EXPERT REVIEWS

Epidemiology of asthma: risk factors for development

Expert Rev. Clin. Immunol. 5(1), 77–95 (2009)

Padmaja Subbarao, Allan Becker, Jeffrey R Brook, Denise Daley, Piush J Mandhane, Gregory E Miller, Stuart E Turvey and Malcolm R Sears†; on behalf of the CHILD study investigators

†*Author for correspondence*
St Joseph's Healthcare,
50 Charlton Ave East, Hamilton,
Ontario, L8N 4A6, Canada
Tel.: +1 905 522 1155
ext. 33286
Fax: +1 905 521 6132
searsm@mcmaster.ca

This comprehensive review of the recent literature was undertaken to determine the current state of knowledge of the risk factors involved in the development of asthma in order to focus investigations in a proposed new longitudinal birth cohort study. The origins of asthma appear to lie in the prenatal and early postnatal period, and renewed investigations in this period with long-term close follow-up and objective phenotypic characterization will help to unravel the role of the multiple putative environmental factors in the development of asthma. It is only after understanding these effects that one can hope to design rational prevention studies for asthma.

Keywords: allergy • asthma • child • cohort study • environment • epidemiology • genetics • lung function • phenotype • risk factor

Asthma is a chronic inflammatory condition of the airways characterized by recurrent symptoms of variable airflow limitation. Risk factors for the development of asthma have been studied with intensity due to the dramatic increase in its worldwide prevalence during the last half of the last century. Two major research initiatives among children and young adults, the International Study of Asthma and Allergies in Childhood (ISAAC) [1] and the European Community Respiratory Health Survey (ECRHS) [2], have established international and regional prevalence data. These studies show low prevalence rates (2–4%) in Asian countries (particularly China and India) and high rates (15–20%) in the UK, Canada, Australia, New Zealand and other 'Westernized' countries [1–4]. Repeated surveys using ISAAC [3] and ECRHS [4] allow the study of temporal trends within and across populations. The regional variability and temporal trends of the 'asthma epidemic' have stimulated many studies, including observations in migrating populations [5] and in Germany after reunification [6], which strongly suggest that environmental factors determine the expression of asthma among genetically similar populations. These studies have also shown a continued increase in asthma prevalence in the younger age groups as well as in developing countries, with a leveling off of asthma prevalence rates in countries where the baseline prevalence was already high [3].

Longitudinal studies have likewise examined risk factors predicting the development as well as the persistence, remission or relapse of asthma from infancy, through childhood, to middle-age. Mature population-based birth cohort studies, such as those commenced in the UK, Australia and New Zealand several decades ago, have provided a useful understanding of adult outcomes of childhood asthma. More-recent birth cohort studies have focused on the early years of life, as evidence accrues suggesting that events and exposures *in utero*, in early infancy and during the preschool years play a major role in the development of the various phenotypes of asthma.

An extensive literature review was undertaken through 2006–2007 by Canadian investigators developing the recently launched Canadian Healthy Infant Longitudinal Development (CHILD) study (see Acknowledgements). That review has been updated with recent literature to summarize known and putative risk factors associated with the development of childhood allergy and asthma. After introducing the multiple phenotypes of asthma, we review the environmental (i.e., indoor air, outdoor air, nutrition and gut colonization, respiratory infections, and psychosocial environment and stress), genetic (i.e., gene–environment and gene–gene interactions, gender and parent-of-origin effects and epigenetics) and host (i.e., immunity, airway inflammation, lung function and sex and gender effects) risk factors currently considered to influence the initiation and course of asthma.

 10.1586/1744666X.5.1.77 ISSN 1744-666X

Phenotypes of childhood asthma

The heterogeneous phenotypes of childhood asthma differ in their etiology, pathophysiology and presentation. Although a positive family history is common, it is neither sufficient nor necessary for the development of asthma [7]. Environmental triggers may impact asthma differently at different times in life, and the relevant risk factors may change over time. Short-term studies of risk factors may suggest an increased likelihood of asthma, but the same risk factors may be associated with a decreased risk when follow up extends over a longer time, or *vice versa*. There is overlap between different wheezing phenotypes in early childhood, only some of which persist as asthma in later childhood and adulthood.

Although almost 50% of preschool children wheeze, by school age, only a minority are diagnosed with 'true' asthma [8,9]. Commonly described wheezing phenotypes in early infancy and childhood are transient, nonatopic , late-onset and persistent wheezing [10]. Only transient wheezing in early infancy has been well characterized by decreased airflow rates on pulmonary function testing at birth [8,11,12]. The other three phenotypes are described primarily by their age of onset in cohort studies, and their genesis in early infancy is largely unknown. Transient wheezing is characterized by onset within the first year of life, and resolution by mid-childhood with no pulmonary function sequelae. By contrast, the majority of persistent 'wheezers' (i.e., asthma sufferers) experience their first symptoms before the age of 3 years, and by 3 years old have abnormal lung function, which persists to adulthood [8–10]. By adolescence, most children with asthma are atopic. Among children with nonatopic and late-onset wheezing, some remit, while others develop persistent symptoms and atopy [13]. Finally, most of these phenotypes have been identified *post hoc* in longitudinal epidemiological studies where descriptions are offered at various ages. However, recent evidence throws into question the idea of 'remission' of asthma, even in the absence of symptoms, as children with a previous diagnosis of asthma have increased airway inflammation [14], perhaps explaining the increased relapse rate in adults of childhood atopic asthma [15]. Distinguishing between these different phenotypes is critical to understanding the role of risk factors and timing of exposure.

Environmental risk factors

Pregnant mothers, infants and young children are exposed to many environmental factors that can be inhaled (indoor and outdoor air), absorbed transdermally (house dust) and ingested, as well as to microbial milieu (infections, gastrointestinal [GI] flora, bacterial and/or mold toxins) and the psychosocial family environment. Prenatal environmental exposures can influence immune development and potentially alter the risk for allergic responses to allergens. Figure 1 illustrates the final common pathway via immunological responses (innate and acquired) related to genetic and environmental factors.

Indoor air

Infants and toddlers spend over 80% of their time indoors, where allergens, microbes and chemical pollutants are ubiquitous. Early-life aeroallergen sensitization, primarily to indoor allergens [16], especially house dust mites, is associated with an increased risk of asthma [17–20]. Where house dust mite levels are low, sensitization to mould (e.g., *Alternaria* spp.) or cat dander is associated with an increased risk of asthma [21,22]. Exposure to bacterial endotoxins is associated with increased early childhood wheezing [23–26] and elevated inflammatory responses during respiratory viral infections [27], but paradoxically, with later-life reduction in atopy in some subpopulations [26,28–31]. Response to endotoxin exposure depends on the expression of cell-surface receptors; however, the complex interactions of exposures, genetics and morbidity remain unclear. The relation between β(1,3)-D-glucans, the nonallergenic water-insoluble constituents of the cell walls of most fungi that accumulate in dust [32,33], and health effects are conflicting in relation to upper airway irritation, lung function, airway hyper-responsiveness and inflammation, and risk of atopy [33–36]. The relative contributions of the factors that are associated with an increased microbial burden in the home, such as dampness and dust loading, are poorly resolved, and causality remains controversial [37–39].

Children in homes with an increased surface area of plastic flooring and wall coverings, and increased airborne concentrations of common organic chemicals, such as formaldehyde and volatile/semivolatile organic compounds (VOC/SVOCs), for example phthalates, have more wheeze and physician-diagnosed allergies and asthma [40–44]. Peak exposures to these chemicals can be transient and difficult to characterize as new products are introduced into the indoor environment, although some partitioning onto dust and other permanent surfaces may increase chronic exposure. Exposure to indoor airborne dust is associated with increased respiratory symptoms and irritation of mucous membranes, effects which may, in part, be due to volatile compounds adsorbed to dust particles. The importance of these exposures, often highly transient in nature (i.e., periodic large acute exposures) is not known, but could explain some nonatopic childhood asthma [45]. Furthermore, occupational exposures to household cleaning products have been associated with increased respiratory symptoms [46]. An association has been noted between the use of cleaning sprays more than 4 days per week and adult asthma [47]. A relationship between household use of cleaning products and asthma development in children has not been demonstrated.

Environmental tobacco smoke (ETS), affecting the indoor environment in at least 20% of homes in Canada, is associated with increased respiratory morbidity, and is a risk factor for wheeze in early life, particularly maternal smoking in pregnancy [48–51]. Prenatal ETS exposure is consistently associated with early childhood wheezing [48,52–54], with a dose–response relationship between exposure and decreased airway caliber [55,56]. ETS exposure has also been associated with cord-blood cytokine responses [50,57] and exhaled nitric oxide (NO) production in newborns [58]. Human nasal challenge models show an adjuvant effect for this and other oxidizing pollutants [59], although mechanisms through which ETS increases morbidity, and the populations who are most vulnerable to it, remain to be identified. Oxides of nitrogen from indoor combustion [60,61] and exposure to trichloramines in indoor swimming pools [62,63] have also been implicated in irritant-induced asthma.

Outdoor air

The outdoor environment may impact respiratory health directly, as well as by infiltration. Exposure to ambient (outdoor) air pollutants (particulate matter [PM], nitrogen dioxide [NO_2], sulphur dioxide [SO_2], ozone and carbon monoxide [CO]), as well as to VOCs, SVOCs and aeroallergens, can cause significant respiratory morbidity [64–67]. PM with a mean aerodynamic diameter of less than 2.5 µm ($PM_{2.5}$) typically exists in a liquid/solid phase as reactive nitrates, sulphates, organics, trace metals and inorganic ('elemental') carbon. These particles also contain water and take up water vapor, and occur as mixtures with complex morphology [68–70]. Coarse particles, (2.5 µm to 10–15 µm in diameter) are more readily inhaled, with greater deposition in the nose and upper respiratory tract, initiating biological responses. These particles are most often associated with crustal dust, road dust and salt, aeroallergens and organic dust related to natural vegetation and/or the handling of agricultural products (e.g., grain or soy bean).

Ambient pollutants also exhibit a direct impact on indoor air quality [60]. Correlations between ambient and personal exposures to $PM_{2.5}$ and sulphates support the significant contribution of ambient sources to overall exposures. Ambient $PM_{2.5}$ contributes more than 50% of the indoor $PM_{2.5}$ levels [71,72]. Variations in outdoor pollution levels, providing a surrogate for potential indoor air quality, have been estimated using Geographic Information Systems (GIS) [73,74] and linked to health outcomes, including respiratory end points [75,76].

Data on the effects of pollutants in infancy are limited. Ambient exposures to SO_2, NO_2, CO and $PM_{2.5}$ have been associated with decreased intrauterine growth [77,78], while exposures to NO_2, $PM_{1.0}$, $PM_{2.5}$ and PM_{10} have been associated with decreases in lung function in children aged 7–10 years [79–82]. Ambient, but not indoor-generated, $PM_{2.5}$ increased levels of expired NO, indicative of airway inflammation [83].

Traffic-related chemical compounds can be readily detected in house dust [84]. Emissions include diesel and spark ignition particulate (e.g., elemental carbon and specific organic carbon species, such as polyaromatic hydrocarbons, alkanes and acids; metals such as iron, zinc and barium; and tire wear [e.g., latex and benzothiasols]); many of these are ultrafine particles (measured as total particle count below 0.1 µm). Exposure to ultrafine particles is assessed by modeling rather than by direct measurement. Questionnaire and GIS studies have found increased asthma prevalence, cough and wheeze in children living in proximity to heavy vehicle traffic [73,74,85–87].

Interactions between exposure to chemicals and aeroallergens have been demonstrated for several combinations of the contaminants. However, further studies are needed to examine those indoors (e.g., allergens, moulds, endotoxins, ETS, volatile and semivolatile compounds) concomitantly with common outdoor particulate and gaseous chemical exposure and proximity to sources (e.g., traveling in traffic) to assess the risks and interactions of many of the common environmental factors.

Nutrition & gut colonization

Theories on asthma development suggest that infants at high risk for asthma have a delayed maturation in their IFN-γ responses and are thought to later become 'Th2-skewed' [50,88], possibly due to a failure to expose the developing infant's immune system to adequate protective bacterial exposures. The gut immune system represents the largest mass of lymphoid tissue in the body, and the initial colonization of the intestine is critical in the development of the total body mucosal immune response [89]. The critical periods of colonization and presentation of intestinal microbiota may even occur prenatally [90], but certainly occur soon after birth and again after weaning to solid foods. The indigenous bacteria contribute to the anti-inflammatory response of the mucosal immune system. The establishment of the normal microbiota provides the host with a significant antigen challenge and a strong immune stimulatory effect for the maturation of the gut-associated lymphoid tissue and the development of tolerance [91]. Epidemiological and animal studies support an association between the composition of the indigenous intestinal microbiota and atopic sensitization and symptoms [92]. Infants with atopic sensitization at 1 year of age harbor more clostridia and less bifidobacteria in their stools at 3 weeks of age than nonsensitized infants destined to develop atopic sensitization [93]. Currently, there are conflicting data regarding the role of the introduction of probiotics to prevent allergy [90,94–96], most likely due to inadequate information about the critical timing of exposure. Further investigations are needed to explore the effect of diet on the diversity of gut microbiota and the resulting immunologic stimulation [97].

Studies examining prenatal nutrient levels or dietary interventions and the subsequent development of atopic disease have focused on foods with anti-inflammatory properties (e.g., n-3 fatty acids), antioxidants such as vitamin E and zinc, and vitamin D. Increased fish or fish oil intake during pregnancy was associated with a reduced risk of developing atopic disease (eczema and atopic wheeze) up to the age of 6 years [98–100], while increased prenatal vitamin E and zinc levels have been associated with a reduced risk of developing wheeze up to the age of 5 years [101–103]. However, maternal exclusion diets (e.g., cow's

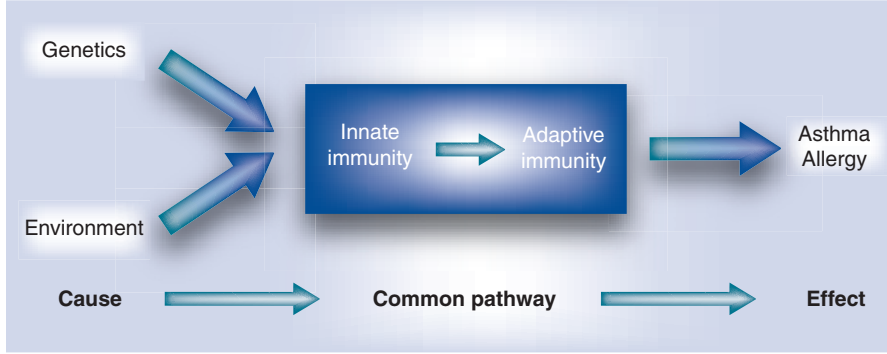

Figure 1. Innate and acquired immunity: the final common pathway.

milk or eggs) during pregnancy have failed to show a protective effect against the development of atopic disease in infancy [104–107]. Among recent studies examining the role of vitamin D in respiratory disease, two found an inverse relationship between maternal vitamin D levels and wheeze in early life [108,109], but no relationship with atopy or asthma symptoms in later life. The role of dietary methyl donors, and possibly other nutritional factors, has not been well studied, particularly as it may influence gene expression through epigenetic mechanisms.

Furthermore, obesity in both children and adults has been shown, in cross-sectional and case-controlled studies, to be associated with an increased prevalence of asthma [110–118]. Prospective studies have also demonstrated a dose–response relationship between asthma incidence and increasing BMI [119–125]. Many of the longitudinal studies controlled for the individuals' diet and level of physical activity. The influence of obesity on the development of asthma is greater in women than in men [119,126]. A relationship between BMI and asthma is also observed among children [127,128], although some studies have failed to demonstrate an association [129]. It is unclear whether the underlying asthma is the cause of obesity through increased inactivity, as observed in some studies [130], or whether obesity itself can cause asthma.

The influence of breastfeeding on the risk of childhood atopy and asthma remains controversial. Some studies show protection [131–133], while others report increased allergy and asthma among breast-fed children [100,131–135]. A meta-analysis and several other studies found that exclusive breastfeeding for at least 3 months was associated with decreased asthma between 2 and 5 years of age, with the effect greatest among those with a parental history of atopy [133,136,137]. While exclusivity and duration of breastfeeding, together with the length of follow up, are often invoked to explain these differences, one study suggested that the increased risk of childhood atopy and asthma associated with breastfeeding occurred predominantly in atopic infants of mothers with a history of asthma [135]. These findings and results obtained from animal studies suggest that maternal transfer of some immune-mediating factor(s) through breast milk may influence the development of asthma [138].

A recent review examining the relationships between breastfeeding and the epidemiology of allergic diseases noted that birth cohort studies, case–control studies and one cluster-randomized intervention trial, have generally failed to demonstrate a protective effect of breastfeeding on the outcomes of atopic dermatitis, allergic sensitization, wheezing or asthma [139]. Difficulties in interpretation relate to the absence of nonbreastfed controls or reference groups in some studies, meaning outcomes can only be compared between different durations of breastfeeding. Studies with a nonbreastfed control group suggest there may be an increased risk for atopy and asthma associated with breastfeeding, and that prolonged breastfeeding eventually reduces this increased risk. The family history, gender of the child, and the presence of other risk factors for allergy and asthma may also influence the outcome. Early reduction in childhood wheezing may reflect protection from viral infections, but allergies and asthma at later ages may be increased. A recent report from the Avon Longitudinal Study of Parents and Children (ALSPAC) confirmed this early beneficial effect, but found no long-term protection, nor an adverse effect in either gender, irrespective of family history [39].

Respiratory infections

Viral lower respiratory tract infection (LRTI) impacts early childhood wheezing. Whether LRTI promotes aeroallergen sensitization causing persistent asthma is controversial, as there is evidence suggesting that childhood viral infections might be pathogenic in some children but protective in others [140–144]. Infants of allergic or asthmatic mothers have a relatively persistent maturational defect in Th1 cytokine synthesis in the first year of life, which may play a role in the development of persistent or severe viral infections [145]. Severe viral LRTI in genetically susceptible infants already sensitized to inhalant allergens may lead to deviation towards Th2 responses, promoting asthma. It is unclear whether these effects of LTRI are virus-specific (e.g., respiratory syncytial virus [RSV] or rhinovirus) or whether synergistic exposures with allergens can induce asthma even in individuals who are not genetically susceptible. Gene interactions with environmental exposures (e.g., allergens, air pollution, ETS and diet) modulate the host response to infections [146,147]. A small sample size, cross-sectional analysis, lack of precise case definition and/or incomplete microbial assessment in previous studies make correlations between infections and outcomes difficult to ascertain [148,149]. Early childhood infections are associated with early wheezing [147] but it is unclear whether infection alone affects the development of persistent asthma. Repeated LRTIs may impact infants already at risk for asthma due to family history or atopy [13,150]. Severe infection with certain viruses, such as RSV [144] and rhinovirus [151], may play a role in persistent wheezing, while other studies suggest this has no effect [152]. Considered as a proxy for viral infections, daycare attendance is associated with an increased incidence of early wheeze but a decreased incidence of persistent wheeze [153].

Antibiotic use has been associated with early wheezing and asthma in several studies [154–156], possibly by immunologic stimulation via changes in bowel flora, but Kummeling found no coincident increase in eczema or atopy despite increased wheezing rates, arguing against this mechanism [154]. The association of wheezing/asthma with increased antibiotic use could also represent a surrogate marker for increased numbers of infections (perhaps viral) in early life.

Family size, as well as the number and order of siblings may impact asthma. The hygiene hypothesis posits that increasing numbers of infections and bacteria stimulate the developing immune system toward a nonasthmatic phenotype [157,158]. This may be mimicked in the real world by large family sizes, in which later-born children in large families would be at a lower risk than first-born children. Although this theory has been supported in studies of allergy prevalence [159,160], it has been partially refuted by recent studies of asthma prevalence suggesting that, although large family size (>4 children) is associated with a decreased risk, birth order is not involved [161,162]. In addition, not only allergic but also autoimmune and other chronic inflammatory diseases are increasing [163], which is difficult to explain by the hygiene hypothesis alone.

Psychosocial environment & stress

Socioeconomic status (SES) influences physical health throughout life [164–166]. Children of parents with a lower SES have more-severe asthma, higher rates of hospitalization and more days in bed due to asthma [167–172]. Findings with respect to asthma prevalence are mixed, with some studies reporting that the disease is more common among low SES families [173,174] and others reporting the opposite [175,176]. Thus, it remains unclear whether low SES increases or decreases vulnerability to the development of asthma. The association may hinge on how SES is measured and the specific outcome assessed; for example, some studies report associations of lower SES with greater airway obstruction and symptoms but not with a diagnosis of asthma [169,172]. Low SES may render children vulnerable to the airway pathology that drives asthma, but at the same time reduce the likelihood they will access medical systems and receive a diagnosis.

Life stress may also contribute to the development and expression of allergy and asthma [177,178]. Several biological processes are known to link neural, endocrine and immune responses, providing biologically plausible pathways that begin to explain how psychosocial factors influence the development of the disease [177,179]. Parental stress has been prospectively associated with wheezing in infancy [180], and family difficulties have been linked to asthma onset [181,182]. Children whose caregivers report high levels of stress and have difficulties parenting are at greatest risk for asthma [183]. A recent database study of almost 14,000 children in Manitoba found that prolonged exposure to maternal distress during the first 7 years of life was associated with a 1.3-fold increased risk of asthma diagnosis [184]. Collectively, these data suggest that the psychosocial environment children are reared in may shape their risk for developing asthma.

There is also evidence that links stress with the expression of asthma [185]. In an 18-month prospective study of children with asthma, acute life stressors, such as the death of a close family member, increased the risk of an attack twofold. The impact of these events was accentuated in the context of ongoing chronic stressors. Children exposed to high levels of acute and chronic stress showed a threefold increase in risk for an attack in the following 2 weeks [186,187]. Tightly controlled projects in animals substantiate the effects of stress on disease [188].

The mechanisms underlying these effects are becoming more fully understood. Life stress elicits negative emotional states such as fear, anger and sadness, which in turn set into motion a series of biological and behavioral adaptations, constituting the stress response [189]. While the function of the stress response is to support coping activities such as 'fight or flight', it may at the same time heighten vulnerability to wheezing, allergy and asthma. Behaviorally, when facing life stress, people are more likely to smoke cigarettes, drink alcohol and eat poorly. Biologically, life stress typically activates the hypothalamic-pituitary-adrenocortical and sympathetic-adrenal-medullary axes, leading to the secretion of cortisol, epinephrine and norepinephrine [190]. These hormones are able to bind to receptors on immune/inflammatory cells and modify their functions [191]. In patients with asthma, or those at risk for it, life stress is thought to bring about symptoms by amplifying the magnitude of the airway inflammatory response to irritants, allergens and infections [179].

In a birth cohort study of children with a family history of atopy, parental stress was prospectively associated with heightened IgE expression, as well as accentuated cytokine production following *in vitro* stimulation with common allergens at 2 years of age [192]. Life stress has also been concurrently associated with heightened *in vitro* production of a variety of Th2 cytokines and elevated circulating eosinophils in asthmatic children [179,193,194]. It is also linked with diminished expression of genes that regulate airway responsiveness and inflammation. Children who simultaneously experienced acute events and chronic stress showed a 5.5-fold reduction in glucocorticoid receptor mRNA and a 9.5-fold reduction in β_2-adrenergic receptor mRNA relative to children without comparable stressor exposure [195]. To the extent that the downregulation of receptors diminishes sensitivity to the anti-inflammatory properties of glucocorticoids, or the bronchodilator properties of β-agonists, this process could explain increased asthma morbidity associated with stress.

Genetic risk factors

Family and twin studies indicate that genetics plays an important role in the causation of asthma and allergy [196]. There are three popular approaches to the identification of genetic susceptibility variants:

- The association/candidate gene approach looks for polymorphisms in a known gene believed to be involved in the disease pathogenesis and compares the allele frequencies in cases and controls; this requires prior knowledge to identify appropriate candidates;
- The traditional linkage approach utilizes several hundred markers throughout the genome and identifies candidate regions shared between affected family members. Candidate regions are then scrutinized for the presence of disease loci. Linkage studies require no prior information and can provide new avenues for research, but the regions identified are often large and include many possible candidate genes;
- The most recent approach is the genome-wide association study (GWAS), which uses hundreds of thousands of markers called single nucleotide polymorphisms (SNPs) to identify SNPs associated with disease phenotypes using family-based or case-controlled association methods. GWAS studies require no prior information and, because they use hundreds of thousands of SNPs, they can target specific candidate genes and narrow regions for investigation.

These approaches have identified putative susceptibility genes for asthma in several different populations, but results have varied substantially from study to study.

Linkage scans have been performed in 11 different populations [197], and 18 genomic regions have been identified that probably harbour asthma/atopy genes, with consistently replicated regions on chromosomes 5q, 2q, 13q, 6q and 12q. Thousands

of association studies have been conducted and, in 2006, over 100 genes had been reported to be associated with asthma in at least one or more studies [198,199]. In the last few years there has been exponential growth in both the number of association studies being conducted and the number of genes reported to be associated with asthma-related traits; to date, over 180 genes have been reported to be associated with asthma and its related traits.

While there is no shortage of positive findings and candidate genes, replication of the findings has proven difficult. Reviews of the published literature have highlighted a number of questionable genotype-phenotype associations, mainly from underpowered studies [199,200] and, as a result, the majority of positive associations are never replicated in subsequent studies [200,201]. However, small sample sizes and lack of statistical power do not fully explain replication failures, as the architecture of genetic susceptibility to asthma and its related phenotypes is complex.

Identifying subgroups with common underlying risk factors can create genetic homogeneity, increasing the power and precision of an association study. For example, the first and only GWAS study for asthma was completed in 2007 [202], identifying *ORMDL3* as a new candidate gene, now replicated in multiple studies [203–206]. However, *ORMDL3* is specific to childhood asthma and the association is not seen in individuals whose asthma symptoms started after the age of 7 years [202]. Similarly, null mutations in the filaggrin (*FLG*) gene associated with atopic dermatitis, eczema and asthma have been replicated in multiple studies [207–211]. A recent meta-analysis of *FLG* mutations indicates that the risk of atopic dermatitis exceeds that for mutations in any other candidate gene identified to date [212]. However, *FLG* is rarely associated with asthma in the absence of atopic dermatitis and is secondary to allergic sensitization. *FLG* mutations do not seem to play a role in nonatopic asthma. The *ORMDL3* association with childhood asthma and *FLG's* association with atopic asthma are strong indicators that genetic susceptibility may be phenotype specific.

Challenges in replicating genetic-association studies across studies and populations include the extensive heterogeneity in the phenotypic presentation and incidence and prevalence patterns, which vary by age and gender [213,214]. These differences are so pronounced that childhood and adult asthmas have been suggested to be different diseases, both in their phenotypic presentation and genetic predisposition [214,215]. Additionally, it is likely that interactions (e.g., environment, gene–gene, age and gender) and epigenetic effects are involved in the pathogenesis of asthma and its related traits. The well-reported interaction between *CD14* variants and endotoxin exposure illustrates the complexity of gene–environment interactions in determining asthma and allergic phenotypes [216].

Gene–environment & gene–gene interactions

Potential mechanisms for the development of asthma and allergies are:

- Genetic predisposition alone, either through one or several interacting genes;
- Environmental exposures independent of genetics;
- The combination of genetic predisposition and environmental exposures, considered the most likely mechanism.

Evidence for gene–environment interactions is accumulating at a rapid pace, and a variety of environmental agonists have been implicated, including animals, endotoxins, ETS, viruses, daycare, ozone and farm exposures. One of the most consistent and frequently reported gene–environment interaction studied includes *CD14* and the -159 C to T variant (rs2569190) in the promoter region of the gene and exposures to endotoxins. Over 200 studies have examined the association between *CD14* and asthma-related phenotypes [216], with inconsistent results. These inconsistent results may be explained by a complex dose-dependent environmental interaction with endotoxin exposure in early life, whereby the T allele can either confer protection or increase risk, with the direction of the effect determined by the level of endotoxin exposure. This type of interaction may obscure or confound results from studies that include only the main genetic or environmental effects, and underscores the need to include interactions in statistical models for complex disease phenotypes such as asthma. An excellent review by Martinez covers the depth and breadth of the literature regarding *CD14* interactions [216], while a full review of gene–environment interactions can be found in a paper by Yang *et al.* [217]. Vercelli provides additional information regarding the complex nature of gene–environment interactions, with special attention paid to the immunological aspects [218].

In addition to environmental interactions, there is an increasing focus on gene–gene interactions, with numerous reports of genes associated with both gene–environmental and gene–gene interactions, making it difficult to separate gene–gene interactions from gene–environment interactions. Both gene–gene and gene–environment interactions involving the same genetic variant may occur, and future analyses will focus on determining if gene–environment or gene–gene interactions, or a complex synergy of both, are involved. Genetic pathways that regulate cell signaling, cytokine and innate immune responses are comprised of a number of genes, and the analysis of these pathways may provide additional insights into the mechanisms involved, as recently reviewed by Vercelli [218].

Gender & parent-of-origin effects

In childhood, asthma and atopy are more prevalent in males than in females, but this distribution changes during puberty [213,214]. Reported gender-dependent effects in the literature, including linkage of thymic stromal lymphopoietin (*TSLP)* to lower IgE levels in girls [219] and estrogen receptor 1 (*ESR1*) associations with asthma in females [220], have promoted speculation that, in the future, there may be sex-specific treatments for asthma and its related phenotypes [220].

There are sex-specific effects of the *in utero* environment and other environmental risk factors. The prevalence of early-onset and persistent asthma among children with *in utero* exposure to tobacco smoke and null genotypes at the glutathione S-transferase (GST) M1 locus varies depending upon whether the fetus is male or female [221].

Growing evidence suggests that imprinting (the phenomenon whereby the effect of a particular allele on disease risk varies according to the parental source of the allele) may play a role in the development of asthma and related phenotypes. Maternal transmission of risk alleles may be more important than paternal transmission for many allergic phenotypes [222–224]. The association between *COL29A1* and atopic dermatitis [225], which demonstrates a strong maternal transmission pattern, is the most-recent and statistically compelling study to support the imprinting hypothesis. The importance and extent of imprinting, and its impact on asthma and related phenotypes, is not known, as few studies have the capability to study these effects. In order to test for parent-of-origin effects, studies need to genotype parents to determine the origin of the alleles, either through inference or by direct methods. Studies of imprinting effects will be challenging and it is likely that international collaboration will be necessary to adequately examine these effects.

Epigenetics

Epigenetics is the study of modifications to DNA and its accompanying histone molecules, which influence the transcription of genes. Although the sequence of the human genome is the same in all cells of an individual, the epigenome differs from tissue to tissue, and changes in response to the cell's environment. More broadly, epigenetic modifications are seen as the mechanism through which the environment, both social and physical, influences gene expression, serving as a bridge from environments to genomes and, more broadly, from nurture to nature. Epigenetic control of gene expression occurs in two main ways. The first type of epigenetic modification is DNA methylation; cytosine residues in the regulatory region of genes can be methylated by specific enzymes and these regions are sensed by proteins that turn gene expression on or off through regulating chromatin structure. The second and more complex alteration is chemical modification of the histones around which chromosomal DNA is wrapped. More than 20 chemical tags can be attached to, or removed from, histones to produce an open or closed chromatin structure and influence access of the transcription machinery. Epigenetic modifications to DNA are believed to be responsible for the phenotypic differences that develop over time between monozygotic twins [226]. It has been suggested that it is principally through epigenetic modification of DNA that lifestyle and chemical exposures affect susceptibility to diseases [227].

Epigenetic modifications to DNA can be inherited and/or acquired. Environmental factors during pregnancy that could result in epigenetic modifications include nutrition/diet (e.g., folic acid and vitamin B_{12}), smoking, exposure to microbial products, maternal stress and maternal care [228,229]. Modifications of fetal genes during pregnancy may influence the risk for allergy and asthma. Another critical window for epigenetic modification occurs during early life, when environmental factors can modify the child's genome to potentially cause and/or prolong allergy and asthma. This emerging field holds immense promise in helping us to understand both the environmental contribution to asthma susceptibility as well as the importance of the timing and dosage of environmental exposures, as recently reviewed by Miller and Ho [230].

Host risk factors

Immunity

Allergen sensitization, the result of adaptive immunity to common aeroallergens, is a major risk factor for persistent asthma. The hygiene hypothesis posits that exposure to microbial agents at a young age (perhaps even *in utero*) may protect the fetus from the development of atopy and asthma [157,158]; thus, the dramatic rise in atopic diseases over the last several decades could be explained by decreased environmental exposures to microbial agents. The immune mechanisms responsible have yet to be fully delineated. Initial simplistic renditions of this hypothesis stated that T cells isolated from the cord blood of newborns showed a Th2-type response when challenged with common environmental allergens [88], and subsequent exposure to the wide range of microbial pathogens (which was an inevitable childhood occurrence in previous generations) supports the generation of Th1-type responses to environmental allergens. However, recent environmental changes, including improvements in sanitation, reductions in childhood infections and decreases in family sizes, abrogate the switch to an 'allergy-protective' Th1-dominated response. Emerging data cast doubt on this simple interpretation of the hygiene hypothesis; in that, infections *per se* cannot explain the epidemiological patterns of modulation of allergic disease (e.g., high prevalence rates for allergy and asthma in some South-American countries). In addition, not only allergic but also autoimmune and other chronic inflammatory diseases are also increasing [163], which is difficult to explain by the hygiene hypothesis alone. On the other hand, exposure to specific allergens very early in life might be a major contributing factor to the development of allergies later in life. In short, the decision of a pro- versus anti-allergic immune response is probably made very early in life, possibly even *in utero*.

In adults, the immunology of established atopy and asthma has been well characterized, specifically involving expansion of Th2 cells with the production of IL-4 and IL-5, which promote IgE- and eosinophil-associated allergy [231]. Early perinatal studies of atopic development that focused on adaptive immunity have shown immune deviation towards a Th2 preponderance in early normal life [232,233]; failure to redirect this deviation to a more Th1-based (particularly IFN-γ-based) immune system may lead to atopy. However, newborns at high risk for atopic development are born with relative decreases in both Th2 and Th1 immunity, indicating that a more complex cascade of events is responsible for clinically manifest atopic disease than Th2 predominance [233].

While adaptive immune responses are clearly important in immune deviation, little is understood about the potential importance of the innate immune reactions involved. This may prove to be pivotal, insofar as the innate immune system provides the first interface with the microbial world [234] and is central to the initiation of an inflammatory allergic reaction [235]. Toll-like receptors (TLRs) are essential for innate recognition of critical molecular patterns present on microorganisms

(e.g., pathogen-associated molecular patterns [PAMPs]) [236]. The response of TLRs to PAMPS differs from host to host. Early evidence suggests differences in the TLR sequence (polymorphisms) may result in alterations of expression and/or function of the allergic immune response [237,238]. Nevertheless, both TLR expression on, and the effects on function of, immune/inflammatory cells have yet to be examined in a comprehensive study that includes detailed assessment of the environment and the genetic makeup of the individual.

Natural killer T (NKT) cells are key actors at the interface between innate and acquired immunity [239]. Upon activation by glycolipid antigens, NKT cells rapidly secrete both Th1 and Th2 cytokines, affecting the development of later immune responses. Initial studies in mice identified a central role for invariant NKT cells for the development of allergen-induced airway hyper-reactivity [240]. However, the contribution of this immunoregulatory cell population in human asthma remains unclear [241,242].

The immune system is the earliest final common pathway in allergy and asthma (Figure 1). A thorough immunological assessment is essential to link candidate genetic and environmental influences to molecular cause-and-effect relationships for asthma and allergy, and to identify early prognostic markers for potential future atopic disease and even potential therapeutic targets for early intervention.

Airway inflammation

Airway inflammation is a key component of asthma [243]. Although not truly a risk factor for the development of asthma, it clearly plays a role in helping to define the wheezing phenotype and thus categorize the risk of persistence of wheeze. In adults with persistent asthma, airway inflammation is characterized by increased eosinophils in induced sputum [244–247]. However, in early childhood, even amongst those who will develop persistent wheezing, airway inflammation may be nonspecific or neutrophilic in nature [248,249], and may represent immaturity of the infant immune system and lung defenses [248,250,251]. NO is a useful marker of airway inflammation in asthma [252,253]. Exhaled nitric oxide (FE_{NO}) levels have been shown to correlate with sputum and peripheral eosinophilia and airway hyper-responsiveness in older children with asthma [254]. The discriminative ability of FE_{NO} has been studied amongst preschool children and, although it was higher amongst children with atopy or diagnosed asthma, it was unable to clearly differentiate between wheezing phenotypes [252], although those who likely had high levels of eosinophilia had higher levels of FE_{NO} [255]. Studies also support its usefulness in infants with recurrent wheezing to document airway inflammation [256–258]. Furthermore, among infants, exhaled NO (eNO) levels are elevated in those with wheezing secondary to airway inflammation upregulation of induced NO synthase (iNOS). There is some evidence that eNO levels are elevated at birth in infants at risk for asthma (e.g., male infants of asthmatic/atopic mothers and who are exposed to cigarette smoke), suggesting that not only does airway inflammation begin early in life but also that maternal and environmental factors can interact prenatally, inducing changes in the developing fetus that may predispose them to develop wheezing [58]. Measures of airway inflammation that differentiate between types of inflammation, such as eNO [259] and possibly exhaled breath-condensate markers, may be useful not only for documenting the type of airway inflammation but also for following its evolution in synchrony with immune-system and lung development to elucidate the role of environmental exposures in the pathogenesis of different wheezing phenotypes. iNOS expression in the placenta is correlated with postnatal exhaled NO in infants, and genetic variants in iNOS may influence NO production [260,261] and modulate the response to environmental stimuli [262]. For example, exposures to ETS and viruses cause neutrophil migration and epithelial inflammatory responses in individuals not susceptible to asthma. Though both are associated with wheezing phenotypes, eNO is decreased in infants exposed to ETS in the absence of atopy; thus, differentiation of the types of inflammation may be useful to distinguish the various wheezing phenotypes [58]. Recent studies also suggest that a more-generalized inflammatory response, as measured by highly sensitive C-reactive protein levels, is also associated with asthma and wheeze [263].

Lung function

Decrements in lung function have been associated with asthma diagnosis [264] and may predict asthma in later life [265,266]. Furthermore, some have argued that poor lung function may be a risk factor for airway hyper-responsiveness [267].

The link between birth weight and forced expiratory volume in 1 s (FEV_1) has been shown in several studies [268,269]. Furthermore, other cohorts have linked undernutrition during fetal life, manifested by small-for-gestational age births, to adult lung-function decrements [270,271]. Studies during the Dutch famine suggested that the period of greatest risk was early-to-mid gestation [271]. Further work found that lung function impairment in early life is not only related to birth weight but also has a complex causal relationship with social disadvantage [272–274]. Tracking of these initial decrements in lung function during early life [274] has been implicated as a risk factor for further lung injury via viral infection mechanisms [275] and asthma [276]. The Tucson cohort has demonstrated similar tracking of infant lung function into adult life [277].

Taken together, the findings from these studies support the hypothesis that early prenatal factors may play a role in determining the final lung function attained in adult life. Given that poor lung function is associated with increased asthma diagnosis [264], it is difficult to determine whether poor lung growth is a risk factor for asthma or is the outcome of asthma, and, until further data are known on the development of the lung pathology of asthma, this remains uncertain.

Furthermore, whether the insult occurs in infancy or later in childhood, the association of low lung function is clear and well established. Children with persistent wheezing (and diagnosed asthma) that continues into adulthood have a fixed decrement in lung function starting from as early as the age of 7 years, compared with children with transient or no wheezing [9,278]. More

recently, preschool studies have documented abnormal lung function in children with persistent wheezing as young as 3 years old [10]. However, some data in infants who develop persistent wheezing display normal lung functioning shortly after birth, suggesting a critical period of exposure within the first few years of life before the development of these persistent abnormalities in expiratory flows [8,279]. By contrast, infants who later exhibit early transient wheezing have decreased airflow at birth [8,280]. Maternal smoking with *in-utero* nicotine exposure correlates with this lung dysfunction [8,55,56], but effects of other exposures are less well studied.

Cigarette smoking is associated with the greatest effect on adult lung function and decline in lung function, with smoking associated with a 28–50ml/year loss in FEV_1 [281]. This effect on FEV_1 seems to be influenced by gender, as the effect is greater amongst adolescent girls than boys [282], but reverses in middle age when the effects are greater in men than in women [281]. Both pregnancy and early infancy have been shown to be risk periods for the effects of ETS [55,272–274]. Other work has suggested that exposure to cigarette smoke may be particularly detrimental to lung growth in populations who are already disadvantaged due to poor rates of fetal growth [270]. Cigarette smoke has been shown to have a direct detrimental effect on lung function at birth [283] and in the early years of life [273].

Air pollution is also associated with poor lung function in childhood. The Children's Health study estimated that exposure to NO_2 led to the greatest loss of lung function development during adolescence and decreased the final lung function at 18 years of age by approximately 100 ml [82]. Other studies by the same group showed a dose-response relationship between increasing levels of pollution (e.g., NO_2 and PM_{10}) and decreased annual growth in FEV_1 in adolescence (8 ml/year) [264]. Air pollution has been shown to have a doubling effect on all-cause mortality when combined with socioeconomic status [284], suggesting that the detrimental effect of air pollution may not be acting through direct mechanisms alone.

Chronic conditions such as asthma have an even greater effect on lung function; chronic asthma was associated with a deficit in lung function growth in the range of 5–25 ml/year [285]. The combination of smoking and asthma had an additive effect, with a decline in lung function of 24 ml/year [286]. Again, this effect was influenced by gender, with males (decline: 48–57 ml/year) more affected than females (decline: 31–36ml/year) in middle age. However, longitudinal studies of asthma, such as among the Dunedin [9] and Melbourne cohorts [278], suggest that significant loss of lung function occurs in early childhood. Not only does loss of lung function growth occur before school-age in children with persistent asthma, lung function in these children remains persistently lower than in their nonasthmatic counterparts. Strunk and colleagues likewise found similar results in the USA, suggesting that the injury in asthma occurs at or before the age of 6 years, with no significant effects on annual lung function velocities thereafter [287].

Impairment in adult lung function has been associated with increased mortality for a wide range of diseases [288] and morbidities, including an increased risk for chronic obstructive pulmonary disease [289]. Decrements in lung function have been linked to a number of factors, including cigarette smoking, air pollution, LRTI and asthma diagnosis [264].

Sex or gender effects

Changes in asthma incidence and severity for females occur around puberty and menopause [290–292]. Until the age of 13–14 years, the incidence and prevalence of asthma is greater in boys than in girls [115,291,292]. Studies throughout puberty support a greater incidence of asthma among both adolescent and young adult females [291,293], and a greater proportion of males with remission of asthma. Before the age of 12 years, boys have more-severe asthma than girls [294], with higher hospitalization rates [295]. By contrast, adult females have more-severe asthma than males, with more asthma-related hospitalizations [296], and they regain control of their asthma more slowly [297], resulting in longer hospital stays [298] and higher rehospitalization rates [299].

The influence of some environmental risk factors, such as allergens, may be modified by gender. The influence of obesity on the development of asthma is greater in women than in men and is not influenced by caloric intake or physical activity [119]. One paper reported that 18% of adult asthmatic women had negative skin-prick tests (IgE < 100 iU/ml) and reported eosinophilia in less than 5% of patients, which were characteristics of only 2.3% of asthmatic men [16], suggesting different disease mechanisms between genders. Interactions have been found between the specific parental (maternal vs paternal) history of atopy, breastfeeding and sex of the child on the risk of developing asthma and atopy [300]. Among high-risk groups in that study, breastfeeding had little effect, whilst among low-risk groups there was a significant interaction between gender and breastfeeding.

Expert commentary & five-year view

This review of recent literature was undertaken to determine the current state of knowledge of the risk factors involved in the development of asthma in order to focus investigations in a proposed new longitudinal birth cohort study. The increased prevalence of allergy and asthma in children over recent decades suggests environmental exposures may be critical. The origins of asthma appear to lie in the prenatal and early postnatal period. Renewed investigations in this period with long-term close follow up and objective phenotypic characterization are needed to understand the role of the multiple putative environmental factors in asthma. Recent advances in genetics and the emerging field of epigenetics should enhance our understanding of gene–environment interactions that increase susceptibility to asthma, as well as the importance of the timing and dose of relevant environmental exposures.

Patterns of development of allergy, airway inflammation and lung-function abnormalities that may predict future asthma have not been well assessed in early infancy. Environmental risk factors may interact with one another, and further studies examining indoor exposures (e.g., allergens, molds, endotoxins, ETS and volatile and semivolatile compounds) concomitantly with outdoor particulate and gaseous chemical exposures, as well as proximity to

sources (e.g., traveling in traffic), are needed to assess the risks and the effects of interactions of common environmental factors.

Measurements that differentiate between the types of airway inflammation, such as exhaled NO and exhaled breath-condensate markers, may be useful not only to document the type of airway inflammation but also to follow its evolution in synchrony with immunological and physical lung development. Prenatal factors likely play a substantial role in determining adult lung function. Poor lung function is associated with increased asthma diagnosis; hence, it is difficult to determine whether poor lung growth is a risk factor for, or the outcome of, asthma. Until further data emerge on the development of lung pathology in asthma, this will remain uncertain.

Comprehensive immunological assessments are essential to link candidate genetic and environmental influences to molecular cause-and-effect relationships, and to identify early prognostic markers for potential future atopic disease and therapeutic targets for intervention. While adaptive immune responses are clearly important in immune deviation, little is understood about the potential importance of the innate immune reactions involved. This may prove to be pivotal, insofar as the innate immune system provides the first interface with the microbial world and is central to the initiation of an inflammatory allergic reaction. Further investigation is needed to explore the effect of diet on the diversity of gut microbiota and the resulting immunologic stimulation.

Whether SES and living circumstances are as relevant to the incidence of allergy and asthma as they are to the expression, severity and management of these diseases, and whether stressful life experiences temporally precede biological and behavioral changes, remain unclear. Further studies are required to determine whether and how these factors prospectively contribute to the development and progression of wheezing, allergy and asthma.

In summary, the effects of a broad range of environmental exposures, host factors and genetics in the promotion of specific immune and proinflammatory responses and the development of clinical allergic diseases and asthma have been explored, but much still remains uncertain. Studies that include objective measures of chemical and microbial contaminant exposures, combined with candidate genetic variation and clearly defined health outcomes, are needed to assess interactions between genes, the environment and age-of-exposure interactions. It is only after determining the factors promoting the development of asthma that one can hope to design rational studies of interventions for its prevention.

Acknowledgements

This work was carried out on behalf of the Canadian Healthy Infant Longitudinal Development (CHILD) Study Investigators:

R Allen, Simon Fraser University, Vancouver, Canada; A Becker, University of Manitoba, Winnipeg, Canada; D Befus, University of Alberta, Edmonton, Canada; M Brauer, University of British Columbia, Vancouver, Canada; J Brook, Environment Canada, Toronto, Canada; M Cyr, McMaster University, Hamilton, Canada; E Chen, University of British Columbia, Vancouver, Canada; D Daley, James Hogg iCAPTURE Centre, Vancouver, Canada; S Dell, Hospital for Sick Children, Toronto, Canada; J Denburg, McMaster University, Hamilton, Canada; S Elliott, McMaster University, Hamilton, Canada; H Grasemann, Hospital for Sick Children, Toronto, Canada; K HayGlass, University of Manitoba, Winnipeg, Canada; R Hegele, James Hogg iCAPTURE Centre, Vancouver, Canada; L Holness, St. Michael's Hospital, Toronto, Canada; M Kobor, University of British Columbia, Vancouver, Canada; T Kollmann, University of British Columbia, Vancouver, Canada; A Kozyrskyj, University of Alberta, Edmonton, Canada; C Laprise, Chicoutimi University Hospital, Quebec, Canada; M Larché, McMaster University,

Key issues

- Asthma is a heterogeneous disease with overlapping phenotypes, the differentiation of which is critical to understanding the risk factors and their timing, particularly as environmental risk factors may impact asthma differently *in utero*, in early infancy and during the preschool years.
- While many genes have been associated with asthma, and replication has proved difficult, *ORMDL3* is now replicated in multiple studies and appears to be specific to childhood asthma, while *FLG* is associated with atopic asthma, suggesting genetic susceptibility may be phenotype specific.
- Gene–environment interactions are probably involved in the development of asthma in relation to the impact of animals, endotoxins, environmental tobacco smoke, viruses, daycare, ozone and farm exposures.
- Environmental factors may modify the genome in early life (epigenetics) to potentially cause or prolong allergy and asthma.
- Male children have a higher incidence and greater severity of asthma, and a greater prevalence of atopy; the sex differences reverse during puberty.
- Environmental tobacco smoke is a risk factor for wheeze in early life, particularly maternal smoking during pregnancy, and exposure may be particularly detrimental to lung growth in those already disadvantaged due to poor rates of fetal growth.
- Childhood viral infections might be pathogenic for asthma in some children but protective in others, while antibiotic use has been associated with early wheezing and asthma.
- Socioeconomic factors, parental stress and family difficulties have been associated with wheezing in infancy and with the onset of asthma.
- Abnormal lung function associated with persistent wheezing is evident as young as the age of 3 years, despite normal lung function shortly after birth, suggesting a critical period of risk within the first few years of life.
- Nutritional risk factors remain controversial, especially the influence of breastfeeding, while maternal exclusion diets during pregnancy have failed to protect against development of infant atopy. Obesity is now firmly associated with an increased prevalence of asthma.

Hamilton, Canada; W Lou, University of Toronto, Toronto, Canada; P Mandhane, University of Alberta, Edmonton, Canada; G Miller, University of British Columbia, Vancouver, Canada; R Moqbel, University of Manitoba, Winnipeg, Canada; P Paré, James Hogg iCAPTURE Centre, Vancouver , Canada; C Ramsey, University of Manitoba, Winnipeg, Canada; F Ratjen, Hospital for Sick Children, Toronto, Canada; A Sandford, James Hogg iCAPTURE Centre, Vancouver, Canada; J Scott, University of Toronto, Toronto, Canada; M Sears, McMaster University, Hamilton, Canada; F Silverman, University of Toronto, Toronto, Canada; P Subbarao, Hospital for Sick Children, Toronto, Canada; S Tebbutt, James Hogg iCAPTURE Centre, Vancouver, Canada; T Takaro, Simon Fraser University, Vancouver, Canada; P Tang, University of British Columbia, Vancouver, Canada; T To, Hospital for Sick Children, Toronto, Canada and S Turvey, University of British Columbia, Vancouver, Canada.

Financial & competing interests disclosure

This work was supported by The Allergy, Genes and Environment Network of Centers of Excellence(AllerGen NCE Inc.), a national multidisciplinary research network whose common aim is to support research, networking, commercialization, knowledge mobilization and capacity-building activities that contribute to reducing the morbidity, mortality and socioeconomic impact of allergic disease. AllerGen is a member of the federal Networks of Centres of Excellence Canada programme, a joint initiative of the Natural Sciences and Engineering Research Council, the Canadian Institutes of Health Research, the Social Sciences and Humanities Research Council and Industry Canada. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as:
• of interest

1 Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. *Lancet* 351(9111), 1225–1232 (1998).

2 Janson C, Anto J, Burney P *et al.* The European Community Respiratory Health Survey: what are the main results so far? European Community Respiratory Health Survey II. *Eur. Respir. J.* 18(3), 598–611 (2001).

• **The first two references taken together give a good overview of the prevalence and worldwide distribution of asthma symptoms.**

3 Asher MI, Montefort S, Bjorksten B *et al.* Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases one and three repeat multicountry cross-sectional surveys. *Lancet* 368(9537), 733–743 (2006).

• **Excellent paper looking at the changes in global regional prevalence over the last decade in asthma and allergy symptoms.**

4 Zock JP, Heinrich J, Jarvis D *et al.* Distribution and determinants of house dust mite allergens in Europe: the European Community Respiratory Health Survey II. *J. Allergy Clin. Immunol.* 118(3), 682–690 (2006).

5 Cohen RT, Canino GJ, Bird HR, Shen S, Rosner BA, Celedon JC. Area of residence, birthplace, and asthma in Puerto-Rican children. *Chest* 131(5), 1331–1338 (2007).

6 von ME, Martinez FD, Fritzsch C, Nicolai T, Roell G, Thiemann HH. Prevalence of asthma and atopy in two areas of West and East Germany. *Am. J. Respir. Crit. Care Med.* 149(2 Pt 1), 358–364 (1994).

7 Burke W, Fesinmeyer M, Reed K, Hampson L, Carlsten C. Family history as a predictor of asthma risk. *Am. J. Prev. Med.* 24(2), 160–169 (2003).

• **Interesting paper examining the risk conferred by family history on asthma diagnosis.**

8 Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. *N. Engl. J. Med.* 332(3), 133–138 (1995).

• **One of the first papers to attempt to answer the question of whether lung dysfunction in asthma precedes symptoms.**

9 Sears MR, Greene JM, Willan AR *et al.* A longitudinal, population-based, cohort study of childhood asthma followed to adulthood. *N. Engl. J. Med.* 349(15), 1414–1422 (2003).

• **One of a very few prospective general cohort studies that follows children with lung function measurements over three decades, giving the natural history of asthma over time.**

10 Lowe LA, Simpson A, Woodcock A, Morris J, Murray CS, Custovic A. Wheeze phenotypes and lung function in preschool children. *Am. J. Respir. Crit. Care Med.* 171(3), 231–237 (2005).

11 Stick SM, Burton PR, Gurrin L, Sly PD, Le Souef PN. Effects of maternal smoking during pregnancy and a family history of asthma on respiratory function in newborn infants. *Lancet* 348(9034), 1060–1064 (1996).

12 Young S, Arnott J, O'Keeffe PT, Le Souef PN, Landau LI. The association between early life lung function and wheezing during the first 2 yrs of life. *Eur. Resp. J.* 15(1), 151–157 (2000).

13 Illi S, von Mutius E, Lau S, Niggemann B, Gruber C, Wahn U. Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study. *Lancet* 368(9537), 763–770 (2006).

14 Warke TJ, Fitch PS, Brown V *et al.* Outgrown asthma does not mean no airways inflammation. *Eur. Respir. J.* 19(2), 284–287 (2002).

15 Bronnimann S, Burrows B. A prospective study of the natural history of asthma. Remission and relapse rates. *Chest* 90(4), 480–484 (1986).

16 Oryszczyn MP, Bouzigon E, Maccario J *et al.* Interrelationships of quantitative asthma-related phenotypes in the epidemiological study on the genetics and environment of asthma, bronchial hyperresponsiveness, and atopy. *J. Allergy Clin. Immunol.* 119(1), 57–63 (2007).

17 Arshad SH, Tariq SM, Matthews S, Hakim E. Sensitization to common allergens and its association with allergic disorders at age 4 years: a whole population birth cohort study. *Pediatrics* 108(2), E33 (2001).

18 Sears MR, Burrows B, Herbison GP, Holdaway MD, Flannery EM. Atopy in childhood. II. Relationship to airway responsiveness, hay fever and asthma. *Clin. Exp. Allergy* 23(11), 949–956 (1993).

19 Bergmann RL, Edenharter G, Bergmann KE *et al.* Atopic dermatitis in early infancy predicts allergic airway disease at 5 years. *Clin. Exp. Allergy* 28(8), 965–970 (1998).

20 Sears MR, Herbison GP, Holdaway MD, Hewitt CJ, Flannery EM, Silva PA. The relative risks of sensitivity to grass pollen, house dust mite and cat dander in the development of childhood asthma. *Clin. Exp. Allergy* 19(4), 419–424 (1989).

21 Perzanowski MS, Sporik R, Squillace SP *et al.* Association of sensitization to *Alternaria* allergens with asthma among school-age children. *J. Allergy Clin. Immunol.* 101(5), 626–632 (1998).

22 Halonen M, Stern DA, Wright AL, Taussig LM, Martinez FD. *Alternaria* as a major allergen for asthma in children raised in a desert environment. *Am. J. Respir. Crit. Care Med.* 155(4), 1356–1361 (1997).

23 Tavernier GO, Fletcher GD, Francis HC *et al.* Endotoxin exposure in asthmatic children and matched healthy controls: results of IPEADAM study. *Indoor Air* 15(Suppl. 10), 25–32 (2005).

24 Dales R, Miller D, Ruest K, Guay M, Judek S. Airborne endotoxin is associated with respiratory illness in the first 2 years of life. *Environ. Health Perspect.* 114(4), 610–614 (2006).

25 Tavernier G, Fletcher G, Gee I *et al.* IPEADAM study: indoor endotoxin exposure, family status, and some housing characteristics in English children. *J. Allergy Clin. Immunol.* 117(3), 656–662 (2006).

26 Perzanowski MS, Miller RL, Thorne PS *et al.* Endotoxin in inner-city homes: associations with wheeze and eczema in early childhood. *J. Allergy Clin. Immunol.* 117(5), 1082–1089 (2006).

27 Foster S, Bedford KJ, Gould ME, Coward WR, Hewitt CR. Respiratory syncytial virus infection and virus-induced inflammation are modified by contaminants of indoor air. *Immunology* 108(1), 109–115 (2003).

28 de Meer G., Toelle BG, Ng K, Tovey E, Marks GB. Presence and timing of cat ownership by age 18 and the effect on atopy and asthma at age 28. *J. Allergy Clin. Immunol.* 113(3), 433–438 (2004).

29 Douwes J, van SR, Doekes G *et al.* Does early indoor microbial exposure reduce the risk of asthma? The Prevention and Incidence of Asthma and Mite Allergy birth cohort study. *J. Allergy Clin. Immunol.* 117(5), 1067–1073 (2006).

30 Michel O. Systemic and local airways inflammatory response to endotoxin. *Toxicology* 152(1–3), 25–30 (2000).

31 Waser M, Schierl R, von ME *et al.* Determinants of endotoxin levels in living environments of farmers' children and their peers from rural areas. *Clin. Exp. Allergy* 34(3), 389–397 (2004).

32 Foto M, Plett J, Berghout J, Miller JD. Modification of the *Limulus amebocyte* lysate assay for the analysis of glucan in indoor environments. *Anal. Bioanal. Chem.* 379(1), 156–162 (2004).

33 Rylander R, Lin RH. (1→>3)-β-D-glucan – relationship to indoor air-related symptoms, allergy and asthma. *Toxicology* 152(1–3), 47–52 (2000).

34 Douwes J. (1→3)-β-D-glucans and respiratory health: a review of the scientific evidence. *Indoor Air* 15(3), 160–169 (2005).

35 Rylander R. Airborne (1→3)-β-D-glucan and airway disease in a day-care center before and after renovation. *Arch. Environ. Health* 52(4), 281–285 (1997).

36 Beijer L, Thorn J, Rylander R. Effects after inhalation of (1→3)-β-D-glucan and relation to mould exposure in the home. *Mediators Inflamm.* 11(3), 149–153 (2002).

37 Dales RE, Miller D, McMullen E. Indoor air quality and health: validity and determinants of reported home dampness and moulds. *Int. J. Epidemiol.* 26(1), 120–125 (1997).

38 Dales RE, Miller D. Residential fungal contamination and health: microbial cohabitants as covariates. *Environ. Health Perspect.* 107(Suppl. 3), 481–483 (1999).

39 Elliott L, Arbes SJ, Harvey ES *et al.* Dust weight and asthma prevalence in the National Survey of Lead and Allergens in Housing (NSLAH). *Environ. Health Perspect.* 115(2), 215–220 (2007).

40 Dales R, Raizenne M. Residential exposure to volatile organic compounds and asthma. *J. Asthma* 41(3), 259–270 (2004).

41 Bornehag CG, Lundgren B, Weschler CJ, Sigsgaard T, Hagerhed-Engman L, Sundell J. Phthalates in indoor dust and their association with building characteristics. *Environ. Health Perspect.* 113(10), 1399–1404 (2005).

42 Bornehag CG, Sundell J, Weschler CJ *et al.* The association between asthma and allergic symptoms in children and phthalates in house dust: a nested case-control study. *Environ. Health Perspect.* 112(14), 1393–1397 (2004).

43 Hoppin JA, Ulmer R, London SJ. Phthalate exposure and pulmonary function. *Environ. Health Perspect.* 112(5), 571–574 (2004).

44 Oie L, Hersoug LG, Madsen JO. Residential exposure to plasticizers and its possible role in the pathogenesis of asthma. *Environ. Health Perspect.* 105(9), 972–978 (1997).

45 Douwes J, Gibson P, Pekkanen J, Pearce N. Non-eosinophilic asthma: importance and possible mechanisms. *Thorax* 57(7), 643–648 (2002).

46 Medina-Ramon M, Zock JP, Kogevinas M *et al.* Short-term respiratory effects of cleaning exposures in female domestic cleaners. *Eur. Respir. J.* 27(6), 1196–1203 (2006).

47 Zock JP, Plana E, Jarvis D *et al.* The use of household cleaning sprays and adult asthma: an international longitudinal study. *Am. J. Respir. Crit. Care Med.* 176(8), 735–741 (2007).

48 Lau S, Nickel R, Niggemann B *et al.* The development of childhood asthma: lessons from the German Multicentre Allergy Study (MAS). *Paediatr. Respir. Rev.* 3(3), 265–272 (2002).

49 Jaakkola JJ, Nafstad P, Magnus P. Environmental tobacco smoke, parental atopy, and childhood asthma. *Environ. Health Perspect.* 109(6), 579–582 (2001).

50 Macaubas C, de Klerk NH, Holt BJ *et al.* Association between antenatal cytokine production and the development of atopy and asthma at age 6 years. *Lancet* 362(9391), 1192–1197 (2003).

51 Sears MR, Holdaway MD, Flannery EM, Herbison GP, Silva PA. Parental and neonatal risk factors for atopy, airway hyper-responsiveness, and asthma. *Arch. Dis. Child.* 75(5), 392–398 (1996).

52 Stein RT, Holberg CJ, Sherrill D *et al.* Influence of parental smoking on respiratory symptoms during the first decade of life: the Tucson Children's Respiratory Study. *Am. J. Epidemiol.* 149(11), 1030–1037 (1999).

53 Lewis S, Richards D, Bynner J, Butler N, Britton J. Prospective study of risk factors for early and persistent wheezing in childhood. *Eur. Respir. J.* 8(3), 349–356 (1995).

54 Tariq SM, Hakim EA, Matthews SM, Arshad SH. Influence of smoking on asthmatic symptoms and allergen sensitisation in early childhood. *Postgrad. Med. J.* 76(901), 694–699 (2000).

55 Dezateux C, Stocks J, Dundas I, Fletcher ME. Impaired airway function and wheezing in infancy: the influence of maternal smoking and a genetic predisposition to asthma. *Am. J. Resp. Crit. Care Med.* 159(2), 403–410 (1999).

56 Lodrup Carlsen KC. The environment and childhood asthma (ECA) study in Oslo: ECA-1 and ECA-2. *Pediatr. Allergy Immunol.* 13(Suppl. 15), 29–31 (2002).

57 Devereux G, Barker RN, Seaton A. Antenatal determinants of neonatal immune responses to allergens. *Clin. Exp. Allergy* 32(1), 43–50 (2002).

58 Frey U, Kuehni C, Roiha H *et al.* Maternal atopic disease modifies effects of prenatal risk factors on exhaled nitric oxide in infants. *Am. J. Respir. Crit. Care Med.* 170(3), 260–265 (2004).

59 Gilliland FD, Li YF, Gong H Jr, Diaz-Sanchez D. Glutathione S-transferases M1 and P1 prevent aggravation of allergic responses by secondhand smoke. *Am. J. Respir. Crit. Care Med.* 174(12), 1335–1341 (2006).

60 Zota A, Adamkiewicz G, Levy JI, Spengler JD. Ventilation in public housing: implications for indoor nitrogen dioxide concentrations. *Indoor Air* 15(6), 393–401 (2005).

61 Emenius G, Svartengren M, Korsgaard J, Nordvall L, Pershagen G, Wickman M. Building characteristics, indoor air quality and recurrent wheezing in very young children (BAMSE). *Indoor Air* 14(1), 34–42 (2004).

62 Bernard A, Carbonnelle S, de Burbure C, Michel O, Nickmilder M. Chlorinated pool attendance, atopy, and the risk of asthma during childhood. *Environ. Health Perspect.* 114(10), 1567–1573 (2006).

63 Bernard A, Carbonnelle S, Michel O *et al.* Lung hyperpermeability and asthma prevalence in schoolchildren: unexpected associations with the attendance at indoor chlorinated swimming pools. *Occup. Environ. Med.* 60(6), 385–394 (2003).

64 Kunzli N, Kaiser R, Medina S *et al.* Public-health impact of outdoor and traffic-related air pollution: a European assessment. *Lancet* 356(9232), 795–801 (2000).

65 Brunekreef B, Holgate ST. Air pollution and health. *Lancet* 360(9341), 1233–1242 (2002).

66 Dockery DW, Pope CA, III, Xu X *et al.* An association between air pollution and mortality in six US cities. *N. Engl. J. Med.* 329(24), 1753–1759 (1993).

67 Nicolai T. Pollution, environmental factors and childhood respiratory allergic disease. *Toxicology* 181–182, 317–321 (2002).

68 Urch B, Brook JR, Wasserstein D *et al.* Relative contributions of $PM_{2.5}$ chemical constituents to acute arterial vasoconstriction in humans. *Inhal. Toxicol.* 16(6–7), 345–352 (2004).

69 Lunden MM, Thatcher TL, Hering SV, Brown NJ. Use of time- and chemically resolved particulate data to characterize the infiltration of outdoor $PM_{2.5}$ into a residence in the San Joaquin Valley. *Environ. Sci. Technol.* 37(20), 4724–4732 (2003).

70 Nel A. Atmosphere. Air pollution-related illness: effects of particles. *Science* 308(5723), 804–806 (2005).

71 Wilson WE, Brauer M. Estimation of ambient and non-ambient components of particulate matter exposure from a personal monitoring panel study. *J. Expo. Sci. Environ. Epidemiol.* 16(3), 264–274 (2006).

72 Weisel CP, Zhang J, Turpin BJ *et al.* Relationships of Indoor, Outdoor, and Personal Air (RIOPA). Part I. Collection methods and descriptive analyses. *Res. Rep. Health Eff. Inst.*(130 Pt 1), 1–107 (2005).

73 Nicolai T, Carr D, Weiland SK *et al.* Urban traffic and pollutant exposure related to respiratory outcomes and atopy in a large sample of children. *Eur. Respir. J.* 21(6), 956–963 (2003).

74 English P, Neutra R, Scalf R, Sullivan M, Waller L, Zhu L. Examining associations between childhood asthma and traffic flow using a geographic information system. *Environ. Health Perspect.* 107(9), 761–767 (1999).

75 Gauderman WJ. Air pollution and children – an unhealthy mix. *N. Engl. J. Med.* 355(1), 78–79 (2006).

76 Brauer M, Hoek G, Smit HA *et al.* Air pollution and development of asthma, allergy and infections in a birth cohort. *Eur. Respir. J.* 29(5), 879–888 (2007).

77 Liu S, Krewski D, Shi Y, Chen Y, Burnett RT. Association between maternal exposure to ambient air pollutants during pregnancy and fetal growth restriction. *J. Expo. Sci. Environ. Epidemiol.* 17, 426–432 (2006).

78 Liu S, Krewski D, Shi Y, Chen Y, Burnett RT. Association between gaseous ambient air pollutants and adverse pregnancy outcomes in Vancouver, Canada. *Environ. Health Perspect.* 111(14), 1773–1778 (2003).

79 Moshammer H, Hutter HP, Hauck H, Neuberger M. Low levels of air pollution induce changes of lung function in a panel of schoolchildren. *Eur. Respir. J.* 27(6), 1138–1143 (2006).

80 Gauderman WJ, McConnell R, Gilliland F *et al.* Association between air pollution and lung function growth in Southern California children. *Am. J. Respir. Crit. Care Med.* 162(4 Pt 1), 1383–1390 (2000).

81 Gauderman WJ, Gilliland GF, Vora H *et al.* Association between air pollution and lung function growth in Southern California children: results from a second cohort. *Am. J. Respir. Crit. Care Med.* 166(1), 76–84 (2002).

82 Gauderman WJ, Avol E, Gilliland F *et al.* The effect of air pollution on lung development from 10 to 18 years of age. *N. Engl. J. Med.* 351(11), 1057–1067 (2004).

83 Koenig JQ, Mar TF, Allen RW *et al.* Pulmonary effects of indoor- and outdoor-generated particles in children with asthma. *Environ. Health Perspect.* 113(4), 499–503 (2005).

84 Brook JR, Graham L, Charland JP *et al.* Investigation of the motor vehicle exhaust contribution to primary fine particle organic carbon in urban air. *Atmos. Environ.* 41(1), 119–135 (2007).

85 Brauer M, Hoek G, Van VP *et al.* Air pollution from traffic and the development of respiratory infections and asthmatic and allergic symptoms in children. *Am. J. Respir. Crit. Care Med.* 166(8), 1092–1098 (2002).

86 Van VP, Knape M, de HJ, Janssen N, Harssema H, Brunekreef B. Motor vehicle exhaust and chronic respiratory symptoms in children living near freeways. *Environ. Res.* 74(2), 122–132 (1997).

87 Gauderman WJ, Avol E, Lurmann F *et al.* Childhood asthma and exposure to traffic and nitrogen dioxide. *Epidemiology* 16(6), 737–743 (2005).

88 Prescott SL, Macaubas C, Holt BJ *et al.* Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. *J. Immunol.* 160(10), 4730–4737 (1998).

89 Rautava S, Kalliomaki M, Isolauri E. New therapeutic strategy for combating the increasing burden of allergic disease: Probiotics-A Nutrition, Allergy, Mucosal Immunology and Intestinal Microbiota (NAMI) research group report. *J. Allergy Clin. Immunol.* 116(1), 31–37 (2005).

90 Kukkonen K, Savilahti E, Haahtela T *et al.* Probiotics and prebiotic galacto-oligosaccharides in the prevention of allergic diseases: a randomized, double-blind, placebo-controlled trial. *J. Allergy Clin. Immunol.* 119(1), 192–198 (2007).

91 Newberry RD, Stenson WF, Lorenz RG. Cyclooxygenase-2-dependent arachidonic acid metabolites are essential modulators of the intestinal immune response to dietary antigen. *Nat. Med.* 5(8), 900–906 (1999).

92 Penders J, Stobberingh EE, van den Brandt PA, Thijs C. The role of the intestinal microbiota in the development of atopic disorders. *Allergy* 62(11), 1223–1236 (2007).

93 Kalliomaki M, Kirjavainen P, Eerola E, Kero P, Salminen S, Isolauri E. Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. *J. Allergy Clin. Immunol.* 107(1), 129–134 (2001).

94 Kalliomaki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet* 357(9262), 1076–1079 (2001).

95 Taylor AL, Dunstan JA, Prescott SL. Probiotic supplementation for the first 6 months of life fails to reduce the risk of atopic dermatitis and increases the risk of allergen sensitization in high-risk children: a randomized controlled trial. *J. Allergy Clin. Immunol.* 119(1), 184–191 (2007).

96 Adlerberth I, Strachan DP, Matricardi PM *et al.* Gut microbiota and development of atopic eczema in 3 European birth cohorts. *J. Allergy Clin. Immunol.* 120(2), 343–350 (2007).

97 Eckburg PB, Bik EM, Bernstein CN *et al.* Diversity of the human intestinal microbial flora. *Science* 308(5728), 1635–1638 (2005).

98 Willers SM, Devereux G, Craig LC *et al.* Maternal food consumption during pregnancy and asthma, respiratory and atopic symptoms in 5-year-old children. *Thorax* 62(9), 773–779 (2007).

99 Romieu I, Torrent M, Garcia-Esteban R *et al.* Maternal fish intake during pregnancy and atopy and asthma in infancy. *Clin. Exp. Allergy* 37(4), 518–525 (2007).

100 Mihrshahi S, Ampon R, Webb K *et al.* The association between infant feeding practices and subsequent atopy among children with a family history of asthma. *Clin. Exp. Allergy* 37(5), 671–679 (2007).

101 Litonjua AA, Rifas-Shiman SL, Ly NP *et al.* Maternal antioxidant intake in pregnancy and wheezing illnesses in children at 2 y of age. *Am. J. Clin. Nutr.* 84(4), 903–911 (2006).

102 Devereux G, Turner SW, Craig LC *et al.* Low maternal vitamin E intake during pregnancy is associated with asthma in 5-year-old children. *Am. J. Respir. Crit. Care Med.* 174(5), 499–507 (2006).

103 Martindale S, McNeill G, Devereux G, Campbell D, Russell G, Seaton A. Antioxidant intake in pregnancy in relation to wheeze and eczema in the first two years of life. *Am. J. Respir. Crit. Care Med.* 171(2), 121–128 (2005).

104 Kramer MS, Kakuma R. Maternal dietary antigen avoidance during pregnancy or lactation, or both, for preventing or treating atopic disease in the child. *Cochrane Database Syst. Rev.* 3, CD000133 (2006).

- **Provides a good up-to-date review of the breastfeeding literature.**

105 Falth-Magnusson K, Kjellman NI. Development of atopic disease in babies whose mothers were receiving exclusion diet during pregnancy – a randomized study. *J. Allergy Clin. Immunol.* 80(6), 868–875 (1987).

106 Falth-Magnusson K, Kjellman NI. Allergy prevention by maternal elimination diet during late pregnancy – a 5-year follow-up of a randomized study. *J. Allergy Clin. Immunol.* 89(3), 709–713 (1992).

107 Lilja G, Dannaeus A, Falth-Magnusson K *et al.* Immune response of the atopic woman and foetus: effects of high- and low-dose food allergen intake during late pregnancy. *Clin. Allergy* 18(2), 131–142 (1988).

108 Camargo CA Jr, Rifas-Shiman SL, Litonjua AA *et al.* Maternal intake of vitamin D during pregnancy and risk of recurrent wheeze in children at 3 y of age. *Am. J. Clin. Nutr.* 85(3), 788–795 (2007).

- **One of the first prospective birth cohort studies showing a relationship between prenatal vitamin D intake and wheeze.**

109 Devereux G, Litonjua AA, Turner SW *et al.* Maternal vitamin D intake during pregnancy and early childhood wheezing. *Am. J. Clin. Nutr.* 85(3), 853–859 (2007).

- **Another birth cohort study showing a relationship with prenatal vitamin D intake and wheeze and bronchodilator response at age 5 years.**

110 Beckett WS, Jacobs DR Jr, Yu X, Iribarren C, Williams OD. Asthma is associated with weight gain in females but not males, independent of physical activity. *Am. J. Respir. Crit. Care Med.* 164(11), 2045–2050 (2001).

111 Cassol VE, Rizzato TM, Teche SP *et al.* Obesity and its relationship with asthma prevalence and severity in adolescents from southern Brazil. *J. Asthma* 43(1), 57–60 (2006).

112 Chen Y, Dales R, Krewski D, Breithaupt K. Increased effects of smoking and obesity on asthma among female Canadians: the National Population Health Survey, 1994–1995. *Am. J. Epidemiol.*150(3), 255–262 (1999).

113 Guerra S, Sherrill DL, Bobadilla A, Martinez FD, Barbee RA. The relation of body mass index to asthma, chronic bronchitis, and emphysema. *Chest* 122(4), 1256–1263 (2002).

114 Luder E, Ehrlich RI, Lou WY, Melnik TA, Kattan M. Body mass index and the risk of asthma in adults. *Respir. Med.* 98(1), 29–37 (2004).

115 Ronmark E, Andersson C, Nystrom L, Forsberg B, Jarvholm B, Lundback B. Obesity increases the risk of incident asthma among adults. *Eur. Respir. J.* 25(2), 282–288 (2005).

116 Shaheen SO, Sterne JA, Montgomery SM, Azima H. Birth weight, body mass index and asthma in young adults. *Thorax* 54(5), 396–402 (1999).

117 Sin DD, Jones RL, Man SF. Obesity is a risk factor for dyspnea but not for airflow obstruction. *Arch. Intern. Med.* 162(13), 1477–1481 (2002).

118 Young SY, Gunzenhauser JD, Malone KE, McTiernan A. Body mass index and asthma in the military population of the northwestern United States. *Arch. Intern. Med.* 161(13), 1605–1611 (2001).

119 Camargo CA, Jr., Weiss ST, Zhang S, Willett WC, Speizer FE. Prospective study of body mass index, weight change, and risk of adult-onset asthma in women. *Arch. Intern. Med.* 159(21), 2582–2588 (1999).

120 Chen Y, Dales R, Tang M, Krewski D. Obesity may increase the incidence of asthma in women but not in men: longitudinal observations from the Canadian National Population Health Surveys. *Am. J. Epidemiol.* 155(3), 191–197 (2002).

121 Ford ES, Mannino DM, Redd SC, Mokdad AH, Mott JA. Body mass index and asthma incidence among USA adults. *Eur. Respir. J.* 24(5), 740–744 (2004).

122 Gunnbjornsdottir MI, Omenaas E, Gislason T *et al.* Obesity and nocturnal gastro-oesophageal reflux are related to onset of asthma and respiratory symptoms. *Eur. Respir. J.* 24(1), 116–121 (2004).

123 Huovinen E, Kaprio J, Laitinen LA, Koskenvuo M. Incidence and prevalence of asthma among adult Finnish men and women of the Finnish Twin Cohort from 1975 to 1990, and their relation to hay fever and chronic bronchitis. *Chest* 115(4), 928–936 (1999).

124 Nystad W, Meyer HE, Nafstad P, Tverdal A, Engeland A. Body mass index in relation to adult asthma among 135,000 Norwegian men and women. *Am. J. Epidemiol.* 160(10), 969–976 (2004).

125 Romieu I, Avenel V, Leynaert B, Kauffmann F, Clavel-Chapelon F. Body mass index, change in body silhouette, and risk of asthma in the E3N cohort study. *Am. J Epidemiol.* 158(2), 165–174 (2003).

126 Hancox RJ, Milne BJ, Poulton R *et al.* Sex differences in the relation between body mass index and asthma and atopy in a birth cohort. *Am. J. Respir. Crit. Care Med.* 171(5), 440–445 (2005).

127 Gold DR, Damokosh AI, Dockery DW, Berkey CS. Body-mass index as a predictor of incident asthma in a prospective cohort of children. *Pediatr. Pulmonol.* 36(6), 514–521 (2003).

128 Gilliland FD, Berhane K, Islam T *et al.* Obesity and the risk of newly diagnosed asthma in school-age children. *Am. J. Epidemiol.* 158(5), 406–415 (2003).

129 Chinn S, Rona RJ. Can the increase in body mass index explain the rising trend in asthma in children? *Thorax* 56(11), 845–850 (2001).

130 Firrincieli V, Keller A, Ehrensberger R *et al.* Decreased physical activity among Head Start children with a history of wheezing: use of an accelerometer to measure activity. *Pediatr. Pulmonol.* 40(1), 57–63 (2005).

131 Bergmann RL, Diepgen TL, Kuss O *et al.* Breastfeeding duration is a risk factor for atopic eczema. *Clin. Exp. Allergy* 32(2), 205–209 (2002).

132 Dell S, To T. Breastfeeding and asthma in young children: findings from a population-based study. *Arch. Pediatr. Adolesc. Med.* 155(11), 1261–1265 (2001).

133 Oddy WH. Breastfeeding and asthma in children: findings from a West Australian study. *Breastfeed Rev.* 8(1), 5–11 (2000).

134 Sears MR, Greene JM, Willan AR *et al.* Long-term relation between breastfeeding and development of atopy and asthma in children and young adults: a longitudinal study. *Lancet* 360(9337), 901–907 (2002).

135 Wright AL, Holberg CJ, Taussig LM, Martinez F. Maternal asthma status alters relation of infant feeding to asthma in childhood. *Adv. Exp. Med. Biol.* 478, 131–137 (2000).

136 Gdalevich M, Mimouni D, Mimouni M. Breast-feeding and the risk of bronchial asthma in childhood: a systematic review with meta-analysis of prospective studies. *J. Pediatr.* 139(2), 261–266 (2001).

137 Kull I, Almqvist C, Lilja G, Pershagen G, Wickman M. Breast-feeding reduces the risk of asthma during the first 4 years of life. *J. Allergy Clin. Immunol.* 114(4), 755–760 (2004).

138 Leme AS, Hubeau C, Xiang Y *et al.* Role of breast milk in a mouse model of maternal transmission of asthma susceptibility. *J. Immunol.* 176(2), 762–769 (2006).

139 Duncan JM, Sears MR. Breastfeeding and allergies: time for a change in paradigm? *Curr. Opin. Allergy Clin. Immunol.* 8(5), 398–405 (2008).

140 Lemanske RF Jr, Busse WW. Asthma: factors underlying inception, exacerbation, and disease progression. *J. Allergy Clin. Immunol.* 117(2 Suppl. Mini-Primer), S456–S461 (2006).

141 Arruda LK, Sole D, Baena-Cagnani CE, Naspitz CK. Risk factors for asthma and atopy. *Curr. Opin. Allergy Clin. Immunol.* 5(2), 153–159 (2005).

142 Martinez FD. Role of viral infections in the inception of asthma and allergies during childhood: could they be protective? *Thorax* 49(12), 1189–1191 (1994).

143 Stein RT, Sherrill D, Morgan WJ *et al.* Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. *Lancet* 354(9178), 541–545 (1999).

144 Sigurs N, Gustafsson PM, Bjarnason R *et al.* Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13. *Am. J. Respir. Crit. Care Med.* 171(2), 137–141 (2005).

145 Martinez FD, Holt PG. Role of microbial burden in aetiology of allergy and asthma. *Lancet* 354(Suppl. 2), SII12–SII15 (1999).

146 Gern JE, Brooks GD, Meyer P *et al.* Bidirectional interactions between viral respiratory illnesses and cytokine responses in the first year of life. *J. Allergy Clin. Immunol.* 117(1), 72–78 (2006).

147 Friedlander SL, Jackson DJ, Gangnon RE *et al.* Viral infections, cytokine dysregulation and the origins of childhood asthma and allergic diseases. *Pediatr. Infect. Dis. J.* 24(Suppl. 11), S170–S176 (2005).

148 Becker A, Watson W, Ferguson A, Dimich-Ward H, Chan-Yeung M. The Canadian asthma primary prevention study: outcomes at 2 years of age. *J. Allergy Clin. Immunol.* 113(4), 650–656 (2004).

149 Chan-Yeung M, Ferguson A, Watson W *et al.* The Canadian childhood asthma primary prevention study: outcomes at 7 years of age. *J. Allergy Clin. Immunol.* 116(1), 49–55 (2005).

- **Study of intervention to reduce asthma incidence that followed children to 7 years of age.**

150 Oddy WH, de Klerk NH, Sly PD, Holt PG. The effects of respiratory infections, atopy, and breastfeeding on childhood asthma. *Eur. Respir. J.* 19(5), 899–905 (2002).

151 Lemanske RF, Jr., Jackson DJ, Gangnon RE *et al.* Rhinovirus illnesses during infancy predict subsequent childhood wheezing. *J. Allergy Clin. Immunol.* 116(3), 571–577 (2005).

- **Interesting paper examining the role of viral infections in the development of asthma and wheezing.**

152 Joshi P, Shaw A, Kakakios A, Isaacs D. Interferon-γ levels in nasopharyngeal secretions of infants with respiratory syncytial virus and other respiratory viral infections. *Clin. Exp. Immunol.* 131(1), 143–147 (2003).

153 Celedon JC, Litonjua AA, Ryan L, Weiss ST, Gold DR. Day care attendance, respiratory tract illnesses, wheezing, asthma, and total serum IgE level in early childhood. *Arch. Pediatr. Adolesc. Med.* 156(3), 241–245 (2002).

154 Kummeling I, Stelma FF, Dagnelie PC *et al.* Early life exposure to antibiotics and the subsequent development of eczema, wheeze, and allergic sensitization in the first 2 years of life: the KOALA Birth Cohort Study. *Pediatrics* 119(1), e225-e231 (2007).

155 Alm B, Erdes L, Mollborg P *et al.* Neonatal antibiotic treatment is a risk factor for early wheezing. *Pediatrics* 121(4), 697–702 (2008).

156 Kozyrskyj AL, Ernst P, Becker AB. Increased risk of childhood asthma from antibiotic use in early life. *Chest* 131(6), 1753–1759 (2007).

157 Schaub B, Lauener R, von Mutius E. The many faces of the hygiene hypothesis. *J. Allergy Clin. Immunol.* 117(5), 969–977 (2006).

158 Strachan DP. Hay fever, hygiene, and household size. *BMJ* 299(6710), 1259–1260 (1989).

159 Matricardi PM, Franzinelli F, Franco A *et al.* Sibship size, birth order, and atopy in 11,371 Italian young men. *J. Allergy Clin. Immunol.* 101(4 Pt 1), 439–444 (1998).

160 Kinra S, Davey SG, Jeffreys M, Gunnell D, Galobardes B, McCarron P. Association between sibship size and allergic diseases in the Glasgow Alumni Study. *Thorax* 61(1), 48–53 (2006).

161 Goldberg S, Israeli E, Schwartz S *et al.* Asthma prevalence, family size, and birth order. *Chest* 131(6), 1747–1752 (2007).

162 Bernsen RM, de Jongste JC, van der Wouden JC. Birth order and sibship size as independent risk factors for asthma, allergy, and eczema. *Pediatr. Allergy Immunol.* 14(6), 464–469 (2003).

163 Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. *N. Engl. J. Med.* 347(12), 911–920 (2002).

164 Adler NE, Boyce T, Chesney MA *et al.* Socioeconomic status and health. The challenge of the gradient. *Am. Psychol.* 49(1), 15–24 (1994).

165 Berkman LF, Kawachi I. *Social Epidemiology*. Oxford University Press, NY, USA (2000).

166 Marmot M. Economic and social determinants of disease. *Bull. World Health Organ.* 79(10), 988–989 (2001).

167 Amre DK, Infante-Rivard C, Gautrin D, Malo JL. Socioeconomic status and utilization of health care services among asthmatic children. *J. Asthma* 39(7), 625–631 (2002).

168 Claudio L, Tulton L, Doucette J, Landrigan PJ. Socioeconomic factors and asthma hospitalization rates in New York City. *J. Asthma* 36(4), 343–350 (1999).

169 Erzen D, Carriere KC, Dik N *et al.* Income level and asthma prevalence and care patterns. *Am. J. Respir. Crit. Care Med.* 155(3), 1060–1065 (1997).

170 Goodman DC, Stukel TA, Chang CH. Trends in pediatric asthma hospitalization rates: regional and socioeconomic differences. *Pediatrics* 101(2), 208–213 (1998).

171 Halfon N, Newacheck PW. Childhood asthma and poverty: differential impacts and utilization of health services. *Pediatrics* 91(1), 56–61 (1993).

172 Strachan DP, Anderson HR, Limb ES, O'Neill A, Wells N. A national survey of asthma prevalence, severity, and treatment in Great Britain. *Arch. Dis. Child.* 70(3), 174–178 (1994).

173 Litonjua AA, Carey VJ, Weiss ST, Gold DR. Race, socioeconomic factors, and area of residence are associated with asthma prevalence. *Pediatr. Pulmonol.* 28(6), 394–401 (1999).

174 Miller JE. The effects of race/ethnicity and income on early childhood asthma prevalence and health care use. *Am. J. Public Health* 90(3), 428–430 (2000).

175 Peckham C, Butler N. A national study of asthma in childhood. *J. Epidemiol. Community Health* 32(2), 79–85 (1978).

176 Hamman RF, Halil T, Holland WW. Asthma in schoolchildren. Demographic associations and peak expiratory flow rates compared in children with bronchitis. *Br. J. Prev. Soc. Med.* 29(4), 228–238 (1975).

177 Wright RJ, Cohen RT, Cohen S. The impact of stress on the development and expression of atopy. *Curr. Opin. Allergy Clin. Immunol.* 5(1), 23–29 (2005).

• **Excellent review of the role of life stress in the development of atopy and mechanisms underlying this process.**

178 Wright RJ. Stress and atopic disorders. *J. Allergy Clin. Immunol.* 116(6), 1301–1306 (2005).

179 Chen E, Miller GE. Stress and inflammation in exacerbations of asthma. *Brain Behav. Immun.* 21(8), 993–999 (2007).

• **Outlines the putative mechanisms linking stress and asthma.**

180 Wright RJ, Cohen S, Carey V, Weiss ST, Gold DR. Parental stress as a predictor of wheezing in infancy: a prospective birth-cohort study. *Am. J. Respir. Crit. Care Med.* 165(3), 358–365 (2002).

181 Mrazek DA, Klinnert M, Mrazek PJ *et al.* Prediction of early-onset asthma in genetically at-risk children. *Pediatr. Pulmonol.* 27(2), 85–94 (1999).

182 Klinnert MD, Nelson HS, Price MR, Adinoff AD, Leung DY, Mrazek DA. Onset and persistence of childhood asthma: predictors from infancy. *Pediatrics* 108(4), E69 (2001).

183 Klinnert MD, Mrazek PJ, Mrazek DA. Early asthma onset: the interaction between family stressors and adaptive parenting. *Psychiatry* 57(1), 51–61 (1994).

184 Kozyrskyj AL, Mai XM, McGrath P, Hayglass KT, Becker AB, Macneil B. Continued exposure to maternal distress in early life is associated with an increased risk of childhood asthma. *Am. J. Respir. Crit. Care Med.* 177(2), 142–147 (2008).

185 Bloomberg GR, Chen E. The relationship of psychologic stress with childhood asthma. *Immunol. Allergy Clin. North Am.* 25(1), 83–105 (2005).

186 Sandberg S, Jarvenpaa S, Penttinen A, Paton JY, McCann DC. Asthma exacerbations in children immediately following stressful life events: a Cox's hierarchical regression. *Thorax* 59(12), 1046–1051 (2004).

187 Sandberg S, Paton JY, Ahola S *et al.* The role of acute and chronic stress in asthma attacks in children. *Lancet* 356(9234), 982–987 (2000).

188 Chida Y, Sudo N, Sonoda J, Hiramoto T, Kubo C. Early-life psychological stress exacerbates adult mouse asthma via the hypothalamus-pituitary-adrenal axis. *Am. J. Respir. Crit. Care Med.* 175(4), 316–322 (2007).

189 Lazarus RS. *Stress, Appraisal and Coping*. Springer Publishing Company, NY, USA (1984).

190 McEwen BS. Protective and damaging effects of stress mediators. *N. Engl. J. Med.* 338(3), 171–179 (1998).

191 Webster JI, Tonelli L, Sternberg EM. Neuroendocrine regulation of immunity. *Ann. Rev. Immunol.* 20, 125–163 (2002).

192 Wright RJ, Finn P, Contreras JP *et al.* Chronic caregiver stress and IgE expression, allergen-induced proliferation, and cytokine profiles in a birth cohort predisposed to atopy. *J. Allergy Clin. Immunol.* 113(6), 1051–1057 (2004).

193 Kang DH, Coe CL, McCarthy DO *et al.* Cytokine profiles of stimulated blood lymphocytes in asthmatic and healthy adolescents across the school year. *J. Interferon Cytokine Res.* 17(8), 481–487 (1997).

194 Kang DH, Fox C. Th1 and Th2 cytokine responses to academic stress. *Res. Nurs. Health* 24(4), 245–257 (2001).

195 Miller GE, Chen E. Life stress and diminished expression of genes encoding glucocorticoid receptor and β2-adrenergic receptor in children with asthma. *Proc. Natl Acad. Sci. USA* 103(14), 5496–5501 (2006).

196 Duffy DL, Martin NG, Battistutta D, Hopper JL, Mathews JD. Genetics of asthma and hay fever in Australian twins. *Am. Rev. Respir. Dis.* 142(6 Pt 1), 1351–1358 (1990).

197 Hoffjan S, Ober C. Present status on the genetic studies of asthma. *Curr. Opin. Immunol.* 14(6), 709–717 (2002).

198 Hoffjan S, Nicolae D, Ober C. Association studies for asthma and atopic diseases: a comprehensive review of the literature. *Respir. Res.* 4, 14 (2003).

199 Ober C, Hoffjan S. Asthma genetics 2006: the long and winding road to gene discovery. *Genes Immun.* 7(2), 95–100 (2006).

• **Key review of genetic association studies and summary of findings.**

200 Chanock SJ, Manolio T, Boehnke M *et al.* Replicating genotype–phenotype associations. *Nature* 447(7145), 655–660 (2007).

201 Hirschhorn JN. Genetic approaches to studying common diseases and complex traits. *Pediatr. Res.* 57(5 Pt 2), R74–R77 (2005).

202 Moffatt MF, Kabesch M, Liang L *et al.* Genetic variants regulating *ORMDL3* expression contribute to the risk of childhood asthma. *Nature* 448(7152), 470–473 (2007).

• **First genome-wide association study of asthma and the identification of *ORMDL3* as a candidate gene.**

203 Galanter J, Choudhry S, Eng C *et al.* *ORMDL3* gene is associated with asthma in three ethnically diverse populations. *Am. J. Respir. Crit. Care Med.* 177(11), 1194–1200 (2008).

204 Hirota T, Harada M, Sakashita M *et al.* Genetic polymorphism regulating *ORM1*-like 3 (*Saccharomyces cerevisiae*) expression is associated with childhood atopic asthma in a Japanese population. *J. Allergy Clin. Immunol.* 121(3), 769–770 (2008).

205 Madore AM, Tremblay K, Hudson TJ, Laprise C. Replication of an association between 17q21 SNPs and asthma in a French-Canadian familial collection. *Hum. Genet.* 123(1), 93–95 (2008).

206 Tavendale R, Macgregor DF, Mukhopadhyay S, Palmer CN. A polymorphism controlling *ORMDL3* expression is associated with asthma that is poorly controlled by current medications. *J. Allergy Clin. Immunol.* 121(4), 860–863 (2008).

207 Basu K, Palmer CN, Lipworth BJ *et al.* Filaggrin null mutations are associated with increased asthma exacerbations in children and young adults. *Allergy* 63(9), 1211–1217 (2008).

208 Enomoto H, Hirata K, Otsuka K *et al.* Filaggrin null mutations are associated with atopic dermatitis and elevated levels of IgE in the Japanese population: a family and case-control study. *J. Hum. Genet.* 53(7), 615–621 (2008).

209 Irvine AD. Fleshing out filaggrin phenotypes. *J. Invest. Dermatol.* 127(3), 504–507 (2007).

210 Morar N, Cookson WO, Harper JI, Moffatt MF. Filaggrin mutations in children with severe atopic dermatitis. *J. Invest. Dermatol.* 127(7), 1667–1672 (2007).

211 Palmer CN, Ismail T, Lee SP *et al.* Filaggrin null mutations are associated with increased asthma severity in children and young adults. *J. Allergy Clin. Immunol.* 120(1), 64–68 (2007).

212 Baurecht H, Irvine AD, Novak N *et al.* Toward a major risk factor for atopic eczema: meta-analysis of filaggrin polymorphism data. *J. Allergy Clin. Immunol.* 120(6), 1406–1412 (2007).

213 Nicolai T, Pereszlenyiova-Bliznakova L, Illi S, Reinhardt D, von Mutius E. Longitudinal follow-up of the changing gender ratio in asthma from childhood to adulthood: role of delayed manifestation in girls. *Pediatr. Allergy Immunol.* 14(4), 280–283 (2003).

214 Bottema RW, Reijmerink NE, Koppelman GH, Kerkhof M, Postma DS. Phenotype definition, age, and gender in the genetics of asthma and atopy. *Immunol. Allergy Clin. North Am.* 25(4), 621–639 (2005).

215 Martinez FD. Gene-environment interactions in asthma: with apologies to William of Ockham. *Proc. Am. Thorac. Soc.* 4(1), 26–31 (2007).

216 Martinez FD. CD14, endotoxin, and asthma risk: actions and interactions. *Proc. Am. Thorac. Soc.* 4(3), 221–225 (2007).

• **Review of *CD14* genetic associations and interactions studies with endotoxin.**

217 Yang IA, Savarimuthu S, Kim ST, Holloway JW, Bell SC, Fong KM. Gene-environmental interaction in asthma. *Curr. Opin. Allergy Clin. Immunol.* 7(1), 75–82 (2007).

• **Review of gene–environment interaction studies.**

218 Vercelli D. Discovering susceptibility genes for asthma and allergy. *Nat. Rev. Immunol.* 8(3), 169–182 (2008).

• **Review of immunological aspects of gene–environment interactions.**

219 Hunninghake GM, Lasky-Su J, Soto-Quiros ME *et al.* Sex-stratified linkage analysis identifies a female-specific locus for IgE to cockroach in Costa Ricans. *Am. J. Respir. Crit. Care Med.* 177(8), 830–836 (2008).

220 Dijkstra A, Howard TD, Vonk JM *et al.* Estrogen receptor 1 polymorphisms are associated with airway hyperresponsiveness and lung function decline, particularly in female subjects with asthma. *J. Allergy Clin. Immunol.* 117(3), 604–611 (2006).

221 Gilliland FD, Berhane K, Li YF, Rappaport EB, Peters JM. Effects of early onset asthma and *in utero* exposure to maternal smoking on childhood lung function. *Am. J. Respir. Crit. Care Med.* 167(6), 917–924 (2003).

222 Cookson WO, Young RP, Sandford AJ *et al.* Maternal inheritance of atopic IgE responsiveness on chromosome 11q. *Lancet* 340(8816), 381–384 (1992).

223 Trabetti E, Patuzzo C, Malerba G *et al.* Association of a lymphotoxin α gene polymorphism and atopy in Italian families. *J. Med. Genet.* 36(4), 323–325 (1999).

224 Lee YA, Wahn U, Kehrt R *et al.* A major susceptibility locus for atopic dermatitis maps to chromosome 3q21. *Nat. Genet.* 26(4), 470–473 (2000).

225 Soderhall C, Marenholz I, Kerscher T *et al.* Variants in a novel epidermal collagen gene (*COL29A1*) are associated with atopic dermatitis. *PLoS Biol.* 5(9), e242 (2007).

• **Paper studying imprinting effects.**

226 Fraga MF, Ballestar E, Paz MF *et al.* Epigenetic differences arise during the lifetime of monozygotic twins. *Proc. Natl Acad. Sci. USA* 102(30), 10604–10609 (2005).

227 Qiu J. Epigenetics: unfinished symphony. *Nature* 441(7090), 143–145 (2006).

228 Dolinoy DC, Weidman JR, Waterland RA, Jirtle RL. Maternal genistein alters coat color and protects Avy mouse offspring from obesity by modifying the fetal epigenome. *Environ. Health Perspect.* 114(4), 567–572 (2006).

229 Waterland RA, Jirtle RL. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Mol. Cell Biol.* 23(15), 5293–5300 (2003).

230 Miller RL, Ho SM. Environmental epigenetics and asthma: current concepts and call for studies. *Am. J. Respir. Crit. Care Med.* 177(6), 567–573 (2008).

• **Review of epigenetic mechanisms.**

231 Cohn L, Herrick C, Niu N, Homer R, Bottomly K. IL-4 promotes airway eosinophilia by suppressing IFN-γ production: defining a novel role for IFN-γ in the regulation of allergic airway inflammation. *J. Immunol.* 166(4), 2760–2767 (2001).

232 Prescott SL, Holt PG. Abnormalities in cord blood mononuclear cytokine production as a predictor of later atopic disease in childhood. *Clin. Exp. Allergy* 28(11), 1313–1316 (1998).

233 Prescott SL, Macaubas C, Smallacombe T, Holt BJ, Sly PD, Holt PG. Development of allergen-specific T-cell memory in atopic and normal children. *Lancet* 353(9148), 196–200 (1999).

234 Turvey SE, Hawn TR. Towards subtlety: understanding the role of Toll-like receptor signaling in susceptibility to human infections. *Clin. Immunol.* 120(1), 1–9 (2006).

235 Horner AA. Toll-like receptor ligands and atopy: a coin with at least two sides. *J. Allergy Clin. Immunol.* 117(5), 1133–1140 (2006).

236 Medzhitov R. Toll-like receptors and innate immunity. *Nat. Rev. Immunol.* 1(2), 135–145 (2001).

237 Tulic MK, Fiset PO, Manoukian JJ *et al.* Role of Toll-like receptor 4 in protection by bacterial lipopolysaccharide in the nasal mucosa of atopic children but not adults. *Lancet* 363(9422), 1689–1697 (2004).

238 Lauener RP, Birchler T, Adamski J *et al.* Expression of CD14 and Toll-like receptor 2 in farmers' and non-farmers' children. *Lancet* 360(9331), 465–466 (2002).

239 Fujii S, Liu K, Smith C, Bonito AJ, Steinman RM. The linkage of innate to adaptive immunity via maturing dendritic cells *in vivo* requires CD40 ligation in addition to antigen presentation and CD80/86 costimulation. *J. Exp. Med.* 199(12), 1607–1618 (2004).

240 Akbari O, Stock P, Meyer E *et al.* Essential role of NKT cells producing IL-4 and IL-13 in the development of allergen-induced airway hyperreactivity. *Nat. Med.* 9(5), 582–588 (2003).

241 Akbari O, Faul JL, Hoyte EG *et al.* $CD4^+$ invariant T-cell-receptor$^+$ natural killer T cells in bronchial asthma. *N. Engl. J. Med.* 354(11), 1117–1129 (2006).

242 Vijayanand P, Seumois G, Pickard C *et al.* Invariant natural killer T cells in asthma and chronic obstructive pulmonary disease. *N. Engl. J. Med.* 356(14), 1410–1422 (2007).

- **Two references (241,242) when taken together explore the possible contribution of natural killer T cells in asthma.**

243 Boulet LP, Becker A, Berube D, Beveridge R, Ernst P. Canadian Asthma Consensus Report, 1999. Canadian Asthma Consensus Group. *CMAJ* 161(Suppl. 11), S1–61 (1999).

244 Louis R, Sele J, Henket M *et al.* Sputum eosinophil count in a large population of patients with mild to moderate steroid-naive asthma: distribution and relationship with methacholine bronchial hyperresponsiveness. *Allergy* 57(10), 907–912 (2002).

245 Pizzichini E, Pizzichini MM, Kidney JC *et al.* Induced sputum, bronchoalveolar lavage and blood from mild asthmatics: inflammatory cells, lymphocyte subsets and soluble markers compared. *Eur. Respir. J.* 11(4), 828–834 (1998).

246 Pizzichini E, Pizzichini MM, Efthimiadis A *et al.* Indices of airway inflammation in induced sputum: reproducibility and validity of cell and fluid-phase measurements. *Am. J. Respir. Crit. Care Med.* 154(2 Pt 1), 308–317 (1996).

247 Pin I, Gibson PG, Kolendowicz R *et al.* Use of induced sputum cell counts to investigate airway inflammation in asthma. *Thorax* 47(1), 25–29 (1992).

248 Krawiec ME, Westcott JY, Chu HW *et al.* Persistent wheezing in very young children is associated with lower respiratory inflammation. *Am. J. Respir. Crit. Care Med.* 163(6), 1338–1343 (2001).

249 Oommen A, Patel R, Browning M, Grigg J. Systemic neutrophil activation in acute preschool viral wheeze. *Arch. Dis. Child.* 88(6), 529–531 (2003).

250 Lex C, Payne DN, Zacharasiewicz A *et al.* Sputum induction in children with difficult asthma: safety, feasibility, and inflammatory cell pattern. *Pediatr. Pulmonol.* 39(4), 318–324 (2005).

251 Saglani S, Nicholson AG, Scallan M *et al.* Investigation of young children with severe recurrent wheeze: any clinical benefit? *Eur. Respir. J.* 27(1), 29–35 (2006).

252 Brussee JE, Smit HA, Kerkhof M *et al.* Exhaled nitric oxide in 4-year-old children: relationship with asthma and atopy. *Eur. Respir. J.* 25(3), 455–461 (2005).

253 Pijnenburg MW, Bakker EM, Hop WC, de Jongste JC. Titrating steroids on exhaled nitric oxide in children with asthma: a randomized controlled trial. *Am. J. Respir. Crit. Care Med.* 172(7), 831–836 (2005).

254 Strunk RC, Szefler SJ, Phillips BR *et al.* Relationship of exhaled nitric oxide to clinical and inflammatory markers of persistent asthma in children. *J. Allergy Clin. Immunol.* 112(5), 883–892 (2003).

255 Moeller A, Diefenbacher C, Lehmann A *et al.* Exhaled nitric oxide distinguishes between subgroups of preschool children with respiratory symptoms. *J. Allergy Clin. Immunol.* 121(3), 705–709 (2008).

256 Wildhaber JH, Hall GL, Stick SM. Measurements of exhaled nitric oxide with the single-breath technique and positive expiratory pressure in infants. *Am. J. Respir. Crit. Care Med.* 159(1), 74–78 (1999).

257 Franklin PJ, Turner SW, Mutch RC, Stick SM. Measuring exhaled nitric oxide in infants during tidal breathing: methodological issues. *Pediatr. Pulmonol.* 37(1), 24–30 (2004).

258 Stick SM. Non-invasive monitoring of airway inflammation. *Med. J. Aust.* 177 (Suppl.), S59–S60 (2002).

259 Gabriele C, Nieuwhof EM, Van Der Wiel EC *et al.* Exhaled nitric oxide differentiates airway diseases in the first two years of life. *Pediatr. Res.* 60(4), 461–465 (2006).

260 Storm van's GK, Wechsler ME, Grasemann H *et al.* Association of a missense mutation in the *NOS3* gene with exhaled nitric oxide levels. *Am. J. Respir. Crit. Care Med.* 168(2), 228–231 (2003).

261 Grasemann H, Storm van's GK, Buscher R, Drazen JM, Ratjen F. Effects of sex and of gene variants in constitutive nitric oxide synthases on exhaled nitric oxide. *Am. J. Respir. Crit. Care Med.* 167(8), 1113–1116 (2003).

262 Franklin P, Dingle P, Stick S. Raised exhaled nitric oxide in healthy children is associated with domestic formaldehyde levels. *Am. J. Respir. Crit. Care Med.* 161(5), 1757–1759 (2000).

263 Takemura M, Matsumoto H, Niimi A *et al.* High sensitivity C-reactive protein in asthma. *Eur. Respir. J.* 27(5), 908–912 (2006).

264 Islam T, Gauderman WJ, Berhane K *et al.* Relationship between air pollution, lung function and asthma in adolescents. *Thorax* 62(11), 957–963 (2007).

265 Toelle BG, Xuan W, Peat JK, Marks GB. Childhood factors that predict asthma in young adulthood. *Eur. Respir. J.* 23(1), 66–70 (2004).

266 Jenkins MA, Hopper JL, Bowes G, Carlin JB, Flander LB, Giles GG. Factors in childhood as predictors of asthma in adult life. *BMJ* 309(6947), 90–93 (1994).

267 Ulrik CS, Backer V. Longitudinal determinants of bronchial responsiveness to inhaled histamine. *Chest* 113(4), 973–979 (1998).

268 Lawlor DA, Ebrahim S, Davey SG, Lawlor DA, Ebrahim S, vey Smith G. Association of birth weight with adult lung function: findings from the British Women's Heart and Health Study and a meta-analysis. *Thorax* 60(10), 851–858 (2005).

269 Orfei L, Strachan DP, Rudnicka AR, Wadsworth ME. Early influences on adult lung function in two national British cohorts. *Arch. Dis. Child.* 93(7), 570–574 (2008).

270 Stein CE, Kumaran K, Fall CH, Shaheen SO, Osmond C, Barker DJ. Relation of fetal growth to adult lung function in South India. *Thorax* 52(10), 895–899 (1997).

271 Lopuhaa CE, Roseboom TJ, Osmond C *et al.* Atopy, lung function, and obstructive airways disease after prenatal exposure to famine. *Thorax* 55(7), 555–561 (2000).

272 Dezateux C, Stocks J, Wade AM, Dundas I, Fletcher ME. Airway function at one year: association with premorbid airway function, wheezing, and maternal smoking. *Thorax* 56(9), 680–686 (2001).

273 Dezateux C, Lum S, Hoo AF, Hawdon J, Costeloe K, Stocks J. Low birth weight for gestation and airway function in infancy: exploring the fetal origins hypothesis. *Thorax* 59(1), 60–66 (2004).

274 Hoo AF, Stocks J, Lum S *et al.* Development of lung function in early life: influence of birth weight in infants of nonsmokers. *Am. J. Respir. Crit. Care Med.* 170(5), 527–533 (2004).

275 Shaheen SO, Sterne JA, Tucker JS *et al.* Birth weight, childhood lower respiratory tract infection, and adult lung function. *Thorax* 53(7), 549–553 (1998).

276 Haland G, Carlsen KC, Sandvik L *et al.* Reduced lung function at birth and the risk of asthma at 10 years of age. *N. Engl. J. Med.* 355(16), 1682–1689 (2006).

277 Stern DA, Morgan WJ, Wright AL, Guerra S, Martinez FD. Poor airway function in early infancy and lung function by age 22 years: a non-selective longitudinal cohort study. *Lancet* 370(9589), 758–764 (2007).

278 Phelan PD, Robertson CF, Olinsky A. The Melbourne Asthma Study: 1964–1999. *J. Allergy Clin. Immunol.* 109(2), 189–194 (2002).

279 Clarke JR, Reese A, Silverman M. Bronchial responsiveness and lung function in infants with lower respiratory tract illness over the first six months of life. *Arch. Dis. Child.* 67(12), 1454–1458 (1992).

280 Turner SW, Palmer LJ, Rye PJ *et al.* The relationship between infant airway function, childhood airway responsiveness, and asthma. *Am. J. Respir. Crit. Care Med.* 169(8), 921–927 (2004).

281 James AL, Palmer LJ, Kicic E *et al.* Decline in lung function in the Busselton Health Study: the effects of asthma and cigarette smoking. *Am. J. Respir. Crit. Care Med.* 171(2), 109–114 (2005).

282 Gold DR, Wang X, Wypij D, Speizer FE, Ware JH, Dockery DW. Effects of cigarette smoking on lung function in adolescent boys and girls. *N. Engl. J. Med.* 335(13), 931–937 (1996).

283 Lodrup Carlsen KC, Jaakkola JJ, Nafstad P *et al. In utero* exposure to cigarette smoking influences lung function at birth. *Eur. Respir. J.* 10(8), 1774–1779 (1997).

284 Finkelstein MM, Jerrett M, DeLuca P *et al.* Relation between income, air pollution and mortality: a cohort study. *CMAJ* 169(5), 397–402 (2003).

285 Ulrik CS. Outcome of asthma: longitudinal changes in lung function. *Eur. Respir. J.* 13(4), 904–918 (1999).

286 Grol MH, Gerritsen J, Vonk JM *et al.* Risk factors for growth and decline of lung function in asthmatic individuals up to age 42 years. A 30-year follow-up study. *Am. J. Respir. Crit. Care Med.* 160(6), 1830–1837 (1999).

287 Strunk RC, Weiss ST, Yates KP *et al.* Mild to moderate asthma affects lung growth in children and adolescents. *J. Allergy Clin. Immunol.* 118(5), 1040–1047 (2006).

288 Hole DJ, Watt GC, vey-Smith G, Hart CL, Gillis CR, Hawthorne VM. Impaired lung function and mortality risk in men and women: findings from the Renfrew and Paisley prospective population study. *BMJ* 313(7059), 711–715 (1996).

289 Burrows B, Knudson RJ, Lebowitz MD. The relationship of childhood respiratory illness to adult obstructive airway disease. *Am. Rev. Respir. Dis.* 115(5), 751–760 (1977).

290 Dodge RR, Burrows B. The prevalence and incidence of asthma and asthma-like symptoms in a general population sample. *Am. Rev. Respir. Dis.* 122(4), 567–575 (1980).

291 Becklake MR, Kauffmann F. Gender differences in airway behaviour over the human life span. *Thorax* 54(12), 1119–1138 (1999).

- **A good overview of some of the observed gender differences in asthma and some of the basic physiological differences that may explain them.**

292 Nicolai T, Illi S, Tenborg J, Kiess W, Mutius E. Puberty and prognosis of asthma and bronchial hyper-reactivity. *Pediatr. Allergy Immunol.* 12(3), 142–148 (2001).

293 Bjornson CL, Mitchell I. Gender differences in asthma in childhood and adolescence. *J. Gend. Specif. Med.* 3(8), 57–61 (2000).

294 Meurer JR, George V, Subichin S, Yauck J, Layde P. Asthma severity among children hospitalized in 1990 and 1995. *Arch. Pediatr. Adolesc. Med.* 154(2), 143–149 (2000).

295 To T, Dick P, Feldman W, Hernandez R. A cohort study on childhood asthma admissions and readmissions. *Pediatrics* 98(2 Pt 1), 191–195 (1996).

296 Chen Y, Stewart P, Johansen H, McRae L, Taylor G. Sex difference in hospitalization due to asthma in relation to age. *J. Clin. Epidemiol.* 56(2), 180–187 (2003).

297 Lin RY, Rehman A. Clinical characteristics of adult asthmatics requiring intubation. *J. Med.* 26(5–6), 261–277 (1995).

298 Skobeloff EM, Spivey WH, St Clair SS, Schoffstall JM. The influence of age and sex on asthma admissions. *JAMA* 268(24), 3437–3440 (1992).

299 Chen Y, Dales R, Stewart P, Johansen H, Scott G, Taylor G. Hospital readmissions for asthma in children and young adults in Canada. *Pediatr. Pulmonol.* 36(1), 22–26 (2003).

300 Mandhane PJ, Greene JM, Cowan JO, Taylor DR, Sears MR. Sex differences in factors associated with childhood- and adolescent-onset wheeze. *Am. J. Respir. Crit. Care Med.* 172(1), 45–54 (2005).

Affiliations

- Padmaja Subbarao
  Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada
- Allan Becker
  University of Manitoba, Winnipeg, Manitoba, Canada
- Jeffrey R Brook
  Environment Canada, Toronto, Ontario, Canada
- Denise Daley
  University of British Columbia, Vancouver, British Columbia, Canada
- Piush J Mandhane
  University of Alberta, Edmonton, Alberta, Canada
- Gregory E Miller
  University of British Columbia, Vancouver, British Columbia, Canada
- Stuart E Turvey
  University of British Columbia, Vancouver, British Columbia, Canada
- Malcolm R Sears
  Professor of Medicine, McMaster University, Hamilton, Ontario, Canada
  and
  Firestone Institute for Respiratory Health, St Joseph's Healthcare, 50 Charlton Avenue East, Hamilton, Ontario, L8N 4A6, Canada
  Tel.: +1 905 522 1155 ext. 33286
  Fax: +1 905 521 6132
  searsm@mcmaster.ca