

The epidemic of asthma and allergy

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In his treatise *On Asthma: Its Pathology And Treatment* first published in 1860, Henry Hyde Salter (Figure 1), a physician at the Charing Cross Hospital, London, differentiated asthma from other causes of breathlessness as 'paroxysmal dyspnoea of a peculiar character with intervals of healthy respiration between attacks'.¹ 6 years later, from an analysis of 150 unpublished cases, he described many of the characteristic features of this disease including hyperresponsiveness to cold air and exercise and attacks provoked by exposure to chemical and mechanical irritants, to particular kinds of air as well as to certain foods and wine.^{2,3} His observations were further enhanced by the use of the spiograph, the earliest record of a water spirometer.⁴ In these publications Salter identified asthma as a spasmodic stricture occurring throughout the conducting airways, and differentiated the condition from bronchial catarrh, recent bronchitis and old bronchitis (Figure 2). He drew special attention to the musical rhonchus that characterized asthmatic bronchoconstriction and indicated that the sibilant bronchi could not be relieved by coughing. Also of great significance was his observation of cells in the asthmatic sputum, which he identified by the presence of a nucleus, nucleolus and cell wall. The identification of eosinophils in sputum had to await the development of eosin by Paul Ehrlich some 15 years later.⁵ Sir William Osler, in his first edition of *Principles and Practice of Medicine*,⁶ likewise drew attention to the factors that could exacerbate asthma including allergens, air pollutants, infections, exercise, weather, food and emotions.

Hyperresponsiveness of the conducting airways, a characteristic feature of all forms of asthma, can be quantified in the laboratory by use of inhalation bronchial provocation tests with such agents as methacholine and histamine. In asthma the dose–response curve to these agonists is displaced to the left in proportion to disease severity, and at high agonist concentrations there is loss of the normal protective plateau (Figure 3). As pointed out by both Salter and Osler, hyperresponsiveness is in part the result of a characteristic type of inflammation that affects the conducting airways and is accompanied by marked structural changes to the airways which include an increase in airway smooth muscle and deposition of matrix leading



Figure 1 Henry Hyde Salter (1823–1871)

to an overall thickening of the airway wall (remodelling). The pathological features of asthma are vividly illustrated by Huber and Koessler in their classic paper of 1922.⁷ These combine to make the airways contract too much and too easily in response to exogenous and endogenous stimuli, as well as contributing to the diurnal variation in airway calibre that is characteristic of the disease.

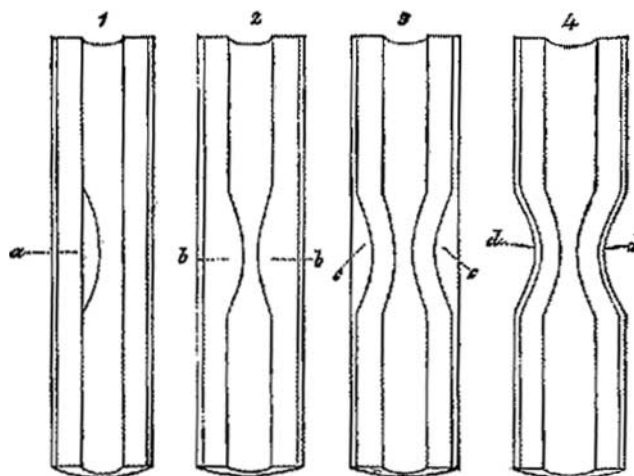


Diagram showing the four ways in which the bronchial tubes may be narrowed. 1. Bronchial catarrh. 2. Recent bronchitis. 3. Old bronchitis. 4. Asthma.

Figure 2 Plate from Salter's *Treatise* (1860) illustrating the different causes of airflow obstruction

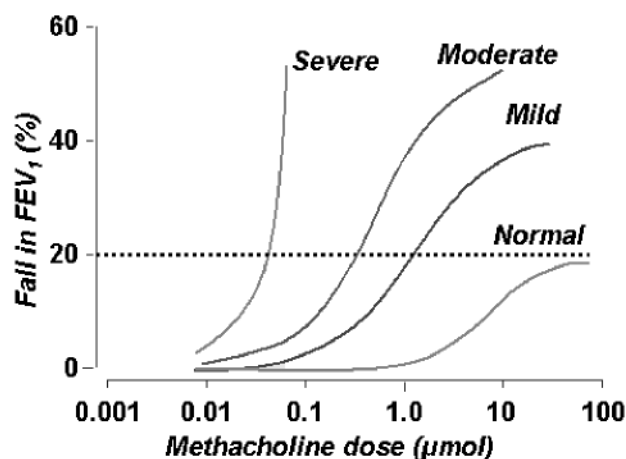


Figure 3 Typical dose-response curves in normal and asthmatic individuals on aerosol bronchial provocation with increasing concentrations of methacholine

Today, fibreoptic bronchoscopy allows ready access to airways, and lavage and mucosal biopsy samples confirm the presence of a special type of inflammation characterized by infiltration of the airway wall with activated T lymphocytes, mast cells, basophils, eosinophils and macrophages. In addition, morphometric studies on airways from patients who have died from or with asthma have quantified the impressive increase in airway smooth muscle that occurs in this disease, along with structural changes that include shedding of epithelial cells and epithelial mucous metaplasia, deposition of collagen and other matrix proteins in the lamina reticularis beneath a normal epithelial basement membrane, increased deposition of proteoglycans and repair collagen throughout the airway wall, and an increase in submucosal microvessels and nerves—all changes tantamount to airway remodelling. The fact that these structural changes occur in early childhood, at the inception of asthma,⁸ indicates that they are fundamental to pathogenesis and occur parallel to, rather than as a consequence of, airway inflammation.⁹

THE RISING TRENDS OF ASTHMA AND ALLERGY

The past three decades have witnessed a spectacular increase in the prevalence of asthma and allergic disease worldwide, especially in those countries with a Western lifestyle.¹⁰ In the International Study of Asthma and Allergy in Children, the highest prevalences of asthma were in Australia, New Zealand and the UK, where in 2003 more than 20% of children aged 13–14 years reported asthma symptoms.¹¹ By contrast, in Central Africa, Central and Eastern Europe and China the prevalence of childhood asthma was less than 5%. The European Community Respiratory Health Survey¹² has revealed similar intercountry differences in prevalence of adult asthma and bronchial hyperresponsiveness. While part of the explanation for these wide differences in disease prevalence may be genetic, in that those countries with the highest disease prevalence were host to large migrations of people from the UK, a critical role for environmental factors in driving the expression of asthma and other allergic diseases is almost certain.¹⁰ This argument is made all the more compelling by the observation that, in countries where prevalence studies have been conducted by identical methods over a span of 10–25 years, the disease prevalence has increased progressively in children, adolescents and adults (Figure 4).¹³

Clues to the environmental factors that drive the rising trends may be had from a closer look at disease mechanisms. With the recognition that gene–environmental interactions are critical to the pathogenesis of allergic disorders such as asthma, there has been a major focus on the immunological and inflammatory mechanisms that underlie the origins of allergy and its progression to allergic inflammation. It was Charles Harrison Blackley (Figure 5) in *Experimental Researches on the Cause and Nature of Catarrhus Aestivus* (1873)¹⁴ who drew our attention to the importance of pollen exposure as a causal factor in hayfever and ‘hay asthma’. Having installed the world’s very first pollen counter on the roof of his house in Manchester, Blackley was able to show clearly that his own symptoms of rhinitis and asthma coincided exactly with the peak increase in the

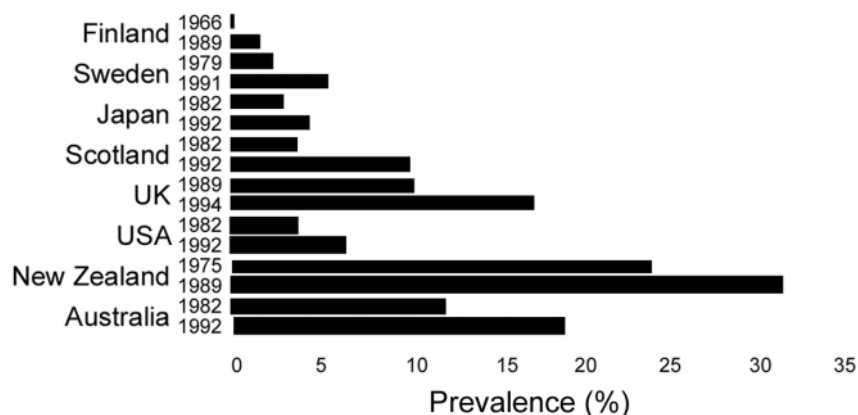


Figure 4 The rising trends in asthma in different countries. The paired prevalence rates in each country were obtained with the same instruments



Figure 5 Charles Harrison Blackley (1820–1900)

count of pollen grains collected over each twenty-four hours across June and July. In the closing paragraph of his monograph¹⁴ he states:

‘I am, as I have intimated, quite aware that other agents may yet be found to produce symptoms not unlike those of hayfever. Amidst the great number of bodies there are with functions similar to those of pollen, it would not be surprising if we should find some that have a similar kind of action; and it is not improbable that among these, we may find the exciting causes of some diseases which are far more formidable than hayfever.’

How right he was. Some 20 years later, Osler drew attention to the importance of house dust as a trigger of

both rhinitis and asthma. The identification of the dust mites *Dermatophagoides pteronyssinus* and *D. farinae* (or rather their faeces) as causal agents led to extensive research on the environmental factors most conducive to dust mite reproduction and survival as well as on the substances that lead to an allergic response. We now know that such allergens, whether in mite faeces or other sources such as pollen grains, fungal hyphae or animal material, may have intrinsic biological properties, including proteolytic enzyme activity, that help them penetrate epithelial barriers and gain access to the mucosal or epidermal tissue where they evoke the allergic response.¹⁵ In countries with a high prevalence of allergic disease, up to 40% of the population are sensitized to common environmental allergens such as grass and tree pollen, dust mite excreta and animal materials, the highest prevalence of sensitization being found in those countries with the greatest incidence of allergic disease.

IMMUNOLOGICAL BASIS FOR ASTHMA AND ALLERGY

Sensitization to allergens usually starts at mucosal or dermal surfaces when the allergen is taken up by antigen presenting cells (APCs; dendritic or Langerhans cells). In genetically susceptible individuals, selective peptides are generated by APCs and presented to naïve T lymphocytes in local lymphoid tissue¹⁶ which then multiply and differentiate into a subtype of T cells designated Th2-like.¹⁷ In addition to being implicated in the pathogenesis of allergy and asthma, Th2-like cells are fundamental to the development of an effective immune response against parasites.¹⁸ It would seem that in the Western world this arm of the immune response has been hijacked by environmental allergens, leading to specific sensitization and allergic disease. A

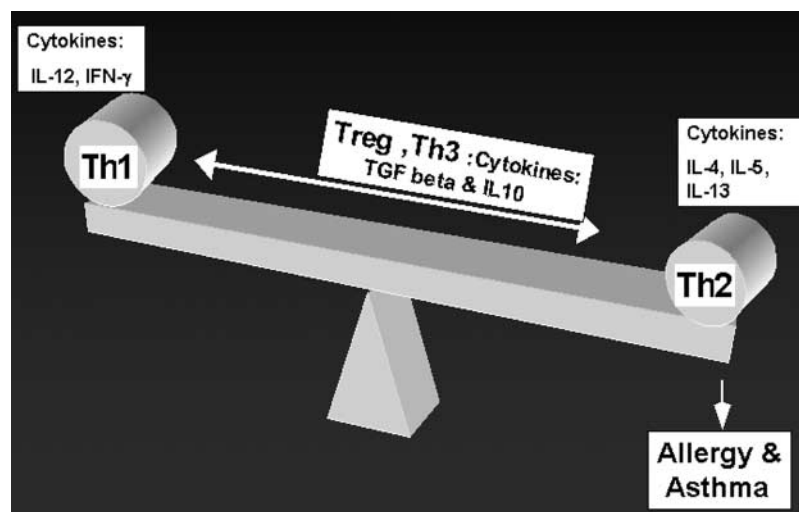


Figure 6 The role of cytokines in directing the balance between T lymphocyte subsets. Th2-like T cells orchestrate the inflammatory response of asthma and allergy. T reg and Th3 inhibit both Th1 and Th2 responses

Box 1 Influence of infection in protecting against allergy

- Strong socioeconomic gradient
- Less allergy in large families
- Less allergy in lastborn siblings
- Less allergy in rural than urban environments
- Less allergy in developing countries
- Less allergy in relation to gastrointestinal infections—e.g. hepatitis A, toxoplasmosis, *Helicobacter pylori*
- Less allergy in children attending daycare centres
- Less allergy in children attending Steiner schools

second set of T lymphocytes designated Th1-like with capacity to secrete interferon γ (IFN- γ) negatively regulates the ability of Th2-like cells to develop. In babies born to families with a strong history of allergic disease, there exists a defect in the Th1 arm of the immune response with a consequent increase in Th2 responsiveness.¹⁹ More recently, additional T lymphocyte subsets designated regulatory T cells (T reg cells) and Th3-like cells have been found to modify the extent of both Th1 and Th2 responses through their ability to secrete anti-inflammatory cytokines, transforming growth factor β (TGF β) and interleukin 10, thereby adding a further level of complexity to T cell mediated immune regulation (Figure 6).²⁰

The expansion of Th2-like T cells occurs in the local lymphoid tissue—i.e. the site of antigen presentation. The net result of this process is the coordinate secretion of a range of small-molecular-weight cytokines encoded in a cluster on chromosome 5q31-34 (including interleukins 3, 4, 5, 6, 9 and 13 and granulocyte-macrophage colony stimulating factor) with the capacity to induce the lymphocytes to switch from making IgM to making the allergic antibody IgE (interleukins 4 and 13), and encourage the migration and maturation of tissue mast cells (interleukins 3, 4, 6, 9, 13). Th2 cytokines also upregulate the expression of specific adhesion molecules (vascular cell adhesion molecule 1 and intercellular adhesion molecule 1) on microvascular endothelial cells which trap and activate passing leukocytes, specifically eosinophils, basophils and monocytes.²¹ The mast cell is particularly important in initiating the allergic response, because cross-linking of the IgE bound to the surface of these cells produces an explosive release of granule-associated and newly formed chemical mediators and cytokines which interact with constituent cells in smooth muscle, nerves, blood vessels and mucus glands to produce the clinical manifestations of allergy.

PREVENTION OF ALLERGY BY REDUCING EXPOSURE TO ALLERGENS

An obvious way to prevent allergic disease in high-risk individuals, or to prevent or reverse symptoms, is to

restrict contact with an offending allergen. In some cases separation of a sensitized individual from an offending allergen (e.g. a laboratory worker sensitized to rodent allergens) can produce a dramatic effect, but in other circumstances the results are less impressive. This is particularly the case for house dust mite avoidance. Some workers argued strongly that encasement of bedding with dust mite impermeable materials, together with measures to reduce dust mite exposure in the bedroom and living area of the house, would have a major impact in reducing sensitization and subsequent development of allergic disease, as well as reducing symptoms in those already sensitized. However, primary prevention studies in infants²² and the use of reasonable (but not exhaustive) dust mite avoidance strategies in adults have proved disappointing.^{23,24} In children with dust mite related asthma and eczema, mite reduction strategies have been reported to be more successful.^{25,26}

THE HYGIENE HYPOTHESIS

Exposure to increased amounts of allergens, such as those derived from house dust mite, may account for some of the increase in allergy seen in countries with a Western lifestyle. In Australia, a rise in dust mite exposure has been linked to the sealing of air conditioned houses and the use of soft furnishings, but increased exposure to allergens cannot explain the large inter-country differences in allergic disease and allergen sensitization, nor the rising trends. In 1989 David Strachan²⁷ stated, on the basis of epidemiological work, that 'the apparent rise [in the prevalence of allergic disease] . . . could be explained if allergic diseases were prevented by infection in early childhood, transmitted by unhygienic contact with older siblings, or acquired prenatally . . .'. Since that time extensive epidemiological research has shown that exposure to microorganisms or their products may account in part for the rising trends in allergic disease. Some the findings are summarized in Box 1. A particularly striking observation is that, compared with children in the general population, children brought up on livestock farms (and thus in frequent contact with farm animals) have a 50–75% reduction in the prevalence of allergic disease such as hayfever in parallel with a reduction in sensitization to common environmental allergens.²⁸ A key question arising from these studies is how exposure to microorganisms is able to protect children from allergic sensitization. Bacterial cell walls contain complex endotoxins such as lipopolysaccharides (Gram-negative bacteria) and muramic acid (Gram-positive bacteria); fungal spores and hyphae contain chitin; bacteria contain unmethylated CpG DNA sequences; and viruses contain double-stranded RNA. Each of these substances is able to stimulate specific toll-like receptors (TLRs) on antigen presenting cells. Viral double-

stranded RNA activates TLR3, lipopolysaccharide activates TLR4 and CpG activates TLR9. Activation of TLRs directs a protective immune response by upregulating the expression of Th1, Th3 and Treg T lymphocytes, thereby inhibiting Th2 mediated allergic sensitization.²⁰ The importance of these mechanisms is illustrated by the work of Braun-Fährlander and colleagues showing that, in children brought up in a rural environment, the endotoxin load in their mattresses is inversely related to the occurrence of hayfever, hayfever symptoms and grass sensitization.²⁹ As an extension of these ideas Donata Vercelli has advanced the concept of the endotoxin 'switch'—whereby in children born of allergic parents who exhibit a defective Th1 response, exposure to the contents of microorganisms through the activation of TLRs (part of the innate immune response) is able to compensate by enhancing Th1-like responses with consequent reduction of Th2 responses.³⁰

With recognition of the importance of TLRs as an integral component of the innate immune response involved in protection against allergic disease, attempts are being made to harness these mechanisms in the form of vaccine development.³¹ When conjugated to the oligonucleotide CpG, the major ragweed allergen *Amb a1* is 100-fold less allergenic than unlinked *Amb a1* in those sensitized to ragweed and has been shown to give total protection against ragweed during the pollen season in the USA.³² Studies such as these, as well as the use of CpG alone or given as a mixture along with an allergen or a peptide derived from the allergen, are being investigated in human clinical trials following very promising results in animals.^{33,34}

IG E AS A THERAPEUTIC TARGET

IgE, originally described as 'reagin' by Prausnitz and Küstner in 1922,³⁵ is the principal trigger for an allergic tissue response on exposure to a specific allergen. Since the molecular identification in 1968 of reagin as the fifth immunoglobulin,^{36,37} IgE has been a major target for development of treatment. IgE directed to specific allergens binds strongly to the high affinity IgE receptor (FCεR1-αβγ₂) present on mast cells and basophils. Cross-linkage of adjacent IgE molecules by prevailing allergen results in dimerization of the receptors and cell activation with secretion of various inflammatory mediators and cytokines.³⁸ Administration of a humanized monoclonal antibody against the Cε3 region of IgE, the component that binds to the α-chain of FCεR1, results in sequestration of circulating IgE³⁹ with eventual loss of IgE binding to cells within tissues.⁴⁰ The anti-IgE itself will not activate IgE bound to its receptors or mast cells or basophils since the epitope against which the antibody is directed is obscured by binding to the FCεR1 receptor (Figure 7).⁴¹ Anti-IgE

(omalizumab) has yielded striking clinical improvement in adults and children with steroid-requiring asthma^{42,43} and has recently been approved for clinical use in the USA. Bronchial biopsies from asthmatic patients receiving omalizumab for twelve weeks demonstrated a pronounced reduction in airway inflammation (including eosinophils) in parallel with a loss of IgE and its receptor from mast cells.⁴⁰ This exciting therapeutic approach to asthma and allergy is now being followed by generation of peptide vaccines with the capacity to induce a therapeutic antibody response to cell IgE Cε3 domain by coupling non-self protein or peptide to self structures. These second-generation vaccines have proven to be highly effective in non-human primate models of allergic disease and are about to enter clinical trials.⁴⁴

TISSUE SUSCEPTIBILITY FACTORS IN ASTHMA

While IgE-dependent sensitization and subsequent allergic inflammation in the lower airways is undoubtedly important in asthma pathogenesis, the picture changes with disease progression: as the disease becomes more severe and chronic (and therefore refractory to corticosteroids) the structural elements of the airway wall, including airway smooth muscle, contribute more to the clinical expression of the disease than does the inflammation. In structural studies a close correlation has been seen between the thickness of the lamina reticularis (beneath the airway epithelium) and the thickness of the airway wall and amount of smooth muscle.⁴⁵ In infant rhesus monkeys, exposure to house dust mite allergens results in thickening of the lamina reticularis and a parallel increase in airway smooth muscle bundle orientation and thickness in the larger airways.⁴⁶ These structural changes are accompanied, in the 'asthmatic' monkeys, by a large increase in airway hyperresponsiveness as well as an increase in mucus-secreting goblet cells in the airway epithelium. If allergen-exposure is discontinued after the first six months of life, there is no reversal of the lamina reticularis thickening or of the accompanying increase in airway smooth muscle, even after 2 years.⁴⁷ This points to the importance of susceptibility genes not only in the development of the allergic response but also in determining the tissue response to allergic processes.

ADAM33—THE FIRST NOVEL ASTHMA GENE

In collaboration between the Genome Therapeutic Corporation, Schering-Plough Research Institute, USA and the University of Southampton, we have undertaken a positional cloning effort to identify novel susceptibility genes involved in the development and progression of asthma (Figure 8).⁴⁸ It has long been known that, although asthma and allergies run in families, bronchial hyperresponsiveness and allergy are separate genetic traits that are

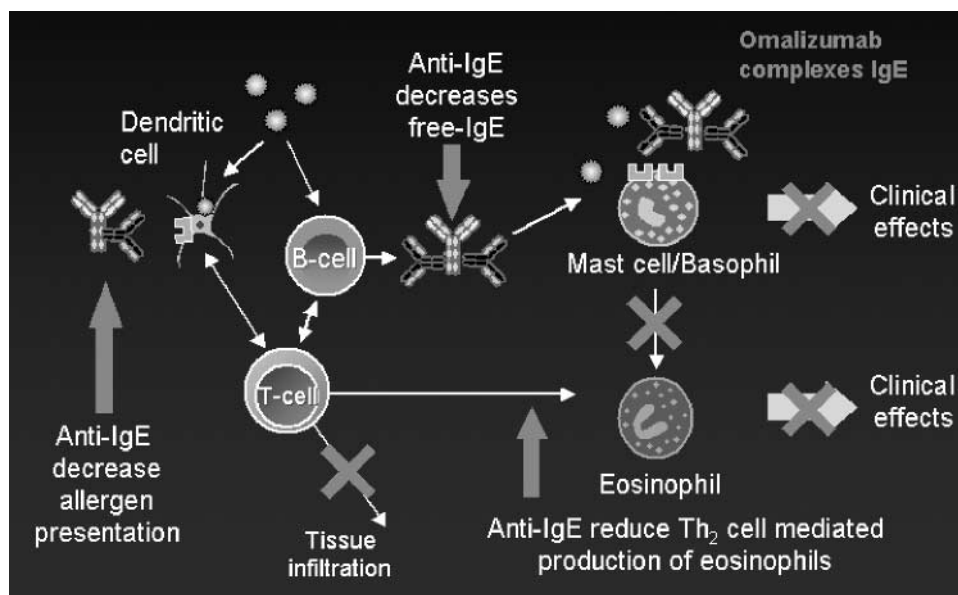


Figure 7 The inhibitory effect of the anti-human IgE monoclonal antibody omalizumab on the allergic cascade

inherited independently. Genetic modelling has shown that asthma is a complex disease involving several genes with moderate effect and important interactions with the environment. In 480 families with two or more affected asthmatic children, a genome-wide screen involving microsatellite markers led to the identification of a major area of linkage between asthma and bronchial hyperresponsiveness on chromosome 20p13. Through physical mapping of the region and identification of the genes underlying the peak of linkage by use of bacterial artificial chromosomes, 40 genes were within the 90% confidence interval. Subsequent case-control and family-based transmission

disequilibrium test (TDT) association mapping led to the identification of a disintegrin and metalloprotease (ADAM) gene as responsible for the linkage signal. *ADAM33* is a complex molecule whose expression is restricted largely to mesenchymal cells including fibroblasts and smooth muscle.^{48,49} It is made up of five domains—activation, proteolytic, adhesion, fusion, and signalling. Single nucleotide polymorphisms that are most strongly associated with asthma in our original study have been shown, in asthmatic adults, to predict rapid decline in lung function over a 20-year interval⁵⁰ and, in children with allergic and asthmatic parents, impaired lung function at ages 3 and 5

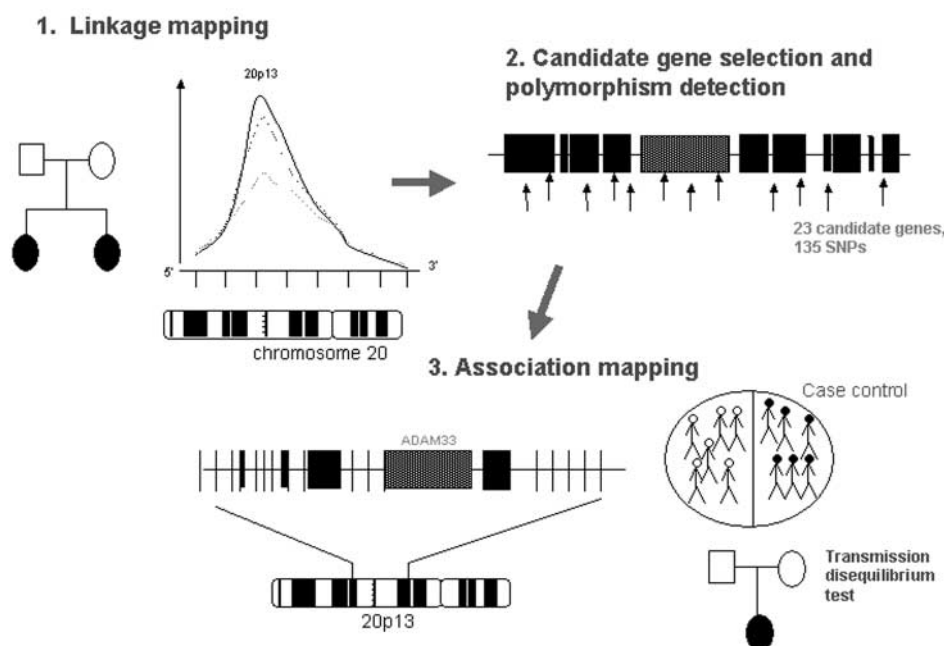


Figure 8 Positional cloning strategy used to identify the novel asthma gene ADAM 33

years.⁵¹ The precise mechanism whereby polymorphic variation in *ADAM33* is associated with asthma is not known, although removal of the gene's function by means of antisense oligonucleotides appears to prevent the differentiation of airway fibroblasts into a contractile phenotype (myofibroblasts) when incubated *in vitro* with the profibrogenic growth factor TGF- β . In addition to *ADAM33*, two asthma genes have been described by the Oxford group—*PHF-11*⁵² and *DPP-12*⁵³—both involved in amplifying IgE and Th2 mediated inflammation.

CONCLUSIONS

From the extraordinarily perceptive clinical and physiological observations made by Salter, Blackley, Osler, Ehrlich, Prausnitz, Küstner the Ishizakas and Johansson, the foundations for the scientific basis of allergic disease and asthma have been laid. The application of modern molecular medicine to well phenotyped patients will undoubtedly lead to the identification of new molecules fundamental to the pathogenesis of complex diseases such as asthma. However, the real challenge for the future is to understand how the changing environment associated with the Western culture is leading to altered expression of these genes and a prevalence of serious allergic disease that in the UK is reaching epidemic proportions.⁵² The increasing recognition that both inflammatory and structural changes are needed in the airways, for asthma to become fully manifest in its chronic form, is opening the debate as to which environmental factors are critical to the inception and progression of the disease in genetically susceptible individuals. It is through understanding of the interplay between these factors that we can hope for better means of prevention and treatment, and even cure.

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