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Quantitative trait locus for body weight identified on rat chromosome 4 in inbred alcohol-preferring and –nonpreferring rats: potential implications for neuropeptide Y and corticotrophin releasing hormone 2

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Abstract

The alcohol-preferring (P) and –nonpreferring (NP) rat lines were developed using bidirectional selective breeding for alcohol consumption (g/kg/day) and alcohol preference (water : ethanol ratio). During a preliminary study, we detected a difference in body weight between inbred P (iP) and inbred NP (iNP) rats that appeared to be associated with the transfer of the Chromosome 4 quantitative trait locus (QTL) seen in the P.NP and NP.P congenic strains. After the initial confirmation that iP rats displayed lower body weight when compared to iNP rats (data not shown), body weight and growth rates of each chromosome 4 reciprocal congenic rat strain (P.NP and NP.P) were measured, and their body weight was consistent with their respective donor strain phenotype, confirming that a quantitative trait locus for body weight mapped to the chromosome 4 interval. Utilizing the newly developed interval-specific congenic strains (ISCS-A and ISCS-B), the QTL interval was further narrowed identifying the following candidate genes of interest: neuropeptide Y (*Npy*), juxtaposed with another zinc finger gene 1 (*Jazf1*), corticotrophin releasing factor receptor 2 (*Crfr2*) and LanC lantibiotic synthetase component C-like 2 (*Lancl2*). These findings indicate that a biologically active variant(s) regulates body weight on rat chromosome 4 in iP and iNP rats. This QTL for body weight was successfully captured in the P.NP and NP.P congenic strains, and interval-specific congenic strains (ISCSs) were subsequently employed to fine-map the QTL interval identifying the following candidate genes of interest: *Npy*, *Jazf1*, *Crfr2* and *Lancl2*. Both *Npy* and *Crfr2* have been previously identified as candidate genes of interest underlying the chromosome 4 QTL for alcohol consumption in iP and iNP rats.

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Keywords

alcoholism; body weight; selective breeding; congenic; interval-specific congenic strain

INTRODUCTION

Alcohol addiction is a complex psychiatric disorder that is influenced by both genetic and environmental factors. The alcohol-preferring (P) and –nonpreferring (NP) rat lines were developed at Indiana University School of Medicine to model high and low voluntary alcohol consumption and preference, respectively (Li et al., 1991). In this model, P rats are considered an animal model of alcoholism displaying the necessary phenotypic criteria (Cicero, 1979). Inbred P (iP) and NP (iNP) lines were later developed that exhibit highly discordant alcohol consumption and preference scores (Carr et al., 1998). Using iP x iNP F2 animals, a genome screen was previously conducted that identified a highly significant QTL (LOD-9.2) for alcohol consumption on rat chromosome 4 (Chr 4) and two suggestive QTLs on rat Chr 3 and 8, respectively (Bice et al., 1998; Carr et al., 1998). In recent years, reciprocal congenic strains have been developed by transferring the iP Chr 4 QTL interval into the iNP line (NP.P) and the iNP Chr 4 QTL interval into the iP line (P.NP), respectively (Carr et al., 2006). These congenic strains display the expected phenotypic differences in alcohol consumption (Carr et al., 2006). Recently, interval-specific congenic strains (ISCSs) were developed by backcrossing P.NP with iNP in order to fine-map the Chr 4 QTL interval. These newly developed ISCSs provide a higher resolution to examine the effect(s) of a biologically active variant(s) on various phenotypes of interest including alcohol drinking behavior as well as body weight.

The identification and characterization of correlative traits can provide additional information about a biological variant(s) associated with a QTL (Spence et al., 2009). To date, several key systems have been identified that modulate both alcohol consumption and body weight including the endogenous opioids, the melanocortins, neuropeptide Y, cholecystokinin, and galanin (Montoro et al., 2012; Thiele et al., 2004; Xu et al., 2011). In addition, linkage analyses have suggested substance abuse disorders and body mass index (BMI) may share some genetic determinants (Ehlers and Wilhelmsen, 2007).

During a preliminary study, we detected a difference in body weight in iP and iNP rats, and these initial results also suggested that this body weight difference may be influenced by the transfer of the Chr 4 QTL as measured in P.NP and NP.P congenic strain (unpublished observation, J.P. Spence, 2010). Therefore, we designed a study to determine whether the Chr 4 region in iP and iNP rats was linked to this variation in body weight. We then further characterized the genetic relationship associated with body weight using the P.NP and NP.P congenic strains, and the newly developed interval specific congenic strains (ISCSs) were employed to fine-map the QTL. These findings may also be relevant to the identification of potential candidate genes underlying the Chr 4 QTL for alcohol consumption in the iP and iNP lines.

MATERIALS AND METHODS

Animals

The animals used for this study included inbred alcohol preferring (iP) and inbred alcohol nonpreferring (iNP) rats (Bice et al., 1998; Lumeng et al., 1977), congenic P.NP and NP.P strains (Carr et al., 2006), and interval-specific congenic strains (ISCSs). The method utilized to develop the interval specific congenic strain (ISCS) is similar to the method previously used to develop the P.NP and NP.P congenic strains (Carr et al., 2006). In brief,

P.NP rats were back-crossed to iP rats, and rats with specific Chr 4 intervals were selected based on the genotype of microsatellites markers. The markers used for genotyping include the following: *D4Rat151, D4Mgh16, D4Rat163, D4Rat33, D4Rat110, D4Rat35, D4Rat173, D4Rat177, D4Rat51, D4Rat55, and D4Rat192. SNPs* identified between iP and iNP in the *Npy, Crfr2,* and *Snca* genes were also used as SNP markers to perform the genotyping (Fig. 3). The P.NP-ISCS-A (hereafter ISCS-A) rat encodes the NP genotype from *D4Mgh16* to *D4Rat173,* and the P.NP-ISCS-B rat encodes the NP genotype from *Snca* to *D3Rat35* (hereafter ISCS-B). These rats were bred and maintained at Indiana University School of Medicine. The experimental protocol used in this study was reviewed and approved by the Indianan University Institutional Animal Care and Use Committee and was carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals. The animals were maintained in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC).

Body weight measurement in alcohol-naïve iP, iNP, P.NP, and NP.P rats

Body weight was measured in alcohol-naïve 6 of each male and female iP (N=12), iNP (N=12), P.NP (N=12), and NP.P (N=12) rats every week from four weeks to 11 or 12 weeks of age. Data were grouped by genotype (i.e., iP, iNP, P.NP, and NP.P) at each time point. To test whether the growth curves were different among groups, areas under the curve minus baseline (AUC) were determined. For each individual, adjacent measurements were connected by a line segment, and t −1 trapezoids were formed (where t is the number of measurements). The sum of all these areas or AUC was used as a response variable (Fitzmaurice et al., 2004). SAS software (version 9) was used to perform AUC analyses.

Body weight measurement in interval-specific congenic strains (ISCSs)

To further narrow the chromosome regions harboring the QTL for body weight on rat Chr 4 in iP and iNP rats, two interval-specific congenic strains were developed by backcrossing the P.NP congenic strain with the iP line. These ISCSs included ISCS-A and ISCS-B (Fig. 3). Body weight was measured in each respective ISCS at 10 to 12 weeks of age. The ISCS-A (experimental group) consisted of both female (N=22) and male (N=23) rats, and the control group iP consisted of both female (N=15) and male (N=10) rats. ISCS-B (experimental group) consisted of female (N=13) and male (N=15) rats, and the control group iP consisted of female (N=35) and iP male (N=23) rats.

RESULTS

Transfer of the Chr 4 QTL interval affects body weight in both male and female P.NP and NP.P congenic strains

At 10 weeks of age, the male and female P.NP congenic strains showed a significant increase in body weight compared to their respective background iP strains ($p<0.05$; Fig. 1A–B). Likewise, the NP.P congenic male and female strains showed a significant decrease in body weight compared to their respective background iNP strains ($p<0.05$; Fig. 1A–B). Growth curves comparing the P.NP and NP.P congenic strains to their respective background strain confirmed these findings. For example, significant growth rate differences were observed in all four comparisons in males using AUC analysis (Fig. 2A): iP verses iNP ($p<0.0008$), iP verses P.NP ($p<0.0001$), iNP verses NP.P ($p<0.0001$), and P.NP verses NP.P ($p<0.0001$). In females (Fig. 2B), significant differences were observed between the iP and P.NP ($p<0.016$) as well as the P.NP and NP.P strains ($p<0.0008$). These findings demonstrate that the body weight of congenic rats follow the phenotype of donor strain.

The QTL for body weight was narrowed to two regions on Chr 4

We recently developed ISCS-A and ISCS-B strains that display overlapping chromosomal regions utilizing marker-assisted backcrossing of the P.NP strain with the iP line (Fig. 3, top panel). This allowed the Chr 4 QTL interval associated with the body weight phenotype to be significantly narrowed. For example, ISCS-A animals showed a significant increase in body weight in both females ($p<0.002$) and males ($p<0.008$) when compared to the iP background. In contrast, neither female nor male ISCS-B rats showed body weight difference (Fig. 3, bottom). These findings clearly indicate that the QTL for body weight was successfully narrowed to these two chromosomal regions. The first region is localized between *D4Mgh151* and alpha-synuclein (*Snca*), and the second region is localized between markers *D4Rat35* and *D4Rat233*.

DISCUSSION

In this study, we characterized body weight in the genetically modified P.NP and NP.P Chr 4 congenic rats as compared to their progenitor iP and iNP lines, respectively. We observed significant differences in body weight that were associated with the transfer of the Chr 4 QTL region. In addition, growth curves that were generated for each respective congenic and inbred line further established that the Chr 4 QTL region in iP and iNP rats that was initially identified for its relevance to alcohol consumption also affected body weight. Furthermore, comparing the interval-specific congenic strains (ISCS-A and ISCS-B) to their progenitor strains, we significantly narrowed the Chr 4 QTL interval for body weight, and we identified four positional candidate genes including neuropeptide Y (*Npy)*, juxtaposed with another zinc finger gene 1*(Jazf1),* corticotrophin releasing factor receptor 2 *(Crfr2)* and LanC lantibiotic synthetase component C-like 2 (*Lancl2*). Interestingly, both *Npy* and *Crfr2* were previously identified as candidate genes for alcohol drinking behavior in the iP and iNP strains (Spence et al., 2005; Spence et al., 2009). In addition to body weight, we speculate that a common variant may be contributing to the differences in both body weight and alcohol consumption associated with the Chr 4 region in iP and iNP rats as a possible pleiotropic effect(s). Finally, human studies demonstrate that functional genetic variation in NPY alters behavior as well as metabolism (Karvonen et al., 1998; Zhou et al., 2008).

In addition to our findings, several other rat strains have been identified that exhibited a divergence in body weight in response to selective breeding for alcohol consumption including the P/NP, HAD1/LAD1 (high/low alcohol drinking), HAD2/LAD2, and AA/ANA (Alko alcohol/Alko non-alcohol) strains (Alam et al., 2005; Apter and Eriksson, 2006). This co-divergence of body weight and alcohol preference may potentially result from a common biologically active variant(s) (Ehlers and Wilhelmsen, 2006). In fact, a recent SNP analysis using recombinant inbred rats indicated that energy metabolism and caloric intake control may influence aspects of alcohol consumption (Tabakoff et al., 2009). Therefore, selective breeding for alcohol-seeking behavior in the P and NP rats may have fixed genes that influence the homeostatic regulation of appetitive and consumptive behaviors.

The present study identified candidate genes of interest that were previously implicated in the modulation of both consummatory and alcohol drinking behavior including *Npy* and *Crfr2*. Npy is a neuromodulator of body weight, feeding, and alcohol-seeking behavior. Relative to iNP rats, iP rats exhibit decreased levels of *Npy* mRNA in various regions of the limbic system (Spence et al., 2005), and *Npy* expression is lower in the cortex and caudate putamen of iP rats compared to P.NP rats (Liang et al., 2010). Previous studies have demonstrated that intracerebroventricular (ICV) administration of NPY decreases alcohol consumption, but increases food consumption in P rats (Cowen et al., 2004; Thiele and Badia-Elder, 2003). Furthermore, an *Npy* knockout study demonstrated that NPY is required for food intake (Sindelar et al., 2002), and ICV administration of NPY increases food intake

in rats (Zarjevski et al., 1993). In addition, the iP and iHAD1 rats display lower levels of *Npy* expression than the iNP and iLAD1 rats, respectively, and both iP and iHAD1 show significantly lower body weights (Alam et al., 2005). These findings suggest that *Npy* expression may contribute to the difference in body weight seen between the iP rats and P.NP rats.

Similar to *Npy*, *Crfr2* has been identified as a potential genetic target underlying the Chr 4 QTL interval, and is known to modulate hypothalamic-pituitary-adrenal (HPA) axis regulation (Bale and Vale, 2004). In the hypothalamus, *Crfr2* has been implicated in controlling food intake (Fekete et al., 2007). Relative to iNP rats, iP rats exhibit decreased levels of *Crfr2* mRNA in various brain regions (unpublished observation, T. Liang, 2011).

Two microarray studies comparing iNP with NP.P (Carr et al., 2007) and iP with P.NP (Liang et al., 2010) identified expression differences in *Jazf1*. *Jazf1* is associated with type 2 diabetes and obesity (Grarup et al., 2008; Zeggini et al., 2008). Differential expression of *Lancl2* was also observed between iP and iNP rats (unpublished observation, T. Liang, 2011). LANCL2 is a possible target for anti-inflammatory and anti-diabetic agents (Lu et al., 2011; Sturla et al., 2009).

In summary, we identified a QTL for body weight on Chr 4 that is co-localized with the previously identified QTL for alcohol consumption observed in an iP x iNP F2 population. By employing Chr 4 reciprocal congenic strains, we confirmed that the Chr 4 QTL region harbors a biologically active variant(s) that influences body weight. Using ISCSs, we successfully fine-mapped the body weight QTL, and identified candidate genes of interest including *Npy*, *Jazf1*, *Crfr2* and *Lancl2*. Our findings suggest that these genes may exhibit a pleiotropic effect(s) on body weight and alcohol drinking behaviors. However, further studies are necessary to establish such a relationship. Future experiments will focus on identifying functional variation in the DNA sequence of these genes to further characterize the gene(s) and pathway(s) associated with these phenotypes.

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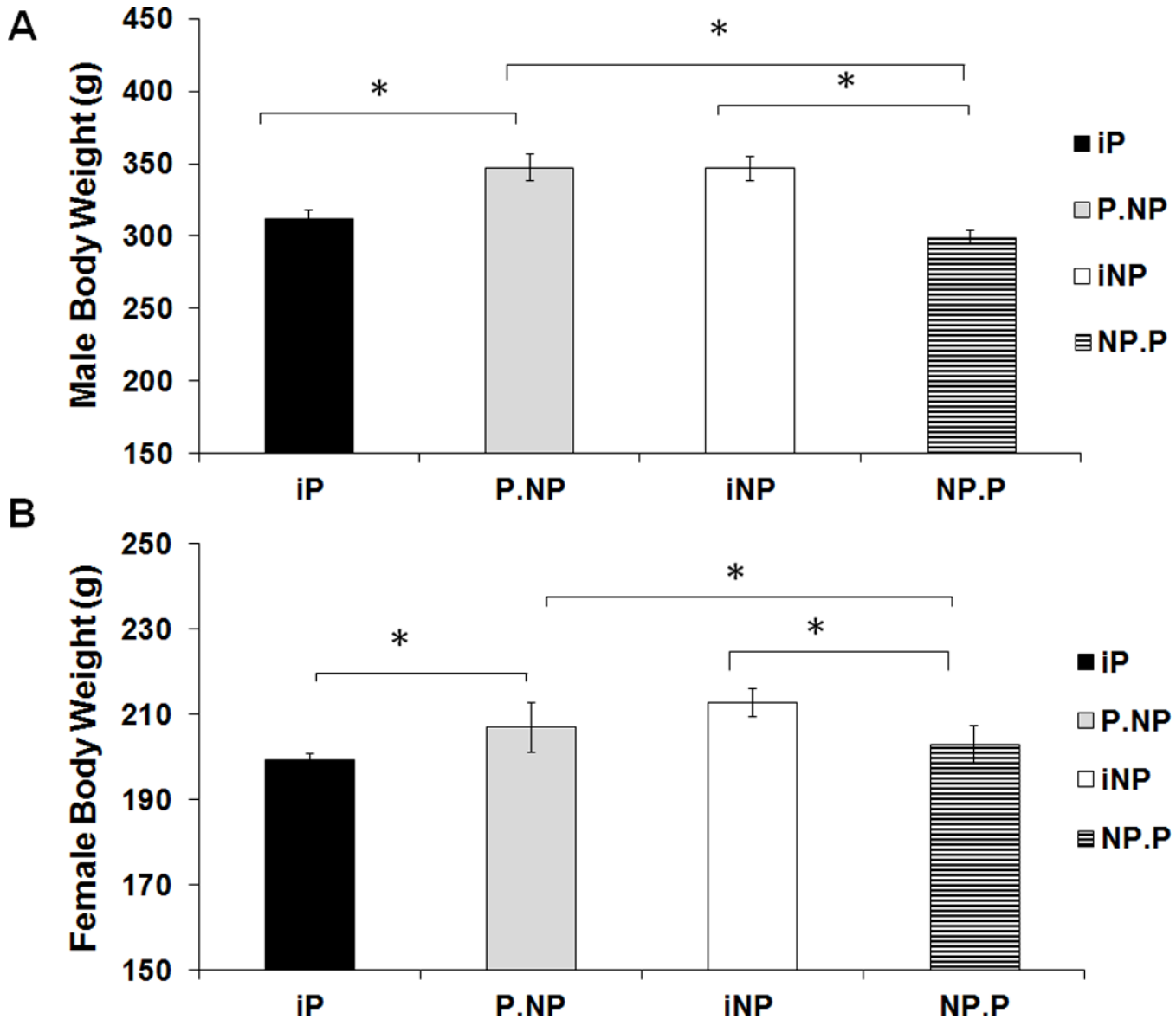

Figure 1.

Male and female body weight at 10 weeks age in iP, iNP, P.NP, and NP.P congenic strains. The effect of the Chr 4 QTL for body weight was detected in both (A) male and (B) female rats. The transfer of the Chr 4 QTL into P.NP rats is associated with increased body weight compared to the iP line, while the transfer of the Chr 4 QTL into NP.P rats leads to decreased body weight compared to the iNP line. Significant body weight differences were also observed between P.NP and NP.P in both male and female rats. * indicates $p<0.05$ and bars show ± SEM.

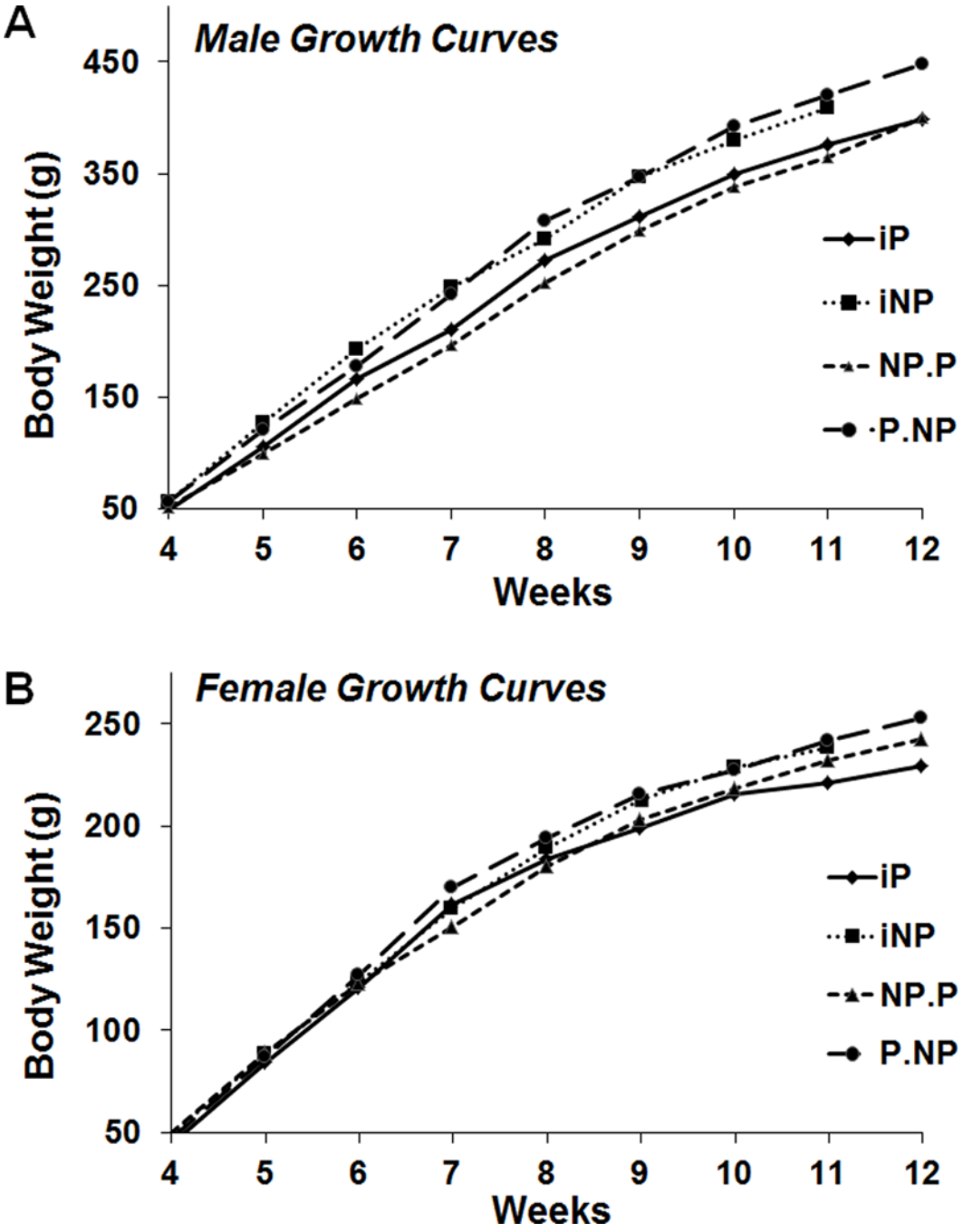

Figure 2.

The growth curves of male and female inbred and congenic rats. The growth rates were determined in both male (A) and female (B) congenic rats. These findings indicate that the growth rate of the congenics P.NP and NP.P is consistent with that of the donor strain iNP and iP, respectively. Further statistical analysis indicated that the body weight changes in the congenic rats are more significant in the male than the female rats.

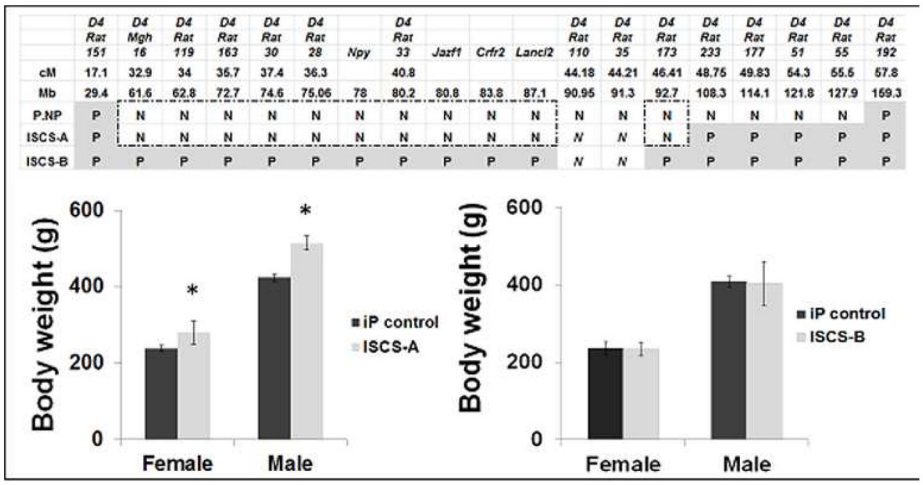

Figure 3.

ISCS-A and ISCS-B strains show differences in body weight compared to their respective iP background. ISCS-A and ISCS-B were generated by back crossing P. NP with iP rats. The transferred Chr 4 genomic region was confirmed by marker-assisted genotyping. Similar to the P.NP strain, the replacement of iP genomic regions with iNP genomic regions in ISCS-A rats significantly increased body weight in both female ($P<0.002$) and male ($P<0.008$) when compared to iP rats. No difference in body weight was detected in the ISCS-B rats compared to their background. The overlapping genomic region (dashed line) contains potential candidate genes. P: alcohol-preferring rat, N: alcohol-nonpreferring rat. P.NP: congenic rat in which the iNP Chr 4 QTL interval was transferred to the iP rat.