

Understanding the complexity of IgE-related phenotypes from childhood to young adulthood: A Mechanisms of the Development of Allergy (MeDALL) Seminar

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Mechanisms of the Development of Allergy (MeDALL), a Seventh Framework Program European Union project, aims to generate novel knowledge on the mechanisms of initiation of allergy. Precise phenotypes of IgE-mediated allergic diseases will be defined in MeDALL. As part of MeDALL, a scientific seminar was held on January 24, 2011, to review current knowledge on the IgE-related phenotypes and to explore how a multidisciplinary effort could result in a new integrative translational approach. This article provides a summary of the meeting. It develops challenges in IgE-related phenotypes and new clinical and epidemiologic approaches to the investigation of allergic phenotypes, including cluster analysis, scale-free models, candidate biomarkers, and IgE microarrays; the particular case of severe asthma was reviewed. Then novel approaches to the IgE-associated phenotypes are reviewed from the individual mechanisms to the systems, including epigenetics, human *in vitro* immunology, systems biology, and animal models. The last chapter deals with the understanding of the population-based IgE-associated phenotypes in children and adolescents, including age effect in terms of maturation, observed effects of early-life exposures and shift of focus from early life to pregnancy, gene-environment interactions, cohort effects, and time trends in patients with allergic diseases. This review helps to define phenotypes of allergic diseases in MeDALL. (J Allergy Clin Immunol 2012;129:943-54.)

Key words: Allergy, Mechanisms of the Development of Allergy, Seventh Framework Program, phenotypes, IgE, asthma

Several mechanisms are involved in allergic diseases, but most patients experience IgE-mediated reactions.^{1,2} The prevalence of allergic disease is increasing globally,³ but in some countries there might be a plateau or even a decrease.⁴ Despite wide

Abbreviations used

FP7: Seventh Framework Program

MeDALL: Mechanisms of the Development of Allergy

Treg: Regulatory T

research efforts in explaining the striking increase in allergy, no satisfactory explanations have been obtained. One of the reasons for the difficulties in understanding the causes of the allergy epidemic might lie in the complexity of allergic diseases and the fact that the components of such complexity have not yet been realized. During the last years, a systems medicine approach has started to emerge as a way to investigate the complexity of human diseases.

In their last research calls, the Seventh Framework Program (FP7) of the European Union has called for projects approaching a large range of interrelated mechanistic aspects of given diseases in an integrative system-based approach. Mechanisms of the Development of Allergy (MeDALL; 2011-14), an FP7 project, aims to generate novel knowledge on the mechanisms of initiation of allergy. A description of the MeDALL rationale, the research architecture, and the relevant methods has been previously reported.⁵ Briefly, MeDALL will use very large standardized datasets from 14 ongoing birth cohorts to define classical and novel IgE-mediated phenotypes. Novel phenotypes will be determined by using unsupervised approaches. These phenotypes will be used for systems biology using transcriptomics, proteomics, and epigenetics. An integrative translational approach will be developed to understand how a network of molecular and environmental factors can lead to complex allergic diseases and their expression in the general population. A more complete view of how MeDALL will first investigate the clinical phenotypes and how these

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TABLE I. A MeDALL framework for a system-based research approach to allergic diseases

1. Establishment of the complexity of IgE-associated allergic diseases
2. Innovative research approaches to the clinical and epidemiologic aspects of the allergic phenotypes
Unsupervised statistical modeling of phenotypes
Candidate biomarkers
IgE microarrays
Severity of allergic diseases
3. Novel approaches to the IgE-associated phenotypes: from the individual mechanisms to the systems
Epigenetics
Human <i>in vitro</i> immunology
Transcriptomics
Animal models
System biology
4. Understanding the population-based IgE-associated phenotypes in children and adolescents
Age effect of maturation
<i>In utero</i> and early-life exposures
Contrasting populations with a similar genetic background
Cohort effects and time trends
5. Ethics of the new approaches in MeDALL

phenotypes will be linked to risk factors and mechanistic data has been provided elsewhere.⁵

An important aspect of research projects adopting a systems medicine approach is how to establish the scientific base, which must necessarily incorporate a wide scope of different issues and at the same time be integrative and reflect the multidisciplinary nature of the research team. The latter is a challenge that goes far beyond establishing the traditional state of the art in a more conventional research project. In responding to this challenge and as part of MeDALL, a scientific seminar was held on January 24, 2011, to review the current knowledge on the IgE-related phenotypes and to explore how a multidisciplinary effort could result in a new integrative translational approach. Following a predefined framework (Table I), a synthesis of the contributions to this MeDALL meeting are presented here.

ESTABLISHING THE COMPLEXITY OF IgE-ASSOCIATED ALLERGIC DISEASES

A relevant and common difficulty in the systems medicine approach is to reach a sufficient agreement among experts coming from different disciplines in use of disease definitions and classification. This is of particular importance in the case of allergic diseases because the use of different allergy-related terms varies widely. The World Allergy Organization nomenclature for allergy (2003) is used in MeDALL and in this document to facilitate a common understanding of terminology (see Table E1 in this article's Online Repository at www.jacionline.org).¹ Allergic diseases are complex and heterogeneous (Table II).^{2,6-16} Asthma, rhinitis, or atopic dermatitis do not represent single disease entities, but several phenotypes might exist.^{6,7,17} These phenotypes vary with age at onset, pattern of disease over time, comorbidities, underlying risk factors, and association with IgE sensitization.

At the forefront of challenges in understanding allergic diseases is the role of IgE sensitization as its fundamental mechanism. In the present review (as in MeDALL), we refer to

TABLE II. Complexity of IgE-associated allergic diseases

1. Major IgE-mediated related phenotypes include rhinitis (and conjunctivitis), ⁶ asthma, ² atopic dermatitis, ⁷ food allergy, and anaphylaxis.
2. Not all sensitized patients present symptoms ⁸ for unclear reasons explained not only by the characteristics of specific IgE.
3. Specific IgE in serum and skin test patterns are not interchangeable in epidemiologic studies ⁹ and might partly represent different phenotypes.
4. The severity of symptoms varies widely from mild to severe and from intermittent to persistent.
5. However, allergy is not always involved in symptoms of these diseases. ^{10,11}
6. Nonallergic mechanisms are intertwined with allergic mechanisms in many diseases.
7. Moreover, diseases tend to cluster, and patients present concomitant or consecutive comorbidities.
8. Respiratory and nonrespiratory allergic phenotypes are interrelated. The atopic march is a classical paradigm, but different consecutive phenotypes can be observed. ¹² Atopic eczema is particularly frequent up to 2 years of age. From the age of 2 years onward, specific IgE levels to inhalant allergens increase together with the presentation of allergic respiratory symptoms. ¹³
9. Rhinitis is a risk factor for asthma both in adults ¹⁴ and children. ¹⁵ However, in adulthood the development of asthma is usually independent of allergy, ¹⁶ whereas in childhood it is often associated with allergy. ¹⁵

IgE-associated disease because allergic disease might respond to mechanisms other than IgE. The MeDALL project looks at the complexity of allergic diseases in children. Although in some diseases, such as asthma, the role of atopy has been shown to be relatively limited among adults,¹⁸ the presence of IgE sensitization in a majority of asthmatic children has not been questioned. Nevertheless, even in children, part of the complexity of asthma and other allergic phenotypes suggests that non-IgE-dependent mechanisms are playing a role.¹⁰

Insufficient knowledge exists concerning phenotypic expression of allergic sensitization profiles, although some data exist.¹⁹ The causes of sex differences in phenotypic expression are still unclear, particularly the shift from a male to female preponderance after puberty. The prevalence and severity of allergic diseases change with age, and marked sex differences exist for the predictive role of specific IgE or the severity of allergic disease at 10 years.²⁰

IgE sensitization and its phenotypic expression seem closely interrelated, and their potential causal links can be nonlinear and bidirectional. Allergic phenotypes are constituted by overlapping separate syndromes with different but still undefined causes and natural histories.

Many allergic conditions appear soon after birth. Although birth cohorts and longitudinal studies have provided valuable information on the development of allergic diseases, it is unlikely that the disease complexity will be resolved with the current types of epidemiologic and mechanistic studies based on traditional causal and predictable relationships. With the current classification, it is impossible to precisely define concurrent and consecutive allergy phenotypes. MeDALL will reconsider allergic disease classification by comparing the classical hypothesis-driven phenotypes with those emerging from the application of data-driven, unsupervised statistical modeling of the existing birth cohort datasets.⁵

TABLE III. Studies using unsupervised models to identify novel phenotypes of asthma and allergy

	Title	Cluster analysis method	Reference
Adults	Cluster analysis and clinical asthma phenotypes	Combined hierarchic/k-means cluster analysis	Haldar et al, 2008 ²⁴
	Distinct clinical phenotypes of airway disease defined by cluster analysis	Hierarchic cluster analysis*	Weatherall et al, 2009 ²⁵
	Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program	Hierarchic cluster analysis	Moore et al, 2010 ²⁶
	Identifying adult asthma phenotypes using a clustering approach	Latent class analysis	Siroux et al, 2011 ²⁷
	Patterns of airway disease and the clinical diagnosis of asthma in the Busselton population	K-means cluster analysis*	Musket al, 2011 ²⁸
Children	Distinguishing phenotypes of childhood wheeze and cough using latent class analysis	Latent class analysis	Spycher et al, 2008 ²⁹
	Associations of wheezing phenotypes in the first 6 years of life with atopy, lung function, and airway responsiveness in midchildhood in the ALSPAC birth cohort	Longitudinal latent class analysis	Henderson et al, 2008 ²²
	Bronchial obstructive phenotypes in the first year of life among Paris birth cohort infants	Partitioning around medoids	Clarisse et al, 2009 ³⁰
	Beyond atopy: multiple patterns of sensitization in relation to asthma in a birth cohort study	Hidden Markov model†	Simpson et al, 2010 ¹⁹
	Overcoming heterogeneity in pediatric asthma: tobacco smoke and asthma characteristics within phenotypic clusters in an African American cohort	Combined hierarchic/k-means cluster analysis	Benton et al, 2010 ³¹
	Comparison of childhood wheezing phenotypes in 2 birth cohorts: ALSPAC and PIAMA	Longitudinal latent class analysis	Savenije et al, 2011 ²³
	Heterogeneity of severe asthma in childhood: confirmation by cluster analysis of children in the National Institutes of Health/ National Heart, Lung, and Blood Institute Severe Asthma Research Program	Hierarchic cluster analysis	Fitzpatrick et al, 2011 ³²

ALSPAC, Avon Longitudinal Study of Parents and Children; PIAMA, Prevention and Incidence of Asthma and Mite Allergy.

*The analyses were not conducted among subjects with asthma but in the general population. These studies were aimed at identifying airway disease phenotypes rather than asthma phenotypes.

†Relates to the identification of phenotypes of sensitization and not asthma phenotypes.

INNOVATIVE RESEARCH APPROACHES TO THE CLINICAL AND EPIDEMIOLOGIC ASPECTS OF ALLERGIC PHENOTYPES

Novel classifications of allergic diseases and asthma need to be identified to distinguish groups of patients presenting homogeneous clinical and prognostic characteristics and responses to treatments. In response to these needs, new clinical and epidemiologic approaches have recently emerged. One of these approaches consists of the application of unsupervised statistical techniques.

Unsupervised statistical modeling of phenotypes

An increasingly popular approach to the investigation of complex phenotypes consists of applying (partly) unsupervised statistical methods, such as cluster analysis or latent class analyses, to a population with a wide distribution of related symptoms, allowing the statistical technique to identify the possible underlying phenotypes (or clusters of symptom expressions).²¹ Most of the studies have either used cluster or latent class analysis (Table III).^{19,22-32} The studies typically begin with no predefined views about the existing phenotypes (see this article's Online Repository at www.jacionline.org).

There are several types of unsupervised statistical modeling, the more commonly used being cluster analysis (hierarchic and k-means) and latent class models. These methods are at an initial step, and studies are not totally consistent. Importantly, application of unsupervised methods require large standardized datasets, something that will be possible within MeDALL with more than 30,000 children from 14 cohorts available and a standardized

follow-up of all cohorts in progress.⁵ The limited literature about unsupervised phenotypes of allergic diseases suggests that some phenotypes seem to be similar between studies (eg, wheeze phenotypes),^{22,23,33} whereas others are similar in their definition but differ by certain characteristics (eg, reduced lung function in transient viral wheeze).^{33,34} Importantly, replication of reported phenotypes is needed but not yet available. Another critical unresolved issue is how to validate the observed phenotypes. This can be performed by comparing the results in 2 independent samples or by testing their predictive performance. Unsupervised statistical methods can be extended to provide clinical algorithms to facilitate identification and classification of the disease in the clinical setting. Although this new approach is still in its infancy, a new paradigm is emerging, similar to the model of data-driven genome analyses. Obviously, the investigation of unsupervised phenotypes is just a first step that needs to be continued with the assessment of their links with the relevant mechanisms and environmental factors included in the research plans of MeDALL.⁵

Candidate biomarkers

Candidate biomarkers have raised remarkable interest in clinical and epidemiologic research.³⁵ They improve the understanding of molecular mechanisms of diseases, identify possible new disease pathways,³⁶ predict models of complex diseases, refine disease phenotypes and guide treatment responses,^{37,38} and possibly lead to personalized medicine.

Thus far, the use of biomarkers in asthma and allergy research has mostly focused on genetics and genomics. However, measurement of multiple protein concentrations in serum and other

biofluids has received increasing attention. Cytokine levels from bronchoalveolar lavage fluid combined with unsupervised statistical approaches helped to differentiate clinical subgroups of asthmatic patients among children³⁹ and adults.⁴⁰ In epidemiology most biomarker studies use serum, plasma, or urine samples because they are easy to collect, process, and store and also carry the strongest implications for intervention at the population level. Molecules involved in different pathways have been used to capture the clinical complexity of disease and the feasibility of linking specific analyte groups to clinical outcomes.^{41,42} Serum biomarkers can change over time, and susceptibility to reverse causality and residual confounding is a potential limitation. Therefore maximizing their contributions to understand the complexity of allergic diseases will require the use of large clinical and epidemiologic cohorts with prospectively collected biorepositories and detailed phenotypic and environmental information. These conditions can be met in the MeDALL consortium.

IgE microarrays

The *in vitro* measurement of specific IgE helps with better understanding of allergic disease and improvement in IgE-related phenotype diagnosis. By using allergen extracts, true allergy cannot be differentiated from cross-reactivity between allergens. This diagnostic uncertainty might lead to phenotypic misclassification and wrong health care decisions.⁴³ The availability of allergen molecules produced by using recombinant technologies allows the determination of IgE-reactivity patterns at a molecular level.⁴⁴ Allergen microarrays^{45,46} allow testing for reactivities to more than 100 allergens in a single step by using a very small serum volume (20 μ L). Measurement of IgE, IgG₁₋₄, IgA, and IgM levels can determine the profiles of allergen-specific antibody responses for all isotypes in subjects from clinically well-defined cohorts. The use of IgE microarrays in combination with phenotypic data and other mechanistic data, as available in MeDALL, will provide opportunities for a better classification of allergic diseases and their interrelationships.

Severe allergic diseases and asthma

Severity is one of the phenotypic characteristics of allergic diseases that has received particular attention. Thus far, most efforts to define severity have been disease specific. By contrast, a uniform definition applicable to all allergic diseases could make it possible to better define the phenotypes of severe allergic diseases. The National Asthma Education and Prevention Program's Expert Panel Report 3⁴⁷ made key suggestions combining impairment, response to treatment, and risks. The uniform definition of severe asthma presented to the World Health Organization⁴⁸ used an approach derived from the National Asthma Education and Prevention Program's Expert Panel Report 3.⁴⁷ It is proposed to generalize the approach of the uniform definition of severe asthma⁴⁶ to allergic and related diseases ascribed to any of the mutually exclusive groups, each carrying different public health messages and challenges (see Fig E1 in this article's Online Repository at www.jacionline.org).

The National Heart, Lung, and Blood Institute has funded investigators using a variety of phenotyping approaches to dissect the heterogeneity of the IgE-related phenotypes, with a particular focus on severe asthma. Severe Asthma Research Program investigators recently performed cluster analyses of data

representing traditional measures of severity, lung function, and asthma control and demonstrated that adult and pediatric asthmatic patients can be grouped into 5 and 4 distinct phenotypes, respectively (Table IV).^{26,32,49-67}

Airway disease severity can be modulated by environmental factors, including viruses, and bacteria. Particularly, *Staphylococcus aureus* colonization is associated with nasal polyposis, whereas staphylococcal superantigens amplify the local T_H2 inflammation.⁶⁸ IgE against staphylococcal enterotoxins (SE-IgE) in nasal polyp tissues is associated with comorbid asthma.⁶⁹ SE-IgE antibodies are more commonly found in sera from patients with severe asthma than in sera from patients with mild asthma.^{70,71} The role of staphylococcal enterotoxins in asthma onset and severity probably starts in early childhood⁷² and increases the risk of asthma at adolescence.⁷³

Identifying meaningful severity-related subphenotypes will provide an important and promising way forward, not only for genetic/epigenetic mapping but also for understanding how environmental and genetic/epigenetic factors interact to influence allergic diseases and asthma susceptibility, expression, and progression to inform the advancement of new treatment strategies. The ultimate aim is to develop disease-modifying treatments and combine them with the ability to tailor them for specific phenotypes.

NOVEL APPROACHES TO THE IgE-ASSOCIATED PHENOTYPES: FROM THE INDIVIDUAL MECHANISMS TO THE SYSTEMS

Whereas in the previous section the focus was on clinical and epidemiologic approaches, this section shifts attention to novel approaches being developed in experimental disciplines.

Epigenetics

The allergy epidemic might have resulted from recent environmental changes interacting with genes. One important mechanism of how gene-environment interactions can affect disease development might be epigenetics. Epigenetics includes a wide range of phenomena, with one of the processes involved in epigenetics being methylation of CpG islands of genes.⁷⁴ Recent advances in the epigenetics and genomics of asthma are described by Koppelman and Nawijn.⁷⁵

Epigenetics might provide clues to several observations crucial in atopy, such as the important role of prenatal and postnatal environmental factors that might program an individual toward atopic disease. For example, epigenetic phenomena might contribute to a T_H1 and T_H2 disbalance.⁷⁶ T_H2 cytokine gene expression in T lymphocytes depends on the methylation status of crucial genes, such as those encoding IL-4 and IFN- γ .

Epigenetics might also be involved in genomic imprinting during embryogenesis, as with the multigenerational transmission of asthma. For example, a grandmother who smoked confers a greater risk for asthma in the grandchild compared with the nonsmoking grandfather independent of parental risks.^{76,77}

Several important issues about the role of DNA methylation in patients with allergic diseases deserve further attention: (1) the role of the methylation pattern at birth in conjunction with *in utero* exposures in the development of atopic diseases; (2) the importance of epigenetic regulation of known atopy genes; (3) the assessment of whether environmental exposures, such as

TABLE IV. Severe Asthma Research Program studies

	Title	Results	Reference
Adults	Characterization of the severe asthma phenotype by the National Heart, Lung, and Blood Institute's Severe Asthma Research Program	Consistent features of severe versus nonsevere asthma is persistent symptoms, increased health care use, and decreased pulmonary function despite treatment with high doses of inhaled or systemic corticosteroids and additional controller medications.	Moore et al, 2007 ⁴⁹
	Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program	Cluster analysis identified 5 distinct clinical phenotypes of asthma and might provide an objective and valid approach to organizing phenotypic complexity and mapping the phenotype to underlying pathobiology.	Moore et al, 2010 ²⁶
	Lung function in adults with stable but severe asthma: air trapping and incomplete reversal of obstruction with bronchodilation	Severe asthma is associated with prominent air trapping and persistent airflow limitation after maximal bronchodilation, suggesting an underlying pathology conducive to airway closure or near closure.	Sorkness et al, 2008 ⁵⁰
	A multivariate analysis of risk factors for the air-trapping asthmatic phenotype, as measured by using quantitative computed tomographic analysis	Air-trapping phenotype identified by quantitative multidetector computed tomography in subjects with asthma is associated with high risk for severe disease.	Busacker et al, 2009 ⁵¹
	Alterations of the arginine metabolome in asthma	Arginine bioavailability is not associated with fraction of exhaled nitric oxide or other inflammatory markers but in patients with severe asthma is strongly correlated with airflow limitation.	Lara et al, 2008 ⁵²
	Airway remodeling measured by using multidetector computed tomography is increased in patients with severe asthma and correlates with pathology	Airway wall thickening identified by means of quantitative multidetector computed tomography is increased in patients with severe asthma and is associated with remodeling measures in biopsy samples of matched airway segments.	Aysola et al, 2008 ⁵³
	Use of exhaled nitric oxide measurement to identify a reactive at-risk phenotype among patients with asthma	High fraction of exhaled nitric oxide level identifies a subgroup of patients with severe asthma with the greatest eosinophilic airway inflammation, with the most severe airflow limitation, and who use emergency care most often.	Dweik et al, 2010 ⁵⁴
	Analyses of asthma severity phenotypes and inflammatory proteins in subjects stratified by sputum granulocytes	Concurrent eosinophilia and neutrophilia in sputum identified an asthma subphenotype associated with worse lung function, symptoms, and increased health care use, and proteomics revealed novel inflammatory mediators.	Hastie et al, 2010 ⁵⁵
	Predicting intermediate phenotypes in asthma by using bronchoalveolar lavage–derived cytokines	Logistic regression and multivariate adaptive regression splines provide equally robust modeling of cytokines assayed in bronchoalveolar lavage fluid to predict intermediate asthma phenotypes.	Brasier et al, 2010 ⁵⁶
	Determinants of exhaled breath condensate pH in a large population with asthma	Exhaled breath condensate pH does not distinguish patient with severe versus those with nonsevere asthma, but values <6.5 might identify unique subgroups and asthma exacerbations.	Liu et al, 2010 ⁵⁷
	Racial differences in biologic predictors of severe asthma: data from the Severe Asthma Research Program	Asthma severity in black subjects, but not white subjects, is linked to family history and IgE levels.	Gamble et al, 2010 ⁵⁸
	Mast cell phenotype, location, and activation in patients with severe asthma: data from the Severe Asthma Research Program	A greater proportion of chymase-positive mast cells in airway submucosa and epithelium with increased prostaglandin D ₂ levels in bronchoalveolar lavage fluid predicts severe asthma and suggests that this chymase-positive mast cell subtype might contribute to severe asthma pathobiology.	Balzar et al, 2011 ⁵⁹
	Epithelial cell proliferation contributes to airway remodeling in patients with severe asthma	Dysregulated epithelium in patients with severe asthma might manifest as cellular hyperplasia, thickening, and desquamation, resulting in accelerated decrease in lung function and airway remodeling.	Cohen et al, 2007 ⁶⁰
	Diminished lipoxin biosynthesis in patients with severe asthma	Imbalance in generation of proinflammatory leukotrienes and counterregulatory lipoxins is present in patients with severe asthma and might contribute to persistent inflammation.	Levy et al, 2005 ⁶¹

(Continued)

TABLE IV. (Continued)

	Title	Results	Reference
Children	Features of severe asthma in school-aged children: atopy and increased exhaled nitric oxide	Children with severe asthma have significantly greater air trapping and airflow limitation during bronchodilator hold and more airflow limitation after maximum bronchodilation, suggesting that at least some children demonstrate patterns of airway physiology resembling those seen in adults with severe asthma.	Fitzpatrick et al, 2006 ⁶²
	Altered airway glutathione homeostasis in children with severe asthma: evidence for oxidant stress	Children with severe refractory asthma exhibit imbalance between glutathione and glutathione disulfide in epithelial lining fluid, resulting in excessive reactive oxygen species formation.	Fitzpatrick et al, 2009 ⁶³
	Increased levels of nitric oxide oxidation products in the epithelial lining fluid of children with persistent asthma	Levels of the nitric oxide oxidation products nitrite, nitrate, and nitrotyrosine are increased in proximal and distal airway epithelial lining fluid in children with persistent asthma, but concentrations do not differ with disease severity and do not consistently correlate with fraction of exhaled nitric oxide.	Fitzpatrick et al, 2009 ⁶⁴
	Association of glutathione oxidation with airway macrophage functional impairment in children with severe asthma	Altered airway and intracellular alveolar macrophage glutathione homeostasis induces alveolar macrophage cellular dysfunction in children with severe asthma, leading to increased apoptosis and impaired alveolar macrophage phagocytosis of infectious particles.	Fitzpatrick et al, 2011 ⁶⁵
	Heterogeneity of severe asthma in childhood: confirmation by cluster analysis of children in the National Institutes of Health/National Heart, Lung, and Blood Institute's Severe Asthma Research Program	Four distinct clusters were identified, which highlights the complexity and heterogeneity of childhood asthma, and unlike previous cluster analyses of asthma in adults, health care use was not a robust discriminator of cluster assignment in children.	Fitzpatrick et al, 2011 ³²
	Progressive airflow limitation as a feature of children with severe asthma	Despite high-dose steroid treatment, children with severe asthma exhibit lung function decrease during adolescent years accompanied by increased frequency of wheezing and asthma symptoms and greater allergic sensitization throughout childhood.	Fitzpatrick and Teague, 2011 ⁶⁶
	Sex dependence of airflow limitation and air trapping in children with severe asthma	Boys versus girls with severe asthma demonstrate greater baseline airflow limitation and air trapping and, unlike girls, have persistent airflow limitation after maximum bronchodilation, suggesting that adult patterns of severe asthma are present in boys but only partially manifest in girls.	Sorkness et al, 2011 ⁶⁷

environmental tobacco smoke or air pollution, can modify this methylation pattern through childhood; and (4) the influence of methylation profiles in protein expression of relevant genes.

Human *in vitro* immunology

Many immunologic mechanisms involved in allergy include T and B cells, dendritic cells, epithelial cells, and structural and inflammatory cells and induce allergen sensitization or tolerance (see Fig E2 in this article's Online Repository at www.jacionline.org).⁷⁸ Peripheral tolerance is characterized by generation of allergen-specific regulatory T (Treg) cells and suppressed proliferative and cytokine responses against allergens⁷⁹ through (1) suppression of antigen-presenting cells that support the generation of effector T_{H2} and T_{H1} cells; (2) suppression of T_{H2} and T_{H1} cells⁸⁰; (3) suppression of mast cells, basophils, and eosinophils; and (4) interaction with resident tissue cells and remodeling.^{78,79} Treg cells have distinct phenotypes and mechanisms of action,^{80,81} which can suppress IgE and induce the noninflammatory antibody isotype IgG₄.⁸² Memory B cells can suppress antigen-specific T-cell responses through cytokine expression.

Better understanding and characterization of tolerance to allergens might identify common links among allergic diseases to re-define the current phenotypes.

Transcriptomics

In asthmatic patients molecular signatures at the RNA or protein levels have been discovered in bronchoalveolar lavage fluid and sputum.^{40,83} This has been extended to metabolomic discrimination between asthma and chronic obstructive pulmonary disease in peripheral blood⁸⁴ or exhaled air.⁸⁵ A gene expression analysis has identified 2 evenly sized and distinct subgroups, "T_{H2}-high" and "T_{H2}-low" asthma, with patients in the latter subgroup indistinguishable from control subjects. Using gene expression profiling in epithelial brushings and quantification of intraepithelial mast cells in endobronchial biopsy specimens, it has been shown that intraepithelial mast cell numbers are increased in subjects with T_{H2}-high asthma and predict responsiveness to inhaled corticosteroids. Snapshots of phenotypic markers might not suffice, and their complex temporal behavior could be highly informative. This has only been examined for physiologic

markers, in which fluctuation of peak expiratory flow rate during months⁸⁶ or attractor analysis of respiratory impedance during minutes⁸⁷ predicts clinical outcomes in patients with asthma and chronic obstructive pulmonary disease.

Systems biology

Allergic diseases are caused by complex gene-environment interactions (risk and protective factors associated with allergens and lifestyle and socioeconomic determinants) acting from fetal life to the elderly. Given functional interdependencies between molecular components, a disease reflects complex network perturbations that link cells, tissues, and organs. This complex network reflects patient-specific biological and clinical effects modulated by prevention and treatment. However, little is known about how allergic diseases cluster at the genetic, molecular, or mechanistic levels.

There has been considerable progress as to the genetic contribution to allergic diseases, such as eczema, asthma, and rhinitis.^{88,89} Genome-wide association studies and meta-analyses of genome-wide association studies have recently been performed and will further elucidate both the common and distinctive pathways that contribute to these clinical allergic diseases, as well as their subphenotypes or clinical characteristics in early childhood and at inception of the disease.⁸⁹ There is a central role for genes in innate immune response pathways that promote the activation and differentiation of T_H2 cells in the pathogenesis of multiple allergic diseases, whereas other variants, such as the 17q21 asthma locus, encoding the *ORMDL3* and *GSDML* genes, is specifically associated with risk for childhood onset asthma yet not that for allergy in early childhood.⁹⁰ Additionally, virus-induced wheeze might also be genetically determined.⁹¹

The complex interactions of genes with the environment and with molecular and downstream mechanisms comprising any mechanism between gene transcription and macrophysiology are studied in a “systems medicine” approach.⁹² Such an approach starts with the collection of global genomic, transcriptomic, proteomic, and metabolomic datasets from patient cohorts and control subjects by using a variety of high-throughput measurement platforms. Multivariate analysis and integration of multiple datasets can then lead to the development of statistical classifier models ranging from the molecular to the organ levels. Such models or “phenotype handprints” are complex biomarkers of predictive value for the characterization of new patients. This is now applied in severe asthma by using Unbiased Biomarkers in Prediction of Respiratory Disease Outcomes.⁹³ The bottleneck in this strategy will be the high-dimensional analysis, for which knowledge management platforms⁹⁴ and strict procedures⁹⁵ are emerging.

Although phenotype handprints can be useful predictors of relevant events in patients, they also represent starting points for the formulation of functional hypotheses on disease mechanisms, pathways, and natural history. Such hypotheses can be tested through perturbation experiments, both computationally and experimentally, using targeted and global technologies in cellular and specifically engineered animal models. Mechanistic models need to provide explicit, probabilistic, or deterministic descriptions of the interdependencies of the disease-relevant components on multiple scales and will inform optimal experimental designs for the analysis of disease mechanisms.

The systems biology loop might be iterated several times until it becomes possible to better characterize human diseases by

using complex biomarkers. It is expected that this approach will facilitate the development of novel diagnostic tools and the design of therapeutic interventions targeting multiple molecular and cellular components, which can be tested and validated in prospective clinical trials.^{92,93,96}

UNDERSTANDING THE POPULATION-BASED IgE-ASSOCIATED PHENOTYPES IN CHILDREN AND ADOLESCENTS

Allergic diseases are the clinical expression of complex gene-environment interactions resulting in immunologic responses, and a simple explanation of recent epidemic trends has not been achieved. Disentangling the respective roles of period and cohort effects on different age groups has proved difficult. This has hampered our understanding of how and in which age period we should intervene to prevent allergic disease development.

Age effect of maturation

Pregnancy is a critical period of developmental immune and tissue programming for future diseases.^{80,97} Epigenetics is the cornerstone of the developmental origins of health and disease hypothesis and is likely to be important in IgE-mediated mechanisms⁹⁸ and in the relationship between air pollution during pregnancy and asthma.⁹⁹ Lung development is vulnerable to gene-environment interactions in preschool children because there is a 10-fold increase in alveoli during the first 4 years of life.¹⁰⁰ The immune system requires even longer to mature.¹⁰¹ Only birth cohorts can investigate prospectively the transgenerational pathways/effects and the relevant time windows in human populations.

Observed effects of early-life exposures and shift of focus from early-life to pregnancy exposure

It was hoped that allergy might be prevented by reducing allergen exposure in early life. However, single-component and multifactorial interventions aimed at the reduction of allergen exposure in combination or not with breast-feeding and diet are not effective.¹⁰²

In utero exposure (eg, maternal tobacco smoke, maternal dietary intake, and air pollution exposure) and even factors before pregnancy, such as maternal overweight status, influence the development of IgE-mediated diseases.^{99,103,104}

Early-life exposure to pollutants, nutrition,¹⁰⁵ or a variety of risk factors, such as paracetamol, are associated with increased risk for allergic diseases, asthma, or both.

Studies of contrasting populations with a similar genetic background

An important opportunity for a better understanding of the determinants of allergic diseases is offered by unique situations in which closely located areas with similar ancestry show a large contrast in disease patterns. This was observed in many studies comparing populations in which living conditions and the socioeconomic gradient differ fundamentally, such as in Eastern and Western Europe in the 1980s or still in Karelian schoolchildren and their mothers.¹⁰⁶⁻¹⁰⁹ Among the Karelian children, allergies and allergic diseases are uncommon in Russian Karelia compared

with Finnish Karelia. Because of the huge economic gap between the 2 areas, the living conditions differ fundamentally, and the socioeconomic gradient between Finnish and Russian Karelia is still one of the widest in the world. Thus far, no in-depth analysis of immunoregulation, including Treg cells,¹¹⁰ has been performed in Finnish and Russian Karelian populations. In addition, the context of allergen exposure, including the quantity and diversity of environmental microbiota, has recently emerged as a central player in the development of tolerance and will be studied in these populations.

Cohort effects and time trends

Course and risk factors of allergic phenotypes are best examined with prospective birth cohorts. To date, asthma and allergy in adolescents are not well understood. Only few prospective birth cohort studies from the Pacific region,¹¹¹ Arizona,¹¹² and Europe¹¹³ have evaluated the course and determinants of asthma beyond 14 years.

Cohort effects, such as maternal prenatal obesity¹¹⁴ or diet,^{115,116} can be studied by using birth cohorts. However, results are still unclear, and only prospective birth cohort studies including investigations at different time periods, as represented in the MeDALL study, will allow us to disentangle the effects of exposures, genes, and critical time periods in the course of IgE-mediated disease to explain the observed time trends in allergic diseases over the past decades.

Ethical aspects of the new approaches in MeDALL

The integration of many approaches and disciplines, the variety of partners involved, and the switch from hypothesis-driven research to data-driven research and unsupervised analyses question the classical research ethical framework.^{117,118} The extent of personal data accumulated prospectively for epidemiologic analyses on large cohorts coupled with large-scale biological approaches, exchange of samples and data across borders, and cross-analyses of different kinds of data from various sources challenges the reality of anonymization of data and biological samples and the way sharing of data is done.^{118,119} Also, the way information on aims of studies and methodologies are given to participants and the definition of what constitutes a result to be communicated to participants need to be reconsidered.¹²⁰⁻¹²² The extent of analyses performed exposes the research teams and consortium to incidental findings that become the norm and are less and less incidental but rather frequent but unsought. The definition of the conditions under which a result must be communicated has to be addressed and redefined.¹²²⁻¹²⁴ Also, the necessity to consider early the ethical aspects of possible policy to be proposed for implementation after research in allergy becomes an imperative.¹²⁵ The importance of all these ethical dimensions is recognized in the MeDALL project, and such topics are addressed in specific tasks that go beyond a classical ethical management of the project.

CONCLUSIONS

Allergic diseases are the most common chronic diseases in the world. A large research effort has been developed to understand the epidemic increase of allergic diseases, but despite some relevant hypotheses, the factors that affect the course of

development of allergy, the period in life in which they are triggered, and its mechanisms remain to be elucidated. MeDALL is an FP7 European Union project started in December 2010 aimed to generate novel knowledge on the mechanisms of initiation of allergy. MeDALL is similar to other projects recently launched by the FP7 calls, addressing complex diseases by means of a system-based approach. MeDALL is a large multidisciplinary consortium aimed to integrate multiple layers of information from large general populations of children to animal models. In this context establishing the comprehensive scientific base of the project is a challenge. As part of the MeDALL launching, a scientific seminar was held on January 24, 2011, to review the current knowledge on the IgE-related phenotypes and to explore how a multidisciplinary effort could result in a new integrative translational approach. The new approaches proposed to investigate the complexity of the allergic phenotypes have been reviewed and provide a synthesis of the state of art for the system-based research of IgE-associated diseases.

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CLUSTER ANALYSIS

Cluster analysis involves a series of statistical techniques for unsupervised classification. The final outcome of a cluster analysis is the classification of subjects into groups or phenotypes based on a set of variables. It is different from principal component analysis or factor analysis, which are data-reduction techniques that summarize a number of original variables into a smaller set of components or factors. By exploring the factor loadings in a factor analysis, one can see how different variables cluster together. In cluster analysis, on the other hand, one clusters individuals and not variables.

There are different types of clustering techniques, such as hierarchic, partitioning, and model-based clustering.^{E1} Hierarchic clustering provides a dendrogram in which one can view the entire process of grouping of the individuals and, based on this, decide the final number of groups in the data. Partitioning methods use algorithms that try to find the final partition of individuals that minimizes some criterion (eg, to minimize the within-group variation). The most widely used of such algorithms in partitioning methods is the k-means. Finally, model-based clustering assumes that the data are generated by a model with a specified form, usually expressed as a mixture of statistical distributions with different parameters (eg, mixture of multivariate normal values for continuous variables or mixture of multivariate Bernoulli for categorical data, also known as latent class analysis).

None of these clustering techniques and analysis decisions are globally better than the others; that is, they might have the best performance in some datasets but not in others.^{E1} Several valid and useful classifications can be obtained from the same dataset. Apart from choosing the clustering method, several other decisions need to be made when performing a cluster analysis, which will influence the final solution. These include what variables need to be included, whether the variables need to be standardized and how, the number of clusters, what metric to use, and whether a dimensionality reduction needs to be performed and how.^{E1-E3} A critical and yet unresolved issue is how to validate the observed phenotypes. A validation of the final classification can be performed by comparing the results in 2 independent samples or by testing their predictive performance. A relevant characteristic is that the unsupervised statistical methods can be extended to provide clinical algorithms that could facilitate the identification and classification of the disease in the clinical setting.

In patients with asthma, several unsupervised modeling studies and scale-free models have been published (Table II). In other allergic diseases, such analyses have not taken place. Several authors have recently approached atopy, wheezing, and asthma in this way.^{E4-E7} Because they are at the initial step in this process, it is not surprising that studies using unsupervised methods are not totally consistent. Some reported phenotypes seem to be similar between studies (eg, atopic persistent wheeze),^{E4,E8} and others are similar in their definition but differ with respect to certain characteristics (eg, reduced lung function in transient viral wheeze).^{E8,E9} The severe asthma phenotype differs in cluster analysis and guideline-defined severity.^{E10} Importantly, replication of reported phenotypes in other populations is needed and has not been performed for most of them. Today, our current understanding of allergic diseases is largely based on traditional disease classification resulting from studies driven by clinical, epidemiologic, and mechanistic hypothesis. However, with an increasing number of studies using unsupervised statistical models instead of testing an *a priori* hypothesis, a new approach is emerging.

Latent scale-free models

Similar to clustering techniques, network representations are an appealing analytic strategy in understanding the complexity of chronic diseases.^{E11} Free-scale models can be used to unravel whether a certain phenotypic dataset has an underlying network structure. This has several advantages, such as enabling a whole array of exhaustive methods for data analysis. Empiric and theoretic studies have shown that real-world networks tend to be sparse and heterogeneous (ie, scale-free networks): there are a few highly interconnected nodes (hubs), and the rest of the nodes have only 1 or 2 links. Networks are modular; that is, they can be decomposed in disjoint subgroups of nodes exchanging many internal links among them and then with the rest of nodes. Then nodes can be seen as phenotypes. Although there are still few attempts to apply network analysis to allergic diseases, a recent study has characterized a potentially tissue-specific gene network (LL module) associated with blood lipid levels, which not only harbors key components of inflammation and allergy, strongly suggesting a role for basophils and mast cells, but also associates with a key single nucleotide polymorphism that regulates serum IgE levels.^{E12}

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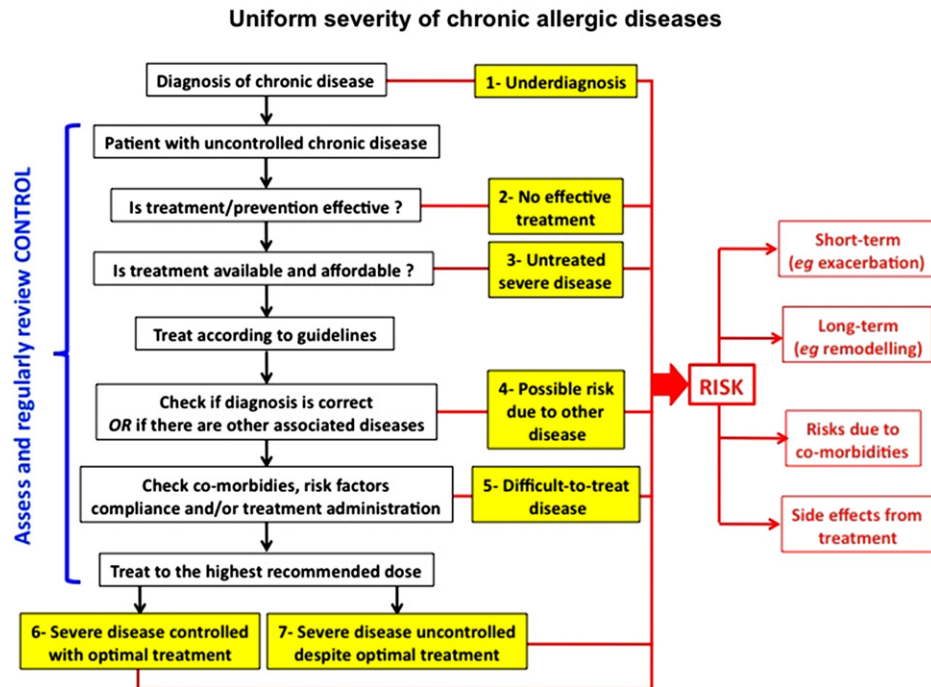


FIG E1. Uniform definition of allergy severity, control, responsiveness to treatment and risk adapted from Bousquet et al.^{E13} Concepts of disease severity, activity, control, and responsiveness to treatment are linked but different. Severity refers to the loss of function of the target organs induced by the disease process or to the occurrence of severe acute exacerbations. Severity can vary over time and requires regular follow-up. Control is the degree to which therapy goals are currently met. Generalizing the approach of the uniform definition of severe asthma presented to the World Health Organization, a uniform severity for chronic allergic diseases (rhinitis, chronic rhinosinusitis, chronic urticaria, and atopic dermatitis) is proposed. This uniform definition will allow us to better define phenotypes of severe allergic (and related) diseases for clinical practice, research (including epidemiology), public health purposes, education, and discovery of novel therapies.

Immune tolerance to allergens

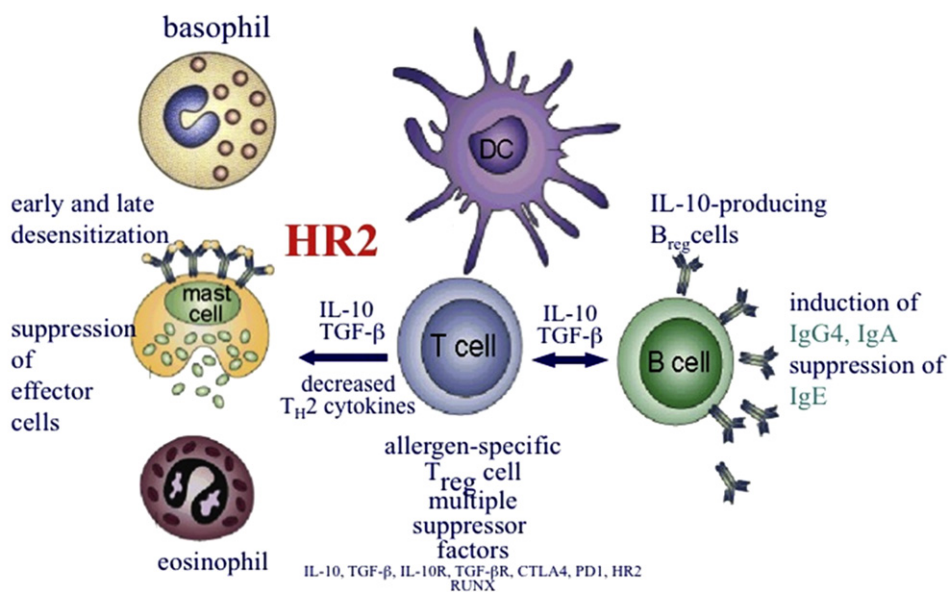


FIG E2. Immune tolerance to allergens adapted from Akdis et al.^{E14} and Klunker et al.^{E15} Treg cells contribute to the control of allergen-specific immune responses in several ways: suppression of antigen-presenting cells that support the generation of effector T_H2 and T_H1 cells; suppression of T_H2 and T_H1 cells; and suppression of mast cells, basophils, and eosinophils. B cells not only produce IgG antibodies, which inhibit allergen-induced activation of mast cells, basophils, and T cells, as well as secondary IgE production, but might also express certain cytokines that can suppress antigen-specific T-cell responses. Histamine receptor 2 (*HR2*), which is highly expressed in T_H2 cells, mediates the effects of histamine, which induces the production of IL-10 by dendritic cells (*DC*) and T_H2 cells and enhances the suppressive activity of TGF- β on T cells. *CTLA4*, Cytotoxic T lymphocyte-associated antigen; *IL-10R*, IL-10 receptor; *PD1*, programmed death-1; *RUNX*, Runt-related transcription factor.

TABLE E1. Definition of allergy and atopy^{E16}

1. "Hypersensitivity should be used to objectively describe reproducible symptoms or signs initiated by exposure to a defined stimulus, at a dose tolerated by healthy persons."
2. "Allergy is a hypersensitivity reaction initiated by specific immunologic mechanisms. When other mechanisms can be proven, as in hypersensitivity to aspirin, the term non-allergic hypersensitivity should be used."
3. "Atopy is a personal or familial tendency, usually in childhood or adolescence, to become sensitized and produce IgE antibodies in response to ordinary exposures to allergens, usually proteins. As a consequence, these persons can have typical symptoms of asthma, rhinoconjunctivitis or eczema. The term atopy should be reserved to describe the genetic predisposition to become IgE-sensitized to [common allergens]."
4. "The term atopy cannot be used until an IgE sensitization has been documented"
5. "The umbrella for a local inflammation of the skin should be dermatitis." It includes "eczema to replace the provisional term atopic eczema/dermatitis syndrome.....contact dermatitis and other forms of dermatitis."
6. "Atopic eczema is eczema in a person of the atopic constitution."

An important component of MeDALL is the comparison of classical (as defined by experts) and novel phenotypes of IgE-related diseases (obtained by means of unsupervised statistical methods) among children included in the participating birth cohorts. For experts to agree on the classical phenotypes, a sufficient agreement on use of disease definitions and classification is needed. This is of particular importance in the case of allergic diseases because the use of different allergy-related terms varies widely. The World Allergy Organization nomenclature for allergy (2003) is used in MeDALL to facilitate common understanding of terminology.^{E16}