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Review Article

Translation of the human genome into clinical allergy

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Abstract

By complete reading of the genome sequence, in the near future we will be able to determine the role of genomic DNA sequence variation among individuals, such a single nucleotide polymorphism (SNP), in the pathogenesis of diseases and responses to drugs. Comprehension of the genome will also accelerate understanding of the transcriptome, the whole transcripts present in a cell. Messages induced by a new therapy, such as an unexpected adverse effects, will not be missed by using such a comprehensive assay. Allergic diseases will be classified into subtypes depending on the impaired or affected molecule. Herein, I introduce our research strategy for genome-wide analysis of SNP related to asthma, granted by the Millennium Genome Project of the Japanese Government, and review the recent results of transcriptome analysis using microarray technology.

Key words: asthma, atopy, genome, microarray, transcriptome.

Introduction

By using the genome sequence,[1–3] it is expected that we will resolve previously unanswered questions, such as the probability of diseases. Now, we have obtained tools for examining what the role of genomic DNA sequence variations among individuals, such a single nucleotide polymorphism (SNP),[4] is in the pathogenesis of diseases and responses to drugs. Comprehension of the genome will also accelerate the understanding of the transcriptome,[5] the whole transcripts present in a cell, and the proteome,[6] the whole protein molecules present in a cell that control cellular function (Fig. 1). Until recently, considerable time and labor was required to measure expression levels of genes, even for 100 transcripts. However, by using the recently developed DNA chip technology (i.e. cDNA microarrays[7] and high-density oligonucleotide probe arrays[8]), such systematic analysis of transcriptomes has become practical. Even in the past few years, such techniques have spread rapidly over many research fields,[7–10] including our own.[11–19] Here, I introduce our research strategy for the genome-wide analysis of SNP related to asthma, granted by the Millennium Genome Project of the Japanese Government, and review the recent results of transcriptome analysis using DNA chip technology.

Strategy for discovering the SNP responsible for asthma and atopy

In contrast with the term 'mutation', which exists in $< 1\%$ of the whole population and often causes serious hereditary diseases, SNP are present in $> 1\%$ of the population and determine common individual variations, such as hair color.[4,20] Single nucleotide polymorphisms are seen in 0.1% (approximately 3 million pairs) of all genomic DNA sequences and are the most common variation. Thus, by examining SNP, it is expected that the probability of onset of common diseases, such as hypertension[21] or drug sensitiviy,[22] can be determined. Mutation due to single nucleotide replacement in the case of an intractable disease and the SNP responsible for drug sensitivity are often accompanied by complete loss of function of the protein. In

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contrast, the SNP responsible for common diseases are accompanied by a mild reduction in the expression level or function of gene products and should be affected by environmental factors (Fig. 2). Of the various single nucleotide replacements responsible for drug sensitivity, in cases in which the gene product is unnecessary for

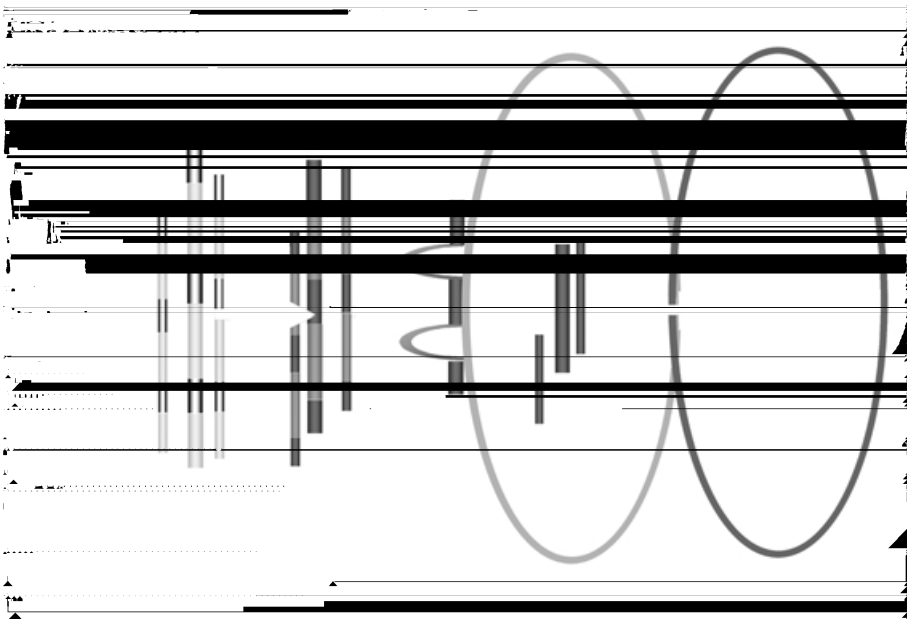

Fig. 1 Genome, transcriptome, proteome. The gene is transcribed and spliced into mRNA (i.e. the transcript). The mRNA is then translated into the protein. The term 'genome' means all the genetic information, including all genes. The term 'transcriptome' means all transcripts expressed in a cell and the term 'proteome' means all the functional elements in a cell.

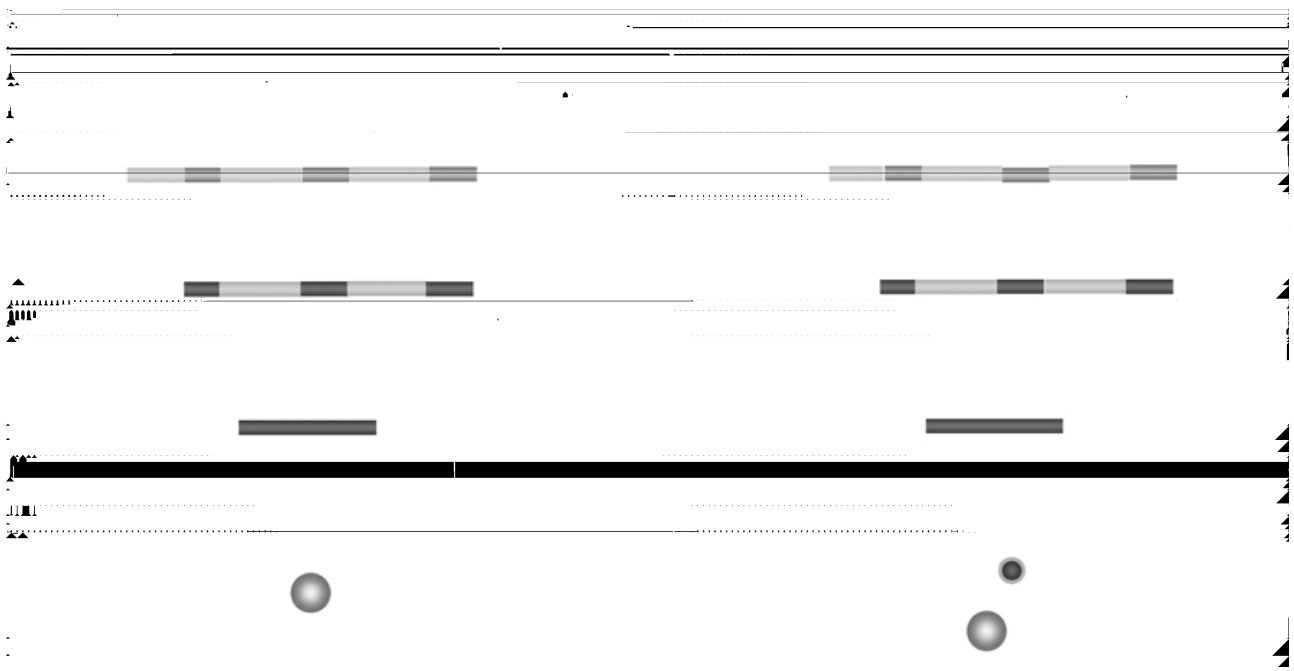

Fig. 2 Effect of single nucleotide replacement. If the protein completely loses its function, it often becomes life threatening. Life-threatening nucleotide replacement inhibits the expansion of family members over the course of the long human history; thus, it should be a rare mutation. If the loss-of-function protein did not affect the survival of our ancestors, the nucleotide replacement can be a common single nucleotide polymorphism. With regard to polygenic common diseases, mild loss of function due to nucleotide replacement may be involved.

human survival, such as in the case of alcohol dehydrogenase, the variation may be quite common.[23] However, when the gene product is essential for survival, the functional loss should be very mild.

The term 'atopy' was coined in 1923 based on the Greek word which means atypical, because it was rare. The original concept of atopy has been explained as an inherited predisposition to immediate-type hypersensitivity reactions to common allergens. However, atopy is currently considered to be acquired often as a result of environmental influences because its prevalence is increasing worldwide. When atopy is defined as one or more positive results for specific IgE antibodies against common allergens using *in vitro* or skin prick tests, the prevalence of atopy is increasing in Japan.[24] In a cohort genomic study for atopy related genes, we found that the prevalence of atopy was 92% in medical students raised in large cities in Japan. In a private company, 88% of workers born in 1971 or later were atopic, whereas only 44% workers born before 1971 were atopic.[25] Children raised on a farm tended to be less sensitized to common allergens and have a lower prevalence of atopic diseases.[26,27] Although the exact mechanisms remain to be determined, recent studies suggest that exposure to endotoxin and other microbial components early in life through contact with livestock may play an important role in predisposing infants to a non-atopic constitution.[28,29] Therefore, this difference in the prevalence of atopy between the young and elderly may be related to the rapid improvements in hygiene in the 1970s in Japan.

Nevertheless, the definition of atopy is crucial in case-control genomic studies of atopic diseases. It may be necessary to define high atopy as serum IgE greater than or equal to 10-fold 1 SD from normal plus multiple positive RAST scores.[30] Bronchial asthma is often considered to represent atopic disease, but is really a group of diseases having similar symptoms with several different pathological factors.[31] For instance, childhood and adult asthma have many different etiologies. There are so many adult patients, but not childhood patients, whose asthma pathology is based on IgE-independent inflammation and inappropriate remodeling of airway tissue following inflammation[32] or aspirin sensitivity.[33] In contrast, rhinovirus infections are common in asthma attacks seen in pediatric patients.[34] Therefore, to detect the SNP on a molecule affecting a certain type of asthma, we should specify what type of asthma by analyzing the clinical information in detail.

Pharmacogenetics of anti-asthma drugs

Drug sensitivity based on genetic variance is considered to be due to at least two different mechanisms. One is the response to the drug and the other is drug metabolism. Considering the drug response, a complete non-responding gene for anti-asthma drugs has not been identified and should not be common because the majority of anti-asthma drugs are related to natural hormones, such as glucocorticosteroids. Most studies regarding glucocorticoid-resistance in asthma and other allergic diseases explain glucocorticoid-resistance as due to competitive actions by inflammatory cytokines. That is, glucocorticoid-resistant patients usually have severe inflammation followed by increased levels of the transcriptional factor nuclear factor (NF)-κB and the glucocorticoid receptor β, a decoy receptor, through cytokine activation.[35]

Regarding β_2-adrenergic receptors, there is a functional SNP affecting the affinity of these receptors for agonists. When asthmatic patients who have an arginine/arginine replacement in the 16th amino acid residue in the β_2-adrenergic receptor receive β_2-adrenergic receptor agonist therapy for 3 months, their lung function is downregulated after withdrawal of the agonist.[36] Although the level of downregulation of β_2-adrenergic receptors is very low, it may sometimes cause asthma death.

In contrast with SNP related to the drug response, many genetic variances are considered to be present in metabolic enzymes, especially in the cytochrome P450 system.[37] We have found that such SNP exist in cytochrome P450 enzymes for glucocorticosteroid metabolism.[38] The SNP present in cytochrome P450 enzymes may be related to the adverse reactions to drugs. It will be important to determine whether such SNP are related to the adverse reactions to glucocorticosteroids, such as glaucoma.

Application of DNA chip technology to the transcriptome project

To understand the functional roles of the entire genome, analysis of the proteome may be necessary. However, a proteome assay is not yet practical, although recent technological developments have made it possible to measure a few hundred proteins at once. In fact, the majority of proteins present in the proteome are still in the 'black box' and their number is speculated to be $>$ 100 000, even if variable immunoglobulins and

T cell receptors are not included. Thus, it remains labor-intensive to analyze even 1% of proteins present in the proteome. In contrast with proteomes, a transcriptome assay has already become practical following rapid developments in microarray technology. Now, there are tools available for measuring the expression levels of 30 000 genes at once. It should be stressed that almost all gene expression levels can be measured using recent DNA chip technology, because the number of genes present in the genome is estimated to be 30 000–40 000. The 'transcriptome' refers to all, not simply many, transcripts expressed in a cell. We will not miss any previously unexpected adverse effects induced by a new therapy by using such a comprehensive assay (Fig. 3).

The transcriptome assay is useful for discovering new diagnostic molecules. We have discovered 770 new genes, which are expected as diagnostic tools or drug targets, by using transcriptome assays in collaboration with Genox Reseach Inc. (Kawasaki Laboratory, Teikyo Univeristy Biotechnology Center, Kanagawa, Japan; http://www.genox.co.jp/).[14–17] The transcriptome assay is also useful for detecting unexpected observations that are often opposite or unrelated to previously published information. We screened for cell-type specific gene expression among the transcriptome in human mast cells compared with other cell types. Of the transcripts for expected mast cell-specific proteins, such as tryptase, major basic protein (MBP), which had been thought to be mostly eosinophil specific, was found to be expressed abundantly by human mast cells.[12] Mast cells obtained from four different sources were found to have high protein levels of MBP in their granules. Without using comprehensive analysis such as DNA chip technology, nobody would have found such an unexpected result.

Animal models of allergic inflammation have been informative. The use of murine models of asthma has increased mainly due to the ability to selectively knock out genes that block specific pathways in the pathogenesis of the disease. However, there is controversy over the relevance of these murine models of asthma.[39,40] It should be noted that murine models of asthma lack the epithelial injury due to the deposition of eosinophil granule components, such as MBP.[40]

Thus, we used GeneChip (Affymetrix, Santa Clara, CA, USA) to compare the transcriptomes of human and mouse mast cells, because rodent mast cells are common experimental tools but are somewhat different from their

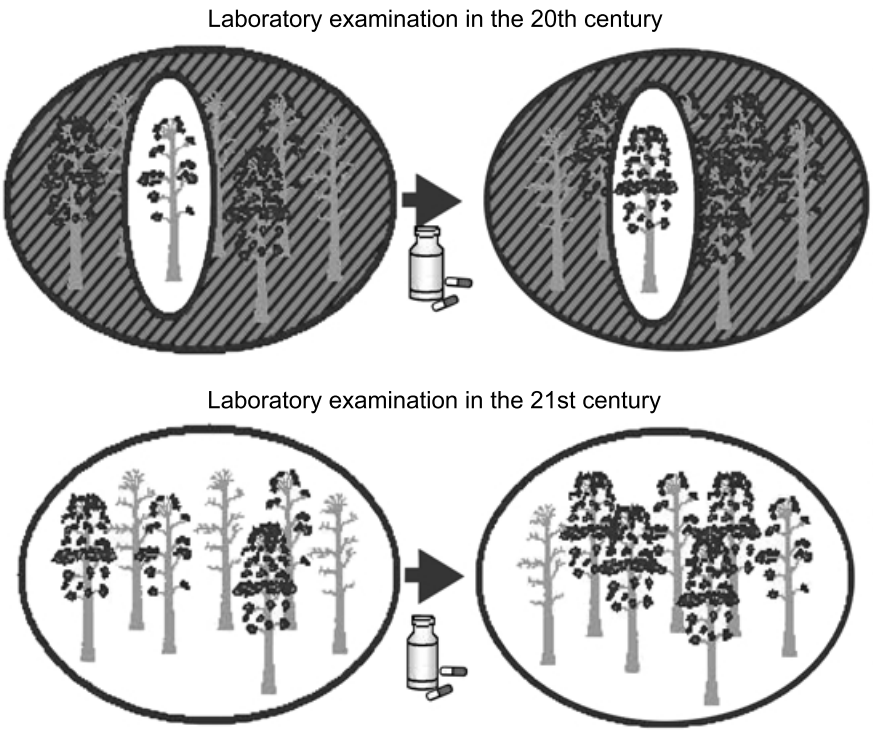

Fig. 3 Impact of genome-wide examination. Transcriptome assay may be useful for detecting previously undiscovered side-effects. The upper part of this figure symbolizes the conventional laboratory examination and the lower part symbolizes new laboratory examinations using the transcriptome or proteome. In the upper part of the figure, the drug was judged to be useful by conventional examination. However, you will find adverse effects by screening all elements, as can be seen in the left-hand-most side tree.

human counterparts in their responses to certain cytokines and drugs. After stimulation via high-affinity IgE receptors (FcεRI), the transcriptional levels of several CC chemokines (i.e. I-309, macrophage inflammatory protein (MIP)-1α and MIP-1β) were found to be markedly upregulated among the transcriptomes of both human and mouse mast cells.[13] These results suggests that mast cells play a crucial role in recruitment of various CC receptor (CCR)-expressing cells into the tissue in an IgE-dependent manner and that FcεRI-mediated induction of several CC chemokines is highly conserved between humans and mice. In contrast, many other genes are expressed in one of either type of mast cells[13] (i.e. the MBP transcript was expressed only by human mast cells). Studies on the function of molecules highly expressed only in mouse cells have to be interpreted carefully with regard to their potential function in humans. Interspecies comparison studies of whole genome expression should be useful for the interpretation of experimental data from animal models of human pathogenesis.

Concluding comments

In the very near future, the probability of asthma, as well as that of other allergic diseases, will be examined in every hospital by screening all SNP and transcriptome information related to the diseases. However, until now, even the most-risky SNP combination related to asthma has only a two- to threefold odds ratio with controls[41] and most SNP research papers deal only with statistical probability. The effect of SNP on the protein structure or expression levels has not usually been investigated. Therefore, the SNP responsible for asthma and other allergic diseases are sometimes different depending on the publication and/or race or community studied. Now, we need to resolve many problems before the clinical application of such genomic information regarding asthma and other allergic diseases. Among these problems, the problem regarding the diagnosis of asthma should be resolved urgently. Allergic diseases will be classified into subtypes depending on the affected molecule. For instance, the diagnosis will be performed based on the affected molecules, such as intercellular adhesion molecule-1-signaling pathway-affected rhinovirus-induced asthma or a disintegrin and metalloproteinase (ADAM)-33-affected glucocorticoid-resistance asthma with hypertrophic airway smooth muscle.

References

1 Venter JC, Adams MD, Myers EW *et al*. The sequence of the human genome. *Science* 2001; **291**: 1304–51.
2 International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. *Nature* 2001; **409**: 860–921.
3 Waterston RH, Lindblad-Toh K, Birney E *et al*. Initial sequencing and comparative analysis of the mouse genome. *Nature* 2002; **420**: 520–62.
4 Cargill M, Altshuler D, Ireland J *et al*. Characterization of single-nucleotide polymorphisms in coding regions of human genes. *Nat. Genet*. 1999; **22**: 231–8.
5 Velculescu VE, Madden SL, Zhang L *et al*. Analysis of human transcriptomes. *Nat. Genet*. 1999; **23**: 387–3.
6 Apweiler R, Biswas M, Fleischmann W *et al*. Proteome analysis database: Online application of InterPro and CluSTr for the functional classification of proteins in whole genomes. *Nucleic Acids Res*. 2001; **29**: 44–8.
7 Duggan DJ, Bittner M, Chen Y, Meltzer P, Trent JM. Expression profiling using cDNA microarrays. *Nat. Genet*. 1999; **21**: 10–14.
8 Lipshutz RJ, Fodor SPA, Gingeras TR, Lockhart DJ. High density synthetic oligonucleotide arrays. *Nat. Genet*. 1999; **21**: 20–4.
9 Fambrough D, McClure K, Kazlauskas A, Lander ES. Diverse signaling pathways activated by growth factor receptors induce broadly overlapping, rather than independent, sets of genes. *Cell* 1999; **97**: 727–41.
10 Alizadeh AA, Eisen MB, Davis RE *et al*. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 2000; **403**: 503–11.
11 Iida M, Matsumoto K, Tomita H *et al*. Selective down-regulation of high affinity IgE receptor (Fc epsilon RI) alpha-chain messenger RNA among transcriptome in cord blood-derived versus adult peripheral blood-derived cultured human mast cells. *Blood* 2001; **97**: 1016–22.
12 Nakajima T, Matsumoto K, Suto H *et al*. Gene expression screening of human mast cells and eosinophils using high-density oligonucleotide probe arrays: Abundant expression of major basic protein in mast cells. *Blood* 2001; **98**: 1127–34.
13 Nakajima T, Inagaki N, Tanaka H *et al*. Marked increase in CC chemokine gene expression in both human and mouse mast cell transcriptomes following Fc epsilon receptor I cross-linking: An interspecies comparison. *Blood* 2002; **100**: 3861–8.
14 Heishi M, Kagaya S, Katsunuma T *et al*. High-density oligonucleotide array analysis of mRNA transcripts in severe atopic dermatitis patients' peripheral blood cells. *Int. Arch. Allergy Immunol*. 2002; **129**: 57–66.
15 Imai Y, Nakada A, Hashida R *et al*. Cloning and characterization of the highly expressed ETEA gene from blood cells of atopic dermatitis. *Biochem. Biophys. Res. Commun*. 2002; **297**: 1282–90.

16 Matsumoto Y, Oshida T, Obayashi I *et al*. Identification of highly expressed genes in peripheral blood T cells from patients with atopic dermatitis. *Int. Arch. Allergy Immunol.* 2002; **129**: 327–40.
17 Ogawa K, Hashida R, Miyagawa M *et al*. Analysis of gene expression in peripheral blood eosinophils from patients with atopic dermatitis and *in vitro* cytokine-stimulated blood eosinophils. *Clin. Exp. Immunol.* 2003 (in press).
18 Saito H, Nakajima T, Matsumoto K. Human mast cell transcriptome project. *Int. Arch. Allergy Immunol.* 2001; **125**: 1–8.
19 Matsumoto K, Saito H. Role of eosinophils in asthma: Sarastro or the Queen of the Night? *Int. Arch. Allergy Immunol.* 2001; **125**: 290–6.
20 Bentley DR The Human Genome Project: An overview. *Med. Res. Rev.* 2000; **20**: 189–96.
21 Martin ER, Lai EH, Gilbert JR *et al*. SNPing away at complex diseases: Analysis of single-nucleotide polymorphisms around APOE in Alzheimer disease. *Am. J. Hum. Genet.* 2000; **67**: 383–94.
22 Sesti F, Abbott GW, Wei J *et al*. A common polymorphism associated with antibiotic-induced cardiac arrhythmia. *Proc. Natl Acad. Sci. USA* 2000; **97**: 10 613–18.
23 Koch HG, McClay J, Loh EW *et al*. Allele association studies with SSR and SNP markers at known physical distances within a 1 Mb region embracing the ALDH2 locus in the Japanese, demonstrates linkage disequilibrium extending up to 400 kb. *Hum. Mol. Genet.* 2000; **9**: 2993–9.
24 Nakagomi T, Itaya H, Tominaga T, Yamaki M, Hisamatsu S, Nakagomi O. Is atopy increasing? *Lancet* 1994; **343**: 121–2.
25 Akasawa A, Matsumoto K, Tomikawa M *et al*. Prevalence and gene expression screening of atopic subjects among medical students in Japan. *J. Allergy Clin. Immunol.* 2001; **107** (Suppl.): S278 (Abstract).
26 von Mutius E. Environmental factors influencing the development and progression of pediatric asthma. *J. Allergy Clin. Immunol.* 2002; **109** (Suppl.): S525–32.
27 Braun Fahrländer C, Gassner M, Grize L *et al*. Prevalence of hay fever and allergic sensitisation in farmer's children and their peers living in the same rural community. SCARPOL team. Swiss Study on Childhood Allergy and Respiratory Symptoms with Respect to Air Pollution. *Clin. Exp. Allergy* 1999; **32**: 28–34.
28 Gereda JE, Leung DYM, Thatayatikom A *et al*. Relation between housedust endotoxin exposure, type 1 T cell development, and allergen sensitisation in infants at high risk of asthma. *Lancet* 2000; **32**: 1680–3.
29 Matricardi PM, Rosmini F, Rapicetta M, Gasbarrini G, Stroffolini T. Atopy, hygiene, and anthroposophic lifestyle. San Marino Study Group. *Lancet* 1999; **354**: 430.
30 Zimmerman B, Forsyth S, Gold M. Highly atopic children formation of IgE antibody to food protein, especially peanut. *J. Allergy Clin. Immunol.* 1989; **83**: 764–70.
31 Pride NB. Asthma. Definition and clinical spectrum. *Br. Med. Bull.* 1992; **48**: 1–9.
32 Jeffery PK. Remodeling in asthma and chronic obstructive lung disease. *Am. J. Respir. Crit. Care Med.* 2001; **164** (Suppl.): S28–38.
33 Sousa AR, Parikh A, Scadding G, Corrigan CJ, Lee TH. Leukotriene-receptor expression on nasal mucosal inflammatory cells in aspirin-sensitive rhinosinusitis. *N. Engl. J. Med.* 2002; **347**: 1493–9.
34 Gern JE, Busse WW. Relationship of viral infections to wheezing illnesses and asthma. *Nat. Rev. Immunol.* 2002; **2**: 132–9.
35 Leung DYM, Bloom JW. Update on glucocorticoid action and resistance. *J. Allergy Clin. Immunol.* 2003; **97**: 1–20.
36 Moore PE, Laporte JD, Abraham JH *et al*. Polymorphism of the β_2-adrenergic receptor gene and desensitization in human airway smooth muscle. *Am. J. Respir. Crit. Care Med.* 2000; **162**: 2117–24.
37 Buters JT, Sakai S, Richter T *et al*. Cytochrome P450 CYP1B1 determines susceptibility to 7,12-dimethylbenz[a]anthracene-induced lymphomas. *Proc. Natl Acad. Sci. USA* 1999; **96**: 1977–82.
38 Koyano S, Kurose K, Ozawa S *et al*. Eleven novel single nucleotide polymorphisms in the NR1I2 (PXR) gene, four of which induce non-synonymous amino acid alterations. *Drug Metab. Pharmacokin.* 2003 (in press).
39 Gelfand EW. Mice are a good model of human airway disease. *Am. J. Respir. Crit. Care Med.* 2002; **166**: 5–6.
40 Persson CGA. Mice are not a good model of human airway disease. *Am. J. Respir. Crit. Care Med.* 2002; **166**: 6–7.
41 Van Eerdewegh P, Little RD, Dupuis J *et al*. Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness. *Nature* 2002; **418**: 426–30.