The fa leptin receptor mutation and the heritability of respiratory frequency in a Brown Norway and Zucker intercross

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Obesity is a major risk factor for a number of chronic respiratory conditions, including hypoventilation and sleep apnea, and for symptoms of dyspnea and exercise intolerance (13). The pathophysiological mechanisms that link obesity and disorders of respiratory control are often linked to mechanical loading of the respiratory system (13); however, evidence exists for a more direct influence on respiratory control by metabolic factors that also lead to the obese state (27). The discovery of leptin (1, 9, 21), an adipocyte-derived hormone that directly regulates both adiposity and energy homeostasis, intensified the interest in the role of molecular mechanisms in the development of obesity and its comorbidities. One primary mechanism of leptin, produced predominantly by white adipose tissue, is to inhibit neuropeptide Y, a potent stimulator of food intake (14). Thus leptin functions in part by decreasing food intake and partitioning metabolic fuels toward utilization and away from storage (9). Human obesity, which is associated with elevated plasma leptin levels, can be considered a state of resistance to leptin. Inheritance of a complete disruption of leptin receptor or of leptin is rare; thus other modifying peptides may lead to leptin resistance (45).

Leptin is also linked to the control of respiration. Mutant mice that constitutively lack circulating leptin display respiratory depression and elevated arterial PCO2 (44). Treatment with exogenous leptin improves the ventilatory response by increasing minute ventilation (Ve), in both sleep and waking states (26), and has lead to the hypothesis that the leptin pathway is one common link between obesity and disorders of respiratory control such as sleep apnea (27).

The most common rat model studied for obesity is the Zucker strain. These animals become obese and hyperleptinemic because they have a missense mutation (Glu296Pro point mutation; fa) in the leptin receptor (12). Homozygotes (fa/fa) with this mutation exhibit markedly diminished responsiveness to leptin and are considered an in vivo surrogate model for leptin resistance, a feature of the human metabolic syndrome (15). One consistent finding in studies comparing lean (fa/wt or wt/wt, where wt represents wild type) and obese (fa/fa) Zucker animals is of a higher respiratory frequency in obese animals (see Refs. 16–18, 22, 24, 36, 40), thus leading to the conclusion that obesity per se induces higher breathing frequencies.

In contrast, another rat strain, the Koletsky, which also bears a mutation in the leptin receptor, shows breathing patterns different from the Zucker; obese Koletsky and Zucker rats, with similar fat accumulation and distribution patterns, respond differently to ventilatory challenges (39). In general, the Koletsky rats showed lower breathing frequencies and tidal volumes (Vt) than the Zucker rats, regardless of the respiratory challenge. This physiologically based experiment, which contrasted breathing patterns in rat strains with the obesity phenotype, demonstrated that obesity by itself may not be sufficient to explain the differences in the breathing rate and depth, leading to the conclusion that other sources of variation, such as strain (genetic) divergence, could be responsible for the dissimilarity in breathing patterns between Zucker and the
Koletsky animals. If genes distinct from the leptin receptor would control ventilation, then phenotypes would segregate independently of obesity in intercrosses where weight and ventilation parameters differ between parental strains.

To determine which of the two competing mechanisms better explains breathing patterns in context of obesity, we intercrossed the Brown Norway (low breathing frequency, nonobese strain with normal/wild-type alleles at the leptin receptor) and the Zucker (moderately high breathing frequency, homozygous for the fa mutation) strains. In addition to the difference in the level of obesity, these two strains demonstrated substantial differences in frequency of respiration in our laboratory’s (40) initial assessment of strain ventilation characteristics; thus this experiment is equivalent to establishment of a dihybrid cross. In physiological terms, we tested characteristics; thus this experiment is equivalent to establishment of a dihybrid cross. In physiological terms, we tested our laboratory’s (40) initial assessment of strain ventilation parameters differ between parental strains.

**MATERIALS AND METHODS**

**Animal breeding and history of the strains.** Two rat strains, Zucker and Brown Norway, were selected for an F2 cross because of the previously described strain differences in ventilatory and metabolic phenotypes (39, 40). The original source of the Brown Norway colony was Harlan Laboratories (Indianapolis, IN), where brother-sister mating was maintained for 23 generations, and further inbred for another generation. Ventilation and metabolism were assessed by whole body plethysmography via the open-circuit method (40). Briefly, the chamber consisted of a 14-cm-diameter Plexiglas cylinder of 8.4 liter volume, with air intake and output ports to allow for different gas mixtures to be flushed through the chamber at a rate of 30 l/min and a low continuous flow of the gas to be drawn through the chamber during the testing period at a rate of 600 ml/min to prevent CO2 buildup and to maintain an ambient chamber temperature. A small opening at the top of the chamber contained a section of PE50 tubing, through which a sample of chamber air was obtained for assessment of oxygen and carbon dioxide concentrations within the chamber for metabolic analysis. Ventilatory parameters evaluated were VE or the total volume of air breathed per minute (in units of ml/min), VT (in units of ml), and breathing frequency (in units of breaths/min); metabolic parameters evaluated were oxygen consumption (VO2; in units of ml/min), carbon dioxide production (VCO2; in units of ml/min), and respiratory quotient (the ratio of VCO2 to VO2). VE was computed as breathing frequency multiplied by the VT.

**Ventilatory parameters were recorded both by strip chart and with an analog-to-digital converter coupled to a computer. Two setups allowed for two animals to be tested at a time.**

**Protocol.** The sequence of testing is done only once per animal so that each animal comes to the testing paradigm without prior experience to the sequence of challenges. Hence, random sources of variance in environment present at each point and behavioral responses may influence trait values. Testing was done between 10:00 AM and 1:00 PM. Each animal was allowed 45 min to acclimate to the chamber. Temperature (via an implanted animal identification-temperature transponder; model IPTT 100, Biomedical Data Systems, Seaford, DE) was measured before and after each testing session. Baseline resting ventilation and carbon dioxide and oxygen concentrations were continuously recorded during this acclimatization period. At the end of the acclimatization period, five measurements were taken over a 15-min period to provide resting values (a1) for ventilation and metabolism (see Table 1). A 5-min presentation of the test gases was conducted in the following order: 10% O2-balance nitrogen (h) and then 100% O2 (o). This was followed by a 20-min rest period in room air and second collection of resting values (a2), after which the animal was then exposed to a 5 min of 7% CO2–93% O2 (c) and then 3% CO2–10% O2-balance nitrogen (b).

**Table 1. Schedule of testing and value collection for each animal**

<table>
<thead>
<tr>
<th>Challenge</th>
<th>Composition</th>
<th>Time Points Measured, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoxia (h)</td>
<td>10% O2-balance N2</td>
<td>1, 2, 3, 4, 5, 5</td>
</tr>
<tr>
<td>Hypoxia (o)</td>
<td>100% O2</td>
<td>1, 1.5, 2, 3, 4, 5</td>
</tr>
<tr>
<td>Hypercapnia (c)</td>
<td>7% CO2-balance O2</td>
<td>1, 2, 3, 4, 5, 5</td>
</tr>
<tr>
<td>Isocapnic hypoxia (b)</td>
<td>10% O2-3% CO2-balanced air</td>
<td>1, 2, 3, 4, 5, 5</td>
</tr>
</tbody>
</table>

*Temperature before collection of Baseline Value and after the last challenge. †Collection of weight and length measures. See text for further explanation of abbreviations. h, Hypoxia (10% O2-0% CO2-balance nitrogen); o, hypoxia (100% O2); c, hypercapnia (9% O2-7% CO2); i, isocapnic hypoxia (10% O2-3% CO2-balance nitrogen).
Our logic for the sequence of testing was as follows. The first sequence tests a traditional hypoxic exposure, without controlling for hypocapnia, within which there might be a complex of responses deemed early (h1 and h2) and late responses (h4 and h5) (34). The timing of data collection was chosen to represent these transitions from peripheral to central chemosensitivity, which in reality may be quite variable. The reoxygenation with oxygen was chosen to allow us to examine also an early response (o1 and o2), e.g., a “Déjours” test (4), which is known to differ among strains (41). Values at a later time (o4 and o5) would be more likely to represent the effect of hyperoxia at rest and thus an inhibition of carotid body activity at rest at a time more remote from hypoxic exposure. The second series of exposures was chosen to test central drive with hyperoxic hypercapnia. Proceeding onto a hypercapnic hypoxic gas mixture introduces complexity, but the intent was to see whether the transition from hyperoxic hypercapnia to hypoxia in the presence of supplemental carbon dioxide to prevent severe hypocapnia might give insight into interactive effects. The time points of interest were therefore the later values (c4–c5, b4–b5).

During the challenges, ventilatory parameters were continuously recorded, and values representative of each challenge were obtained in the last 15–20 s of each minute during the challenge. We used a respiratory-based software program to score breaths. Sniffs or sighs were not included in the calculations of VT and breathing frequency. A mean value under each condition was entered for each animal. At

\[\text{Variance (Brown Norway)} = \sigma_g^2\]

\[\text{Variance (Zucker)} = \sigma_e^2 + \sigma_g^2\]

\[\text{Variance (F1)} = \sigma_g^2 + \frac{1}{2}\sigma_e^2\]

\[\text{Variance (F2)} = \sigma_e^2 + \sigma_g^2 + \frac{1}{4}\sigma_e^2\]

where \(g\) represents the genetic component and \(e\) is an unknown variance value between 0 and 1.

We estimate \(\sigma_g^2\) as a weighted average of variance (Brown Norway) and the absolute value of \(2 \times \text{[variance (F1) − variance (Zucker)]}\) based on sample size. Hence, heritability can be estimated as

\[H^2 = \frac{\text{[variance (F2) − \sigma_g^2]} / \text{[variance (F2)]}}{2}\]

Effect of the fa allele. For each of the traits, we determined the effect of the fa allele by calculating the phenotype mean for each of the three possible genotypes in the F2 generation. ANOVA was used for comparisons of ventilation and its components, metabolism, and derivative values among F2 animals with homozygous wild-type (wt/wt), heterozygous (fa/wt) and homozygous affected (fa/fa) to obtain specific modes of inheritance. Four different models (recessive, dominant, additive, and overdominant) were fitted to the data with the use of the SPSS statistical software package. For example, a recessive model referred to animals with a fa/fa genotype having a trait value significantly different from the fa/wt and wt/wt animals, with the fa allele being recessive to the wild-type allele. The dominant model was very similar to the recessive, with the wild-type (wt/wt) genotype being recessive to the other two groups. In the additive model, the value of the heterozygote (fa/wt) category was fixed exactly midway between the means of the two homozygotes. Finally, to fit a dominance component where each allele interacted in a multiplicative manner, an overdominance model was also examined. Here, the heterozygote (fa/wt) was allowed to assume a value two times greater than the average of the two homozygote classes, such that the joint effect of the two alleles (in the heterozygote) was greater than expected if the effect of each were considered singly (as in each homozygote). The best-fitting ANOVA model was chosen on the basis of which had the largest F statistic. The threshold for reporting a model was a significance level of \(P < 0.05\) or greater. Because models were run on the 53 traits with heritability estimates, a Bonferroni correction was also performed to estimate a conservative level of statistical significance with an \(\alpha = 0.05/53 = 0.000943\) level.

RESULTS

To determine how the fa allele affected heritability of ventilatory traits, 28 Brown Norway rats, 15 Zucker rats, 28 Brown Norway-Zucker F1 hybrids, and 73 F2 progeny were measured for several ventilatory and metabolic parameters. Among the F2 generation, genotyping of the Glu296Pro mutation in the leptin receptor was necessary to distinguish between homozygous (wt/wt) and heterozygous (fa/wt) lean animals. Data were tested for departure from Hardy-Weinberg
Table 2. Covariate-adjusted values for variables that describe the baseline characteristics of the Brown Norway parents, Zucker parents, F1 generation, and the F2 generation rats before respiratory challenges

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>F0 BN</th>
<th>F0 Z</th>
<th>Strain Effect</th>
<th>Sex Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>P Value</td>
<td>P Value</td>
</tr>
<tr>
<td>Age, wk</td>
<td>28</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length, cm</td>
<td>20.25</td>
<td>20.40</td>
<td>&lt;1×10⁻⁵</td>
<td></td>
</tr>
<tr>
<td>Weight, g</td>
<td>208.63</td>
<td>14.87</td>
<td>&lt;1×10⁻⁵</td>
<td></td>
</tr>
<tr>
<td>Lee index, ( \text{g/cm} )</td>
<td>0.29</td>
<td>0.02</td>
<td>&lt;1×10⁻⁵</td>
<td></td>
</tr>
<tr>
<td>( V_{O_2} ), ml/min</td>
<td>5.86</td>
<td>0.07</td>
<td>5×10⁻⁵</td>
<td></td>
</tr>
<tr>
<td>( V_{\dot{O}_2} ), ml/min</td>
<td>3.38</td>
<td>0.11</td>
<td>10¹⁻⁰</td>
<td></td>
</tr>
<tr>
<td>RQ, (( V_{O_2}/V_{\dot{CO}_2} ))</td>
<td>0.63</td>
<td>0.02</td>
<td>10⁻³</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. Brown Norway, Z; Zucker, F0; parental, F1; first filial; F2, second filial; \( V_{O_2} \), \( O_2 \) consumption; \( V_{\dot{O}_2} \), \( \dot{O}_2 \) production; RQ, respiratory quotient; \( f \), breathing frequency; \( V_r \), tidal volume; \( V_e \), minute ventilation; N/A, not applicable. See text for explanation of adjustments. For each covariate, the \( f \)-test was used to evaluate whether statistically significant differences existed in phenotypic values between the F0 BN and F0 Z strains. A P value is reported in this column if the differences were statistically significant at a 5% level. For sex-effect \( P \) values, a \( t \)-test was used to evaluate whether statistically significant differences existed in the phenotypic values between males and females. All animals were included in this analysis. A P value is reported if the differences were significant at the 5% level.

Table 3. Covariate-adjusted values for \( f \), \( V_r \), and \( V_e \) in response to hypoxia among the Brown Norway parents, Zucker parents, F1 generation, and the F2 generation rats

<table>
<thead>
<tr>
<th>( f ), breaths/min</th>
<th>F0 BN</th>
<th>F0 Z</th>
<th>Strain Effect</th>
<th>Sex Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>P Value</td>
<td>P Value</td>
</tr>
<tr>
<td>1 min</td>
<td>101.64</td>
<td>101.62</td>
<td>&lt;1×10⁻⁵</td>
<td></td>
</tr>
<tr>
<td>2 min</td>
<td>112.37</td>
<td>112.75</td>
<td>&lt;1×10⁻⁵</td>
<td></td>
</tr>
<tr>
<td>3 min</td>
<td>120.81</td>
<td>120.98</td>
<td>&lt;1×10⁻⁵</td>
<td></td>
</tr>
<tr>
<td>4 min</td>
<td>129.01</td>
<td>129.21</td>
<td>&lt;1×10⁻⁵</td>
<td></td>
</tr>
<tr>
<td>5 min</td>
<td>132.29</td>
<td>132.05</td>
<td>&lt;1×10⁻⁵</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( V_r ), ml</td>
<td>1.40</td>
<td>1.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( V_e ), ml/min</td>
<td>113.89</td>
<td>113.89</td>
<td>&lt;1×10⁻⁵</td>
<td></td>
</tr>
<tr>
<td></td>
<td>128.63</td>
<td>128.63</td>
<td>&lt;1×10⁻⁵</td>
<td></td>
</tr>
<tr>
<td></td>
<td>120.28</td>
<td>120.28</td>
<td>&lt;1×10⁻⁵</td>
<td></td>
</tr>
<tr>
<td></td>
<td>120.34</td>
<td>120.34</td>
<td>&lt;1×10⁻⁵</td>
<td></td>
</tr>
<tr>
<td></td>
<td>118.46</td>
<td>118.46</td>
<td>&lt;1×10⁻⁵</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. The data were analyzed using a GLM procedure with an \( f \)-test applied pairwise. The \( f \)-test was also used to evaluate whether statistically significant differences existed in phenotypic values between the F0 BN and F0 Z strains. A P value is reported in this column if the differences were statistically significant at a 5% level. For sex-effect \( P \) values, a \( t \)-test was used to evaluate whether statistically significant differences existed in the phenotypic values between males and females. All animals were included in this analysis. A P value is reported if the differences were significant at the 5% level.
Table 4. Covariate adjusted mean values for \( f \), \( V_t \), and \( V_e \) in response to hyperoxia among the Brown Norway parents, Zucker parents, \( F_1 \) generation, and the \( F_2 \) generation rats

<table>
<thead>
<tr>
<th>Strain Effect</th>
<th>Sex Effect</th>
<th>F0 BN</th>
<th>F0 Z</th>
<th>F1</th>
<th>F2 Average</th>
<th>F2 fa/fa</th>
<th>F2 fa/wt</th>
<th>F2 w/wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>( f ), breaths/min</td>
<td>( p ) Value</td>
<td>( f ) Value</td>
<td>( p ) Value</td>
<td>( f ) Value</td>
<td>( f ) Value</td>
<td>( f ) Value</td>
<td>( f ) Value</td>
<td>( f ) Value</td>
</tr>
<tr>
<td>1 min</td>
<td>69.55±2.02</td>
<td>122.54±8.48</td>
<td>&lt;1\times10^{-5}</td>
<td>0.0020</td>
<td>103.36±3.26</td>
<td>119.98±1.19</td>
<td>114.42±2.33</td>
<td>110.56±1.72</td>
</tr>
<tr>
<td>1.5 min</td>
<td>78.74±1.97</td>
<td>115.40±6.81</td>
<td>&lt;1\times10^{-5}</td>
<td>&lt;1\times10^{-5}</td>
<td>104.18±2.24</td>
<td>168.00±1.22</td>
<td>120.00±2.29</td>
<td>115.19±1.88</td>
</tr>
<tr>
<td>2 min</td>
<td>84.04±1.78</td>
<td>114.14±5.58</td>
<td>&lt;1\times10^{-5}</td>
<td>&lt;1\times10^{-5}</td>
<td>106.40±2.15</td>
<td>117.17±1.01</td>
<td>120.11±1.80</td>
<td>115.84±1.58</td>
</tr>
<tr>
<td>3 min</td>
<td>86.70±2.70</td>
<td>120.51±7.40</td>
<td>&lt;1\times10^{-5}</td>
<td>&lt;1\times10^{-5}</td>
<td>105.64±2.59</td>
<td>117.45±1.07</td>
<td>120.84±1.66</td>
<td>115.63±1.74</td>
</tr>
<tr>
<td>4 min</td>
<td>84.29±3.06</td>
<td>107.43±4.95</td>
<td>0.0002</td>
<td>111.38±2.82</td>
<td>113.22±0.87</td>
<td>112.47±1.45</td>
<td>104.65±1.87</td>
<td>106.74±2.37</td>
</tr>
<tr>
<td>5 min</td>
<td>86.67±1.84</td>
<td>103.48±4.99</td>
<td>0.0007</td>
<td>105.18±1.14</td>
<td>113.17±0.37</td>
<td>112.99±0.71</td>
<td>113.47±0.58</td>
<td>113.01±0.78</td>
</tr>
</tbody>
</table>

Values are means ± SD.

F1 animals are 50:50 genomic hybrids between Brown Norwegian female and Zucker male (fa/fa) animals that had trait means that often fell between the means for the two parental strains. As an example, F0 Brown Norway with zero \( fa \) alleles weighed on average 208.63 g vs. 322.29 g for the F0 Zucker with two \( fa \) alleles (Table 2). The F1 animals with one copy of the \( fa \) allele weighed 260.59 g, a value in between the two progenitor strains. Similarly, the value of the mean breathing frequency at rest in the F1, at 112.13 breaths/min, was in between the values for each progenitor strain (Table 2). In the F2 generation, we could follow the segregation of the \( fa \) mutation and determine whether the ventilatory parameters cosegregated with the dosage of the \( fa \) mutation. Therefore, the mean and standard deviations for ventilatory parameters partitioned by leptin receptor genotype in the F2 generation are provided (Tables 2–6). Also provided are the means and standard deviations for all the F2 animals when not differentiated by \( fa \) allele status. Thus we tested the hypothesis that rats bearing one (heterozygote) or two ( homozygote) alleles of the Glu296Pro point mutation (fa) had a uniformly high respiratory

Table 5. Covariate adjusted values for \( f \), \( V_t \), \( V_e \) in response to hypercapnia among the Brown Norway parents, Zucker parents, \( F_1 \) generation, and the \( F_2 \) generation rats

<table>
<thead>
<tr>
<th>Strain Effect</th>
<th>Sex Effect</th>
<th>F0 BN</th>
<th>F0 Z</th>
<th>F1</th>
<th>F2 Average</th>
<th>F2 fa/fa</th>
<th>F2 fa/wt</th>
<th>F2 w/wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>( f ), breaths/min</td>
<td>( p ) Value</td>
<td>( f ) Value</td>
<td>( p ) Value</td>
<td>( f ) Value</td>
<td>( f ) Value</td>
<td>( f ) Value</td>
<td>( f ) Value</td>
<td>( f ) Value</td>
</tr>
<tr>
<td>1 min</td>
<td>89.21±2.09</td>
<td>138.45±10.33</td>
<td>&lt;1\times10^{-5}</td>
<td>0.0002</td>
<td>120.65±1.65</td>
<td>127.25±1.32</td>
<td>127.13±2.28</td>
<td>128.01±2.30</td>
</tr>
<tr>
<td>2 min</td>
<td>96.84±2.26</td>
<td>142.14±9.57</td>
<td>&lt;1\times10^{-5}</td>
<td>&lt;1\times10^{-5}</td>
<td>127.71±2.58</td>
<td>131.85±1.53</td>
<td>131.65±2.06</td>
<td>132.92±3.41</td>
</tr>
<tr>
<td>3 min</td>
<td>102.97±2.49</td>
<td>141.84±8.45</td>
<td>&lt;1\times10^{-5}</td>
<td>&lt;1\times10^{-5}</td>
<td>128.64±1.94</td>
<td>132.91±1.00</td>
<td>133.49±2.00</td>
<td>132.08±1.29</td>
</tr>
<tr>
<td>4 min</td>
<td>104.80±2.82</td>
<td>137.69±9.40</td>
<td>&lt;1\times10^{-5}</td>
<td>&lt;1\times10^{-5}</td>
<td>128.17±1.84</td>
<td>135.11±0.87</td>
<td>135.68±1.68</td>
<td>134.44±1.17</td>
</tr>
<tr>
<td>5 min</td>
<td>109.49±1.86</td>
<td>137.81±6.50</td>
<td>&lt;1\times10^{-5}</td>
<td>&lt;1\times10^{-5}</td>
<td>126.96±1.60</td>
<td>135.93±0.91</td>
<td>137.78±1.76</td>
<td>134.51±1.49</td>
</tr>
<tr>
<td>6 min</td>
<td>106.58±1.75</td>
<td>135.52±6.82</td>
<td>&lt;1\times10^{-5}</td>
<td>&lt;1\times10^{-5}</td>
<td>134.60±2.50</td>
<td>134.47±0.74</td>
<td>132.97±1.24</td>
<td>135.73±1.39</td>
</tr>
</tbody>
</table>

Values are means ± SD.

In the F2 generation, we could follow the segregation of the \( fa \) mutation and determine whether the ventilatory parameters cosegregated with the dosage of the \( fa \) mutation. Therefore, the mean and standard deviations for ventilatory parameters partitioned by leptin receptor genotype in the F2 generation are provided (Tables 2–6). Also provided are the means and standard deviations for all the F2 animals when not differentiated by \( fa \) allele status. Thus we tested the hypothesis that rats bearing one (heterozygote) or two ( homozygote) alleles of the Glu296Pro point mutation (fa) had a uniformly high respiratory frequency at rest, the \( V_t \), and \( V_e \), among the Brown Norway parents, Zucker parents, \( F_1 \) generation, and the \( F_2 \) generation rats.

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frequency during rest and during chemosensory challenges, compared with wild-type animals. Similar hypotheses were tested for VT and VE. The cosegregation of the leptin receptor genotypes and ventilatory parameters can be modeled with dominant, recessive, additive, and overdominant modes of inheritance, as in a two-way cross of a binary character and a segregating locus under a specific mode of inheritance. For example, during the “isocapnic hypoxic” challenge (b.5) the variation at the fa locus explained 20.1% of the total heritability (~0.67 or 67%) under an additive model (P < 0.0009; Table 7). In traits like weight and Lee index, the effect of the fa allele at the leptin receptor locus is expectedly large (>35–45%); in contrast, only 10% of the heritability of values of VCO2 was accounted for by the fa mutation. Generally small contributions of the leptin receptor were observed in ventilatory traits, and this contribution was not consistent within a chemosensory challenge (i.e., at 2, 3, etc. minutes into the challenge). We conclude that inheritance patterns of traits for ventilatory behavior in this intercross are largely unexplained by the fa allele and are likely to represent the contributions of other regions of the rat genome.

As the fa allele was tracked in the cross, we could determine the proportion of variance attributable to the leptin receptor locus under a specific mode of inheritance. For example, during the “isocapnic hypoxic” challenge (b.5) the variation at the fa locus explained 20.1% of the total heritability (~0.67 or 67%) under an additive model (P < 0.0009; Table 7). In traits like weight and Lee index, the effect of the fa allele at the leptin receptor locus is expectedly large (>35–45%); in contrast, only 10% of the heritability of values of VCO2 was accounted for by the fa mutation. Generally small contributions of the leptin receptor were observed in ventilatory traits, and this contribution was not consistent within a chemosensory challenge (i.e., at 2, 3, etc. minutes into the challenge). We conclude that inheritance patterns of traits for ventilatory behavior in this intercross are largely unexplained by the fa allele and are likely to represent the contributions of other regions of the rat genome.

The magnitude and direction of the effects of the fa allele are reported by β (Table 7), which is equivalent to a regression coefficient. This parameter, β, provides an estimate of the effect of one fa allele in the units of measurement for the variables of interest. An example of a rather strong effect is observed in the trait for frequency during the hypoxic challenge (h3; Table 7). In this instance, the variation at the fa locus explained 17.1% of the total heritability (~0.76 or 76%) under an additive model (P = 0.002; Table 7), and animals bearing one copy of the Glu296Pro allele would have an increase in breathing frequency of 13.5 breaths/min, whereas those bearing two copies of the Glu296Pro allele would have an increase of 27 breaths/min 3 min into a hypoxic challenge. Other genetic models were also feasible for some of the traits. For instance, at the second baseline (a2), values for frequency fit a model where each allele interacted in a multiplicative manner (overdominance), such that the mean for the heterozygote was greater than the average of the two homozygote categories.

### Table 6. Covariate adjusted values for f, Vt, and Ve in response to isocapnic hypoxia (10% O2-3% CO2-balance air) among the Brown Norway parents, Zucker parents, F1 generation, and the F2 generation rats

<table>
<thead>
<tr>
<th></th>
<th>F0 BN</th>
<th>F0 Z</th>
<th>Strain Effect</th>
<th>Sex Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>P Value</td>
<td>P Value</td>
</tr>
<tr>
<td>f, breaths/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 min</td>
<td>106.06±1.96</td>
<td>142.67±6.45</td>
<td>&lt;1×10⁻⁵</td>
<td>151.14±3.31</td>
</tr>
<tr>
<td>2 min</td>
<td>117.82±1.75</td>
<td>143.98±5.57</td>
<td>&lt;1×10⁻⁵</td>
<td>151.38±2.66</td>
</tr>
<tr>
<td>3 min</td>
<td>117.89±1.86</td>
<td>144.83±4.22</td>
<td>&lt;1×10⁻⁵</td>
<td>151.65±2.51</td>
</tr>
<tr>
<td>4 min</td>
<td>121.69±2.32</td>
<td>145.74±4.37</td>
<td>&lt;1×10⁻⁵</td>
<td>153.54±2.65</td>
</tr>
<tr>
<td>5 min</td>
<td>123.47±1.44</td>
<td>148.60±4.94</td>
<td>&lt;1×10⁻⁵</td>
<td>144.80±2.10</td>
</tr>
<tr>
<td>Vt, ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 min</td>
<td>1.11±0.03</td>
<td>1.34±0.09</td>
<td>1.34±0.02</td>
<td>1.20±0.03</td>
</tr>
<tr>
<td>2 min</td>
<td>1.15±0.03</td>
<td>1.30±0.08</td>
<td>1.29±0.02</td>
<td>1.19±0.007</td>
</tr>
<tr>
<td>3 min</td>
<td>1.16±0.03</td>
<td>1.28±0.09</td>
<td>1.26±0.03</td>
<td>1.17±0.01</td>
</tr>
<tr>
<td>4 min</td>
<td>1.18±0.03</td>
<td>1.28±0.07</td>
<td>0.0005</td>
<td>1.25±0.02</td>
</tr>
<tr>
<td>5 min</td>
<td>1.14±0.03</td>
<td>1.30±0.08</td>
<td>0.0001</td>
<td>1.27±0.03</td>
</tr>
<tr>
<td>Ve, ml/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 min</td>
<td>119.32±4.77</td>
<td>193.86±17.07</td>
<td>&lt;1×10⁻⁵</td>
<td>205.13±6.85</td>
</tr>
<tr>
<td>2 min</td>
<td>139.24±3.68</td>
<td>190.39±14.09</td>
<td>5×10⁻⁵</td>
<td>189.72±3.38</td>
</tr>
<tr>
<td>3 min</td>
<td>139.07±3.83</td>
<td>188.10±10.75</td>
<td>&lt;1×10⁻⁵</td>
<td>187.39±5.56</td>
</tr>
<tr>
<td>4 min</td>
<td>143.92±6.32</td>
<td>182.62±11.69</td>
<td>2.10</td>
<td>199.96±8.23</td>
</tr>
<tr>
<td>5 min</td>
<td>143.19±6.56</td>
<td>183.68±15.28</td>
<td>5.56</td>
<td>149.25±2.35</td>
</tr>
<tr>
<td>5 min</td>
<td>136.32±4.52</td>
<td>190.51±14.44</td>
<td>7×10⁻⁵</td>
<td>181.49±5.55</td>
</tr>
</tbody>
</table>

Values are means ± SD.
In short, compared with a major effect of the \(fa\) allele on weight and body mass (Lee index), its effect on respiratory frequency, \(V_T\), and \(V\dot{E}\) was small, and models were unpredictable.

**DISCUSSION**

We conducted an intercross of Brown Norway and Zucker strains to explore the inheritance of ventilatory traits (\(V_T\), breathing frequency, and \(V\dot{E}\)) during baseline and chemosensory challenges (hypoxia, hypercapnia, and hyperoxia) in the context of a known mutation producing obesity. In contrast to the strong effects of the \(fa\) allele on weight and body mass, the effect size on ventilatory behavior at rest and during several different chemosensory stimuli was more consistent with a minor or modifying gene effect. At maximum, the leptin receptor locus accounted for \(\sim20.1\%\) of

---

**Table 7. Heritability (inclusive of all genetic effects) estimates and models that explain the effect of the leptin receptor locus**

<table>
<thead>
<tr>
<th>Trait</th>
<th>Heritability (H^2)</th>
<th>Best Fitting Model*</th>
<th>P Value</th>
<th>% Variance Explained by the Model†</th>
<th>(\hat{\beta})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, g</td>
<td>77.5 (9)</td>
<td>Recessive</td>
<td>(4 \times 10^{-4})</td>
<td>47</td>
<td>67.8</td>
</tr>
<tr>
<td>Length, cm</td>
<td>27.6 (3.2)</td>
<td>Additive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lee index</td>
<td>71.8 (8.4)</td>
<td>Recessive</td>
<td>(8 \times 10^{-7})</td>
<td>38</td>
<td>0.02</td>
</tr>
<tr>
<td>(V_{CO_2}), ml/min</td>
<td>54.9 (6.4)</td>
<td>Recessive</td>
<td>0.020</td>
<td>10</td>
<td>0.53</td>
</tr>
<tr>
<td>(V_t) (a1)</td>
<td>11.4 (1.3)</td>
<td>Additive</td>
<td>0.018</td>
<td>6</td>
<td>18.1</td>
</tr>
<tr>
<td>(V_t) (a2)</td>
<td>86.1 (10.0)</td>
<td>Overdominant</td>
<td>0.004</td>
<td>9</td>
<td>-6.3</td>
</tr>
<tr>
<td>(f) (h1)</td>
<td>50.9 (5.9)</td>
<td></td>
<td>0.065</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(V_t) (h1)</td>
<td>76.6 (8.9)</td>
<td></td>
<td>0.115</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(V_e) (h1)</td>
<td>81.1 (9.5)</td>
<td></td>
<td>0.302</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(f) (h2)</td>
<td>80.4 (9.4)</td>
<td>Dominant</td>
<td>0.021</td>
<td>17</td>
<td>18.0</td>
</tr>
<tr>
<td>(V_e) (h2)</td>
<td>3.7 (0.4)</td>
<td></td>
<td>0.418</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(V_e) (h5)</td>
<td>2.5 (0.3)</td>
<td></td>
<td>0.244</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(f) (h3)</td>
<td>76.4 (8.9)</td>
<td>Additive</td>
<td>0.002</td>
<td>17</td>
<td>13.6</td>
</tr>
<tr>
<td>(V_e) (h3)</td>
<td>15.9 (1.9)</td>
<td></td>
<td>0.254</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(f) (h4)</td>
<td>60.5 (7.1)</td>
<td>Additive</td>
<td>0.012</td>
<td>11</td>
<td>13.6</td>
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<tr>
<td>(V_e) (h4)</td>
<td>2.8 (0.3)</td>
<td></td>
<td>0.752</td>
<td></td>
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<tr>
<td>(V_e) (h9)</td>
<td>54.4 (6.3)</td>
<td></td>
<td>0.241</td>
<td></td>
<td></td>
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<tr>
<td>(f) (h45)</td>
<td>11.9 (1.4)</td>
<td></td>
<td>0.131</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(f) (h5)</td>
<td>77.7 (9.1)</td>
<td>Overdominant</td>
<td>0.042</td>
<td>3</td>
<td>-2.1</td>
</tr>
<tr>
<td>(f) (o1)</td>
<td>43.9 (5.1)</td>
<td></td>
<td>0.134</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(V_e) (o1)</td>
<td>66.6 (7.8)</td>
<td></td>
<td>0.660</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(V_e) (o15)</td>
<td>73.6 (8.6)</td>
<td>Overdominant</td>
<td>0.012</td>
<td>7</td>
<td>0.04</td>
</tr>
<tr>
<td>(f) (o15)</td>
<td>10.2 (1.2)</td>
<td>Recessive</td>
<td>0.000</td>
<td>9</td>
<td>7.9</td>
</tr>
<tr>
<td>(f) (o2)</td>
<td>6.3 (0.7)</td>
<td>Recessive</td>
<td>0.005</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>(f) (o5)</td>
<td>43.9 (5.1)</td>
<td></td>
<td>0.068</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(f) (c1)</td>
<td>38.1 (4.4)</td>
<td></td>
<td>0.163</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(V_e) (c1)</td>
<td>84.3 (9.8)</td>
<td>Overdominant</td>
<td>0.002</td>
<td>11</td>
<td>0.08</td>
</tr>
<tr>
<td>(f) (c2)</td>
<td>84.1 (9.8)</td>
<td></td>
<td>0.154</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(V_e) (c2)</td>
<td>4.6 (0.5)</td>
<td></td>
<td>0.011</td>
<td>7</td>
<td>0.07</td>
</tr>
<tr>
<td>(V_e) (c2)</td>
<td>96.4 (11.2)</td>
<td></td>
<td>0.474</td>
<td></td>
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<tr>
<td>(V_e) (c3)</td>
<td>17.8 (2.1)</td>
<td></td>
<td>0.103</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(f) (c4)</td>
<td>58.2 (6.8)</td>
<td></td>
<td>0.103</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(V_e) (c4)</td>
<td>92.1 (10.7)</td>
<td></td>
<td>0.443</td>
<td></td>
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</tr>
<tr>
<td>(V_e) (c4)</td>
<td>59.6 (7.0)</td>
<td></td>
<td>0.201</td>
<td></td>
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</tr>
<tr>
<td>(f) (c45)</td>
<td>75.2 (8.8)</td>
<td></td>
<td>0.065</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(V_e) (c45)</td>
<td>88.0 (10.3)</td>
<td>Overdominant</td>
<td>0.001</td>
<td>11</td>
<td>0.08</td>
</tr>
<tr>
<td>(f) (c5)</td>
<td>6.3 (0.7)</td>
<td>Additive</td>
<td>0.199</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(V_e) (c5)</td>
<td>28.1 (3.3)</td>
<td>Additive</td>
<td>0.002</td>
<td>17</td>
<td>-2.4</td>
</tr>
<tr>
<td>(V_e) (c5)</td>
<td>76.7 (8.9)</td>
<td>Recessive</td>
<td>0.156</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(f) (c5)</td>
<td>38.1 (4.4)</td>
<td>Recessive</td>
<td>0.178</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(f) (b1)</td>
<td>32.8 (3.8)</td>
<td>Recessive</td>
<td>0.002</td>
<td>17</td>
<td>-2.1</td>
</tr>
<tr>
<td>(f) (b1)</td>
<td>43.1 (5.0)</td>
<td></td>
<td>0.244</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(f) (b1)</td>
<td>35.4 (4.1)</td>
<td></td>
<td>0.055</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(f) (b2)</td>
<td>17.7 (2.1)</td>
<td>Recessive</td>
<td>0.005</td>
<td>14</td>
<td>-1.9</td>
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<tr>
<td>(V_e) (b2)</td>
<td>51.6 (6.0)</td>
<td></td>
<td>0.057</td>
<td></td>
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<tr>
<td>(V_e) (b2)</td>
<td>46.7 (5.4)</td>
<td>Additive</td>
<td>0.342</td>
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<tr>
<td>(f) (b3)</td>
<td>78.0 (9.1)</td>
<td>Additive</td>
<td>0.004</td>
<td>15</td>
<td>-1.8</td>
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<tr>
<td>(V_e) (b3)</td>
<td>28.1 (3.2)</td>
<td></td>
<td>0.149</td>
<td></td>
<td></td>
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<tr>
<td>(V_e) (b3)</td>
<td>75.8 (8.8)</td>
<td></td>
<td>0.330</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(f) (b4)</td>
<td>4.9 (0.6)</td>
<td></td>
<td>0.123</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(f) (b45)</td>
<td>67.1 (7.8)</td>
<td>Additive</td>
<td>6 \times 10^{-4}</td>
<td>20</td>
<td>5.4</td>
</tr>
<tr>
<td>(f) (b5)</td>
<td>69.2 (8.1)</td>
<td></td>
<td>0.173</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(H^2\), broad sense heritability (%) and standard error of those estimates. Variables with a heritability of zero were not included. \(\hat{\beta}\), Magnitude and direction of the allelic effect of the leptin receptor in units of the measured variable. *F2 animals were partitioned by leptin receptor genotype, and the model best fitting particular modes of inheritance was tested using ANOVA. Models are provided when the fit has a \(P\) value \(<0.05\). †The amount of variance could be accounted for by allelic variation at the leptin receptor. §Significant after Bonferroni correction (\(\alpha = 0.05/53 = 0.000943\)).
the total heritability in breathing frequency (Table 7; heritability = 67% at 4.5 min into the isocapnic hypoxia challenge). During other chemosensory challenges, even smaller amounts of the variation in the breathing frequency were attributed to the leptin receptor allele. Similar observations can be made about $V_T$ and $V_E$, which were not as consistently heritable as breathing frequency but nevertheless did show occasional evidence of a genetic basis. Based on these data, we conclude that the $fa$ allele is not a major gene for ventilatory behavior in this intercross.

With regard to the traits of weight and mass, as expected, the $fa$ allele acted in a recessive mode (46). Most importantly, but predictably, the leptin receptor accounted for 46.8% of the total heritability for weight (heritability of 77.5%). Within the Zucker strain, not all of the metabolic parameters relating to fat and glucose metabolism and turnover associate with the $fa$ mutation in a dose-response manner (30). Specifically, in a study of Brown Norway-Zucker F1 generation, an action of one $fa$ allele ($fa/wt$) was apparent with regard to increased fat content even at 7 days of life but did not necessarily translate to measurable differences in thermoregulatory thermogenesis and plasma concentrations of insulin and triglycerides found in the $fa/fa$ pups (38). In our study, such detailed measurements of fat distribution and biochemical factors were beyond the scope of the study; however, this serves as an example of the complex actions of the leptin receptor gene and how it might contribute to some of the ventilatory values found in the heterozygotes.

Alternately, obesity mechanisms that act independently of the leptin receptor may influence ventilatory traits. In a previous genome scan of obese apnea and body mass index in Caucasians, Palmer and Redline (29) did not obtain any evidence for the human ortholog of the leptin receptor on 1q32 being linked to either body mass index or to the apnea-hypopnea index. They did, however, determine some evidence for linkage to the apnea-hypopnea index explained by body mass index. Furthermore, they found evidence of linkage to obesity-related traits on chromosomes 2p, 7p, and 12q. In our model, only ~50% of the total heritability for weight was explained by the leptin receptor; the remainder of the variance is probably due to other genes for obesity that are strain specific. In the literature, the actions of leptin and its receptor have been cited extensively in mediating the effects of obesity on ventilation. Our data demonstrate that the genetic architecture of ventilation in the context of obesity is complex, involving many genes (oligogeny) and/or gene-gene (epistatic) interactions. Evidence to sort which other obesity-related genes may influence ventilation await a genome scan of this intercross.

Because the leptin receptor does control some proportion (~20%) of the total heritability in isocapnic hypoxia, we need to consider pleotropic actions of the leptin system and the potential for epistatic effects. To some extent, these issues have been addressed in previous studies. For example, information from knockout mouse models suggests that leptin and leptin receptors affect ventilation. The $ob/ob$ C57BL/6J mouse compared with the null littermate exhibited differences in hypercapnic ventilation before pronounced obesity emerged, but changes in baseline breathing appeared to follow age-dependent increases in body weight (42). In the mouse model of absent leptin, leptin administration improved the hypercapnic response (44). Thus leptin and the leptin system may influence the trajectory of hypercapnic responses independent of fat accumulation and distribution. However, even the presence of obesity is accompanied by differences in ventilatory behavior (32), perhaps because the genetic background of the $db/db$ mouse differs from the $ob/ob$ model, as well as by gender (33). The presence of metabolic derangements such as ketosis would have an effect independent of obesity (32).

Furthermore, the actions of the leptin receptor may modify a number of other systems directly and indirectly involved in respiratory control. There are effects not only on body weight and lipid metabolism but also on other physiological systems, including immune function (7, 25, 35). Relevant to breathing, several systems and pathways are known to be abnormal in Zucker animals. There are differences between lean and fatty Zucker siblings in temperature regulation (19, 20) and in dopaminergic effects on hypoxia (22), as well as in the response to pharmacological interventions, including inhibition of neuronal nitric oxide synthase (23), opioid blockade (16, 36), and a noncompetitive glutamate N-methyl-D-aspartate receptor antagonist (18). Many of these effects are not solely accounted for by differences in respiratory mechanics between lean and obese phenotypes (6). Thus, even the $fa$ allele can have substantial effects on ventilatory behavior and other components of respiratory control that go beyond the known primary targets for leptin in the central nervous system (7).

Finally, the progenitor background appears to have an impact on ventilatory behavior under many conditions of challenge. In the Brown Norway strain, downstream or upstream pathways may differ from those in the Zucker strain in structural or functional mechanisms that are encoded by other parts of the genome. A full genome scan is needed to identify such loci. What is clear is that the obese animals in the F2 generation have ventilatory behaviors that are different from those observed in Zucker obese parental strains. Correspondingly, the $wt/wt$ animals in the F2 generation resemble the Zucker grandparents in ventilatory behavior, which further supports the hypothesis that the obese phenotype is not associated with a unique ventilatory pattern. We suspect ventilatory behavior is a consequence of multiple genes determining some proportion of the variance in ventilation at rest and with chemosensory challenge and that the $fa$ allele and its physiological effects are only one of many components that are manifested by these genetic variants. In summary, our results lead us to conclude that the action of the leptin receptor is insufficient to explain the genetic variation in ventilatory traits.

There are some design limitations. This was a rather small data set, and it was designed specifically to track the $fa$ allele. The power to estimate the effects and/or numbers of other putative genes is limited. Because a cross between any two rat strains represents only a small proportion of the potential genetic variation in this species, our estimates of heritability are only applicable to this cross; other strains may have novel alleles or loci that also modulate ventilation parameters. Second, this was not a reciprocal intercross design because Zucker females homozygotic for the $fa$ allele are very poor breeders (46). Reproductive success with male Zucker homozygotes was more effective when Brown Norway females were exposed to a number of these rats. However, this directional breeding strategy limited us from studying the effects of the Brown Norway Y chromosome and the Zucker XX genotype. Third, because heritability values were estimated from a lim-
ited number of F2 animals across a large number of traits, the precision of the estimates and models are modest at best. Fourth, the Zucker strain is not entirely homozygous (inbred) at all loci; therefore, there was additional variance encountered by such heterozygosity. This necessitated a general approach to the estimation of broad-sense heritability (5). Estimates can be refined to address additive genetic effects and inheritance across multiple alleles with a genome-wide scan, accompanied by measurements in a larger number of F2 animals, as statistical power is improved, the degree of heterozygosity is determined empirically, and modes of inheritance are defined for specific regions of the genome other than the fa locus. Finally, researchers could disentangle the confounding effects in sity and ventilation measures by creating a conditional knockout or knockin for the leptin receptor that can be expressed in selected tissues at particular developmental phases. However, this work is beyond the scope of the present study.

Other, more practical, limitations were related to specific testing procedures, which were implemented based on the need for high throughput at times when litters of particular ages were to be tested, with attention given to uniformity in the testing schedule and high reproducibility of results. Thus animals were brought to the laboratory at a certain time of day and after acclimatization to the testing environment. To encourage animals to be awake during the study, they had 5 h of light exposure before coming to the laboratory, and testing was accomplished within the next 3 h. Although the use of plethysmography raises some unavoidable technical issues, this approach is favorable compared with the potential incompatibility with restraint or anesthetic variance. The variability in an animal for frequency and VT is within the range reported in the literature for rodents (28). In the analysis, problems with the accuracy and reproducibility are accounted for as technical and environmental variance. The identification of strain differences by us and by others indicates that the study of the unanesthetized rodents with body plethysmography is an appropriate first step (8, 11, 43).

Results indicate that attention to the time and manner of collection of phenotype value may be quite important in studies that seek to uncover naturally occurring allelic variations that operate in the regulation of ventilation. For instance, we found that measures of resting breathing taken before and the several minutes after the initial challenge with hypoxia and reoxygenation are modeled with different degrees of heritability. This difference could reflect how genetic background might shape ventilatory behavior during a series of challenges. In a practical sense, one needs to be explicit about the testing paradigm, especially if comparisons are to be made across studies from different laboratories, and the interpretations from linkages studies may need to account for the presence of such differences in proprionecence.

In summary, traits for ventilatory behavior at rest and with chemosensory challenge show inheritance, but the relative influence from the fa locus is not consistent with a major gene effect. Other regions of the rat genome and various patterns of inheritance may contribute to adult ventilatory phenotypes.

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