A novel ATPase on mouse chromosome 7 is a candidate gene for increased body fat

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RECENT STUDIES HAVE MAPPED quantitative trait loci (QTL) that influence murine body weight or body fat content (18, 34–36, 38, 39), including five that map to mouse chromosome (Chr) 7. One of the Chr 7 body weight QTLs (18) is flanked by markers D7Mit62 and D7Mit66, a 12-cM region in distal Chr 7 that includes the tubby (tub), uncoupling protein 2 (ucp2), and uncoupling protein 3 (ucp3) genes. The other four QTLs implicate a more proximal gene or genes near the mouse pink-eyed dilution (p) locus. One of these (18) is linked to D7Mit193-D7Mit82-D7Mit84-D7Mit62, a 10-cM interval that includes p (11). Additional reports (34, 35), using two different genetic crosses, also suggest that a gene or genes near the p locus affects either the body fat or body weight of mice.

Oak Ridge National Laboratory (ORNL) generated and maintains a large collection of mouse stocks that carry radiation-induced chromosomal deletions at the p locus (11, 16, 30). Mice heterozygous for a subset of these homozygous-lethal deletions that extend distally from p (9, 10, 11, 16) become visibly fat as they age. By assessing the body fat of deletion heterozygotes of 30-wk-old mice that inherit the deletion either maternally or paternally, we have deletion-mapped this phenotype to the Gabrb3-Ube3a interval (named p-locus-associated obesity-1, plo1) of mouse Chr 7. DNA sequencing has discovered within this interval only one gene, a member of the third subfamily of P-type ATPases, which we have named p-locus fat-associated ATPase (pfatp).

Northern blot analysis and RT-PCR data show that this ATPase transcript is expressed at high levels in mouse and human tissues with predominant expression in the testis and lower levels of expression in adipose tissue and other organs. We propose this ATPase as the prime candidate for the gene at the plo1 locus modulating body fat content in the mouse. The unusual inheritance pattern of this phenotype suggests either genomic imprinting, known to occur in other local genes (Ube3a, Ipw), or an effect of maternal haploinsufficiency during pregnancy or lactation on body fat in the progeny.

Methods

Mice. Males and females for body fat phenotyping were derived from reciprocal matings p+/p− × p+/p′ and p+/p × p′/p′, where p− (p"extra dark") is a fully viable intermediate allele at p (p7.75M), and p′ (p"lethal") is any one of four Oak Ridge p deletions: p8FDFoD, p7THO-II, p23DFiOD, and p29Fih6 (16, 30). Each deletion stock is maintained by closed-colony random matings, so that the deletions are not congenic on an inbred background; phenotype comparisons are made for different genotypes within each stock as well as between stocks to rule out overriding genetic background influences on the phenotype. The progeny from these crosses are easily visually genotyped, since p+/p′ confers a darker coat color than p+/p′. See Fig. 1 for the relative distal extent of each of these deletions.

Body composition analysis. Body fat measurements were carried out on both male and female deletion heterozygotes from the reciprocal crosses and their p+/p′ littermates as...
Fig. 1. An integrated genetic and physical map of the distal breakpoints of the p30PUb, p30Pud, p8FDFoD, and p23DFoD deletions in murine chromosome 7 (Chr 7). The distal breakpoint for p23DFoD is within the Gabrb3 gene. The yeast artificial chromosome (YAC), Y437H8, and five bacterial artificial chromosomes (BACs), B131k11, BH6, BD5, BA13, and B290h08, covering the critical region are shown. The insert sizes are shown in parentheses. The STS sequence for the distal end of the Y437H8 YAC clone is designated Y437H8.L. The 5‘ end of the pfatp gene is located at the centromeric end of BAC B290h08 and extends 3‘ toward the telomere.

BAC DNA was isolated using the Qiagen Mega Prep Kit (Qiagen, Santa Clarita, CA) using twice the recommended volume for P1, P2, and P3 solutions. All other steps were according to the manufacturer’s instructions. The BAC DNA was further purified through two rounds of CsCl gradient centrifugation (31). About 20% of the DNA was then sheared using a Gene Machine HydroShear on a setting of 8, and the sheared DNA was treated with T4 polymerase to make blunt ends. Subsequently, the DNA was subcloned into EcoR V digested and phosphatased pBSK– (Stratagene, La Jolla, CA). The ligated DNA was electroelaborated into E. coli DH5α cells (GIBCO BRL, Rockville, MD), and putative transformants were selected on Luria broth/ampicillin (100 µg/ml) plates.

Plasmid DNA was isolated on a BioRobot 9600 using the QIAprep 96 Turbo BioRobot Kit (Qiagen). These templates were then sequenced with either the Dye Primer or Big Dye Terminator Kits (PE Biosystems, Foster City, CA), and the products were run on an ABI377 DNA sequencer.

Sequence data were processed and assembled using the Phred, Phrap, and Consed programs on an SGI O2 workstation. Repeat sequences were identified using Repeat Masker (Univ. Washington, Seattle, WA). Additional analyses were performed using either the Wisconsin Package of programs, Version 10 (Genetics Computer Group, Madison, WI), the Genome Channel software (ORNL, Oak Ridge, TN; http://grail.lsd.ornl.gov/channel/index.html), or the NCBI BLAST (http://www.ncbi.nlm.nih.gov) program.

Expression analysis. Total RNAs from the brain, kidney, liver, lung, testis, and abdominal white adipose tissue from C3Hf/RI mice were isolated for Northern blot analysis (8, 31). Human white adipose tissue RNA (2.5 µg/µl) was obtained from Invitrogen, Carlsbad, CA. A 1.2-kb EcoR I–Not I restriction fragment from the mouse expressed sequence tag (EST) (AA116479; Genome Systems) was used as a probe for the Northern analysis of mouse tissues. A preliminary evaluation of differential expression of the portion of the candidate

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A novel ATPase on mouse chromosome 7

RESULTS

Plo1, a locus affecting body fat in mice, maps to the p deletion complex. Comparative body fat analysis showed that both females and males inheriting the p30PUb and p8FDFoD deletions maternally have nearly 1.5–2 times the actual weights of the individual fat pads (Table 1) and the AIs than those inheriting the deletion paternally (Fig. 2, Table 1). Mice heterozygous for either the p8FDFoD or the pTHO-II deletion did not differ in body fat depending on the parental source of the deletion. For deletions p23DFoD and p8FDFoD the body fat content of p/p progeny was also analyzed. Progeny from mothers carrying the p23DFoD mutation, whether p/p or p/p, had similar body fat content and similar AI values, and both body fat content and AI were nearly double for the p/p progeny inheriting the deletion paternally (Fig. 2, Table 1). In contrast, for p8FDFoD, body fat content for both male and female progeny was the same among p/p, p/p, and p/p from maternal inheritance, and p/p from paternal inheritance.

These body fat effects seemed to influence the mass of all adipose depots equally (Table 1). In addition, these effects on body fat were not due to a general increase in somatic growth, since the AI (which normalizes for body size) also showed an effect when the deletion was inherited from a p23DFoD or p30PUb dam, an effect not seen in p8FDFoD or pTHO-II. However, in females that inherited p23DFoD or p30PUb maternally, the kidney, liver, and spleen weights were significantly greater than the female mice that inherited p23DFoD or p30PUb paternally (Table 2); again, this effect was not seen in p8FDFoD or pTHO-II progeny. A similar trend in organ weight was found in the male progeny that inherited p23DFoD or p30PUb maternally, suggesting a

<table>
<thead>
<tr>
<th>Parent Carrying Deletion</th>
<th>Male</th>
<th>Female</th>
</tr>
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<tbody>
<tr>
<td>Sample Size</td>
<td>Body WT, mg</td>
<td>Gonad.</td>
</tr>
<tr>
<td>23DFoD</td>
<td>12</td>
<td>20.00 (6.03, 27.44)</td>
</tr>
<tr>
<td>8FDFoD</td>
<td>10</td>
<td>21.14 (12.76, 37.32)</td>
</tr>
<tr>
<td>p30PUb</td>
<td>15</td>
<td>20.42 (14.03, 27.45)</td>
</tr>
</tbody>
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Values are means ± SE. The gender of the parent from which offspring inherited deletion, or gender of parent carrying the deletion but not passing the deletion to its offspring, is shown. (Sire, Dam). ND, no deletion inherited; Body wt, average of left and right retroperitoneal adipose depot weights; Gonadal, average of left and right gonadal adipose depot weights; RP, average of left and right retroperitoneal adipose depot weights; Adiposity, adipose depot weights/organ weights × 100; ai, p < 0.05.
somatic growth effect of maternal inheritance of these deletions but of lesser degree than the effects on body fat content. These data clearly suggest that the minimal critical region affecting body weight, in the mice described above, lies between the distal breakpoints of p4THO-II and p23DfiOD (Fig. 1).

Pfatp, a putative aminophospholipid transporter, maps to the Plo1 locus. A YAC/BAC contig of the region between Gabrb3 and Ube3a/IPW has been generated. A single YAC, Y437H8, and five BACs, B131k11, BH6, BD5, BA13, and B290h08, cover the entire critical region (Fig. 1). A detailed sequence analysis of all the genomic clones was undertaken. Sequence coverage of about five to six times was achieved for each BAC. Blast sequence similarity search of the National Center for Biotechnology Information (NCBI) nonredundant database with the genomic sequence of BAC B290h08 showed about 99% identity with two mouse ESTs, AF011337 (resequenced and submitted to the database as AA116479) and AF156549. These cDNA sequences of ~1.2 kb and 4.5 kb, respectively, code for a M. musculus putative E1-E2 ATPase mRNA, belonging to the class V of the third subfamily of P-type ATPases (12, 13). We have named this gene “p-locus fat-associated ATPase” (pfatp). The cDNA sequence reported in AF156549 is full length. It shows an open reading frame of 4,526 bp, encoding a protein of 1,508 amino acids. Furthermore, the sequences are homologous with a human 5.5-kb cDNA (AB0111338) of an unknown function, and there is 88% identity for predicted amino acid sequence between the mouse and human cDNAs (15). This human cDNA isolated from brain cDNA library is not full length and maps to human 15q11-q13, which is homologous to the region between Gabrb3 and Ube3a/IPW in mouse Chr 7. Analysis of the DNA sequence from the other BACs identified no unknown transcriptional units.

The mouse EST AA116479 was mapped by Southern blot analysis using the interspecific hybrid genomic DNAs to the p deletion complex (data not shown). The cDNA sequence was then deduced by aligning the sequences of the mouse EST (AF156549) and human EST (AB0111338) ATPases with the genomic sequence from BAC B290h08. This alignment showed that the DNA sequence on BAC B290h08 does not include the 5’ untranslated region and is missing 416 bases at the 5’ end of the gene. The 3’ end, however, is complete. Comparison of the predicted protein sequences from AF156549, AB0111338, and from the deduced cDNA sequence from BAC B290h08, showed that they were identical (Pileup Program; Genetics Computer Group).

Pfatp is expressed in mouse and human white adipose tissue. Northern blot analysis and RT-PCR were carried out to study the expression of this gene in mouse and human tissues. Northern blot analysis using the mouse EST AA116479 as a probe showed a single transcript of ~6.0 kb in the mouse testis RNA (Fig. 3). There was no visible hybridization signal with any of the other mouse tissues (brain, heart, kidney, liver, spleen, small intestine, and the abdominal white adipose tissue). The inheritance of the p deletion from either the dams or the sires showed no effect on the fertility of the animals. These data thus give no information about the functional significance of the expression of pfatp in the testis.

RT-PCR with primers designed from the mouse EST AA116479 was carried out using the human and mouse MTC panel of cDNAs (Clontech), as well as abdominal fat RNAs. The cDNA-specific primers were designed from cDNA sequence that would amplify the sequence specific to this particular subfamily of P-type ATPases. As shown in Fig. 4, this gene is expressed in the mouse and human brain, heart, kidney, liver, lung, skeletal muscle, small intestine, and spleen. In both species it is expressed at the highest level in the testis. Of more
relevance to the body fat phenotype, the gene is expressed in both mouse and human white adipose tissue. In the mouse embryo, the ATPase is expressed at relatively high levels at day 7 of development, and it is found by RT-PCR in a number of human tissues (colon, ovary, leukocyte, prostate, thymus, pancreas, and placenta) that were not tested in the mouse.

We also used the ORN554/ORN555 primers to assess imprinting of this gene in both the testis and abdominal fat in mice inheriting the p30PUb deletion from either the dam or the sire (Fig. 5). Expression levels were the same in mice inheriting the deletion from dam or sire and were not different from animals in the same litter that did not inherit the deletion. Although preliminary, these data suggest that the mRNA expression of pfatp is not imprinted in a simple and direct manner.

**DISCUSSION**

Body fat content analyses of mice heterozygous for a subset of distally extending chromosomal deletions that encompass the pink-eye dilution (p) region of mouse Chr 7 suggest that the phenotype of the p-linked obesity locus (plo1) is due to haploinsufficiency for a novel P-type ATPase mapping between Gabrb3 and Ube3a. These data show that maternal inheritance of either of two of the longest distally extending p deletions, p30PUb or p23DFiOD, results in significantly greater body fat content in adult mice when compared with the inheritance of the same deletion from the father. For two p deletions of lesser distal extent, p4THO-II and p8FDFoD, there is no such effect of maternal inheritance of the deletion. This effect of the maternal inheritance of the longer deletions is primarily observed in the adipose tissue since the AI, which normalizes for changes in the overall body size, is significantly influenced by the parental source of deletion. This effect of maternal inheritance of these deletions on body fat immediately suggests one of two different mechanisms, although others are clearly possible. First, the gene or genes responsible for this trait could be paternally imprinted, so that inheritance of a paternally silenced allele and a deletion would render the pup null for this ATPase. Other genes in this region are known to be imprinted in both mice and humans (26). Since we could not show simple on/off imprinting of the expression of the maternal/paternal alleles by our RT-PCR analysis of testis or adipose tissue RNAs, we assume that the ATPase is not imprinted in a straightforward way. However, these results should be considered preliminary, since exon-specific imprinting or tissue-specific imprinting has been reported (4) and we examined only the imprinting status of these deletion mutants for the 5' end of the transcript.

Second, it may be that the in utero or early weaning environment of pups is altered in heterozygous dams, affecting growth and development throughout the lifetime of the fetus/pup. There are some data that support the idea that maternal haploinsufficiency for these two longest p deletions affects growth or development of her pups. Both male and female p/ppx and p/plp off-spring of female carriers of the p23DFiOD were fat compared with p/plp progeny inheriting the same deletion from the sire. Furthermore, this effect was not found in body fat content analysis among the same genotypes in progeny from carriers of the less extensive p8FDFoD

![Image](https://phphysiolgenomics.physiology.org/32473/fig3.png)
deletion. The in utero and early preweaning environment in rodents has been characterized as a critical period during which nutritional and metabolic manipulations can have permanent effects on the body fat of the adult offspring. For example, reduction of litter size to increase milk supply (27), early overfeeding (37), and early starvation during pregnancy (2) can all have permanent effects on the body fat content in rats. Similarly in humans, gestational diabetes (32), low birth weight (6), and early starvation during pregnancy (28) all have been reported to affect later susceptibility to obesity in the adult. Therefore, it is certainly possible that haploinsufficiency of a gene or genes during pregnancy and lactation could be affecting development of the pups.

\( \text{Pfatp} \) shows a relatively higher level of expression in mouse testis than in white adipose tissue. Lesser expression of \( \text{Pfatp} \) in the adipose tissue should not be underestimated. A similar pattern of expression is reported for the \( \text{agouti} \) gene (5). \( \text{Agouti} \) normally functions to control the differential production of melanin pigments in the hair shaft. Two dominant mutations, lethal yellow (\( \text{Ay} \)) and viable yellow (\( \text{A}^{vy} \)), however, exhibit a phenotype that includes yellow fur, marked obesity, a form of type II diabetes associated with insulin resistance, and an increased susceptibility to tumor formation. It is well documented that the \( \text{agouti} \) gene product antagonizes the binding of \( \alpha \)-melanocortin stimulating hormone (\( \alpha \)-MSH) to the MC1-R melanocortin receptor and blocks the increase in cAMP leading to the default synthesis of pheomelanin. At present, it is not known whether melanocortin receptor antagonism in peripheral tissues contributes to the obesity, insulin resistance, or other pleiotropic phenotypes (22). It is interesting to note that the wild-type \( \text{agouti} \) gene is expressed in human adipose tissue, even though it is not yet clear whether any mutations in the human gene are associated with an obesity-related phenotype (20). These data, coupled with the experiments involving transgenic mice expressing \( \text{agouti} \) under the control of the adipocyte-specific promoter (aP2), clearly suggest a physiological role for \( \text{agouti} \) in adipocytes in both mice and humans (17, 24). The generation of antibodies and conditional knockouts/transgenics of \( \text{Pfatp} \) will help elucidate its functional role in the regulation of body fat in mice.

![Fig. 4. RT-PCR analysis of RNA from mouse (A) and human (B) tissues using primers that generate an 886-bp fragment from the 5’ open reading frame of AA116479. Amplification of \( \beta \)-actin was used as a positive control for these samples. The primers are described in the METHODS. In the mouse, the highest expression was in testis, day 7 embryo (“Embryo 7d”), and adipose tissue. In human, expression was highest in kidney, testis, spleen, colon, and prostate. Sk muscle, skeletal muscle; Small Int, small intestine.](http://physiolgenomics.physiology.org)

![Fig. 5. RT-PCR analysis of white adipose tissue (WAT) and testis RNA from wild-type mice and from mice inheriting the \( p^{dopC} \) deletion from either the dam or the sire. There was no difference in the abundance of the transcript depending upon the gender of the parent from which they inherited the deletion, or deletion status.](http://physiolgenomics.physiology.org)
The P-type ATPases are the most extensively studied membrane transporters (21, 23). Two well-recognized subfamilies have been described: the non-heavy metal ion transporters, e.g., Ca²⁺, Na⁺/K⁺, and H⁺/K⁺, and the heavy metal ion transporters, e.g., Cu²⁺. Recently, a third subfamily proposed to be transbilayer amphipath transporters has been described (12, 13, 33). Seventeen different mammalian genes from this subfamily have been identified. The majority of the DNA sequence for members of this subfamily is characteristic of the P-type ATPases; however, some specific motifs are useful for further classification into five distinct classes of ATPases (12). The mouse cDNA, AF156549, the human homolog, AB0111338, and the deduced cDNA from BAC B290h08 belong to class V of the third subfamily of P-type ATPases. The exact function of these class V ATPases is still not known. Cloning and comparison of a bovine and murine class II ATPase purified from chromaffin granules with the previously identified yeast class I gene, DRS2, suggest that aminophospholipid translocation could be a general function of members of this subfamily (3, 7, 29, 33). Given these data, it is likely that the novel ATPase we have identified and named pfatp is involved in the transport of phospholipids.

With the current experiments, we have not yet proved that pfatp is the gene responsible for modulating body fat content in animals carrying p locus deletions. Our deletion mapping has defined the critical interval for the plo1 locus, and sequence analysis has identified only one transcript from this interval. Based on the sequence coverage of the critical region, the likelihood of detecting another gene is very low. Further, the homology of pfatp with other genes shown to be involved in phospholipid trafficking as well as its expression in the white adipose tissue suggests that it might be involved in lipid metabolism. Although this is certainly reasonable evidence that pfatp is the gene affecting body fat content in the minimal deletion interval, it is possible that there are other genes or parts of genes, or regulatory elements, within the deletion interval. Further studies of the biological role of this ATPase, along with targeted deletion of this candidate, will be necessary to confirm its possible role in the regulation of adipose depot lipid stores.

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