Increased energy expenditure in cystic fibrosis is associated with specific mutations

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1. Measurements of resting metabolic rate were made by open-circuit indirect calorimetry in 78 unrelated cystic fibrosis patients and 30 healthy control subjects. The aims of this study were: (i) to determine the range of variability in resting metabolic rate in cystic fibrosis, (ii) to relate this to pulmonary function and body size, and (iii) to investigate the hypothesis that, in cystic fibrosis, genotype exerts a significant influence on energy requirements.

2. There was no significant difference in age or body weight between patients with cystic fibrosis and control subjects. Resting metabolic rates for control subjects fell within ±10% of predicted values. Fifty-nine per cent of patients with cystic fibrosis had elevated resting metabolic rates (i.e. >111% of predicted). Genotype analysis divided the patients with cystic fibrosis into three groups: AF508 homozygotes, AF508 heterozygotes and others. Patients homozygous for the AF508 allele had a significantly higher resting metabolic rate (121% of predicted, 95% confidence interval 116–126%), compared with other genotypes (P<0.005).

3. There were significant differences in pulmonary function between the groups (P<0.005). However, after adjustment of individual resting metabolic rates for differences in pulmonary function by using analysis of covariance, resting metabolic rates remained significantly higher for AF508 homozygotes than for other genotypes (P<0.05).

4. We conclude that there is a significant contribution to resting metabolic rate in cystic fibrosis associated with specific mutations that is not explained by declining pulmonary function. The increase in resting metabolic rate in patients homozygous for mutations involving a nucleotide-binding fold, which may result from a disruptive effect on ATP binding, indicates a practical implication of genotype identification with the need for effective nutritional intervention and support in this patient subgroup.

INTRODUCTION

Although an elevation in resting metabolic rate (RMR) is documented in cystic fibrosis (CF) [1–3], this is subject to wide variability in onset and in magnitude, and it is becoming clear that this is a multi-factorial trait determined by genetic and non-genetic factors and their interaction. The CF gene is large, extending over 250 kb of genomic DNA, with over 70 mutations having been identified in the gene product, cystic fibrosis transmembrane conductance regulator (CFTR). In CF, homozygotes or compound heterozygotes for two abnormal alleles are clinically affected. The common CF mutation, which involves a phenylalanine deletion at position 508 of the gene product (AF508), is identified in 71% of CF chromosomes in the U.K. [4], and is located in a proposed nucleotide-binding fold (NBF) of CFTR [5]. Recent evidence from Thomas et al. [6] demonstrates that this region binds adenosine nucleotides. The protein contains two proposed nucleotide-binding domains, and both sites may be required for ATP catalysis. The structural alteration induced by mutation in a NBF is therefore of potential importance for the functional regulation of cellular energy metabolism.

Heritability is believed to account for between 11 and 40% of the total variance in RMR between healthy subjects [7, 8]. We have suggested a genetic influence on energy expenditure in CF [9], recognizing two clinical subgroups of CF patients, those with elevated RMR and those whose RMR is normal. Although a weak correlation has been shown between RMR and pulmonary function [1, 2], the recognition of increased RMR in patients with
normal lung function and growth parameters suggests additional factors, including a response to the basic cellular defect. We hypothesized that mutations in the NBFs of the gene product exert an influence on RMR in CF.

METHODS

Subjects

Seventy-eight unrelated CF patients (52 males, 26 females) with a mean age of 11.2 years (range 1.5–28 years) were studied. We used a group of 30 healthy control subjects with a mean age of 14.9 years (range 8–24 years) to standardize our calorimetric methods. All subjects were of British Caucasian origin. Body weight in

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Methods

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RMR

RMR was measured by continuous computerized open-circuit indirect calorimetry, using a ventilated hood (Datex Metabolic Monitor; Datex Instrumentation Corporation, Helsinki, Finland). Subjects aged 5 years and over had measurements made whilst they were supine in a warm room, 12 h post-prandially between 08.00 and 10.00 hours. There was no history of acute infection for at least 4 weeks (although a number of those studied were colonized by Pseudomonas). No medication was given for 12 h before the study. An adaptation period of 15–30 min was allowed before measurement was started. RMR was calculated from O₂ consumption and CO₂ production by using the equation of Weir [17], with measurements at 1 min intervals for a minimum of 30 min. Tests were carried out under close monitoring by a single observer and where extra movements were recorded the subjects were excluded from analysis. In children less than 5 years old (n = 18), measurements were made at home at least 4 h post-prandially, within 2 h of the onset of sleep. These patients were excluded from subsequent analysis of respiratory quotient (RQ). To determine intra-individual variability in RMR and RQ, replicate studies were made on 30 subjects (including 15 control subjects) within 3 months. The measured RMR is expressed as a percentage of predicted, as derived from the Harris–Benedit equations [17a], and the RQ was determined from gas fractions (CO₂/O₂). The individual variability in RMR measurements in the individual subject on repeat assessment is given by a coefficient of variation [18] of 4% for RMR and 3% for RQ.

Statistical analysis

Values in the text and Tables are expressed as means with 95% confidence limits in parentheses. As there was evidence of heterogeneity of variance in RMR values between patients and control subjects, the non-parametric Kruskal–Wallis test and an associated multiple-range procedure [19] were used for this comparison. All other group comparisons (including comparisons of RMR between the patient groups) were performed by using analysis of variance with Newman–Keuls multiple-range procedure [20]. Analysis of co-variance was also used to adjust RMR comparisons between patient groups for the possible confounding effects of BMP, age and pulmonary function. The square of the multiple-correlation coefficient (r²) was used to assess the contribution of genotype to RMR.

RESULTS

We studied 108 subjects (including 30 control subjects). Of the 78 CF patients, 31 (40%) were homozygous for the ΔF508 mutation (ΔF508/ΔF508), 29 (37%) were heterozygous for the AF508 mutation (ΔF508/AF508) and 18 (23%) carried other mutations (N/N genotype). Eight of these latter patients had mutations characterized subsequently. An abnormal RMR has been defined as a

Genomic DNA analysis

DNA was extracted from the leucocytes of CF patients [15]. Samples were randomly coded and sent to the Human Genetics Unit, Western General Hospital, Edinburgh, for analysis. Genomic DNA was amplified by the polymerase chain reaction and the ΔF508 mutation was identified by hybridization of the amplified sequence with allele-specific oligonucleotides or by polyacrylamide-gel electrophoresis as previously described [4, 16]. In patients without this mutation on both chromosomes, subsequent analysis was undertaken for the presence of five other mutations (ΔA507, G551D, R553X, G542X, R117H) [4].

Statistical analysis

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deviation of greater than 11% from the predicted RMR [7]. Fifty-nine per cent of patients had an elevated RMR. The range of RMR values for the control subjects fell within ± 10% of predicted values.

There were no significant differences in age or body size (as indicated by BMP) between genotype groups and control subjects (Table 1). Differences in ventilatory capacity (FEV₁, FVC) were significant between the four groups (P < 0.005); however, there was no significant difference in pulmonary function between the ΔF508/ΔF508 genotype and the ΔF508/N genotype using multiple-range procedure [19]. The CN score (0–38 according to severity) and ventilatory capacity were significantly different between the three patient groups (P < 0.01). The CN score for both the ΔF508/ΔF508 and the ΔF508/N genotypes was 9 (95% confidence interval 7–11) and that for the N/N genotype was 5 (4–6). Our data suggest that ΔF508 heterozygotes had slightly less percentage body fat and less lean body mass (as expressed by MAMC) than other genotype groups (Table 2). Of the 78 CF patients studied, 29 were chronically infected with Pseudomonas aeruginosa and two with P. cepacia. There was a significant difference in the rate of colonization between genotype groups, ΔF508 homozygotes having the highest frequency. Bacteriological data did not, however, appear to correlate with RMR: of 31 patients culturing Pseudomonas in sputum, 13 had normal RMRs. In the analysis of RMR (Table 3), results were significantly higher for the ΔF508/ΔF508 genotype compared with those for other patient groups and control subjects (P < 0.005). The differences in RMR between patient groups alone were significant (P < 0.005), with a mean RMR for ΔF508 homozygotes of 121% of predicted, for ΔF508 heterozygotes of 109% of predicted and for those without the ΔF508 mutation on either chromosome (N/N group) of 104% of predicted. The Newman–Keuls multiple-range procedure confirmed that the significant difference lay in the AF508/AF508 group comparison(s). There was no significant correlation shown between the decline in pulmonary function (FEV₁) and RMR (r = 0.31) in the 48 CF patients old enough to permit testing. In view of differences in pulmonary function (including that indicated by the CN score), and the differences in body size (BMP), percentage body fat, MAMC and age between groups which might influence mean RMR values for genotype groups, we used analysis of co-variance to adjust RMR for differences in these co-variates (r² = 0.14). RMR values remained significantly higher for ΔF508 homozygotes than for the other patient groups after adjustment (P < 0.05). When genotype was added to the regression model that predicted RMR, and additional 20% of the variation in RMR was explained (r² = 0.34). Significantly higher RQ values (Table 3) were measured for all CF patient groups compared with control subjects (P < 0.001), with mean values of 0.87 and 0.80, respectively. These were not accounted for by differences in FEV₁ and FVC between the four groups; however, differences in RQ between the three patient groups were removed when adjustment was made for pulmonary function by analysis of co-variance.

Genomic DNA analysis detected mutations other than ΔF508. We compared the results of extended genotype analysis with clinical and metabolic manifestations of the disease (Table 4). Each combination of compound heterozygote demonstrates a wide variability in the individual patient. The functional consequences of AF508 heterozygosity, where the other mutation is not known, vary widely. The second most common mutation amongst Caucasian CF chromosomes is G551D, which is also

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### Table 1. Anthropometric data and pulmonary function in 108 subjects. Values are means with 95% confidence intervals in parentheses. N denotes an uncharacterized mutation. Statistical significance: *P < 0.005 by analysis of variance on four group means; **Significance difference from values for other genotype groups (P < 0.01) by the Newman–Keuls multiple-range procedure.

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Age (years)</th>
<th>BMP (%)</th>
<th>FEV₁ (%)</th>
<th>FVC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔAF508/ΔAF508 (31)</td>
<td>13.0(10.6–15.4)</td>
<td>95(91–99)</td>
<td>56(48–64)</td>
<td>67(59–74)</td>
</tr>
<tr>
<td>ΔAF508/N (29)</td>
<td>9.7(7.1–12.3)</td>
<td>98(94–101)</td>
<td>63(52–74)</td>
<td>79(69–80)</td>
</tr>
<tr>
<td>N/N (18)</td>
<td>10.9(8.3–13.5)</td>
<td>97(92–102)</td>
<td>97(93–102)**</td>
<td>104(96–109)**</td>
</tr>
<tr>
<td>Control subjects (30)</td>
<td>14.9(13.6–16.1)</td>
<td>103(100–105)</td>
<td>103(99–107)</td>
<td>110(104–115)</td>
</tr>
</tbody>
</table>

### Table 2. Clinical data for patients with CF. Values are means ± SD.

<table>
<thead>
<tr>
<th>Body composition</th>
<th>Fat (% of body weight)</th>
<th>Male</th>
<th>13 ± 4</th>
<th>15 ± 2</th>
<th>16 ± 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>20 ± 5</td>
<td>21 ± 5</td>
<td>17 ± 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAMC (percentile)</td>
<td>20 ± 26</td>
<td>37 ± 28*</td>
<td>25 ± 22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas in sputum</td>
<td>17/31</td>
<td>12/29</td>
<td>2/18</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 3. Influence of the ΔF508 mutation on RMR and RQ.

Values are means with 95% confidence intervals in parentheses. N denotes an uncharacterized mutation. Statistical significance: *P < 0.005 by the Kruskal–Wallis test on patient and control groups, and by analysis of variance on three patient groups only; **P < 0.001 by analysis of variance on four means; †Significantly different from patient and control groups (P < 0.01) by the Kruskal–Wallis multiple-range procedure; ‡Significantly different from other patient groups (P < 0.01) by the Newman–Keuls procedure.

<table>
<thead>
<tr>
<th>Group</th>
<th>RMR* (% of predicted)</th>
<th>RQ**</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔAF508/ΔAF508</td>
<td>121(116–126)††</td>
<td>0.88(0.87–0.89)</td>
</tr>
<tr>
<td>ΔAF508/N</td>
<td>109(103–116)</td>
<td>0.86(0.85–0.87)</td>
</tr>
<tr>
<td>N/N</td>
<td>109(97–111)</td>
<td>0.86(0.85–0.87)</td>
</tr>
<tr>
<td>Control subjects</td>
<td>101(98–104)</td>
<td>0.80(0.75–0.82)††</td>
</tr>
</tbody>
</table>
Table 4. Influence of CF genotype on BMP, CN score and RMR.

<table>
<thead>
<tr>
<th>Genotype (n)</th>
<th>BMP</th>
<th>CN score*</th>
<th>RMR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔF508/ΔF508 (31)</td>
<td>95 (91-99)</td>
<td>9 (6-11)</td>
<td>121 (116-126)</td>
</tr>
<tr>
<td>ΔF508/G551D (1)</td>
<td>93</td>
<td>10</td>
<td>121</td>
</tr>
<tr>
<td>ΔF508/R117H (3)</td>
<td>98 (97-101)</td>
<td>5 (3-6)</td>
<td>111 (100-122)</td>
</tr>
<tr>
<td>ΔF508/R553X (2)</td>
<td>95, 89</td>
<td>12, 11</td>
<td>91, 94</td>
</tr>
<tr>
<td>ΔF508/G542X (2)</td>
<td>79, 97</td>
<td>13, 3</td>
<td>91, 94</td>
</tr>
<tr>
<td>G551D/R117H (1)</td>
<td>103</td>
<td>1</td>
<td>86</td>
</tr>
<tr>
<td>ΔF508/N (20)</td>
<td>100 (97-104)</td>
<td>8 (6-11)</td>
<td>110 (101-118)</td>
</tr>
<tr>
<td>G551D/N (5)</td>
<td>100 (91-110)</td>
<td>7 (4-11)</td>
<td>121 (112-134)</td>
</tr>
<tr>
<td>ΔF507/N (1)</td>
<td>79</td>
<td>14</td>
<td>133</td>
</tr>
<tr>
<td>G542X/N (2)</td>
<td>92, 89</td>
<td>9, 3</td>
<td>96, 87</td>
</tr>
<tr>
<td>N/N (10)</td>
<td>96 (88-105)</td>
<td>4 (2-6)</td>
<td>102 (91-112)</td>
</tr>
</tbody>
</table>

*Severity of radiographic features according to the system of Chrispin & Norman [14], with points awarded to a maximum of 36 (best score=0).

situated in a proposed nucleotide-binding domain [23], and was identified on six chromosomes in six patients. This mutation had a similar effect on RMR to the ΔF508 mutation; however, patients had slightly better CN scores and pulmonary function. Patients (n=8) who were compound heterozygotes for a mutation within a nucleotide-binding domain with a second mutation either outside this functional region (R117H) [21] or a nonsense mutation (R553X [22] or G542X [23]) demonstrated a normal RMR of 100% of predicted (91-109). Four of the five lowest BMPs were recorded in patients carrying the G542X allele, either in combination with ΔF508 or another undetermined allele. RMR was not increased in this sub-group. The basis for their energy imbalance is unclear, but high faecal energy losses may be one explanation.

**DISCUSSION**

We have identified a correlation between genotype and phenotype in CF. Homozygosity for mutation(s) in a proposed nucleotide-binding domain of the gene product CFTR has a significant influence on RMR in CF. Moreover, although our data confirm that the lowest recorded values for FEV₁, FVC, and the highest chronic infection rate by *Pseudomonas*, were found in the ΔF508/ΔF508 genotype group, there was no direct correlation between RMR and these measures of pulmonary function, and no direct association between colonization of the respiratory tract with *Pseudomonas* and an increased RMR. In attempting to relate metabolic requirements to lean body mass, we were constrained by the availability of methods with which to assess body composition. Assessment of body composition in CF has demonstrated a decrease in both body fat and LBM [24], and inadequate accretion of total body potassium has been shown in both presymptomatic infants detected by screening and in patients growing within normal centiles [25]. As prediction equations to calculate the body-fat content of children with CF appear to be invalid [26], we did not use them to derive values for lean body mass, but used MAMC as an index of lean body mass. Including MAMC and percentage body fat in analysis of co-variance, differences in body composition between the CF groups derived from anthropometric data did not explain the variation in RMR. Differences between genetic group means were significant after adjustment for differences in pulmonary function and anthropometric data, with significantly elevated values for RMR in ΔF508 homozygotes irrespective of FEV₁, FVC, percentage body fat and MAMC. Other studies, while recognizing a contribution from the decline in pulmonary function and differences in body composition to measured increases in RMR, have suggested the influence of a basic defect in energy metabolism at the cellular level [1, 2]. The variable clinical course in CF patients has been attributed, at least in part, to specific genotypes at the CF locus [27]. Using multiple-regression analysis we have shown that 20% of the variance in RMR is explained by the presence of the ΔF508 mutation. This value is similar to the percentage variation in RMR attributed to heritability in family studies of healthy subjects [7, 8]. Although exerting a significant influence on RMR independent of pulmonary function, genotype is not, however, the sole determinant of energy requirement in CF and other factors (genetic and/or environmental) are recognized to play a role in the course of the disease.

The ΔF508 mutation has been localized to a putative NBF in the CFTR molecule [5]. Recent studies with synthetic oligopeptides have shown that this region of CFTR does indeed possess adenine-nucleotide-binding activity. Circular dichroism spectroscopy predicts that the deletion of the phenylalanine residue at position 508 would disrupt a β-sheet structure in this region [6]. It has been assumed, but not proven, that bound ATP is hydrolysed and the energy released is used to permit a conformational change in the molecule. Other mutations in the NBF (e.g. G551D) may also have a disruptive effect on ATP binding. It has recently been suggested that CFTR is a tightly regulated chloride channel and that ATP binding and hydrolysis is necessary for conformational change before chloride transport [28]. Our evidence from indirect calorimetry, that specific mutations in the nucleotide-binding domain alter cellular energy requirement, supports this hypothesis.

The recessive nature of CF means that both alleles play a role in determining phenotype and both characters in a compound heterozygote are expected to exert specific effects on the CFTR protein. The functional consequences of rare allelic variants vary widely. That values for some of the ΔF508/N group fell within the range of the ΔF508/ΔF508 group suggests that the other mutation may exert a similar influence to ΔF508 (i.e. that it is situated within the NBF). In particular, the G551D mutation has a similar effect on RMR to the ΔF508 mutation, and both mutations lie within exons 10–11 of the gene and relate to the same functional domain [5, 23]. Differences in pulmonary and anthropometric assessment support the suggestion of independent genetic determinants for various clinical components of the phenotype [29]. Certain known mutations do not confer an increase
in RMR. For example, the R117H mutation is near a transmembrane region of the protein and is probably not involved in nucleotide binding [21]. If the observed phenotype is the result of aberrant ATP binding or hydrolysis, null mutations which prevent synthesis of the CFTR protein (e.g. the stop mutations G542X and R553X) would not result in this phenotype.

Our results have shown a significant influence on RQ in all CF patient groups compared with control subjects, indicating a shift towards an increase in carbohydrate metabolism. This was most marked in patients homozygous for ΔF508, although differences in pulmonary function explained the difference in RQ between genotype groups. An increase in RQ in CF patients has previously been reported [30]. The possibility that this might be a diet-induced response [31] was explored by analysing 7-day weighed intake data on 37 of the patients studied. The average food quotient was 0.87 for patient and control groups and no correlation was shown between RQ and food quotient in regression analysis. Two possible explanations for this increase in RQ are: (i) the glycogen reserve in CF is larger (although there is no evidence of this), or (ii) glycogen reserves are depleted more slowly by earlier mobilization of fat in CF. Evidence for an increased turnover of fatty acids in CF [32, 33] would be consistent with this hypothesis.

In summary, the variable nutritional requirements in CF patients can be partly attributed to specific genotypes at the locus of the CF gene. An increase in RQ indicating altered substrate utilization, however, seems to be a metabolic consequence of CF, irrespective of genotype. At a clinical level these results indicate that specific genotypes are at a greater risk of nutritional compromise. A high RMR combined with elevated fasting RQs resulting in RQ/food quotient imbalance has implications not only for intervention, but in planning current and future strategies for nutritional supplementation in patients with CF.

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