the better the outlook; cessation <10 years before the end of the study did not appear to help. Sex was not a significant risk factor for COPD (most of the subjects who performed spirometric tests were women), but age at entry to the study was, presumably because older smokers had a history of more pack years at entry than younger smokers.

The authors emphasise that the incidence of COPD was unexpectedly high; it must have been over 25%, given that only 48% of those alive at 25 years underwent spirometric tests, and those who did not were almost certainly in worse health than those who did. Furthermore, 109 participants (more than 1% of the total) died of COPD during the observation period and were therefore unavailable for testing. This is distinctly higher than the commonly quoted figure of 15%—that is, 15% of smokers develop symptomatic COPD, a point recently emphasised by another editorial comment.[3]

The estimate of 15% of smokers developing COPD is usually attributed to Fletcher *et al*[4] whose work forms the basis of most of our current understanding of the disease. I am unable to locate an unequivocal statement in their book quoting this number, but I believe that there are some circumstances that might explain a difference between the purported views of Fletcher and the findings of the Copenhagen City Heart Study. Firstly, most of the COPD in the latter study was ''moderate''—that is, the participants had FEV_1 values of 50–79% of the predicted normal—so it is likely that many of these were not very symptomatic. Secondly, COPD in the early to mid 1960s was somewhat different from today; in my experience, deaths and hospital admissions caused by COPD were not uncommon in men aged 55–65, which is not the case today. If one imagines the rate of loss of FEV_1 necessary to culminate in values of 0.5–1.0 l at the age of 55, assuming the subject started smoking in late adolescence, the result is spectacular and rare today. This is a somewhat long winded way of saying that the COPD ''sensitivity'' of the Copenhagen Heart Study was very likely considerably greater than that of Fletcher *et al*.

Lokke *et al*[1] emphasise that the high incidence of COPD which they observed was due to the length of their study. The mean age at study entry was about 45 years and, given a 25 year follow up period, many of their participants must have been over 70. The message is that many smokers develop airways obstruction if they live long enough and continue to smoke, and that the number that do so is increasing because of a decline in competing mortality. I heartily agree with this conclusion. If a smoker has an FEV_1 of 4.0 l at the age of 30, his FEV_1 will have to decline by about 120 ml/year to develop respiratory failure (FEV_1 = 1.0 l) at the age of 55, which is uncommon. On the other hand, if the FEV_1 declines at a rate of 60 ml/year—the rate of decline of an ''average'' smoker[4 5]—respiratory failure is likely to occur at the age of 80 in an ''average'' smoker. It is not uncommon for smokers to reach 80 years of age nowadays.

An argument can therefore be made that many (perhaps most) smokers are ''susceptible'' to COPD if they live long enough. Indeed, the definition of susceptibility may need to be examined. Furthermore, the GOLD definition of COPD as an ''abnormal'' response to inhaled toxins may need revision, or at least a rethink. This is not to say that people's propensity to develop airways obstruction in response to smoking does not vary; approximately 65% of continuous smokers in the Copenhagen City Heart Study had FEV_1/FVC >70% after 25 years. There are such differences, and it is important—though difficult—to work out their mechanisms.

Thorax 2006;**61**:924–925.
doi: 10.1136/thx.2006.065623

Correspondence to: Dr N R Anthonisen, University of Manitoba, Respiratory Hospital, Winnipeg, Canada R3A 1R8; nanthonisen@exchange.hsc.mb.ca

Competing interests: none.

REFERENCES

1 **Lokke A**, Lange P, Scharling H, *et al*. Developing COPD: a 25 year follow up study of the general population. *Thorax* 2006;**61**:935–9.
2 **Celli BR**, MacNee W, Agusti A, *et al*. Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. *Eur Respir J* 2004;**23**:932–46.
3 **Rennard SI**, Vestbo J. The dangerous underestimate of 15%. *Lancet* 2006;**367**:1216–9.
4 **Fletcher CM**, Peto R, Tinker C, *et al*. *The natural history of chronic bronchitis and emphysema*. Oxford: Oxford University Press, 1976.
5 **Anthonisen NR**, Connett JE, Murray RP, for the Lung Health Study Research Group. Smoking and lung function in Lung Health Study participants after 11 years. *Am J Respir Crit Care Med* 2002;**166**:675–9.

Polymorphisms and asthma exacerbations

Genetic variability of the β_2 adrenergic receptor and asthma exacerbations

S B Liggett

Predictive value of genetic tests for asthma exacerbations

The treatment of asthma remains a formidable task for clinicians, despite the development of new treatment options such as long acting β agonists, potent inhaled corticosteroids, and leukotriene receptor antagonists. The complexities in treatment arise not only from temporal changes in the clinical status of patients (such as viral infections and allergy exposures), but also from the well recognised variability between individuals in the response to treatment. This variability is found with the most widely used treatment for asthma—the β agonists—which act to dilate constricted airways by binding to the β_2 adrenoceptor on airway smooth muscle. It has been suggested that up to 50% of the variability in response to β agonists between individuals has a genetic basis.[1] Beta agonists evoke smooth muscle relaxation in airways that have become constricted from virtually any spasmogen, so it is no wonder they have a central role in treating asthma.

However, several aspects of β_2 adrenoceptor signalling have been lurking in the shadows for many years. Firstly, in virtually every cell type that has been studied, chronic exposure (~8 hours or more) to β agonists results in a loss of receptor expression with accompanying desensitisation of the cellular response.[2] At the physiological level, desensitisation could be manifested as tachyphylaxis. Clinically, tachyphylaxis to β agonists could lead to a variety of outcomes such as progressive loss of the bronchodilating effect, a loss of the protective effect against bronchoconstriction, and increased exacerbations. It has also been recognised for over 25 years that corticosteroids increase β_2 adrenoceptor expression,[3] which could mitigate against agonist promoted downregulation.[4] The clinical response to corticosteroids in asthma may therefore involve both anti-inflammatory effects which reduce local spasmogens,

as well as improvement in β_2 adrenoceptor signalling.

As these molecular studies were underway, epidemiological[5–7] and clinical[8–10] studies were beginning to reveal potential adverse effects of β agonists, particularly with regular usage compared with an as-needed schedule. One of the most recently published studies involved approximately 26 000 asthmatics treated with salmeterol or placebo (the SMART study).[11] Small but statistically significant increases in several secondary mortality related end points were noted in the salmeterol treatment group. This may have been due to an excess in events in African Americans treated with salmeterol.

The genomic era had its impact on β agonist pharmacology, beginning in 1993 when we defined three non-synonymous polymorphisms of the β_2 adrenoceptor coding region.[12] Subsequent studies in transfected cells[13] and endogenously expressing airway smooth muscle cells[14] indicated that the two common polymorphisms (at amino acid positions 16 and 27) have an impact on agonist promoted down-regulation of receptor number. Furthermore, the variation at position 16 (where either Arg or Gly can be found) appeared to dominate the down-regulation effect. What was not clear at that time was how these in vitro findings would be manifested in patients. A reasonable hypothesis was that the position 16 polymorphisms influence tachyphylaxis to chronic β agonists, and this has been explored using a number of different approaches. For example, Taylor *et al*[15] retrospectively examined asthma exacerbations and peak expiratory flow rates (PEFR) in a three-way crossover study of 157 patients receiving regularly scheduled placebo, salbutamol (albuterol), or salmeterol and potential relationships to Arg or Gly16. In those treated with salbutamol, asthma exacerbations were greater in Arg16 homozygous patients than in Gly16 homozygotes, and Arg16 homozygotes treated with salbutamol had a higher exacerbation rate than Arg16 homozygotes treated with placebo. There was a strong trend towards lower PEFR in Arg16 homozygotes, but this did not reach statistical significance. The exacerbation rates during salmeterol treatment were low for all genotypes and did not differ between genotypes.

At about the same time, Israel *et al*[16] published results from a retrospective analysis of a trial of as-needed versus regularly scheduled salbutamol in 190 mild asthmatics. The primary finding centred around changes in the PEFR. In the Arg16 group receiving regular salbutamol, a decrease of 30.5 l/min in morning PEFR was observed. In contrast, neither Arg16 patients on as-needed salbutamol nor Gly16 patients receiving regularly scheduled salbutamol had a decrease in PEFR. Regular salbutamol treatment in Arg16 homozygotes was associated with a decrease of 23.8 l/min in morning PEFR compared with Gly homozygotes. A somewhat similar association was found in a subsequent prospective trial with regularly scheduled salbutamol[17] with a genotype attributable treatment difference of 24 l/min. Although retrospective and with a rather small number of subjects, another recent analysis has shown little benefit in patients with the Arg16 genotype during salmeterol treatment, either with or without concomitant inhaled corticosteroids.[18] Taken together, these studies point to a segment of the population—those homozygous for Arg16—who do not appear to benefit and/or may deteriorate with chronic β agonists.

It is worth noting that the aforementioned studies had relatively strict enrolment criteria and, as such, there was some degree of homogeneity in the patient populations within each study. This is a typical study design in that investigators attempted to minimise the noise from non-genetic heterogeneity. In the current issue of *Thorax*, Palmer *et al*[19] have explored this important concept in a different manner. These investigators considered a more ''real life'' situation, using a cross section of children and young adults with asthma (546 patients) from asthma clinics in Tayside, Scotland. Of these, 164 were receiving regular salmeterol with an inhaled corticosteroid and 351 were receiving as-needed salbutamol. These two groups represented approximately 95% of the β agonist treatment regimens. When all patients were considered, the odds ratio associated with Arg16 and asthma exacerbations was 2.05 ($p = 0.010$) but, in salmeterol treated patients, the odds ratio associated with Arg16 homozygosity and exacerbations was 3.40 ($p = 0.022$). In contrast, in patients not being treated with salmeterol (which essentially represents those on as-needed salbutamol), the odds ratio for exacerbations associated with Arg16 was not statistically significant (regardless of whether homozygous status or a co-dominant model was used).

These results are therefore consistent with the previously cited studies, all pointing to Arg16 polymorphism as a genetic marker for a poor response to chronic β agonist treatment in asthma. In addition, like the aforementioned study,[18] this phenotype is present during inhaled corticosteroid treatment. The potential ramifications of this concept are significant in that the frequency of homozygous Arg16 in the population ranges from ~15% in white ethnic groups to ~35% in those of Asian descent. What is not really clear from any of these studies is what percentage of the variability in a phenotype can be attributed to the position 16 polymorphism. If it is only a small fraction (10% or less), one has to wonder whether, alone, knowing the genotype at position 16 has any clinical usefulness. Similarly, what is the sensitivity and specificity of the test? The predictive value of genetic tests can potentially be increased if the gene has more than one polymorphism and only certain combinations of these polymorphisms (haplotypes) are responsible for a given cellular response. Genotyping at these multiple sites can therefore identify certain combinations more precisely. On the other hand, if a single polymorphism is the only variant that is controlling the phenotype, then partitioning the population into further subgroups will not improve the predictive power. For the intronless β_2 adrenoceptor gene, there are a number of polymorphic sites in the 5′ flanking region (up to nucleic acid −1023) which are found in 12 distinct haplotypes[20] (there may be additional polymorphic sites further upstream or in the 3′ untranslated region of the gene). Of the 12 haplotypes, five have frequencies of ~5% or more in one or more ethnic groups. As can be seen in table 1, if an individual has an adenine (A, which encodes for arginine) at nucleic acid position 46 (amino acid 16), their haplotype could be #1 or #4. This is particularly so for those of African descent in whom haplotype #1 has a frequency of 25%. Furthermore, an individual with a guanine (G, which encodes for glycine) at position 46 could have haplotype #2 or #6. Also note the approximately sevenfold difference in the frequency of haplotype #2 between white ethnic groups and African Americans, and the ~5% frequency of haplotype #9 which is exclusively found in African Americans. Within the β_2 adrenoceptor gene there is therefore substantial genetic variability outside the coding region. Adding these additional polymorphisms to the coding polymorphisms or the use of haplotypes may improve the predictive power. In addition, the β_2 adrenoceptor pathway has a number of signalling elements such as G_s, adenylyl cyclase, G-protein coupled receptor kinases, and protein kinase A. Genetic variability in any of these elements could also contribute to β agonist phenotypes.[21]

It is quite likely that a set of variants from multiple genes acts to set β agonist

Table 1 Localisation of single nuclear polymorphisms and identification of haplotypes of the β_2 adrenoceptor gene

	Nucleotide													Frequency (%)		
Haplotype*	−1023	−709	−654	−468	−406	−367	−47	−20	46	79	252	491	523	Ca	AA	As
1	A	C	G	C	C	T	T	T	A	C	G	C	C	0.7	25.0	12.5
2	A	C	G	G	C	C	C	C	G	G	G	C	C	48.3	6.3	10.0
3	G	A	A	C	C	T	T	T	A	C	G	C	C	0.7	0.0	0.0
4	G	C	A	C	C	T	T	T	A	C	G	C	C	33.0	29.7	45.0
5	G	C	A	C	C	T	T	T	G	C	G	C	C	1.4	0.0	0.0
6	G	C	G	C	C	T	T	T	G	C	A	C	A	13.2	31.3	30.0
7	G	C	G	C	C	T	T	T	G	C	A	T	A	1.0	1.6	0.0
8	G	C	A	C	C	T	T	T	A	C	A	C	A	0.7	0.0	0.0
9	A	C	G	C	T	T	T	T	A	C	G	C	C	0.0	4.7	0.0
10	G	C	G	C	C	T	T	T	G	C	A	C	C	0.7	0.0	0.0
11	G	C	G	C	C	T	T	T	G	C	G	C	C	0.3	0.0	2.5
12	A	C	G	G	C	T	T	T	A	C	G	C	C	0.0	1.6	0.0
Location	5′	5′	5′	5′	5′	5′	AA19 BUP T=Cys C=Arg	5′	AA16 G=Gly A=Arg	AA27 C=Gln G=Glu	Syn	AA164 C=Thr T=Ile	Syn			

*The haplotype number is based on the original description of β_2AR haplotypes as presented in Drysdale *et al*.[20]
Ca, Caucasian; AA, African American; As, Asian; BUP, β-adrenergic upstream peptide.

responsiveness. Ultimately, then, a ''score card'' may be developed which uses genetic variants from multiple genes and has substantial predictive power such that treatment can be individualised for asthma patients.

Thorax 2006;**61**:925–927.
doi: 10.1136/thx.2006.064006

Correspondence to: Dr S B Liggett, Cardiopulmonary Genomics Program, University of Maryland School of Medicine, 20 Penn Street, Baltimore, MD 21201-1075, USA; sligg001@umaryland.edu

Funding: none.

Competing interests: none.

REFERENCES

1 **Drazen JM**, Silverman EK, Lee TH. Heterogeneity of therapeutic responses in asthma. *Br Med Bull* 2000;**56**:1054–70.
2 **Green SA**, Liggett SB. G protein coupled receptor signalling in the lung. In: Liggett S, Meyers D, eds. *The genetics of asthma*. New York: Marcel Dekker, 1996:67–90.
3 **Fraser CM**, Venter JC. The synthesis of beta-adrenergic receptors in cultured human lung cells: induction by glucocorticoids. *Biochem Biophys Res Commun* 1980;**94**:390–7.
4 **Brodde OE**, Brinkmann M, Schemuth R, *et al*. Terbutaline-induced desensitization of human lymphocyte beta 2-adrenoceptors. Accelerated restoration of beta-adrenoceptor responsiveness by prednisone and ketotifen. *J Clin Invest* 1985;**76**:1096–101.
5 **Fraser PM**, Speizer FE, Waters SD, *et al*. The circumstances preceding death from asthma in young people in 1968 to 1969. *Br J Dis Chest* 1971;**65**:71–84.
6 **Pearce N**, Grainger J, Atkinson M, *et al*. Case-control study of prescribed fenoterol and death from asthma in New Zealand, 1977–81. *Thorax* 1990;**45**:170–5.
7 **Grainger J**, Woodman K, Peace N, *et al*. Prescribed fenoterol and death from asthma in New Zealand, 1981–7: a further case-control study. *Thorax* 1991;**46**:105–11.
8 **Van-Metre TE**. Adverse effects of inhalation of excessive amounts of nebulized isoproterenol in status asthmaticus. *J Allergy* 1969;**43**:101–13.
9 **Harvey JE**, Tattersfield AE. Airway response to salbutamol: effect of regular salbutamol inhalations in normal, atopic, and asthmatic subjects. *Thorax* 1982;**37**:280–7.
10 **Sears MR**, Taylor DR, Print CG, *et al*. Regular inhaled beta-agonist treatment in bronchial asthma. *Lancet* 1990;**336**:1391–6.
11 **Nelson HS**, Weiss ST, Bleecker ER, *et al*. The Salmeterol Multicenter Asthma Research Trial: a comparison of usual pharmacotherapy for asthma or usual pharmacotherapy plus salmeterol. *Chest* 2006;**129**:15–26.
12 **Reihsaus E**, Innis M, MacIntyre N, *et al*. Mutations in the gene encoding for the β_2-adrenergic receptor in normal and asthmatic subjects. *Am J Respir Cell Mol Biol* 1993;**8**:334–9.
13 **Green S**, Turki J, Innis M, *et al*. Amino-terminal polymorphisms of the human β_2-adrenergic receptor impart distinct agonist-promoted regulatory properties. *Biochemistry* 1994;**33**:9414–9.
14 **Green SA**, Turki J, Bejarano P, *et al*. Influence of β_2-adrenergic receptor genotypes on signal transduction in human airway smooth muscle cells. *Am J Respir Cell Mol Biol* 1995;**13**:25–33.
15 **Taylor DR**, Drazen JM, Herbison GP, *et al*. Asthma exacerbations during long term β agonist use: influence of β_2 adrenoceptor polymorphism. *Thorax* 2000;**55**:762–7.
16 **Israel E**, Drazen JM, Liggett SB, *et al*. The effect of polymorphisms of the β_2-adrenergic receptor on the response to regular use of albuterol in asthma. *Am J Respir Crit Care Med* 2000;**162**:75–80.
17 **Israel E**, Chinchilli VM, Ford JG, *et al*. Use of regularly scheduled albuterol treatment in asthma: genotype-stratified, randomised, placebo-controlled cross-over trial. *Lancet* 2004;**364**:1505–12.
18 **Wechsler ME**, Lehman E, Lazarus SC, *et al*. Beta-adrenergic receptor polymorphisms and response to salmeterol. *Am J Respir Crit Care Med* 2006;**173**:519–26.
19 **Palmer CNA**, Lipworth BJ, Lee S, *et al*. Arginine-16 β_2 adrenoceptor genotype predisposes to exacerbations in young asthmatics taking regular salmeterol. *Thorax* 2006;**61**:940–4.
20 **Drysdale CM**, McGraw DW, Stack CB, *et al*. Complex promoter and coding region β_2-adrenergic receptor haplotypes alter receptor expression and predict in vivo responsiveness. *Proc Natl Acad Sci USA* 2000;**97**:10483–8.
21 **Tantisira KG**, Small KM, Litonjua AA, *et al*. Molecular properties and pharmacogenetics of a polymorphism of adenylyl cyclase 9 in asthma: interaction between β-agonist and corticosteroid pathways. *Hum Mol Genet* 2005;**14**:1671–7.