Objective: The objective was to test the hypothesis that the BK2R gene polymorphism is a determinant of fat-free mass and quadriceps strength in patients with COPD.

Design: In a cross-sectional design we determined BK2R genotype, fat-free mass, and quadriceps strength in 110 COPD patients with a mean (±SD) predicted forced expiratory volume in 1 s of 34.3 ± 16.4% and in 104 healthy age-matched control subjects.

Results: The mean (±SD) fat-free mass index (in kg/m²) was significantly lower in 37 patients homozygous for the +9 allele than in carriers of the −9 allele (15.7 ± 1.8 compared with 16.7 ± 2.3; P = 0.038); the same pattern was true for quadriceps maximal voluntary force (30.8 ± 10.4 and 36.4 ± 12.8 kg; P = 0.02), respectively. No significant effect of BK2R genotype on inspiratory muscle strength or on any variable in control subjects was observed. There was no interaction between the effect of the BK2R and ACE genotypes on quadriceps strength.

Conclusions: The genotype associated with reduced BK2R expression is associated with reduced fat-free mass and quadriceps strength in COPD. However, alterations in the activity at the BK2R do not seem to account for the previously identified association of quadriceps strength with ACE genotype.

Key Words Gene polymorphism, bradykinin, angiotensin-converting enzyme, respiratory muscle, quadriceps, magnetic stimulation

INTRODUCTION

Reduced skeletal muscle mass occurs commonly in patients with advanced chronic obstructive pulmonary disease (COPD) and is associated with both impaired exercise capacity (1) and increased mortality (2–4). Although a variety of underlying mechanisms have been postulated (including disuse atrophy, systemic inflammation, and hypoxia), the fundamental pathogenesis of such changes remains obscure (5).

Angiotensin-converting enzyme (ACE) is a key component of the circulating endocrine renin-angiotensin system (RAS) that plays a role in circulatory homeostasis through the synthesis of pressor angiotensin II and degradation of vasodilator kinins (6). However, ACE is also expressed in diverse human tissues, where it may serve a variety of roles (7, 8). The absence (deletion, D allele) rather than the presence (insertion, I allele) of a 287–base pair fragment in the human ACE gene is associated with elevated concentrations of both circulating (9) and tissue (10) ACE.

ACE is expressed in skeletal muscle, where it may influence its function: the ACE D allele has been associated with elite “sprint” athletic performance (11, 12) and preserved quadriceps muscle strength in COPD patients (13). Whether such effects are mediated through increased synthesis of angiotensin II or through increased kinin degradation is unknown. However, bradykinin is synthesized in muscle during exercise (14) and may influence cellular growth either directly (15–17) or indirectly through altered skeletal muscle blood flow (18) or metabolism (19–24).

Many of the actions of bradykinin are mediated through the bradykinin type 2 receptor (BK2R), whose gene demonstrates a common polymorphism in which the absence (−9) of a 9-base pair repeat is associated with greater gene transcription (25) and higher messenger RNA expression of the receptor (26). The −9 allele has been shown to be associated with greater skeletal muscle metabolic efficiency (ratio of internal work performed to...
external work measured) (27) and the highest kinin activity genotype combination (ACE II/BK2R –9/–9) was the most efficient. Meanwhile, greater left ventricular growth responses have been identified in those genotypes associated with the low kinin activity (BK2R +/–9 or ACE DD) genotype (28, 29).

We hypothesized that the preserving influence of the D allele of the ACE genotype on skeletal muscle in COPD might be mediated through altered kinin activity and that skeletal muscle would therefore be relatively preserved in patients with the low BK2R expression (+/–9) genotype in our patient cohort (13).

SUBJECTS AND METHODS

Subjects

The study was approved by The Ethics Committee of The Royal Brompton Hospital. All subjects provided informed written consent in accordance with the Helsinki Declaration. Details about the recruitment of patient and control populations were described elsewhere (13). Patients with COPD consistent with the Global Initiative for Chronic Obstructive Lung Disease criteria were recruited from hospital clinics. Patients with significant comorbidity, including a diagnosis of diabetes, malignancy, heart failure, and neuromuscular disease, were excluded. Pulmonary function was assessed with the use of a Jaeger CompactLab system (Jaeger, Hoechberg, Germany). Healthy control subjects were recruited by advertisement in local newspapers. They had to be free of cardiorespiratory disease and to have spirometric measurements within normal limits. Data from these 2 populations were previously published (13), but the current article includes a number of additional subjects recruited after enrollment for that original study ended. Fat-free mass (FFM) was measured by using bioelectrical impedance analysis (Bodystat 1500; Bodystat, Isle of Man, United Kingdom) and either a device’s internal algorithm for control subjects. FFM was normalized for height to produce the FFM index (FFMI; in kg/m2). The signal was amplified and passed to a computer running LabView software (National Instruments, Austin, TX). Force was measured by using bioelectrical impedance analysis (Bodystat) and either a device’s internal algorithm for control subjects. FFM was measured by using bioelectrical impedance analysis (Bodystat 1500; Bodystat, Isle of Man, United Kingdom) and either a device’s internal algorithm for control subjects. FFM was normalized for height to produce the FFM index (FFMI; in kg/m2). Maximum sniff nasal pressure was used as a measure of inspiratory muscle strength (31).

Genotyping

Genomic leukocyte DNA was extracted by salting out. BK2R genotype was ascertained by polymerase chain reaction (PCR) amplification with the use of forward 5’-TCTGGCTTCTGGGCTCGAG-3’ and reverse 5’-AGCGGCATGGGCACTTCAGT-3’ primers (25). ACE genotype was determined by PCR with the use of a published 3-primer method that included an I-specific oligonucleotide (32). Two technicians, blind to subject characteristics, independently confirmed the genotypes. If discrepancies occurred, they were resolved by repeat genotyping.

Quadriiceps strength

Isometric quadriceps strength (QMVC) was measured while the subjects were seated upright in a chair attempting to extend their legs against an inextensible strap attached at the ankle (33). The signal was amplified and passed to a computer running LabView software (National Instruments, Austin, TX). Force generated was visible online, and the subjects received vigorous encouragement. The best of ≥3 efforts was taken. Quadriceps strength was expressed as an absolute value, corrected for body weight (33) and as strength per kilogram FFM. In some patients, magnetic nerve stimulation was used to give an additional nonvolitional measure of quadriceps strength. For this measurement, 2 linked Magstim 200 Monopulse stimulator units (Magstim Ltd, Whitland, United Kingdom) were discharged simultaneously through a 70-mm branding iron coil placed over the femoral nerve (with subjects supine), and the resulting quadriceps twitch force (TwQ) was measured (34).

Statistical analysis

Statistical analysis was conducted by using STATA (version 8.0; Stata Corp, College Station, TX). Hardy-Weinberg equilibrium was examined for both genotypes with the use of chi-squared tests. Differences in continuous variables between individual genotypes were considered by using Bonferroni-Dunn tests for normally distributed variables; Kruskal-Wallis testing was used as a nonparametric alternative. A Scheffe test was used to compare +9 homozygotes with the other 2 genotypes combined. Linear trend analysis was used to investigate the presence of a trend in FFMI or quadriceps strength across genotypes. Differences in categorical variables by genotype were considered with the use of chi-squared tests. The relation between BK2R genotype and FFMI and QMVC was analyzed by using analysis of covariance with a stepwise modeling approach. In addition, models forcing in possible confounders were considered. Possible confounders included in the model were disease severity [percentage predicted forced expiratory volume in 1 s (FEV1), and carbon monoxide transfer factor (TLco)], ACE genotype, and sex. Interactions were also considered in the model as appropriate. A 2-tailed P value >0.05 was considered to be significant.

RESULTS

One hundred ten patients with COPD were studied (FEV1: 34.3 ± 16.4% of predicted), whose genotype distribution (n = 37 +9/+9, 47 +9/–9, 26 –9/–9) was similar to that of 104 control subjects (n = 27 +9/+9, 50 +9/–9, and 27 –9/–9, respectively). ACE genotype was independent of the BK2R genotype (P > 0.5), and both genotypes were in Hardy-Weinberg equilibrium in both patients and control subjects (P > 0.4). Subject characteristics are described in Table 1. Thirteen patients were taking maintenance oral prednisolone (<10 mg/d), and 80 (73%) were taking inhalant corticosteroids; neither variable differing between genotypes.

Control subjects

Control subjects had a significantly higher FFMI (17.3 ± 2.8) than did the COPD patients (16.3 ± 2.2; P = 0.008) and a greater QMVC (44.0 ± 12.9 kg) than did the COPD patients (34.5 ± 12.3 kg; P < 0.0001) (Table 1). No variable measured in the control subjects was related to either BK2R or ACE genotype.

COPD patients

FFMI varied according to BK2R genotype: +9/+9, 15.7 ± 1.8; –9/+9, 16.9 ± 2.2; and –9/–9, 16.2 ± 2.3 (P = 0.045, ANOVA) and was significantly lower in patients who were +9 homozygotes: 15.7 ± 1.8 compared with 16.6 ± 2.3 (P = 0.038) (Figure 1). By stepwise analysis, transfer factor was also retained as an independent predictor of FFMI, but measures of airflow obstruction, hyperinflation, sex, and ACE genotype were not.
QMVC was significantly lower in +9 homozygotes than in patients with one or more −9 alleles: QMVC 30.8 ± 10.4 compared with 36.4 ± 12.8 kg (P = 0.02) (Table 2). BK2R genotype, ACE genotype, % of predicted TLCO, and sex (but not % of predicted FEV1) were retained as independent predictors of QMVC (P = 0.02, 0.03, 0.002, and <0.0001, respectively). There was no evidence that any of the variables included in this model significantly altered the effect of BK2R on QMVC (P > 0.2 for all).

FFMI was strongly correlated with QMVC (P < 0.00005), but there was no evidence that the relation between BK2R and QMVC varied with FFMI (P = 0.98). However, when FFMI was

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Control subjects (n = 104)</th>
<th>Patients (n = 110)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male:female)</td>
<td>48:56</td>
<td>78:32</td>
<td>0.0002</td>
</tr>
<tr>
<td>Age (y)</td>
<td>61.8 ± 8.5²</td>
<td>63.5 ± 9.4</td>
<td>0.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.7 ± 4.0</td>
<td>23.9 ± 4.7</td>
<td>0.2</td>
</tr>
<tr>
<td>FFMI (kg/m²)</td>
<td>17.3 ± 2.8</td>
<td>16.3 ± 2.2</td>
<td>0.008</td>
</tr>
<tr>
<td>FEV₁ (% of predicted)</td>
<td>103.2 ± 14.6</td>
<td>34.3 ± 16.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>QMVC (% of predicted)</td>
<td>83.6 ± 18.5</td>
<td>66.4 ± 19.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>QMVC/FFMI</td>
<td>0.89 ± 0.18</td>
<td>0.72 ± 0.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>QMVC (kg)</td>
<td>44.0 ± 12.9</td>
<td>34.5 ± 12.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SNiP (cm H₂O)</td>
<td>93.1 ± 22.2</td>
<td>63.8 ± 19.1</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

¹ FFMI, fat-free mass index; FEV₁, forced expiratory volume in 1 s; QMVC, quadriceps maximum voluntary contraction; SNiP, sniff nasal pressure; FFM, fat-free mass.
² x ± SD (all such values).

**FIGURE 1.** Fat-free mass index (FFMI) according to bradykinin type 2 receptor (BK2R) genotype in 110 patients (n = 37 +9/+9, n = 47 +9/−9, and n = 26 −9/−9) with chronic obstructive pulmonary disease (P = 0.045, ANOVA). Box values encompass 75th and 25th percentiles; the whiskers represent the 90th and 10th percentiles. *Significantly different from heterozygotes, P = 0.015 (Bonferroni-Dunn post hoc test). **Significantly different from the other 2 genotypes combined, P = 0.038 (Scheffe test).
TABLE 2
Comparison of chronic obstructive pulmonary disease patients homozygous for the +9 allele and the carriers of the −9 allele

<table>
<thead>
<tr>
<th></th>
<th>−9 Carriers (n = 73)</th>
<th>+9 Homozygotes (n = 37)</th>
<th>P²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male:female)</td>
<td>52:21</td>
<td>26:11</td>
<td>0.9</td>
</tr>
<tr>
<td>Age (y)</td>
<td>63.4 ± 9.8</td>
<td>63.8 ± 8.8</td>
<td>0.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.1 ± 5.0</td>
<td>23.5 ± 4.1</td>
<td>0.5</td>
</tr>
<tr>
<td>FFMI (kg/m²)</td>
<td>16.7 ± 2.3</td>
<td>15.7 ± 1.8</td>
<td>0.038</td>
</tr>
<tr>
<td>QMVC (kg)</td>
<td>36.4 ± 12.8</td>
<td>30.8 ± 10.4</td>
<td>0.02</td>
</tr>
<tr>
<td>QMVC (% of predicted)</td>
<td>69.1 ± 19.1</td>
<td>61.0 ± 20.0</td>
<td>0.04</td>
</tr>
<tr>
<td>TWQ (kPa)</td>
<td>9.4 ± 1.5</td>
<td>9.8 ± 1.3</td>
<td>0.96</td>
</tr>
<tr>
<td>SNiP (cm H₂O)</td>
<td>15.2 ± 2.3</td>
<td>16.5 ± 2.1</td>
<td>0.3</td>
</tr>
<tr>
<td>GLucose (mmol/L)</td>
<td>5.7 ± 1.0</td>
<td>5.9 ± 2.1</td>
<td>0.67</td>
</tr>
<tr>
<td>TWQ/FFM</td>
<td>0.24 ± 0.7</td>
<td>0.18 ± 0.7</td>
<td>0.011</td>
</tr>
</tbody>
</table>

¹FFMI, fat-free mass index; QMVC, quadriceps maximum voluntary contraction; FEV₁, forced expiratory volume in 1 s; TLCO, carbon monoxide transfer factor; TLC, total lung capacity; Pa, partial pressure in arterial blood; SNiP, sniff nasal pressure; TWQ, quadriceps twitch force in response to magnetic femoral nerve stimulation; FFMI, fat-free mass.
²Scheffe test.
³± SD (all such values).
⁴Random blood glucose values were available for forty −9 carriers and eighteen +9 homozygotes.

Included in the model, the association of BK₂R with QMVC was no longer statistically significant (P = 0.15).

TWQ was measured in 39 patients: n = 20 +9/+9, n = 15 +9/−9, and n = 4 −9/−9. It was significantly lower in +9 homozygotes than in those with one or more −9 alleles: 8.7 ± 3.3 compared with 11.1 ± 3.3 kg (P = 0.04). There was a trend for increasing TWQ in those with the −9 allele across genotypes: 9.3 ± 3.3, 11.1 ± 3.6, and 11.5 ± 5.0 kg for +9/+, +9/−, and −9/−, respectively, but this change was not significant (P for linear trend = 0.051) (Figure 2). The association was stronger for TWQ expressed as a function of FFMI across genotypes (P for linear trend = 0.007). There was no evidence of any interaction between the effects of BK₂R genotype and ACE genotype on TWQ (P = 0.4) or TWQ/FFM (P = 0.6).

There was no relation between BK₂R genotype and either pulmonary function or respiratory muscle strength. The relation between quadriceps strength and ACE gene polymorphism described in our previous article (13) remained significant in the expanded cohort. The D allele was associated with greater strength by linear trend against QMVC (P = 0.05), percentage predicted QMVC (P = 0.013), and QMVC/FFM (P = 0.003).

DISCUSSION

The main finding of this study was that patients with COPD who were +9 homozygotes had a lower FFMI than did those patients with one or more −9 alleles. We found reduced quadriceps strength in these patients, but, in contrast with the situation for ACE polymorphism, BK₂R genotype was not a predictor of quadriceps strength when the data were adjusted for FFMI. In addition, we did not identify any interaction between BK₂R and ACE genotypes in determining subject phenotype; in fact, the association observed was the opposite of that which would have been predicted had ACE gene polymorphism modulated skeletal muscle strength through the bradykinin pathway. Taken together, the data suggest that BK₂R polymorphism is a determinant of FFMI in patients with COPD with secondary effects on quadriceps strength, whereas data from ourselves (13) and others (35) support a local role for ACE genotype in determining quadriceps strength in COPD.

Loss of muscle mass is a serious development in COPD and confers a poor prognosis. Thus, for example, in the Copenhagen City Heart Study the combination of a BMI < 20 and an FEV₁ < 50% conferred an almost 20-fold increased risk of dying over a 17-y period of follow-up (3). In addition, reversal of nutritional depletion materially improves survival (36). Therefore, we believe that our observation offers an insight into the loss of FFMI in patients with COPD that could offer therapeutic opportunities by increasing kinin activity at the muscle level.
These data support a possible independent role for kinin activity in regulating skeletal muscle form and function in COPD patients. However, this finding should be interpreted with caution, because the association of the BK2R genotype with receptor expression may vary in health and disease. Furthermore, angiotensin II is known to increase the expression of BK2R receptors, which further complicates the issue (37). Bradykinin is not known to influence fiber type in the manner that angiotensin II does, with high concentrations of the latter favoring fast-twitch fibers (38–41).

The data presented in this article do not allow us to reach any definite conclusions about the mechanism by which BK2R genotype is associated with FFMI in COPD. Interestingly, the +9/+9 genotype has been shown to be associated with reduced metabolic efficiency during exercise in healthy subjects (27). In our study, COPD +9 homozygotes had a reduced FFMI as well as a reduced quadriceps strength. This may reflect a greater tendency toward protein catabolism in the face of the increased metabolic demands in patients with COPD (42). Another possible mechanism of action could be through insulin resistance. Bradykinin promotes glucose uptake in muscle (18, 43), the absence of the BK2R gene is associated with insulin resistance in mice (44), and the action of ACE inhibitors to increase whole-body insulin sensitivity is attenuated by BK2R blockade (45). One of the actions of insulin is the inhibition of proteolysis, and an association has been shown between proteolysis and insulin resistance in patients with cystic fibrosis (46). Moreover, amino acid metabolism is altered in COPD (47) with an increase in whole-body protein turnover (48). Thus, insulin resistance may account for our finding of reduced lean body mass in +9/+9 homozygotes with COPD. In a proportion of the patients studied, random blood glucose measurements taken as part of routine clinical care were available and did not differ by genotype. Clearly, the measurement of blood glucose is not a surrogate for the direct measurement of insulin resistance; therefore, any relation between insulin resistance, BK2R genotype, and FFMI remains speculative, but we believe that it may prove to be a productive area for future studies.

Although the relation between BK2R genotype and strength is partly mediated by changes in muscle bulk, the finding of an association between genotype and QMVC corrected for body weight as well as TwQ/FFM suggests that this need not be the sole explanation. More research is needed to establish whether there is a loss of specific force as well. A further limitation of this study is that we measured only whole-body FFMI and not regional muscle mass or quadriceps cross-sectional area. This made it impossible to be certain whether the reduction in quadriceps strength that we observed in the +9 homozygotes was due to a loss of muscle bulk or a loss of specific force (force per unit of cross-sectional area). The fact that both strength and FFMI were lower in patients who were +9 homozygotes than in the other genotype groups favors the former explanation as does the fact that the relation between BK2R and QMVC ceased to be significant if FFMI was included in the statistical model.

Finally, the observation that quadriceps but not respiratory muscle strength is related to genotype supports the view that susceptibility to deconditioning in COPD depends on the demands made on the muscle locally, because the locomotor muscles experience detraining, whereas the respiratory muscles must work against an increased load (49). The absence of any relation between genotype and FFMI in the control subjects suggests that the presence of disease-related factors, such as disuse or circulating cytokines, is necessary for it to have an effect. It remains to be seen whether the relation we observed in patients with COPD will be replicated in other conditions that are characterized by a loss of skeletal muscle bulk, including heart failure, and particularly in conditions in which disuse and immobility are major factors at play such as after fractures and strokes.

NSH, AHN, JM, HM, and MIP were involved in the study design. NSH and AHN collected the data. JP and KIE performed the genetic analyses. EH performed the statistical analyses. NSH analyzed the data with statistical input from EH and wrote the first draft of the manuscript. All authors contributed to subsequent drafts of the manuscript and approved the final version. NSH, AHN, JM, JP, KIE, EH, and MIP had no conflicts of interest. HM acts as a consultant to Ark Therapeutics, which has an interest in the role of ACE in the genesis of cachexia (including muscle wasting). This subject matter relates obliquely to the subject area of this article, ie, ACE and muscle function.

REFERENCES


