have been found to carry the R1162X mutation. Sequencing of all 27 CFTR exons, including flanking intronic regions, and a search for larger rearrangements were undertaken in the remaining 18 DNA samples. Calling all the patients back for further familial genetic studies could not be considered and we assumed that two identified mutations are located in trans. ORs were calculated and proportions were compared with prior probability using the likelihood ratio test and assuming an expected carrier rate of 1/25 (4%) in the Belgian general population.

Mean age (±SD) at the time of DNA sampling was 58.9 (±14.2) years. Bronchiectasis were present in 14/18 patients. Sweat chloride values between 40–59 mmol/l and 30–39 mmol/l were observed in 1 (5.5%) and 6 patients (35%), respectively. A total of 18 putative mutations were identified in 17/36 alleles (table 1), most of which were mild/uncommon. The CFTR mutation carrier frequency was much higher in patients with ABPA (12/18, 67%) than expected in the general population (p < 0.0001; OR 48.0, 95% CI 5.2 to 445.3). The probability of bearing two CFTR mutations was even more strikingly different (p<0.0001; OR 714, 95% CI 25 to 6797).

This study considerably extends previous findings by demonstrating a strong link between ABPA in adults and CFTR mutations. Although not altering the message, limitations of this work include the small population size which is inherent to the rarity of ABPA, the absence of DNA testing in parents and the dilemma of the clinical relevance of putative CFTR mutations. The hitherto best studied CFTR-related disorders are congenital bilateral absence of the vas deferens (CBAVD) and idiopathic chronic pancreatitis (ICP). It has been estimated that 85% of patients with CBAVD and 30% of those with ICP carry at least one CFTR mutation while ~50% and 10–15%, respectively, are compound heterozygous, with the F508del mutation and IVS8-T5 variant being most frequently detected. The present study supports the concept that ABPA in pancreatic-sufficient adults is a CFTR-related disorder, with rare class IV–V mutations being mostly found and IVS8-T5 not seeming to play a significant role. Moreover, as ABPA is usually associated with bronchiectasis, a major phenotypic feature of CF, appropriate investigations to exclude milder forms of CF are warranted in these patients.

**Vitamin D and COPD: seasonal variation is important**

Janssens et al. have demonstrated the relationship between vitamin D status and lung function in patients with chronic obstructive pulmonary disease (COPD). However, in their study there was only one assessment of vitamin D status per patient. Given the minimal component of diet to vitamin D status (serum 25-hydroxyvitamin D [25(OH)D] concentration), it is mostly determined by sunlight exposure and has a seasonal variation in healthy individuals.

In a study of 24 patients with COPD (mean (SD) age 69 (5.3) years and smoking history 43 (15.8) pack-years) with measurements undertaken in the same individuals at the end of summer (August/September) and winter (March/April), we have also shown that vitamin D status correlated with forced expiratory volume in 1 s (FEV1) (r=0.486, p=0.016), which remained significant (p=0.048) when corrected for age, gender, body mass index and activity level. However, we also showed that the relationship between spirometry (FEV1 and forced vital capacity (FVC)) and vitamin D status was stronger in the winter (FEV1: r=0.451, p=0.027; FVC: r=0.567, p=0.078) than in the summer (FEV1: r=0.599, p=0.053; FVC: r=0.264, p=0.215). For spirometry there were non-significant seasonal trends with a mean difference of 0.051 (95% CI –0.15 to 0.12) and 0.071 (95% CI –1.1 to 0.25) for FEV1 and FVC, respectively (table 1).

Janssens et al. showed that patients with COPD have poorer vitamin D status than healthy smokers. Our results confirmed this finding and, additionally, showed differences between the vitamin D status both in summer and winter compared with age-, gender-, month- and geographical region-matched controls from the UK National Diet and Nutrition Survey (NDNS). For the winter values the mean difference was 11.9 nmol/l (95% CI 7.4 to 16.3) (p<0.0001) and for summer values the mean difference was 20.9 nmol/l (95% CI 15.5 to 26.3) (p<0.0001). Furthermore, in the winter there were three subjects who had 25(OH)D concentrations >50 nmol/l but none with a concentration >75 nmol/l, a concentration regarded by many as being appropriate to define vitamin D sufficiency. In the summer, eight patients had a 25(OH)D concentration >50 nmol/l and only one patient had a concentration >75 nmol/l. We did not find any association between oily fish intake and vitamin D status.

Sunlight exposure is also determined by the distance from the equator and therefore the findings from one region may not represent those of another. In a study which evaluated the vitamin D status in patients with asthma compared with healthy controls (which showed no significant difference in status between these groups), the serum 25(OH)D concentration was twice as high in patients from Aberdeen (latitude 57°N) than in those from Norwich (latitude 52°N).

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**Table 1 Mean (SD) values for summer and winter measurements**

<table>
<thead>
<tr>
<th>Vitamin D (nmol/l)</th>
<th>PTH (µg/l)</th>
<th>Calcium (mmol/l)</th>
<th>Corrected calcium (mmol/l)</th>
<th>FEV1 (l)</th>
<th>FVC (l)</th>
<th>PEF (l/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Winter</strong></td>
<td>35.1 (10.5)</td>
<td>5.4 (1.7)</td>
<td>2.4 (0.1)</td>
<td>2.4 (0.1)</td>
<td>1.18 (0.41)</td>
<td>2.66 (0.86)</td>
</tr>
<tr>
<td><strong>Summer</strong></td>
<td>49.3 (13.0)</td>
<td>4.7 (1.6)</td>
<td>2.4 (0.1)</td>
<td>2.4 (0.1)</td>
<td>1.23 (0.43)</td>
<td>2.73 (0.72)</td>
</tr>
<tr>
<td><strong>p Value</strong></td>
<td>0.000</td>
<td>0.018</td>
<td>0.239</td>
<td>0.375</td>
<td>0.121</td>
<td>0.420</td>
</tr>
</tbody>
</table>

There was a significant seasonal difference for vitamin D and parathyroid hormone. FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; PEF, peak expiratory flow; PTH, parathormone.
β-agonist safety and the elephant in the room?

We read with interest the recent paper by Weatherall et al who presented data from a meta-analysis of the relative safety of long-acting β-agonists (LABAs). In 2008 the FDA reported on the safety of LABAs and concluded that the nature and magnitude of risk needs to be confirmed. When adverse outcomes are reported relatively infrequently in clinical trials, it is difficult to interpret the value of such analysis and a more thorough examination of these index cases may yield more meaningful information about individual risks per se.

β-agonist safety and the elephant in the room?

Polymorphisms in the β2-adrenoceptor have long been linked to differences in response to β2-agonists after chronic dosing. In asthma, the prevalence of the genotype Arg-Arg-16 is approximately 15% and of Arg-Gly-16 is 45%. In patients with asthma using frequent β-agonists, there is an increased risk of asthma exacerbation per copy of Arg16 allele (OR 1.64; 95% CI 1.22 to 2.20; p=0.001).3 Could this be the elephant in the room for better understanding risk?

In a recent multicentre study assessing salmeterol as an add-on to inhaled corticosteroids in genotype-selected patients (the LARGE study), patients with the Gly-Gly-16 genotype had a 2.4-fold greater improvement in bronchial hyper-reactivity (BHR) with salmeterol than with placebo (p<0.0001), while patients with the Arg-Arg genotype had no such benefit (p=0.87).4 Lee et al have previously shown similar differences in response to BHR for patients with the Gly-Gly genotype compared with individuals carrying either one or two copies of Arg16 (table 1).5 Furthermore, this paper demonstrated differences in patients with Arg16 in their response to the full agonist formoterol compared with the partial agonist salmeterol. These findings are in keeping with the discussion by Weatherall et al which suggested that there may be different risks in different drugs from the same drug class.

It is common practice for respiratory physicians to measure levels of thiopurine methyltransferase before administration of the drug azathioprine in pulmonary fibrosis. This test identifies individuals with genetic polymorphisms which predispose to an adverse reaction to the drug. Perhaps the time has come to join up the dots and start assessing individual risk to β2-agonists rather than continuing to count cases.

Table 1 Change in bronchial hyper-reactivity as a doubling dilution shift for formoterol and salmeterol in genotype-specific patients as change from placebo after chronic dosing

<table>
<thead>
<tr>
<th>Drug</th>
<th>Genotype</th>
<th>Doubling dilution (dd) shift</th>
<th>&gt;1 dd (improvement)</th>
<th>&gt;1 dd (worsening)</th>
<th>&gt;1 dd (no change)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formoterol</td>
<td>Arg-Arg and Arg-Gly</td>
<td>17%</td>
<td>23%</td>
<td>60%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gly-Gly</td>
<td>27%</td>
<td>44%</td>
<td>29%</td>
<td></td>
</tr>
<tr>
<td>Salmeterol</td>
<td>Arg-Arg and Arg-Gly</td>
<td>35%</td>
<td>46%</td>
<td>18%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gly-Gly</td>
<td>50%</td>
<td>39%</td>
<td>11%</td>
<td></td>
</tr>
</tbody>
</table>

After data from Lee et al.

Provenance and peer review
Not commissioned; not externally peer reviewed.

Accepted 9 March 2010
Published Online First 7 June 2010

REFERENCES

Author’s response
We thank the authors for their interest in our paper.1 They raise an interesting and pertinent point about identifying patients at greater risk of β-agonist toxicity, a group in whom either the dose of β-agonist should be lowered, or in whom our point about co-prescription of inhaled corticosteroids is even more relevant.2 Unfortunately, we feel that the question about whether these patients could be identified in the context of randomised controlled trials, in which mortality is very rare and important morbid outcomes are relatively uncommon, is that it would be impossible to demonstrate a difference in these rare outcomes stratified by genetic status. This of course amounts to a similar problem as experienced by subgroup analysis of randomised controlled trials, namely a lack of statistical power to detect important differences. Another way of examining whether genotype affects morbid outcomes related to medication use...