Genetic control of differential baseline breathing pattern

CLARKE G. TANKERSLEY,1 ROBERT S. FITZGERALD,1 ROY C. LEVITT,2 WAYNE A. MITZNER,1 SUSAN L. EWART,2 and STEVEN R. KLEEBERGER1
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TANKERSLEY, Clarke G., Robert S. Fitzgerald, Roy C. Levitt, Wayne A. Mitzner, Susan L. Ewart, and Steven R. Kleeberger. Genetic control of differential baseline breathing pattern. J. Appl. Physiol. 82(3): 874–881, 1997.—The purpose of the present study was to determine the genetic control of baseline breathing pattern by examining the mode of inheritance between two inbred murine strains with differential breathing characteristics. Specifically, the rapid, shallow phenotype of the C57BL/6J (B6) strain is consistently distinct from the slow, deep phenotype of the C3H/HeJ (C3) strain. The response distributions of segregant and nonsegregant progeny were compared with the two progenitor strains to determine the mode of inheritance for each ventilatory characteristic. The BXH recombinant inbred (RI) strains derived from the B6 and C3 progenitors were examined to establish strain distribution patterns for each ventilatory trait. To establish the mode of inheritance, baseline breathing frequency (f), tidal volume, and inspiratory time (Ti) were measured five times in each of 178 mature male animals from the two progenitor strains and their progeny by using whole body plethysmography. With respect to f and Ti, the two progenitor strains were consistently distinct, and segregation analyses of the inheritance pattern suggest that the most parsimonious genetic model for response distributions of f and Ti is a two-loci model. In similar experiments conducted on 82 mature male animals from 12 BXH RI strains, each parental phenotype was represented by one or more of the RI strains. Intermediate phenotypes emerged to confirm the likelihood that parental strain differences in f and Ti were determined by more than one locus. Taken together, these studies suggest that the phenotypic difference in baseline respiratory timing between male B6 and C3 mice is best explained by a genetic model that considers at least two loci as major determinants.

inquiring frequencies among many mammalian species including mice and humans. Although many advances have been made since Quetelet (21) to improve our understanding of the complex interaction among neural, mechanical, and other factors that control baseline f, little attention has been given to the contribution of genetic determinants.

Recently, Tankersley et al. (29) described significant variation among eight inbred strains of mice with respect to baseline f. The variability observed between these strains was significantly greater than within each strain, suggesting that genetic determinants account for a significant proportion of variation in f. The general purpose of the present study was to examine more closely the role of genetic constituents in controlling baseline breathing pattern and respiratory timing. Two inbred strains, which were phenotypically distinct with respect to baseline breathing characteristics, were used to determine the mechanism of genetic regulation of baseline f. If the data support this hypothesis in mice, we propose that the genetic regulation of breathing may be determined by similar mechanisms in humans (8, 24).

Two inbred strains, namely, C57BL/6J (B6) and C3H/HeJ (C3), demonstrate consistent and distinctively diverse breathing patterns while animals are unanesthetized and unrestrained (29). Specifically, the B6 strain adopts a rapid, shallow breathing pattern relative to the slow, deep pattern characteristic of the C3 strain. This phenotypic variation in f and tidal volume (VT) observed between strains occurs without significant differences in minute ventilation. In the present study, we sought to determine the best fitting genetic model to explain the response distributions of the two progenitor strains and their nonsegregant (i.e., B6C3F1) and segregant (i.e., the two backcross and intercross; B6C3F2) progeny. In addition, recombinant inbred (RI) strains derived from B6 and C3 progenitors (i.e., BXH RI) were examined to further support the enumeration of loci controlling strain differences in baseline breathing pattern (25, 28, 31).

METHODS

Animals. Reproductively mature male and female B6 and C3 inbred parental strains, and the B6C3F1/J (i.e., female B6 × male C3, F1) progeny were purchased from Jackson Laboratories (Bar Harbor, ME) to conduct breeding studies. Backcross (i.e., female B6 × male B6C3F1, BXB6; female C3 × male B6C3F1, BXC3) and intercross (i.e., B6C3F2 or F2) progeny were generated in the animal facilities at Johns Hopkins University. Animals from each of the 12 available BXH RI strains were also procured from Jackson Laboratories. All animals were weaned within 4–5 wk, and water and chow (Agway Pro-Lab RMH 1000) were provided ad libitum. From the breeding colonies, male backcross and intercross

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Tom Quetelet was among the first to report the baseline breathing pattern of normal healthy human subjects and to articulate the conditions (e.g., age, gender, wakefulness) that cause variation in respiratory rate (21). Since then many studies have investigated the neural and mechanical processes that control f (e.g., Refs. 18, 19) and have defined conditions that modify these mechanisms. Generally, central neural modulation initiates the generation of the breathing rhythm, whereas peripheral pulmonary stretch receptors delineate the timing of the inspiratory phase of each tidal breath (2, 13, 26). Another principle central to the control of f is the notion that optimal respiratory pattern is regulated to maximize both breathing efficiency in terms of mechanical cost as well as alveolar ventilation to facilitate gas exchange (19). A number of studies (e.g., Refs. 1, 5) have demonstrated relationships between predicted and observed optimal breathing frequencies among many mammalian species including mice and humans. Although many advances have been made since Quetelet (21) to improve our understanding of the complex interaction among neural, mechanical, and other factors that control baseline f, little attention has been given to the contribution of genetic determinants.

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METHODS

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progeny were randomly selected, placed in cages containing 4–6 animals, and housed for an additional 4–12 wk before testing. At least 48 h before the day of each experiment, mature, male mice were placed in cages set under a laminar flow hood with high-efficiency particulate-filtered air. All experiments were performed between 0900 and 1800 h, and the environment during the experiments as well as animal handling were highly standardized.

Whole body plethysmography. In quiescent animals, baseline ventilatory function was assessed five times during room air conditions (inspiratory fraction of O₂ = 0.21; inspiratory fraction of CO₂ = 0.0003) by using whole body plethysmography (7, 10, 11) modified for unanesthetized, unrestrained mice (29, 30). These intermittent measurements represented a portion of a broader study investigating acute ventilatory responses to a standard hypoxic/hypercapnic challenge protocol (29). Each animal was permitted to acclimate within the chamber for at least 30 min before the beginning of the protocol. Chamber temperature was maintained within the thermoneutral zone for mice (i.e., 26–28°C) and was recorded during each ventilatory measurement by using a T-type thermocouple. Compressed air was humidified (90% relative humidity) and directed through the chamber at a flow rate of 300 ml/min. After the animal became quiescent, f, Vr, and inspiratory time (TI) were recorded on a strip-chart recorder (model 7D polygraph, Grass). At a constant chamber volume, changes in pressure due to inspiratory and expiratory time periods were quantified using differential pressure transducer (model 8510B-2, Endevco). The inspired and expired limbs of each tidal breath were averaged, and body temperature account for 5% error in computing baseline VT. From a total of 178 experiments, the ventilatory measurements and were maintained within 1% of normal room air conditions. Each animal was weighed after this protocol.

Data analysis. The analog signal generated from the pressure transducer was also recorded as a digital input by using a data-acquisition system (Keithley Instruments) and a dedicated computer. Data were acquired at an input frequency of 100 Hz, and peak inspiration and expiration were determined from 16 consecutive tidal breaths. On the rare occasion during which data were not secured by computer f, Vr, and TI were estimated from four tidal breaths within a 6-s strip-chart recording, as described elsewhere (29). Least squares regression analysis was used to compare the two methods of acquiring ventilatory data, and suitable reproducibility ($r^2 = 0.99$) was established for each ventilatory measurement. Pressure transducer calibrations were performed daily using a 50-µl gastight syringe while chamber temperature was maintained similar to the experimental ambient conditions.

In computation of Vr, the amplitude of the inspiratory and expiratory limbs of each tidal breath was averaged, and body temperature of each animal was assumed to be constant at 37°C. Although it is unknown whether strain differences exist in resting body temperature among the B6 and C3 strains, their progeny, and the BXH R1 strains, variations of 1–2°C in body temperature account for <5% error in computing baseline VT. Minute ventilation (Ve) was calculated as the product of f and Vr. Expiratory time (Te) was determined from total respiratory time (TI) minus TI, mean inspiratory flow was calculated as the ratio of Vr to TI (Vr/TI), and the duty cycle was computed as the ratio of TI to Tt (TI/Tt). In exploratory studies, the respiratory timing was verified by positioning a strain gauge on the lateral aspect of the chest wall in anesthetized mice and assessing the minimum and maximum excursions of the analog signal generated by the strain gauge. These experiments demonstrated that the moment of end inspiration and end expiration during baseline and challenged breathing coincided with the maximum and minimum excursions of the chest wall, respectively.

Data analysis. For each individual animal, ventilatory data reported in Figs. 1–3 represent the average of five repeated measurements. The statistical procedures were initiated by examining the variance among the progenitors and their progeny by a one-way analysis of variance. Means comparisons were performed to test for statistical differences between each offspring class and the progenitor strains by using Duncan’s multiple-range test and were considered significant at the α-level of 0.01. Genetic statistical procedures regarding segregation analyses and the use of RI strains are described in the Appendix.

RESULTS Ventilatory responses of progenitors and their progeny. From a total of 178 experiments, the ventilatory data (i.e., means, SE, and ranges) of the B6 and C3 progenitors and their segregant and nonsegregant progeny are reported in Table 1. The B6 and C3 progenitors

Table 1. Baseline ventilatory characteristics of B6 and C3 progenitor strains and segregant and nonsegregant progeny

<table>
<thead>
<tr>
<th>Strain</th>
<th>n</th>
<th>Age, days</th>
<th>Wt, g</th>
<th>f, Hz</th>
<th>Vr, µl/g</th>
<th>Ve, ml·min⁻¹·g⁻¹</th>
<th>Ti, ms</th>
<th>Te, ms</th>
<th>Ti/Te,%</th>
<th>Vr/Ti, µl·g⁻¹·s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6J</td>
<td>20</td>
<td>78 ± 4 (58–112)</td>
<td>26.4 ± 0.8 (18.9–31.9)</td>
<td>2.81 ± 0.08* (2.16–3.31)</td>
<td>7.0 ± 0.2* (5.7–9.1)</td>
<td>1.18 ± 0.05 (0.88–1.67)</td>
<td>111 ± 2* (92–129)</td>
<td>250 ± 10* (169–359)</td>
<td>31.6 ± 0.9 (21.8–38.2)</td>
<td>64 ± 4* (49–87)</td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td>20</td>
<td>82 ± 4 (58–115)</td>
<td>25.2 ± 0.6 (19.9–30.6)</td>
<td>1.80 ± 0.04 (1.53–2.14)</td>
<td>9.9 ± 0.3 (6.6–12.5)</td>
<td>1.06 ± 0.03 (0.91–1.27)</td>
<td>188 ± 3 (165–208)</td>
<td>374 ± 11 (301–462)</td>
<td>33.9 ± 0.8 (28.8–42.5)</td>
<td>53 ± 2 (32–73)</td>
</tr>
<tr>
<td>B6C3F₁</td>
<td>14</td>
<td>61 ± 1†‡ (53–76)</td>
<td>25.4 ± 0.2 (21.9–27.4)</td>
<td>3.00 ± 0.12†‡ (2.42–3.79)</td>
<td>6.0 ± 0.2†‡ (5.2–7.2)</td>
<td>1.08 ± 0.06 (0.77–1.42)</td>
<td>126 ± 3†‡ (108–146)</td>
<td>215 ± 11†‡ (156–279)</td>
<td>37.1 ± 1.0†‡ (32.3–43.7)</td>
<td>48 ± 2†‡ (40–62)</td>
</tr>
<tr>
<td>BXB6</td>
<td>35</td>
<td>73 ± 2 (53–87)</td>
<td>25.7 ± 0.4 (21.3–29.1)</td>
<td>2.72 ± 0.08†‡ (2.08–4.01)</td>
<td>7.1 ± 0.2†‡ (3.9–8.8)</td>
<td>1.15 ± 0.04 (0.66–1.88)</td>
<td>131 ± 3†‡ (103–177)</td>
<td>247 ± 9†‡ (147–358)</td>
<td>25.7–48.7†‡ (35–86)</td>
<td>55 ± 2†‡ (40–62)</td>
</tr>
<tr>
<td>BXC3</td>
<td>20</td>
<td>75 ± 1 (71–86)</td>
<td>28.2 ± 0.6‡ (25.6–35.6)</td>
<td>2.26 ± 0.06†‡ (1.92–2.67)</td>
<td>7.3 ± 0.2†‡ (5.4–8.8)</td>
<td>0.98 ± 0.03 (0.80–1.21)</td>
<td>156 ± 3†‡ (136–186)</td>
<td>293 ± 9†‡ (227–358)</td>
<td>25.8–48.7†‡ (40–54)</td>
<td>47 ± 1†‡ (38–60)</td>
</tr>
<tr>
<td>B6C3F₂</td>
<td>69</td>
<td>80 ± 2 (61–112)</td>
<td>28.5 ± 0.6‡ (22.2–42.9)</td>
<td>2.43 ± 0.06†‡ (1.86–3.95)</td>
<td>7.9 ± 0.2†‡ (5.7–11.9)</td>
<td>1.16 ± 0.03 (0.54–1.99)</td>
<td>146 ± 3†‡ (109–218)</td>
<td>267 ± 6†‡ (137–422)</td>
<td>26.3–45.9†‡ (29–82)</td>
<td>55 ± 2†‡ (32–73)</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of animals. Nos. in parentheses, range. f, Breathing frequency; Vr, tidal volume; Ve, minute ventilation; Ti, inspiratory time; Te, expiratory time; Ti, total respiratory time. Significantly different: C57BL/6J (B6) vs. C3H/HeJ (C3) strains, *P < 0.01; offspring class vs. B6, †P < 0.05; offspring class vs. C3 strain, ‡P < 0.01.
were similar with respect to age, body weight, baseline $V_E$ and the respiratory duty cycle (i.e., $T_I/T_T$). In contrast, baseline $f$, $V_T$, $T_I$, and $T_E$, and mean inspiratory flow (i.e., $V_I/T_I$) were significantly ($P < 0.01$) different between the strains. There was no overlap between the progenitor strains in the range of responses for $f$ and $T_I$.

For the other ventilatory traits, the response distributions of the progenitors could not be consistently classified as typical of either progenitor. A parallel response appeared to exist between the strains. There was no overlap between the B6 and C3 progenitor strains in the range of responses for $f$ and $T_I$. The response distributions did not conform to the majority of ventilatory characteristics for the F1 progeny, although there were several significant ($P < 0.01$) differences. The breathing pattern of the F1 progeny was significantly ($P < 0.01$) different from that of the C3 progenitor with respect to $f$, $V_T$, $T_I$, and $T_E$, and significantly ($P < 0.01$) different from the B6 progenitor in terms of $V_T$, $T_I$, $T_I/T_T$, and $V_T/T_I$. With the exception of baseline $f$, these data suggest that the majority of ventilatory characteristics for the F1 progeny differed when compared with both the B6 or C3 progenitors. A parallel response appeared to exist between the F1 progeny and the B6 progenitor for baseline $f$ (Fig. 1). If the rapid breathing phenotype is qualitatively defined based on the "upper limit" of the C3 response distribution (i.e., $\approx 2.05$ Hz in Fig. 1), the distributions of all individual responses for the F1 and BXB6 strains were not different from those of the B6 strain. The individual responses of the BXC3 progeny were divided into rapid and slow phenotypes, and a ratio of 14:6 was not significantly different from a 50:50 proportion predictive of Mendelian inheritance ($\chi^2 = 3.20$; $P > 0.05$). The response distribution of the F2 progeny was divided into rapid and slow phenotypes in a ratio of 57:12, which was not significantly different from a 75:25 proportion ($\chi^2 = 2.13$; $P > 0.05$).

As shown in Fig. 2, the response distributions of two progenitor strains for baseline $T_I$ were distinct, yet intermediate phenotypes were demonstrated in each segregant and nonsegregant population. More specifically, the $T_I$ response distributions for each offspring class were significantly ($P < 0.01$) different from both the B6 and C3 progenitor strains (Table 1). Because intermediate phenotypes were detected among the segregant and nonsegregant progeny for baseline $T_I$, the response distributions did not conform to the proportions predictive of Mendelian inheritance. The consequence of a greater $T_I$ response for each offspring class relative to the B6 progenitor accounted for a significantly ($P < 0.01$) attenuated mean inspiratory flow (i.e., $V_I/T_I$) in the groups of progeny compared with that in parental B6 mice.

Statistical Analysis of Genetic Epidemiology (SAGE). To test a single-gene hypothesis more rigorously, quantitative genetic models were considered (see APPENDIX regarding segregation analysis). One-locus, mixed-loci, and polygenic general models were rejected in the segregation analysis of baseline $f$ because the likelihood values associated with these models differed significantly from the unrestricted model based on the $\chi^2$ goodness-of-fit test (Table 2). Only the two-loci model did not demonstrate a significant ($P > 0.05$) difference from the unrestricted model. This indicated that the observed response distributions for baseline $f$ were not significantly different from expected criteria defining two-loci models. With the use of Akaike's information criterion (AIC), the most parsimonious model for baseline $f$ appeared to be the two equal- and additive-loci model. In a similar way, the segregation analysis for baseline $T_I$ rejected the one-locus, mixed-loci, and polygenic general models; that is, the general two-loci

![Fig. 1. Inheritance pattern of baseline breathing frequency for B6 and C3 progenitors and their segregant and nonsegregant progeny (C57BL/6, C3H/He, B6C3F1, BXB6, BXC3, B6C3F2 strains). Bars are shown for means ± SE of parental strains and F1 offspring.](image)

![Fig. 2. Inheritance pattern of baseline inspiratory time for B6 and C3 progenitors and their filial and backcross progeny (as defined in Fig. 1). Bars are shown for means ± SE of parental strains and F1 offspring.](image)

<table>
<thead>
<tr>
<th>Genetic Hypothesis</th>
<th>Log Likelihood Parameters</th>
<th>AIC</th>
<th>$\chi^2$ df</th>
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<tbody>
<tr>
<td>Baseline $f$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two unlinked-loci general</td>
<td>579.27 10</td>
<td>-1,138.54 4</td>
<td>4.65* 4</td>
</tr>
<tr>
<td>Two unlinked additive</td>
<td>576.17 6</td>
<td>-1,139.41 8</td>
<td>11.78* 8</td>
</tr>
<tr>
<td>Two unlinked equal to additive</td>
<td>575.18 4</td>
<td>-1,142.37 10</td>
<td>12.82* 10</td>
</tr>
<tr>
<td>One-locus general</td>
<td>555.50 4</td>
<td>-1,103.00 10</td>
<td>52.18 10</td>
</tr>
<tr>
<td>Polygenic general</td>
<td>431.61 6</td>
<td>-851.22 299.96 8</td>
<td>299.96</td>
</tr>
</tbody>
</table>

| Baseline $T_I$           |                           |       |             |
| Two unlinked-loci general| 740.04 10                 | -1,460.07 4 | 5.29* 4 |
| One-locus general        | 704.07 4                  | -1,400.14 10 | 77.23 10 |
| Polygenic general        | 579.27 6                  | -1,146.53 326.84 8 | 326.84 |

SAGE, Statistical Analysis for Genetic Epidemiology; AIC, Akaike's information criterion; df, degrees of freedom. *Not significantly different relative to unrestricted model, $P > 0.05$. 

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model was not significantly (P > 0.05) different from the unrestricted model (Table 2).

Ventilatory responses of BXH RI strains. The baseline ventilatory results of the 82 individuals making up the BXH RI strain-distribution pattern (SDP) are summarized in Table 3 (see APPENDIX regarding the use of RI strains). The BXH RI strains were comparable with the B6 and C3 progenitor strains in terms of age, body weight, baseline VE, and Ti/TT. With respect to f, VT, Ti, and TE, the responses for the BXH RI strains encompassed ranges similar to those of the progenitor strains.

The baseline breathing pattern of the BXH 8 strain was not different from the C3 progenitor strain. In contrast, the BXH 8 strain was significantly (P < 0.01) different relative to the B6 progenitor in terms of f, VT, Ti, and TE. The breathing patterns of BXH 3, 4, 7, 11, and 12 strains were not different from the B6 progenitor strain but were significantly (P < 0.01) different from the C3 progenitor in terms of f, VT, Ti, and TE. The Ti responses of BXH 3 and 11 strains were also significantly (P < 0.01) different from those of the B6 progenitor. There were no differences among the B6 progenitor strain and BXH 9 and 10 strains with respect to f, Ti, and TE, and these RI strains were significantly (P < 0.01) different from the C3 progenitor.

The baseline ventilatory responses of the BXH 2, 6, and 19 strains were intermediate relative to the progenitors. These strains were significantly (P < 0.01) different from the progenitor strains with respect to f and Ti. In addition, BXH 2 differed significantly (P < 0.01) from the B6 progenitor in terms of VT. With respect to TE, both BXH 2 and 6 differed significantly (P < 0.01) from the B6 progenitor, and BXH 19 differed significantly (P < 0.01) from the C3 progenitor. The ventilatory characteristics of the BXH 14 strain were unique relative to the other BXH RI strains in terms of f, VT, Ti, TE, and Ti/TT. The baseline f response of the BXH 14 strain was significantly (P < 0.01) greater relative to the two progenitor strains and every BXH RI strain with exception of BXH 12.

In Fig. 3, A and B, the BXH RI SDPs are depicted for baseline f and Ti, respectively. The interstrain variance (genetic) of both f and Ti was significantly (P < 0.01) greater than the within-strain variance (environmental). As shown in Fig. 3A, the f response of one RI strain (i.e., BXH 8) resembled that of the C3 progenitor, and seven RI strains (i.e., BXH 3, 4, 7, 9–12) resembled that of the B6 progenitor. Four RI strains represent alternative phenotypes; that is, the f response of the BXH 2, 6, and 19 strains were intermediate, and the f response of the BXH 14 strain exceeded that of the B6 progenitor. In Fig. 3B, one RI strain (i.e., BXH 8) resembled the C3 progenitor, and six RI strains (i.e., BXH 4, 7, 9, 10, 12, and 14) resembled the B6 progenitor in terms of the Ti response. Five RI strains (i.e., BXH 2, 3, 6, 11, and 19) had intermediate responses.
Although \( f \) and \( T_i \) are independent measurements, these variables of respiratory timing are physiologically linked. To examine the relationship between baseline \( f \) and \( T_i \), the two breathing characteristics of the BXH RI strains relative to the progenitor strains were represented in a cosegregation plot (Fig. 4). By classifying the BXH RI strains on the basis of both \( f \) and \( T_i \), three distinct phenotypes for the respiratory timing emerged: C3-like (BXH 8), B6-like (BXH 3, 4, 7, 9–12, and 14), and intermediate responses (BXH 2, 6, and 19). The presence of three distinct phenotypes in the BXH RI set is consistent with a two-loci model.

**DISCUSSION**

Although the precise genetic interaction underlying the mechanisms of baseline breathing pattern remains to be elucidated, the simplest genetic model to explain the present results suggests that as few as two genes determine the differences in respiratory timing between male B6 and C3 strains of mice (Table 2). This conclusion is strengthened by the presence of an intermediate phenotype in the BXH RI SDPs, which differed from both the B6 and C3 progenitors (Fig. 4). If the two-gene model proposed in the present study is supported by molecular approaches to mapping of specific genes in the mouse genome, a distinct possibility exists that a limited number of genes are present in the human genome that control differences in individual breathing patterns (8, 24). This hypothesis is based on the high degree of homology between the genomes of the mouse and human species.

Qualitative and quantitative genetic strategies were employed in the present study to enumerate and begin to position the genes determining baseline respiratory timing. These strategies have been used to unravel the genetic control of other complex physiological traits relevant to respiratory physiology (e.g., Refs. 14–16). These studies have led to a broader understanding of the precise molecular basis of the trait in question (e.g., Ref. 6). In the design of the present study, several important controls such as age, weight, and gender were considered. The groups of progenitors, offspring, and RI strains were generally comparable within certain limits; that is, the results of the present study extend to male animals within a 8–16 wk age range. Each animal's baseline breathing characteristics were evaluated in a quiescent state while the animal was unanesthetized and unrestrained. Ventilatory responses were measured five times during intermittent room air exposure within a standard laboratory hypoxic/hypercapnic challenge protocol (29). These measurement criteria were considered an important foundation in assessing the genetic control of baseline breathing characteristics.

We initiated our genetic analyses by using a qualitative approach to test each ventilatory characteristic against a classical Mendelian hypothesis. This requires that the responses of the offspring be classified relative to the parental phenotypes. Under this paradigm, the response distribution for baseline \( f \) was the only ventilatory trait that conformed to the proportions predictive of Mendelian inheritance (Fig. 1). Recently, more sophisticated statistical analyses (e.g., SAGE; Ref. 22) permit the evaluation of continuous data by a quantitative genetic approach. Segregation analysis by using SAGE allows one to assess the genetic control of complex traits by considering one-locus, two-loci, mixed-loci, and polygenic models. This strategy was initiated by establishing variance homogeneity and eliminating sources of covariance. For both baseline \( f \) and \( T_i \), the one-locus, mixed-loci, and polygenic models were rejected by this analysis. These results suggest that the strain differences in baseline \( f \) and \( T_i \) are determined by as few as two genes (Table 2). The development and discovery of many phenotypic and genotypic associations have succeeded in much the same way. Cystic fibrosis, for example, was once thought to be an expression of a single gene mutation but now is believed to be a trait determined by more than one gene (12).

The number of genes can also be estimated by evaluating how the phenotypes of each BXH RI strain compare to the progenitor strains. As shown in Fig. 3, there are four distinct phenotypes for baseline \( f \) and three distinct phenotypes for baseline \( T_i \). Although the timing of the inspiratory phase is mechanistically linked to the timing of the total respiratory cycle (i.e., the duty cycle), it is not significantly different among the progenitor and the BXH RI strains, with the exception of BXH 14), the SDPs for these two traits are not altogether qualitatively concordant. This might suggest that, in addition to the two genes that control these traits (as determined by SAGE), other loci may impact on the phenotypic outcome of the baseline ventilatory response. This explanation is supported by the evidence of a fourth phenotype for baseline \( f \) (i.e., BXH 14). In Fig. 4, baseline \( f \) is depicted as a function of baseline \( T_i \) in a cosegregation plot. Qualitatively, three distinct phenotypes are apparent, supporting the results from our quantitative analyses that used SAGE. Therefore,
a two-loci model appears to be the simplest model to explain a major proportion of the parental strain differences in respiratory timing at baseline. Presently, it is unknown which mechanisms might result in differences in baseline respiratory timing between B6 and C3 mice. The f among groups of mice from the present study were similar to a number of previous reports (e.g., Refs. 5, 11). Crosfill and Widdicombe (5) and others (e.g., Refs. 1, 2) suggest that the observed breathing frequencies of different species are remarkably similar to the optimal f required to minimize the mechanical work of breathing. These investigators further suggest that the time constant for mice is very low relative to other species, which allows for rapid changes in lung volume; i.e., the optimal f for mice was \( \sim 2 \text{ Hz} \) (5). From the present study, the separation in the f phenotypes between the C3 and B6 progenitors occurs between 2.0 and 2.1 Hz (Fig. 1). Likewise, the response range of f for the entire group of mice examined rarely exceeded (23 of 260 individuals) the upper or lower limits estimated for a 10% change in the work of breathing (Ref. 5; Table 1; Fig. 1). Therefore, it appears that genetic mechanisms that control baseline f must operate within certain physiological and mechanical constraints determined by natural selection. The remarkable similarity among the groups with respect to the baseline \( \dot{V}_E \) underscores this point.

Otis et al. (20) summarized the frequency-dependent mechanical properties of the lung from another theoretical viewpoint. These investigators surmised that pulmonary compliance can be inversely proportional to f because of variation in regional distribution of ventilation and altered regional time constants. In the consideration of those individual animals that exceeded the theoretical upper limit for f (i.e., proposed by Crosfill and Widdicombe; Ref. 5) in the present study, 14 of the 23 individuals originated from three groups, namely, the F1, BXB6, and BXH 14 strains. Coincidentally, the \( \dot{V}_E \) was abnormally shortened in these individuals relative to the other groups of mice, suggesting that, if expiration results primarily from passive recoil, the pulmonary compliance of these individuals may be unusually low (2). An alternative explanation might involve strain differences in pulmonary stretch receptors affecting such lung volumes as functional reserve capacity by influencing inspiratory braking mechanisms (13). Although it is difficult to infer strain differences in mechanical properties from the results of the present study, genetic control of structural differences (4, 8, 14, 18) may explain a portion of the respiratory timing variation between progenitor strains.

Differences in lung morphometric parameters have also been described (14) among inbred progenitor strains and their offspring. For instance, the lung volume differences for a given transpulmonary pressure have been illustrated to occur between the B6 and DBA/2J (D2) progenitors. Our laboratory has reported the f of B6 mice to be significantly greater than the D2 strain under similar conditions as the present study (29). This strain difference in breathing pattern was due to a prolonged inspiratory phase and a greater respiratory duty cycle in D2 mice relative to the B6 strain. The B6 and D2 ventilatory data were consistent with the morphometric data and the possible strain differences in compliant properties. On the basis of these associations and the data presented in Table 1, if there were structural differences among the progenitor strains, the C3 mice would hypothetically demonstrate greater lung compliance and lung volume relative to the B6 strain. One strategy to investigate the genetic role of functional and structural interactions between B6 and C3 strains would be to measure individual pressure volume characteristics coincident with variation in baseline breathing pattern among segregant offspring. If the data supported our hypothesis, phenotypic differences in lung compliance would cosegregate with differences in f.

It has long been understood that baseline f is modified by central neurally mediated cholinergic mechanisms. Particularly, conditional variation in f such as state-dependent depression of ventilation has been shown to involve cholinergic mechanisms (17). Recent evidence suggests that the B6 strain is genetically deficient of several cholinergic enzymes including acetylcholinesterase (AChE) in various regions of the brain (e.g., Refs. 3, 23) relative to the D2 strain. It remains controversial whether these biochemical differences accompany strain variation in cholinergic neuronal density. One potential mechanism to explain strain differences in breathing characteristics noted in the present study might involve an AChE deficiency in the B6 strain relative to the C3 strain. This explanation is consistent with the present results, given the hypothesis that the greater f of B6 compared with C3 mice may be related to a lower inhibitory effect of cholinergic stimulation via an AChE deficiency. Furthermore, one might expect that potential strain differences in cholinergic neuronal function may influence the depression in f that occurs with sleep in a strain-specific way. Future studies will be conducted to better understand the extent to which cholinergic mechanisms are involved in regulating strain differences in respiratory timing at baseline.

In summary, the data suggest that polymorphisms in as few as two autosomal genes explain the differences in respiratory timing at baseline between B6 and C3 mice. One hypothesis to test in future studies is the genetic role in establishing strain differences with respect to structural or mechanical properties fundamental to baseline breathing characteristics. Another hypothesis is the genetic control of central neurally mediated cholinergic processes that may determine respiratory timing mechanisms. Ultimately, these studies should lead to a greater understanding of the genetic mechanisms central to the normal breathing characteristics in humans.

APPENDIX

Animals

Inbred mice strains are the product of 20 or more generations of brother-sister matings, and within a strain, individu-
als are genetically identical and homozygous at essentially all loci (32). As a result of this homozygosity, observed within-strain phenotypic variances may be attributed to environmental factors, whereas the between-strain variance is explained by genetic factors. Thus within-strain variance can be minimized by stringently controlling the environment.

Segregation Analysis

The method of Elston (9, 27) was used to estimate the number of genes that segregated with each ventilatory variable. A data-analysis program (CLUSTR; SAGE) (22) was used to estimate group means and variances. The program also identified the transformation parameters that best normalized the data in the genetically homogeneous groups (B6, C3, F1); i.e., the variances of the three groups were made homogeneous. This power transformation (−0.6612 for baseline TI) was applied to the entire data set (B6, C3, F1, F2, and both backcrosses) in subsequent segregation analyses. The genetic models examined in the BCROSS program of SAGE (version 2.2, 1994) included one-locus, two-loci, mixed-loci, and polygenic models (22). Comparisons were made between each inheritance model and the unrestricted (free) model to determine whether the restrictions placed on a model significantly decreased its likelihood. In the segregation-analysis program, parameters that could be restricted included any of the group means, common variances, littermate covariances, and the recombination fraction between two loci. Any restriction placed on a model lowers the maximum likelihood of that model, regardless of the goodness of fit. In addition to the log likelihood, AIC was used to normalize this reduction in maximum likelihood across models in which different restrictions were applied. The AIC was computed by using the number of restrictions placed on a model and the log likelihood of that model as shown in the following formula

\[
AIC = -2 \times (\text{log likelihood} - \text{no. of restrictions})
\]

BXH RI Strains Analysis

The BXH RI strains are propagated by inbreeding (i.e., brother-sister matings) randomly selected B6C3F1 progeny. After 20 or more generations, BXH RI strains represent stable segregant progeny of the B6 and C3 progenitor strains, and presumably, receive 50% of their autosomal genes from each progenitor strain. On the basis of this property, the phenotypes for any given trait that differ between the progenitor strains are equally likely to be transmitted to any one RI strain. If “new” phenotypes emerge in the RI set (i.e., different from both progenitors), this suggests that more than one locus controls the response (31). Therefore, the BXH RI SDP can be used to estimate the number of loci controlling a particular ventilatory trait (25). The responses of the twelve RI and two progenitor strains were examined by a one-way analysis of variance, and mean comparisons between each RI strain and the two progenitor strains were performed as described above. The BXH RI strains were also used in cosegregation analysis to evaluate phenotypic associations between traits for baseline breathing. If two traits cosegregate in an RI set, this infers that the traits are controlled by common genetic mechanisms.

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