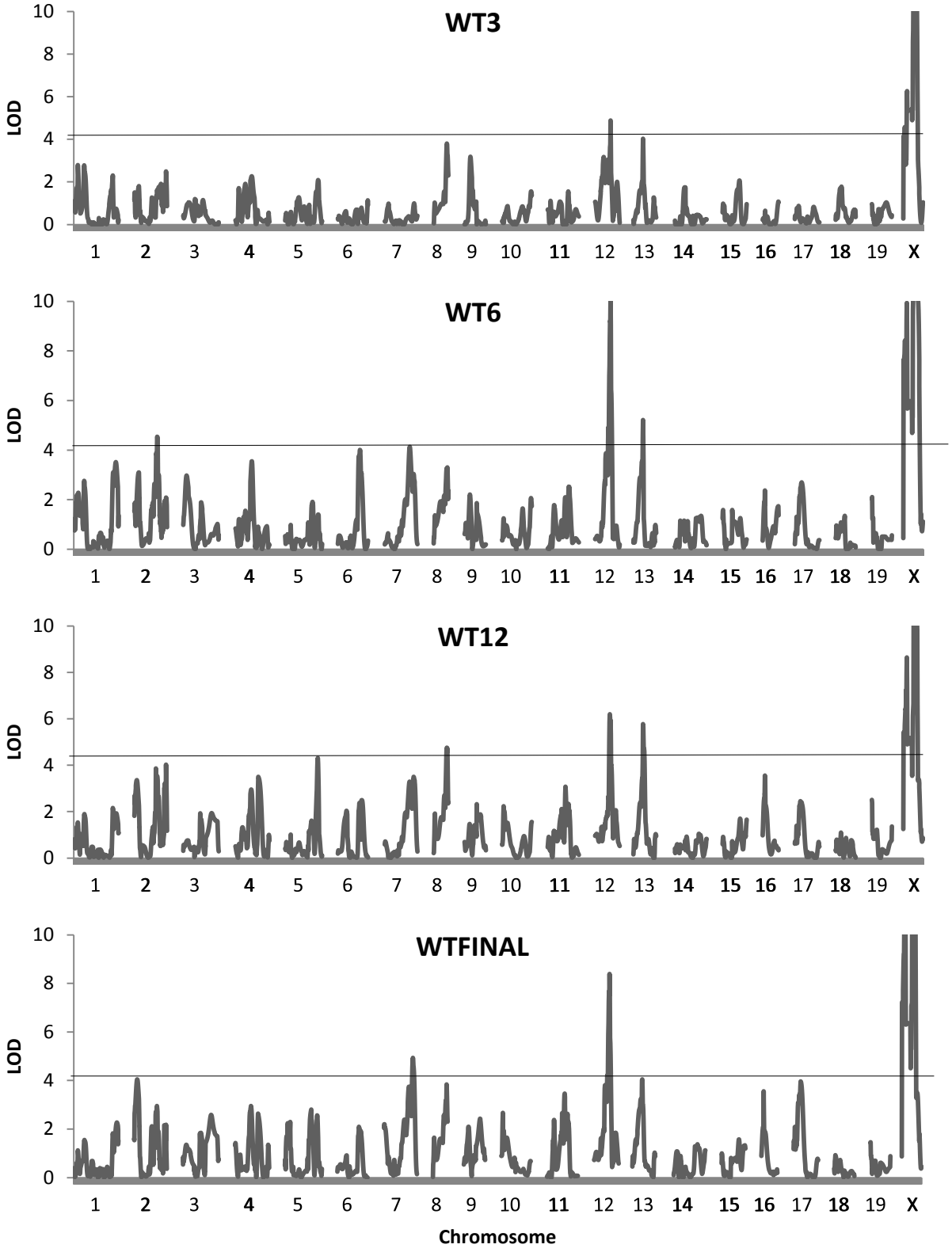

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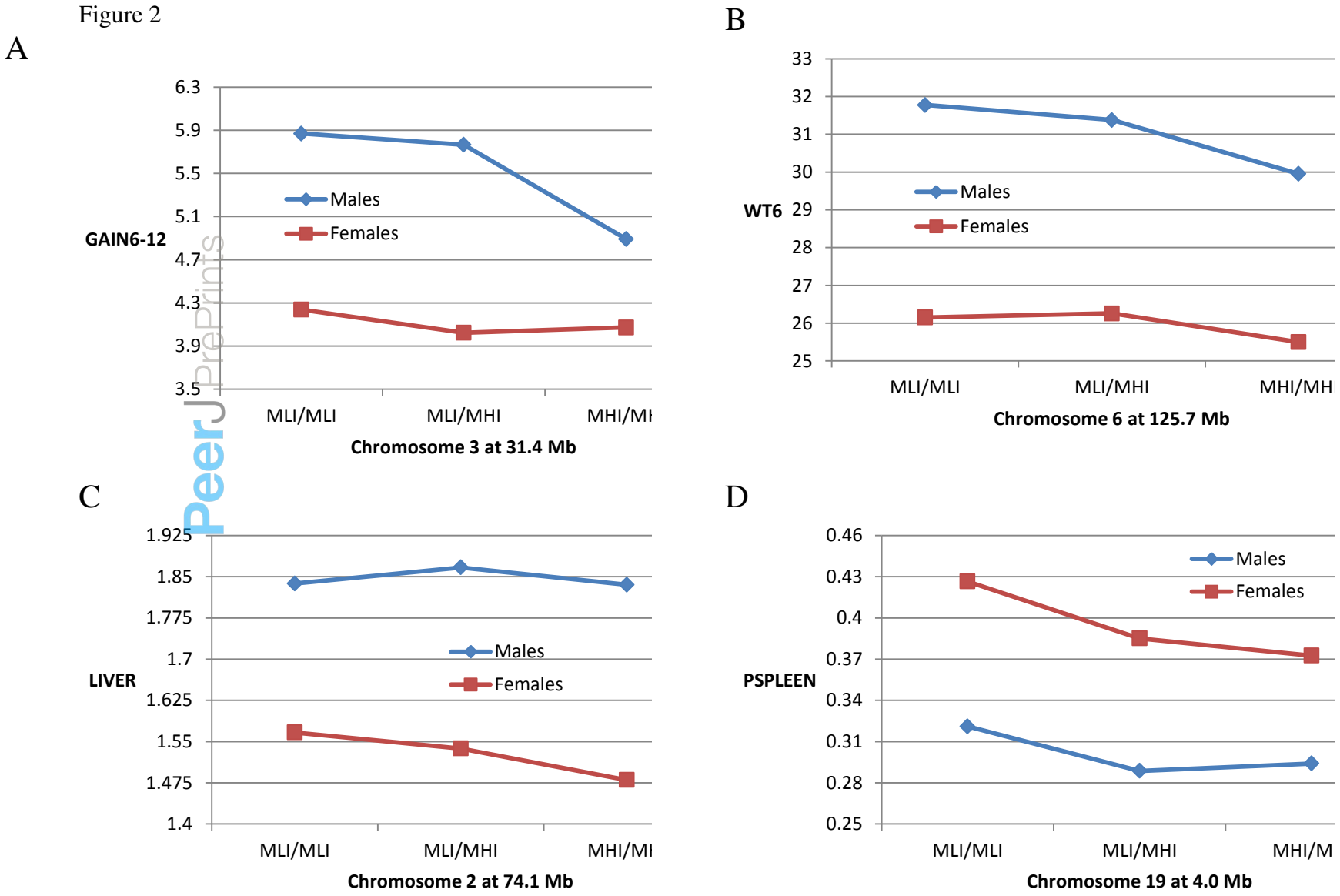
Figure 2



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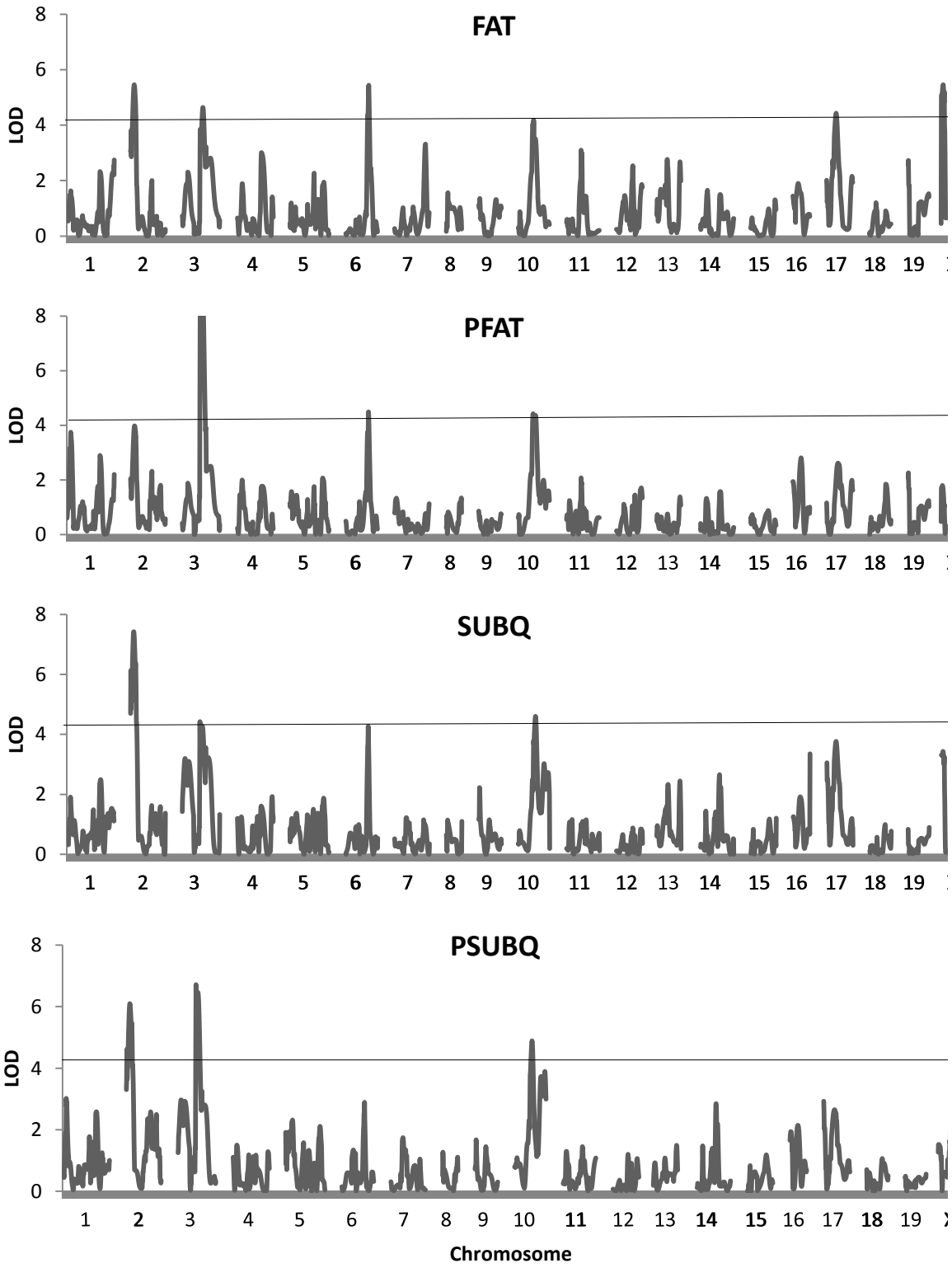
858 Figure 3



859

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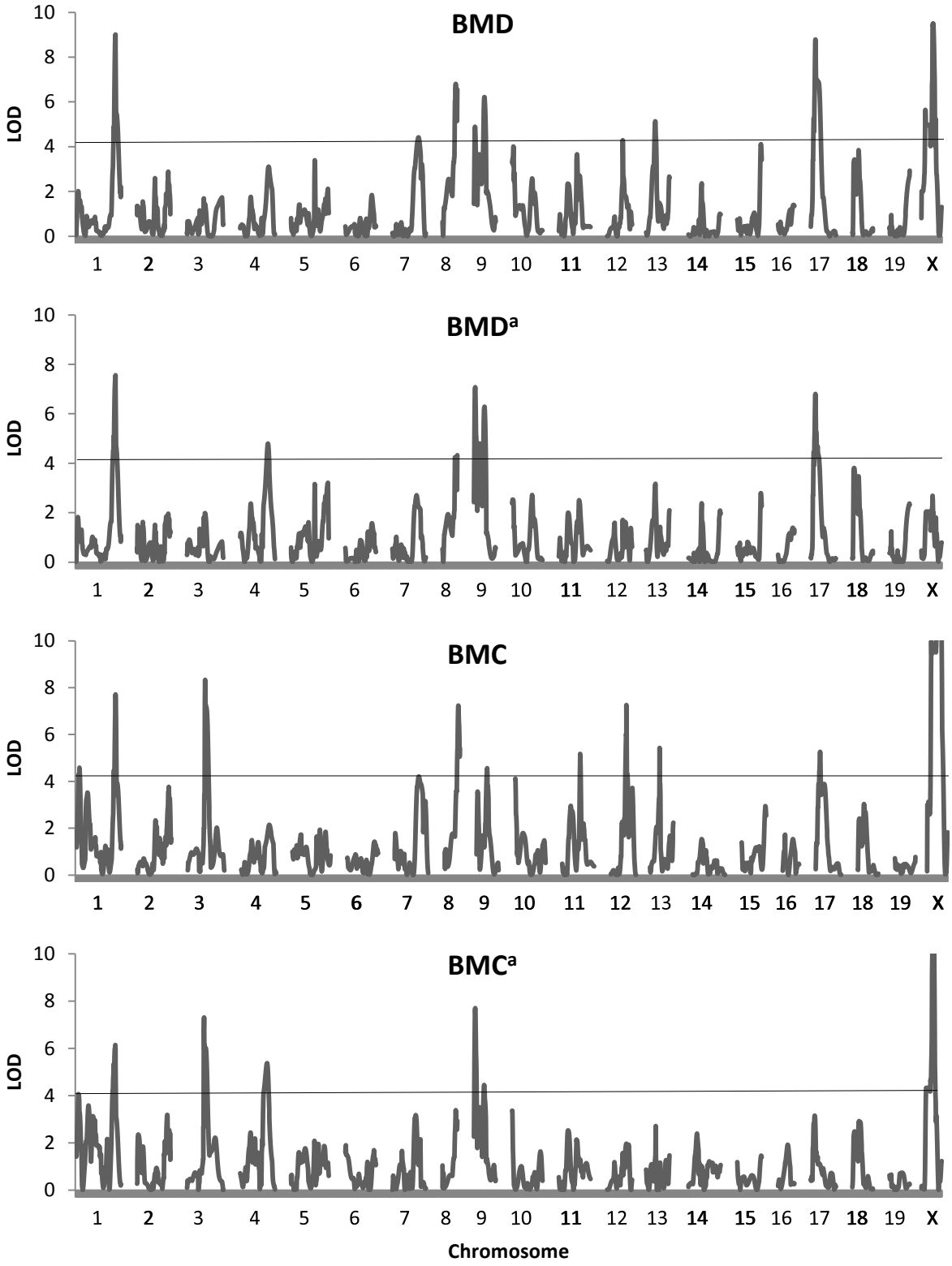
860 Figure 4
861



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Appendix 1. SNP markers used with their chromosome and coordinates.

Marker	Chromosome	Coordinate	Marker	Chromosome	Coordinate
rs3677817	1	5.197303	rs3695581	1	118.500635
rs13475712	1	7.41187	rs3667720	1	120.697988
rs3726952	1	12.906283	rs13476089	1	122.457718
rs13475735	1	15.147784	rs3697826	1	124.896697
rs3658044	1	19.498836	rs13476098	1	126.400694
rs3711079	1	22.156681	rs6355835	1	130.487043
rs4222215	1	24.234159	rs3713473	1	131.948608
rs13475771	1	25.676777	rs3700475	1	138.09948
rs3677683	1	27.510356	CEL-1_140588762	1	140.588762
rs6237824	1	30.982111	rs13476147	1	142.551146
UT_1_35.224766	1	34.822105	rs6186115	1	144.615698
rs13475816	1	37.880203	rs6364156	1	146.112228
rs13475821	1	39.844439	gnf01.149.342	1	149.831572
mCV23591750	1	51.617463	rs13476187	1	154.18791
rs8254826	1	61.776437	rs8242852	1	171.128307
rs3716105	1	61.943426	rs3143355	1	175.96311
rs6356603	1	75.36232	rs13476259	1	177.399967
rs6321468	1	78.484909	rs6301437	1	179.84146
rs3667200	1	78.622208	CEL-1_181947877	1	181.947877
rs13475972	1	89.01772	rs3693165	1	183.213131
rs13475960	1	89.120328	rs6154379	1	184.815448
rs6250696	1	89.196129	rs13476290	1	186.816908
rs13475973	1	89.403365	gnf01.195.387	1	191.348177
gnf01.089.691	1	89.659368	rs4222922	1	193.166401
rs3022827	1	90.567434	rs6246360	1	194.9579
rs13475982	1	92.258955	rs13476318	2	3.076675
rs13475988	1	93.553382	rs13476330	2	5.79421
rs13475989	1	93.819709	rs6240512	2	10.929543
rs13475991	1	94.403364	rs13476352	2	13.387323
rs3675505	1	95.019059	rs8250941	2	25.427137
rs6342650	1	96.400818	rs3718405	2	27.249691
rs6358447	1	97.506398	rs6181760	2	27.847598
rs13476003	1	97.677389	rs13476429	2	35.357114
CEL-1_98681809	1	98.681809	rs13476553	2	66.906537
CEL-1_98799654	1	98.799654	rs13476556	2	67.617045
gnf01.099.019	1	99.03077	rs6371268	2	68.927682
rs3695980	1	99.464143	rs13476560	2	69.403391
rs13476012	1	99.797518	rs13476563	2	70.070631
rs13476014	1	100.146917	rs3682843	2	71.050924
rs3685663	1	100.268247	rs3670752	2	71.26882
rs3717264	1	103.113538	rs3683059	2	75.399681
rs3664662	1	104.472398	rs6248415	2	76.480252
rs3664301	1	107.059245	mCV25095764	2	76.990271
rs3685919	1	109.634975	rs3711780	2	77.245946
rs3725409	1	116.434018	CEL-2_79237503	2	79.237503
			rs13476594	2	79.662978

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rs3722345	2	80.509747	rs6363066	3	57.943185
rs13476639	2	92.720804	rs6239288	3	60.673679
rs4223268	2	93.313855	rs3696955	3	63.295533
rs13476663	2	99.952945	rs6224355	3	66.3279
CEL-2_100344390	2	100.34439	rs13477165	3	66.436519
rs6249987	2	100.974556	rs6226544	3	67.856811
rs13476667	2	101.477932	CEL-3_68001820	3	68.00182
rs13476669	2	101.877243	rs13477178	3	70.089276
rs6155648	2	102.059025	rs6198234	3	70.36143
rs3700286	2	102.525512	CEL-3_70552044	3	70.552044
rs3143810	2	103.177373	CEL-3_70697605	3	70.697605
rs3674721	2	104.594142	rs3698109	3	71.209119
mCV25337624	2	105.399199	rs6264454	3	71.858927
rs13476684	2	105.859705	rs3715136	3	72.730362
rs13476689	2	107.356662	rs13477190	3	73.392003
rs13476693	2	109.285116	rs13477210	3	77.646357
rs3022892	2	109.891529	rs3715352	3	78.618892
rs13476697	2	110.552699	rs13477215	3	78.622271
rs13476700	2	111.672465	rs3659866	3	81.285486
rs3693678	2	112.367495	rs6305129	3	81.508202
rs3701250	2	114.662573	gnf03.079.138	3	82.509078
rs6276129	2	116.113235	rs13477233	3	83.880528
rs3677413	2	116.603199	rs3708227	3	84.422484
rs3723406	2	117.650393	rs6376008	3	87.149248
rs13476728	2	118.595838	rs13477244	3	87.394444
rs6340352	2	121.051368	rs3722681	3	110.165817
rs13476746	2	122.660089	rs6242665	3	111.412955
rs13476755	2	124.470148	rs6214597	3	117.350554
rs3697020	2	125.287975	rs13477498	3	153.484447
rs6411422	2	128.122514	rs6331755	3	156.678486
rs6161193	2	129.466095	rs3667025	3	158.614152
rs3699051	2	132.104826	rs13477528	3	160.447976
rs13476788	2	135.061275	rs13477534	4	4.046388
rs13476794	2	136.490987	rs13477546	4	7.682681
rs3710324	2	136.973991	rs13477592	4	19.653411
rs13476805	2	139.473083	rs13477599	4	21.606001
rs6303304	2	141.192338	rs13477617	4	27.105003
rs6360457	2	141.929558	rs13477637	4	33.888067
gnf02.141.261	2	142.837835	rs13477662	4	39.882315
rs6195594	2	143.346715	CZECH-4_46713961	4	46.713961
rs3696870	2	147.404525	rs3676423	4	51.03541
rs13476827	2	148.284246	UT_4_57.645957	4	57.161636
rs3676033	2	149.197838	rs13477735	4	60.185456
rs3719352	3	10.752146	rs13477741	4	62.556359
rs3694133	3	12.562659	CEL-4_74066970	4	74.06697
rs6398851	3	14.447458	rs6258088	4	80.891223
rs13476992	3	16.59583	rs13477813	4	82.905304
rs13477043	3	31.257023	rs3726736	4	102.512069
rs13477046	3	32.50179			

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rs3670382	4	109.18677	rs13478508	5	122.876611
rs3694396	4	112.812399	rs13478521	5	125.897723
rs3696331	4	115.756451	rs3661159	5	128.187011
rs3726907	4	118.140601	rs13478546	5	133.978719
gnf04.117.960	4	120.989378	rs6334078	5	136.117818
rs3671259	4	123.272188	rs6319445	5	139.022464
rs13477972	4	126.623471	rs3023061	5	141.390786
rs6268364	4	149.902058	rs6191249	5	143.668186
rs3693138	4	152.591465	rs3668534	5	145.8327
rs3693087	4	154.007691	rs3692702	5	147.670924
rs13478092	5	3.601413	rs3655269	6	17.7151
rs13478104	5	7.984264	rs13478656	6	21.755667
rs3714258	5	11.417181	rs3684860	6	48.907491
CEL-5_14611794	5	14.611794	rs3023069	6	52.190471
gnf05.014.723	5	19.14048	rs13478761	6	53.702681
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rs13478145	5	23.049118	rs3698364	6	81.140583
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rs3663141	5	114.196024	rs13479234	7	35.506778

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CEL-7_36545579	7	36.545579	rs6174757	9	68.381485
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gnf07.050.858	7	44.11275	rs13480285	9	76.908789
rs6388842	7	44.912991	rs13480308	9	83.133092
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rs13479274	7	45.712494	rs3725272	9	95.000478
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rs13479277	7	46.313106	rs6377847	9	100.692014
rs6160140	7	53.312532	rs13480436	9	114.857586
rs3705155	7	55.55709	rs6302293	9	118.240473
rs6405142	7	55.615198	gnf09.117.044	9	119.540217
rs13479317	7	56.524353	gnf10.004.219	10	3.088178
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rs13479334	7	60.382457	rs13480525	10	18.959895
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rs3716002	7	61.171781	rs13480629	10	67.390746
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rs3726906	8	58.201849	rs13480839	11	4.125911
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rs3712611	8	65.261034	rs13480859	11	8.373216
rs13479813	8	66.929486	rs13480869	11	10.898579
rs3690549	8	76.12653	mCV23851630	11	13.163316
rs6296891	8	79.15201	CEL-11_15345124	11	15.345124
rs8236770	8	82.939075	rs13480889	11	17.360319
rs13479880	8	86.040626	rs3678321	11	19.522724
rs13480026	8	123.079487	rs6367881	11	26.054403
rs4227456	8	124.053484	rs3657760	11	32.856509
rs3711756	9	34.437206	rs13480965	11	35.564301
rs3669224	9	37.029046	rs13480997	11	43.490965
rs13480151	9	40.150719	mCV23044839	11	65.492498
rs13480160	9	42.760021	UT_11_68.607315	11	68.648705
CEL-9_49033157	9	49.033157	gnf11.079.978	11	75.081947
gnf09.058.846	9	64.854283	rs13481119	11	79.159755

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rs13481123	11	81.870816	rs6209128	13	51.80716
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rs13481154	11	90.012989	rs3700819	13	56.358945
rs3714299	11	92.73468	mCV22624058	13	58.311395
rs3710148	11	96.196554	CEL-13_60831741	13	60.831741
rs3695865	11	101.350115	rs6179438	13	68.057256
rs6384437	11	103.833223	rs13481878	13	70.156974
rs13481226	11	109.851386	rs3669221	13	80.367567
rs13481233	11	111.472921	rs13481918	13	81.984151
rs13481264	11	119.95554	rs3655061	13	86.241392
rs13481276	12	3.64518	rs4230027	13	97.200857
rs3699421	12	3.922406	rs3705092	13	100.020595
rs3655333	12	23.704034	rs13481992	13	102.442609
rs13481380	12	27.085682	rs13464858	13	105.049971
rs6187012	12	28.579503	rs13482011	13	107.432985
rs6223000	12	29.204179	rs4230094	13	110.039545
gnf12.033.545	12	30.14931	rs3657414	13	113.498433
rs6243157	12	32.296827	rs6340768	14	7.266803
rs3689063	12	50.059458	rs13482084	14	8.848582
rs13481465	12	50.509782	rs3719629	14	12.874743
rs3662939	12	54.082727	gnf14.019.954	14	22.731865
rs13481480	12	54.343362	rs13482122	14	27.054167
rs3674641	12	55.129109	rs6155573	14	29.837255
rs13481486	12	55.941001	rs3722090	14	32.565225
rs3721804	12	56.413174	mCV23384307	14	41.762771
rs13481488	12	56.527832	rs13482179	14	43.710903
rs13481499	12	59.639662	rs3140262	14	45.774656
rs3686891	12	60.735544	rs6392664	14	47.364134
rs4137680	12	61.533001	rs13482194	14	47.801247
rs3686378	12	63.477601	rs13482214	14	53.590138
rs13481522	12	66.97148	rs6161506	14	56.25048
rs13481531	12	69.626283	rs13482225	14	56.692261
mCV23169261	12	72.254494	rs6179045	14	57.017412
rs6318521	12	74.957568	CEL-14_65598536	14	65.598536
rs3696951	12	77.991846	rs3668373	14	66.081018
rs8259763	12	82.491932	rs6156908	14	66.473686
rs13481583	12	84.94215	gnf14.069.419	14	66.681374
rs13481589	12	86.779666	UT_14_66.802733	14	66.99281
rs3679514	12	92.576929	rs13482259	14	68.392545
rs3698001	12	95.092951	rs6298191	14	69.251728
gnf12.101.501	12	97.964429	CEL-14_71690454	14	71.690454
CEL-12_101776500	12	101.7765	rs3023412	14	72.111198
rs13481650	12	105.932708	CEL-14_72264521	14	72.264521
rs6198959	13	11.008611	rs6325141	14	73.875166
rs3721858	13	16.752051	rs13482273	14	74.295489
rs13481715	13	18.523821	rs13482301	14	81.862571
rs13481743	13	31.273115	rs3725470	14	82.281581
rs3727136	13	33.688045	gnf14.085.610	14	82.796068
gnf13.045.330	13	46.738859	rs6395984	14	82.944063

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rs6407863	14	83.212623	rs3702484	17	9.103503
rs13482306	14	83.42809	mCV22941359	17	9.24339
rs3706761	14	83.865561	rs3662575	17	9.388595
rs3655019	14	83.873573	rs6309949	17	10.69913
rs6291434	14	83.88723	rs3674166	17	11.214621
rs6176735	14	84.102001	rs3674900	17	11.922826
rs6299927	14	84.309814	gnf17.011.487	17	12.376655
rs4139735	14	84.482998	rs3723317	17	12.406402
rs13482309	14	84.549347	rs6270865	17	15.307109
CEL-14_85152539	14	85.152539	rs3667748	17	15.457982
rs13482311	14	85.28705	rs3721884	17	16.225206
rs13482312	14	85.438583	rs4231344	17	22.269867
rs3718262	14	85.692909	rs3724223	17	32.068525
rs13482313	14	85.873792	UT_17_33.238924	17	32.099572
rs13482314	14	86.050411	rs8242408	17	32.690515
rs3708779	14	103.068	mhcTNFa7	17	33.6972
rs3683221	14	104.867978	rs3682923	17	34.343989
rs3707842	14	111.967694	rs6298471	17	35.059374
rs13482409	14	114.979966	rs13482974	17	36.98639
rs13482416	14	117.011917	rs8254221	17	38.94185
rs13482418	15	3.49896	mCV25197172	17	41.0809
rs3693019	15	7.948918	rs8273969	17	52.02091
rs3714169	15	21.816884	rs13483042	17	53.781849
rs13482490	15	26.813067	mCV22888090	17	61.947366
rs6210607	15	30.253148	rs3675634	17	69.670995
rs6187312	15	31.266214	rs13483139	17	83.294259
CEL-15_58115663	15	58.115663	rs3667051	17	88.400292
rs3701449	15	60.283849	CEL-17_91401354	17	91.401354
rs13482605	15	62.435922	rs13483179	17	93.216106
rs13482618	15	66.126656	rs13483210	18	12.147024
rs3697744	15	87.041436	rs13483217	18	14.478102
rs3716673	15	92.579012	rs13483230	18	17.674697
rs6285067	15	95.741617	rs3656185	18	19.941646
rs13482734	15	99.184112	rs3691362	18	30.05309
rs3690173	15	101.419686	rs3714096	18	36.136675
mCV25142331	N/A	N/A	rs6302276	18	39.612385
rs4166445	16	28.119031	rs13483319	18	41.351739
rs4168890	16	31.10743	gnf18.043.155	18	45.21264
rs4170974	16	32.825259	rs6334596	18	55.31767
rs4172915	16	35.504262	rs13483378	18	57.789927
rs4174174	16	37.637201	rs6364818	18	59.921604
rs4175353	16	38.902653	rs6350869	18	61.07351
rs4197416	16	66.683459	rs6346101	18	69.04019
rs3656592	16	87.019209	rs13483433	18	72.937807
rs4217061	16	90.320114	rs13483466	18	82.712912
rs4220668	16	95.392761	rs13483472	18	84.261455
rs3164088	16	97.1111	rs13483500	19	3.744813
rs3694629	17	6.436726	rs3671671	19	4.208173
			rs3713033	19	4.818261

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rs13483505	19	4.836043	rs13483831	X	67.285372
rs13483510	19	6.160915	rs13483834	X	67.880612
rs13483511	19	6.331751	CEL-X_68179178	X	68.179178
rs3023477	19	7.133376	CEL-X_68645226	X	68.645226
CEL-19_8529644	19	8.529644	rs13483838	X	68.74927
UT_19_10.709331	19	9.813271	CEL-X_71104123	X	71.104123
rs6163293	19	9.899424	CEL-X_71438949	X	71.438949
rs3700209	19	10.19721	rs13483849	X	71.752528
rs3688406	19	11.531705	CEL-X_72627341	X	72.627341
rs6237846	19	12.452484	CEL-X_72697823	X	72.697823
CEL-19_12595293	19	12.595293	CEL-X_73027245	X	73.027245
CEL-19_12911424	19	12.911424	gnfX.070.167	X	73.485959
rs3692733	19	13.056443	rs13483858	X	73.756646
rs3694570	19	13.056716	CEL-X_74073918	X	74.073918
rs3662712	19	15.964901	rs13483862	X	74.580193
rs3669192	19	16.124735	rs13483863	X	74.827617
rs3686467	19	16.159229	CEL-X_74985293	X	74.985293
rs8267310	19	16.991104	CEL-X_75125049	X	75.125049
rs13483555	19	19.177412	rs13483864	X	75.392167
gnf19.017.711	19	19.28395	rs13483877	X	78.499387
rs3720318	19	19.458954	rs13483803	X	81.225989
rs13483557	19	19.479228	gnfX.076.619	X	84.227028
rs13483563	19	20.842526	rs13483888	X	86.725106
rs6392565	19	21.461474	rs13483898	X	89.327593
rs3672759	19	21.707322	CEL-X_91222960	X	91.22296
rs3653630	19	21.729294	CEL-X_94143306	X	94.143306
rs13483571	19	23.62697	rs13483927	X	97.613903
rs13483577	19	25.63107	rs13483935	X	101.057496
rs3090325	19	26.007713	rs13483941	X	102.845364
rs13483669	19	51.559161	rs6221690	X	121.042456
rs6257938	19	52.744319	rs13484004	X	123.782683
CEL-X_44311522	X	44.311522	CEL-X_125736335	X	125.736335
rs6411410	X	44.405254	rs13484023	X	127.710219
rs13483753	X	44.656596	rs13484031	X	129.847872
rs13483756	X	45.475262	rs13484040	X	131.961674
rs13483757	X	45.612522	rs13484043	X	132.699243
rs13483771	X	51.03826	rs13484094	X	147.274623
CEL-X_51185805	X	51.185805	rs6365259	X	150.148872
rs13483777	X	52.741694	CEL-X_158112484	X	158.112484
rs13483778	X	53.039702			

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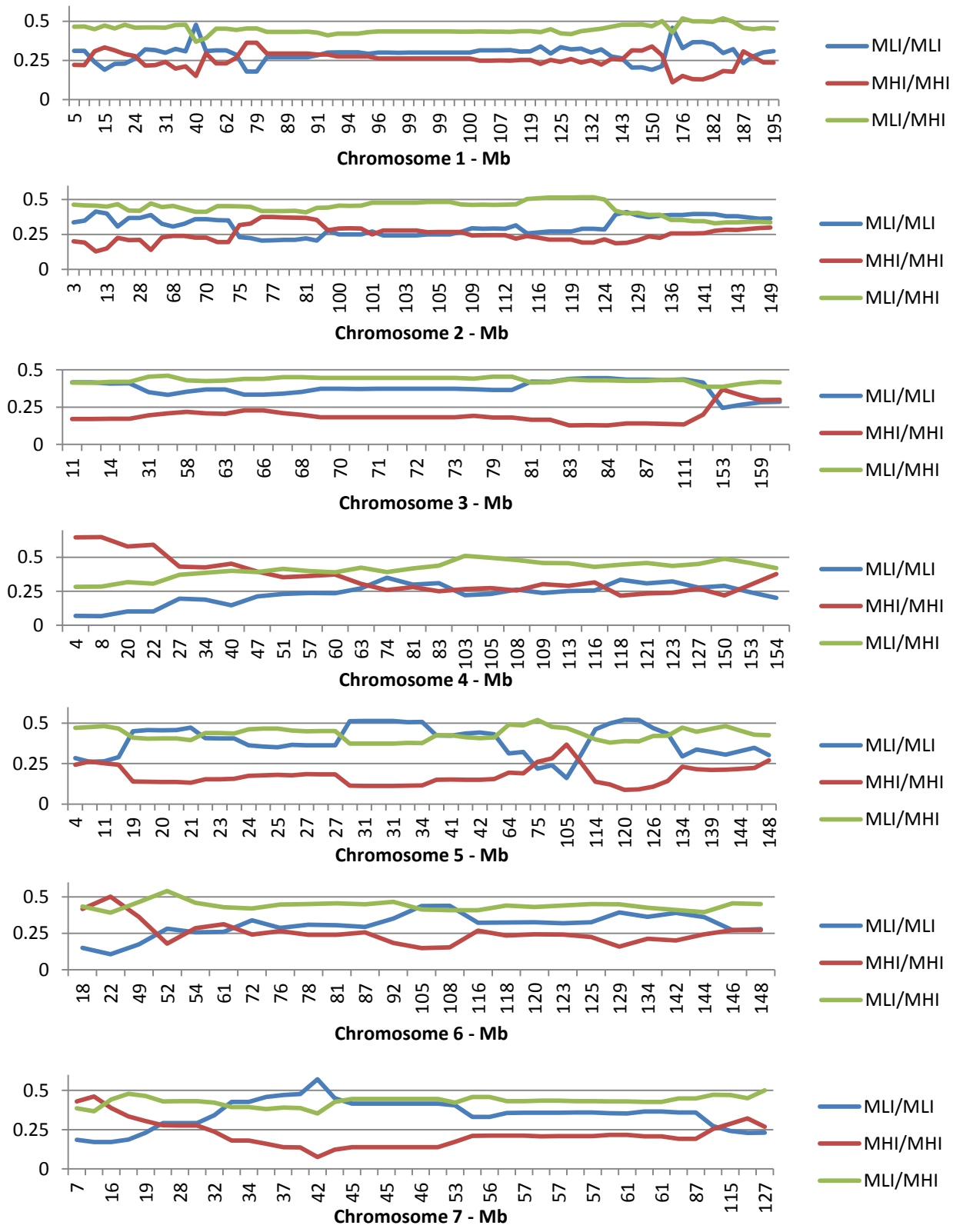
Appendix 2. MLI/MLI, MHI/MHI, and MLI/MHI genotype frequencies at each marker on all chromosomes. Mb = megabases.



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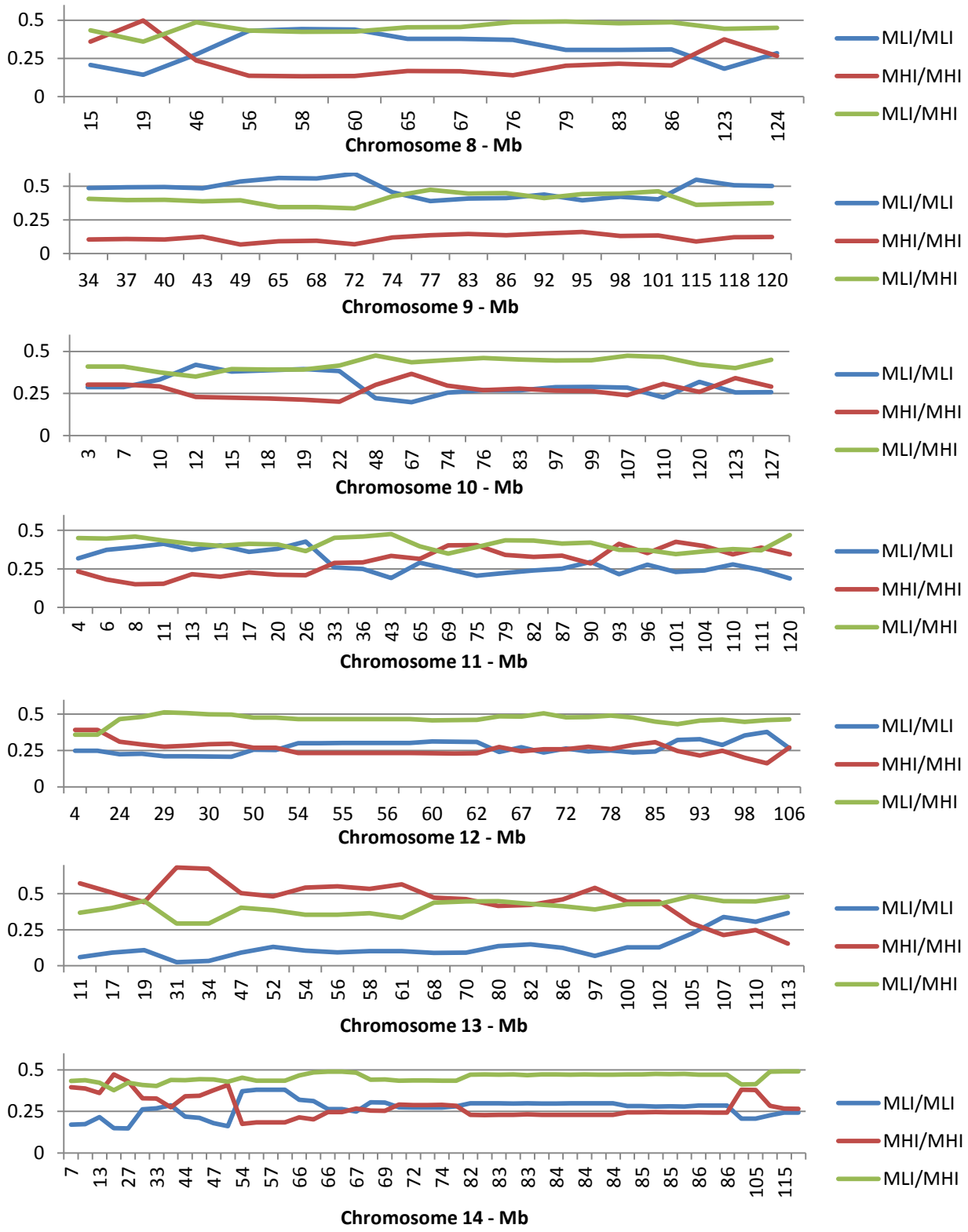

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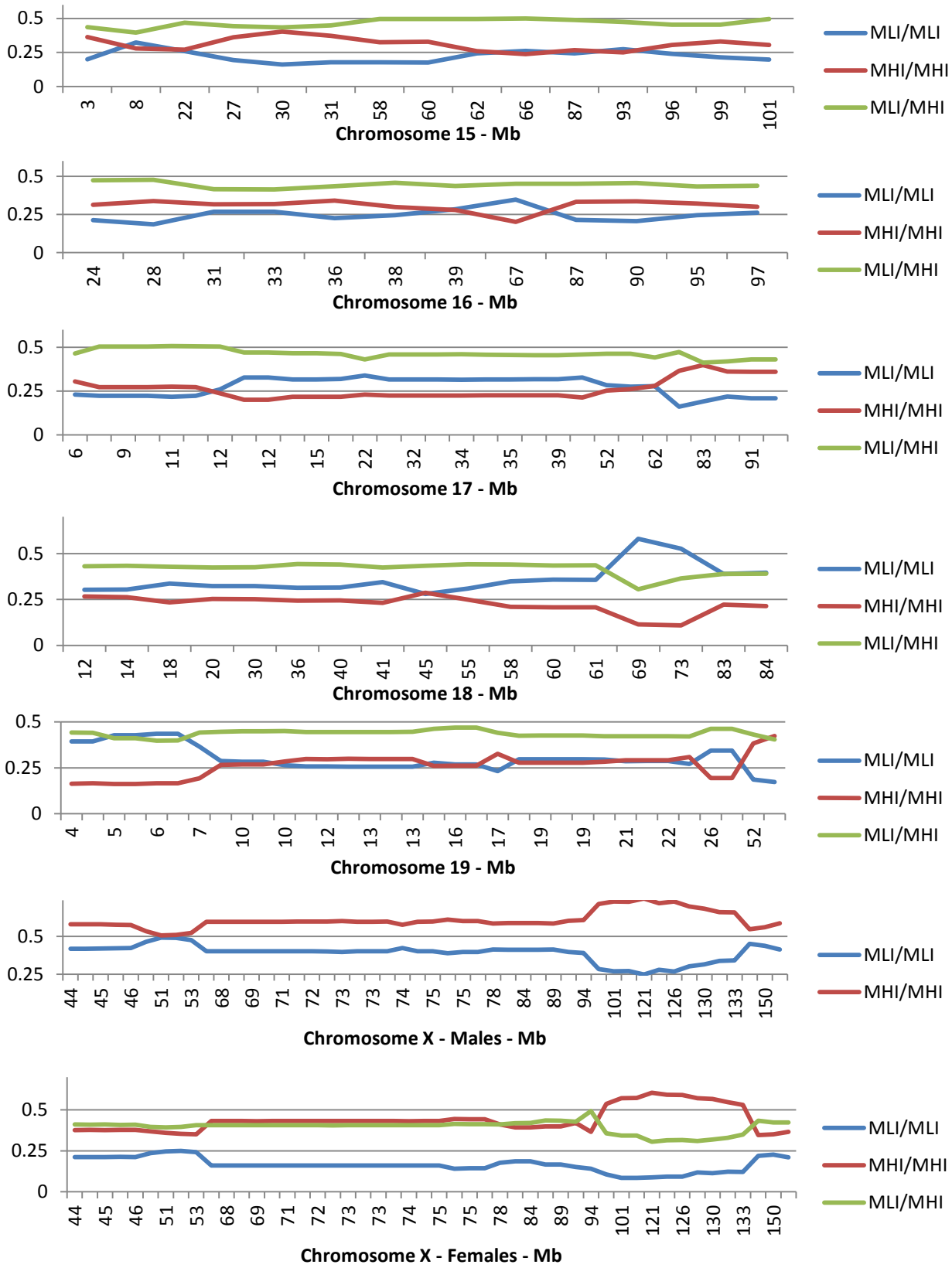

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