

# Mechanisms of the Development of Allergy (MeDALL) Study: A Systems Medicine Approach to Understand Allergic Diseases

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## ABSTRACT

Mechanisms of the Development of Allergy (MeDALL) was a European Union-Seventh Framework Programme for Research and Technological Development project aimed to apply the systems medicine approach to improve the understanding of allergic diseases. It was based on existing birth cohorts to redefine classical phenotypes and identify novel phenotypes of immunoglobulin (Ig)E-associated asthma, allergic rhinitis and dermatitis. The project included a wide range of population-based, clinical and mechanistic studies applying systems medicine. MeDALL demonstrated the existence of the multimorbidity of eczema, rhinitis, and asthma both in IgE-sensitized and non-sensitized children and integrated the evidence on multimorbidities and polysensitization in a new framework of allergic diseases. Innovative approaches included unsupervised statistical modelling, computational analysis of the topology of the protein interaction network and the IgE MeDALL allergen-chip. Mechanistic studies included testing candidate biomarkers, epigenetics, and transcriptomics. Assessment of individual and environmental factors included puberty, maternal smoking and pregnancy. (BRN Rev. 2019;5(3):169-83)

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## INTRODUCTION

The mechanisms underlying allergic diseases are complex and insufficiently understood, some of them associated with an immunoglobulin (IgE) immune response. These IgE associated allergic diseases are complex multifactorial disorders, with both genetic and environmental interactions determining disease expression and leading to different, frequently co-existing, phenotypes. Both IgE and non-IgE associated allergic diseases are linked by complex and currently insufficiently defined interrelationships which occur during childhood and persist throughout life. The limited understanding of allergic diseases has prevented to control the worldwide epidemic of allergic diseases that has occurred over the past decades.

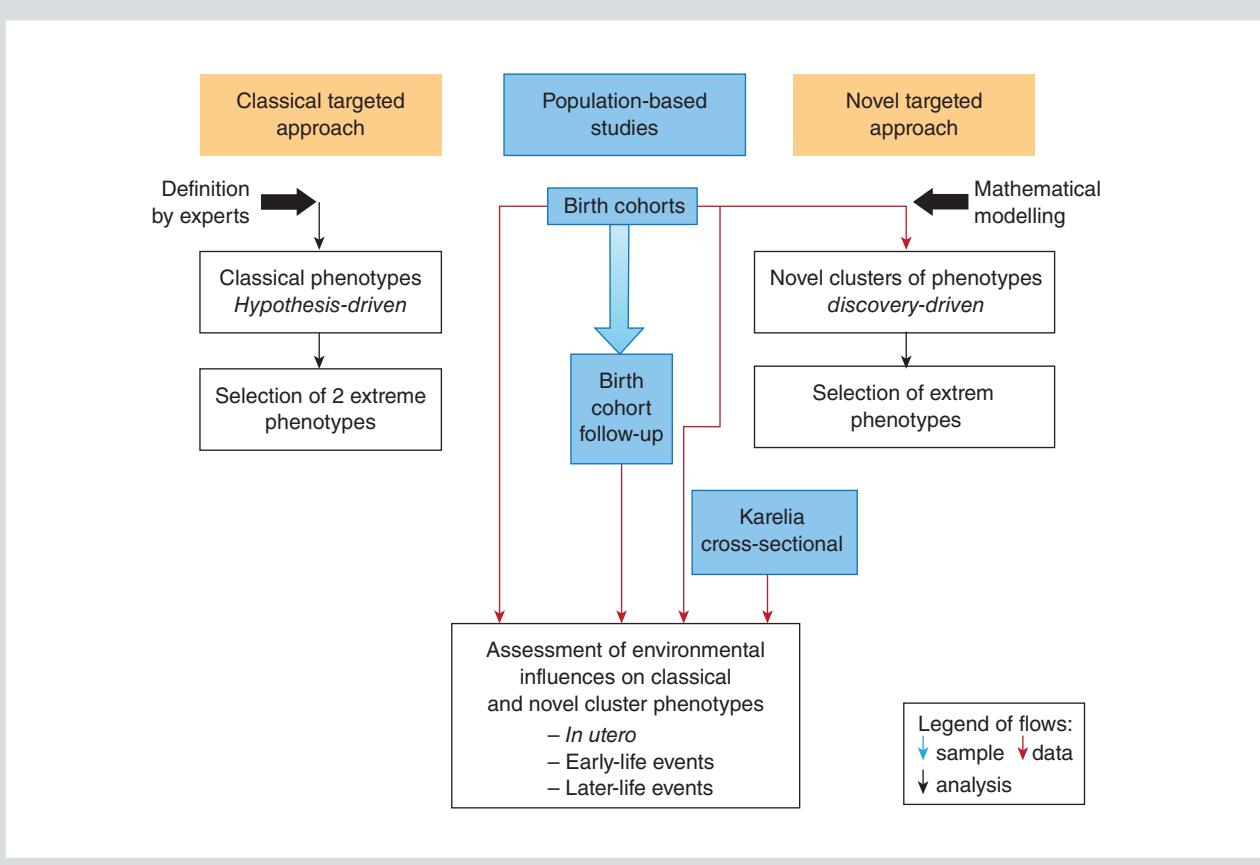
The Mechanisms of the Development of Allergy (MeDALL) project emerged in 2010 in response to a call for proposals from the European Union Seventh Framework Programme (FP7) for Research and Technological Development, aimed at adopting a systems medicine approach to improve the understanding of the mechanisms of allergic diseases (i.e. FP7 HEALTH.2010.2.4.5-1-Investigation of the mechanisms of initiation of allergic response, genetic predisposition, biomarkers and identification of targets for therapy). The authors of this article coordinated the preparation of a proposal that was finally funded (Grant Agreement no: 261357; 2010-2014, <https://cordis.europa.eu/project/rcn/96850/factsheet/en>). In previous publications we reported the conceptual bases of MeDALL<sup>1</sup>, the main characteristics of the project<sup>2</sup> as well as a review of its main results<sup>3,4</sup>. The present paper describes the main characteristics of MeDALL, with special emphasis on its novel methodological aspects, the advances in the understanding of the

multimorbidities of allergic diseases, as well as an update of its main results regarding genetic and molecular mechanisms. Inspired by recent advances in systems biology and complexity sciences, systems medicine still is in its early stages. A general hypothesis underlying the application of systems approaches in medicine is that the interplay between multiple genetic and environmental factors results in deregulation of complex molecular networks. Systems medicine aims to understand this complexity by using an integrative approach to identify new opportunities to improve prevention, clinical diagnostics and therapy<sup>5</sup>. The systems medicine approach involves large-scale integration of existing knowledge with newly acquired multidimensional data, including bio-banking and clinical, phenotypic, environmental, lifestyle, biologic, and -omics data. The recent advances in systems medicine have opened new opportunities in the field of allergic diseases<sup>6,7</sup>. The MeDALL project was pioneer in adopting a systems medicine approach to understand allergic diseases from early childhood to young adulthood<sup>1,2</sup> by linking epidemiologic, clinical, and basic research in birth cohorts<sup>8</sup> and including genome-wide association studies (GWASs) as well as transcriptomic, epigenetic, and targeted proteomic studies (Fig. 1, Table 1). This approach was possible due to the multidisciplinary nature of MeDALL which included 23 partner institutions and a multidisciplinary network of experts.

## PROJECT'S MAIN CHARACTERISTICS

### Building on previous networks of birth cohorts

Long-term birth cohort studies are essential for understanding the life course and



**FIGURE 1.** Mechanisms of the Development of ALLergy (MeDALL): an integrated approach from phenotypes to systems medicine (reproduced with permission from Bousquet J, et al.<sup>2</sup>).

childhood predictors of allergy and the complex interplay between genes and environment, including air quality, lifestyle, and socio-economic determinants<sup>9</sup>. More than 150 cohorts focusing on asthma and allergy have been initiated in the world over the past 30 years<sup>8</sup>. Since 2004, several research initiatives funded under the EU Framework Program for Research and Technological Development (FP6 and FP7) have attempted to identify, compare, and evaluate pooling data from existing European birth cohorts<sup>10-16</sup>. In the United Kingdom, the Study Team for Early Life Asthma Research (STELAR)<sup>17</sup> developed a network of birth cohorts to share data on asthma.

## MeDALL strategy to integrate heterogeneous data from different birth cohorts

MeDALL included the historical data collected by fourteen European birth cohorts. An important difficulty was the heterogeneity of questionnaires and definitions of symptoms and diseases as well as different periods and ages at which data had been obtained. MeDALL approached this difficulty through different strategies.

First, a systematic review of the literature was instrumental to establish agreed definitions

**TABLE 1.** Mechanisms of the Development of ALLergy (MeDALL) framework for a system-based research approach to allergic diseases

1. Establishment of the complexity of immunoglobulin (Ig)E-associated allergic diseases.
2. Innovative research approaches to the clinical and epidemiologic aspects of the allergic phenotypes: <ul style="list-style-type: none"> <li>– Unsupervised statistical modelling of phenotypes.</li> <li>– Candidate biomarkers.</li> <li>– IgE microarrays.</li> <li>– Severity of allergic diseases.</li> </ul>
3. Novel approaches to the IgE-associated phenotypes from the individual mechanisms to the systems: <ul style="list-style-type: none"> <li>– Epigenetics.</li> <li>– Human <i>in vitro</i> immunology.</li> <li>– Transcriptomics.</li> <li>– Animal models.</li> <li>– System biology.</li> </ul>
4. Understanding the population-based IgE-associated phenotypes in children and adolescents: <ul style="list-style-type: none"> <li>– Age effect of maturation.</li> <li>– <i>In utero</i> and early-life exposures.</li> <li>– Contrasting populations with a similar genetic background.</li> <li>– Cohort effects and time trends.</li> </ul>
5. Ethics of the new approaches in Mechanisms of the Development of ALLergy (MeDALL).

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of phenotypes<sup>18</sup>. The review included 197 studies and identified up to 33 different phenotypes of allergic diseases reported from studies using very different methods and often lacking objective phenotypic measures. This review showed the need to standardise definitions and terminology as the basis for data harmonisation.

Second, existing historical data from fourteen birth cohorts, including three to twenty follow-ups, from nine European countries were harmonised<sup>19</sup>. The harmonisation process followed six steps: an expert panel; identification of candidate variables; an agreed reference definition for each candidate variable; assessment of the compatibility of each cohort variable with its reference definition (inferential equivalence) and classification of the inferential equivalence as complete, partial, or

impossible; a workshop to agree on the reference definitions and classifications of inferential equivalence; and, data preparation and delivery through a knowledge management portal. The panel finally agreed on 137 reference definitions and examined the inferential equivalence of more than 3,500 cohort variables to their corresponding reference definitions, showing that about 70% of these variables could be considered as completely equivalent.

Third, a harmonised MeDALL Core Questionnaire (MeDALL-CQ) was developed to guarantee the homogeneity of newly collected data<sup>20</sup>. The process included the agreement on a set of core variables from fourteen birth cohorts, achieving a consensus on questionnaire items as well as its translation and back-translation from English into eight other languages. As a result, three harmonised MeDALL core questionnaires (two for parents of children aged 4-9 and 14-18, and one for adolescents aged 14-18) were developed and used for a harmonised follow-up assessment of eleven European birth cohorts on asthma and allergies with over 13,000 children.

Fourth, a knowledge-management platform was established. Developing a MeDALL knowledge-management platform to integrate data available from all participating birth cohorts and make it available for MeDALL analysis and collaborations was an important step. The MeDALL knowledge base integrates historical and newly collected data from 44,010 participants on 398 clinical and phenotypic attributes (harmonised from 7,495 individual cohort variables) and 160 different follow-ups in 25 different time points between pregnancy and age 20 years, as well as information about available samples (> 30,000 samples from blood, plasma,

serum, deoxyribonucleic acid [DNA], ribonucleic acid [RNA], and leukocytes). Making the MeDALL database fully operative and widely available has proven to be a very demanding task that would have needed targeted funding for a longer period of time. A different strategy, that does not include harmonisation, is the STELAR Asthma e-Lab<sup>17</sup>, an established database focusing on asthma data from UK-based birth cohorts.

## New allergen microarray technology

With a few selected allergens, it is possible to characterise allergy in epidemiologic studies. However, to understand the complexity of allergic diseases it is necessary to assess IgE sensitization in a more comprehensive way including a large range of different allergenic molecules. A “MeDALL allergen-chip” was developed including a collection of 170 allergen molecules used for the reliable detection of IgE and other isotypes of allergen-specific antibody signatures showing a higher sensitivity than the traditional ImmunoCAP system and skin prick testing<sup>21,22</sup>. So far, the MeDALL chip has been used in several studies, improving the understanding of the allergic nature of asthma, rhinitis, and eczema in children (Table 2).

## The use of bioinformatics and the integration of clinical and -omics data

An important aim of MeDALL was the use of bioinformatics tools and the integration of clinical and mechanistic data as part of the systems medicine approach. One type of strategy was the use of new bioinformatics methods together with the classical epidemiologic

**TABLE 2.** Studies using the Mechanisms of the Development of ALLergy (MeDALL) immunoglobulin (Ig)E microarray

1. IgE reactivity to the pathogenesis-related class 10 (PR-10) protein family and allergic rhinitis to birch pollen (ARbp) from early childhood up to the age of 16 years<sup>28</sup>.
2. IgE sensitization patterns to cat and dog allergen molecules during childhood and symptoms to these furry animals up to age of 16 years<sup>31</sup>.
3. Variability in allergen-specific IgE and IgG frequencies in adults living in different regions in France<sup>32</sup>.
4. Identification of a multimorbidity-polysensitization phenotype<sup>30</sup>.
5. IgE reactivity to a few allergens early in life to identify children with a high risk of asthma and/or rhinitis at age 16 years<sup>33</sup>.

and statistical tools. The application of unsupervised statistical models to identify novel allergic phenotypes was complemented with a sensitivity analysis testing alternative hypothesis-free grouping methods using hierarchical clustering with Ward’s method, latent class analysis and self-organising maps<sup>23</sup>. Another type of approach was the development of a computational *in silico* model of multimorbidity to identify those shared genes and pathways that could explain the multimorbidity of asthma, rhinitis and eczema<sup>24</sup>. Regarding the integration of the MeDALL available -omics data, including GWASs, DNA methylation, transcriptomics and targeted proteomics, the potential of MeDALL has been shown by studies on some asthma genes like chitinase-3-like protein 1 [CHI3L1]<sup>25</sup> and interleukin (IL)-1 receptor-like 1 [IL1RL1]<sup>26</sup>.

## UNDERSTANDING THE COMPLEXITY OF THE CLINICAL PHENOTYPES OF ALLERGIC DISEASES

Allergic diseases like asthma, allergic rhinitis, dermatitis or food allergy are usually

approached as separate entities which itself involve substantial heterogeneity. Thus, it is not surprising that the dominant approach to understanding their complexity has been to segregate each disease in separate entities. The systematic review of allergic diseases performed in MeDALL did show that about 45% of the 197 included studies considered multimorbidity in a way or another<sup>18</sup>. However, none of the studies had assessed the multimorbidity with robust methods and the magnitude of the multimorbidity was not known. MeDALL was the first population-based study to assess systematically the multimorbidity of eczema, rhinitis, and asthma and to quantify the net excess of multimorbidity. The assessment included cross-sectional and longitudinal models<sup>27</sup> and involved 16,147 children aged 4 years and 11,080 children aged 8 years, from twelve birth cohorts. The results of these models showed that the coexistence of eczema, rhinitis, and asthma in the same child is more common than expected by chance, with 50% of the observed multimorbidity being a net excess of multimorbidity, not attributable to chance. In particular, the absolute excess of any multimorbidity was 1.6% for children aged 4 years and 2.2% for children aged 8 years; 44% of the observed multimorbidity at age 4 years and 50% at age 8 years was not a result of chance. It also showed that all the possible combinations of the three diseases were more common than expected by chance with their clustering being statistically significant. A similar pattern of multimorbidity was observed in both age groups.

The hypothesis that the symptoms of asthma, rhinitis and eczema co-occur in the same children more often than expected by chance

was also tested using machine-learning methods as alluded to above<sup>23</sup>. The study involved 17,209 children at age 4 years and 14,585 children at age 8 years from seven birth cohorts. Two groups of children were identified as the optimal way to cluster the data at both age periods. One group, that was considered asymptomatic, included 70% and 79% of children at 4 and 8 years of age respectively. The second group, including the remaining children, was considered a symptomatic group and exhibited more frequent symptoms and sensitization. The symptomatic group included about 30% of children between 4 and 8 years, suggesting that a large proportion of children in the general population could be at risk of developing multimorbidity. Almost all children with multimorbidity as identified in the study using canonical epidemiological methods were classified in the symptomatic group when using unsupervised methods.

These findings were to some extent in contrast to the dominant emphasis on the heterogeneity of the individual allergic entities and the need to segregate these entities into more specific subgroups, highlighting the need of new approaches, like the diseasesome and the integrative systems biology models, to understand how these different diseases are linked to each other and why they tend to occur in the same children.

## The role of IgE sensitization in the multimorbidity

The longitudinal model of multimorbidity between 4 and 8 years of age, also showed that a net excess of multimorbidity was present both in IgE-sensitized and non-sensitized

children and that IgE sensitization accounted only for 38% of multimorbidity<sup>27</sup>. The same pattern of IgE and non-IgE multimorbidity was observed in the unsupervised model<sup>23</sup>. While IgE sensitization was more prevalent in the symptomatic group, the distribution of symptoms in the two groups did not change when IgE sensitization was included or excluded from the model. In addition, it was also shown that polysensitization increased the risk of developing multimorbidity as compared with monosensitization.

One limitation of our studies based on existing historical data of the birth cohorts was that a low number of allergens had been tested. In MeDALL we had access to a new allergen chip encompassing more than 170 allergens. However, the new chip could only be performed in a limited number of children thus precluding the assessment of multimorbidity in a sufficiently large sample.

In the Children, Allergy, Milieu, Stockholm, Epidemiology (Swedish abbreviation for BAMSE) cohort 779 children were tested at 4, 8, and 16 years of age with the MeDALL chip to birch proteins and cross-reactive pathogen-related class 10 proteins<sup>28</sup>. The results showed that children with higher levels of Bet v 1-specific IgE or increasing numbers of IgE-reactive pathogen-related class 10 (PR-10) proteins at age 4 years had an increased risk of incidence and persistence of allergic rhinitis to birch pollen up to age 16 years. Compared to 4-year-old asymptomatic children monosensitized to Bet v 1 allergens, children who in addition to Bet v 1 were polysensitized to one or more PR-10 proteins had an increased risk to develop allergic rhinitis between 8 and 16 years of age. This is potentially important for multimorbidity

since, as we showed in MeDALL, rhinitis at the age of 4 increases the risk of asthma and multimorbidity at the age of 8 years.

The assessment of the role of polysensitization on multimorbidity was further extended to other age groups including toddlers, adolescents and adults. Regarding to the latter, the relationship between early sensitization and allergic morbidity in early life was studied in the Pollution and Asthma Risk: an Infant Study (PARIS) population-based birth cohort<sup>29</sup>. Allergic sensitization to twelve food allergens and four inhalant allergens was assessed at 18 months. Using cluster analysis, 1525 infants were grouped into three groups: not or rarely sensitized (89.2%), mainly sensitized to one or few allergens (9.2%) and polysensitized (1.6%). The prevalence of doctor-diagnosed asthma, rhinitis, eczema, food allergy and multimorbidity at 2 years showed a statistically significant increase from group one to three. At 6 years, multimorbidity was significantly more frequent in the pauci-sensitized and polysensitized groups.

Regarding older age groups, IgE sensitization patterns towards a broad panel of aeroallergen components were assessed in adolescents and adults from BAMSE and Epidemiological Study of the Genetics and Environment of Asthma (EGEA) cohorts, with a focus on asthma and rhinitis multimorbidity<sup>30</sup>. The IgE reactivity to 64 micro-arrayed aeroallergen molecules was determined with the MeDALL-chip. As compared to subjects without any allergic diseases, the adjusted ratio of the mean number of IgE-reactive molecules was higher in those with asthma and rhinitis (multimorbidity) than in those with either asthma or rhinitis alone. These results suggest that the poly-sensitization and multimorbidity phenotype

seems to be generalizable to various ages and allergenic environments and could be associated with specific mechanisms.

Altogether, the results of these and other MeDALL studies<sup>31-33</sup> suggest that there are relevant clinical and immunologic differences between non-IgE sensitized, monosensitized and polysensitized subjects. The integration of multimorbidities and polysensitization could help to improve the understanding of genetic and epigenetic mechanisms and pathways of allergy and improve the management of allergic diseases. Under this framework, asthma, rhinitis and dermatitis are considered manifestations of a common systemic immune imbalance with an embryological mesodermal origin together with specific patterns of remodelling either from ectodermal or endodermal origin allowing to propose a new classification of IgE-mediated allergic diseases<sup>34</sup>.

## Other risk factors associated with multimorbidity

Risk factors that are associated with several diseases will increase the risk of multimorbidity between these diseases, even if their molecular mechanisms are totally independent. MeDALL authors assessed the associations of parental smoking from foetal life through adolescence with asthma and rhinoconjunctivitis during childhood and adolescence in 10,860 participants. Any maternal exposure to smoking during pregnancy was associated with early transient asthma, whereas maternal smoking of  $\geq 10$  cigarettes/day during pregnancy was associated with persistent asthma and persistent rhinoconjunctivitis. However, tobacco smoke exposure

during foetal life, infancy, childhood, and adolescence was not associated with adolescent-onset asthma or rhinoconjunctivitis<sup>35</sup>. We also assessed the influence of puberty showing that current allergic multimorbidity prevalence was higher among boys than girls especially in earlier childhood; these differences decreased as the participants grew older to smaller or disappeared between males and females, suggesting that sex-shift towards females after puberty was strongest in subjects with multimorbidity of asthma and rhinitis<sup>36</sup>.

## UNDERSTANDING THE GENETIC AND MOLECULAR MECHANISMS OF ALLERGIC DISEASES

Improving the understanding of the genetic and epigenetic mechanisms of the allergic diseases and its multimorbidity was an important goal of MeDALL. Several types of studies were conducted. An effort was made to adopt a 2-stage approach including discovery and validation and to conduct integrative analyses both in the birth cohorts and using *in silico* models.

## *In silico* computational genetic model

Studies alluded to above showed that in 4 to 8-year-old children, asthma, rhinitis and eczema occurred in the same children more often than expected by chance<sup>27</sup> and that symptoms of the three diseases clustered together in a multimorbidity group<sup>23</sup>. Following these results, an *in silico* study was conducted to test the hypothesis that asthma, rhinitis

and eczema shared associated genes more frequently than expected by chance<sup>24</sup> and that these genes could be relevant to explain the multimorbidity. The *in silico* study showed the existence of shared genes exhibiting a significant degree of interconnectedness in the interaction network. The shared genes signalled 15 different pathways involved in the multimorbidity of asthma, eczema and rhinitis, including IL4 signalling and GATA3-related pathways. The study also reported new candidate genes potentially contributing to multimorbidity and supported our previous findings that asthma, allergic rhinitis and dermatitis tend to cluster in the same subjects<sup>24</sup>.

## DNA methylation studies

It has been shown that epigenetic mechanisms, including methylation, may contribute to the development of childhood asthma and potentially be relevant in other allergic diseases. Two large DNA methylation studies were performed. One was an epigenome-wide methylation study showing 27 seven methylated CpG sites associated with asthma, 14 of them being replicated<sup>37</sup>. Lower methylation levels were observed at all associated loci across childhood from age 4 to 16 years in participants with asthma, but not in cord blood at birth. Whole-blood transcriptional signatures associated with these CpG sites indicated increased activation of eosinophils, effector and memory cytotoxic (CD8) T cells and natural killer cells, and reduced number of naive T cells.

A second study with a larger sample size was part of the Pregnancy And Childhood

Epigenetics (PACE) consortium, consisting of an epigenome-wide meta-analysis of newborns and school-age children<sup>38</sup>. The study assessed CpG methylation in blood to identify differentially methylated regions (DMRs). In new-borns, 9 CpGs and 35 regions were differentially methylated in relation to asthma development. In children, 179 CpGs and 36 differentially methylated regions were identified. Pathway analyses highlighted enrichment for asthma-relevant immune processes and gene expression correlated with methylation at most loci.

The assessment of DNA methylation also provided an opportunity to test the role of environmental exposures. As part of the PACE consortium, some MeDALL cohorts contributed to a meta-analysis to assess the association between maternal smoking in pregnancy and new-born blood DNA methylation<sup>39</sup>. Over 6,000 CpGs were differentially methylated in relation to maternal smoking at genome-wide statistical significance, including 2,965 CpGs corresponding to 2,017 genes not previously related to smoking and methylation. Some of these genes have been implicated in genetic studies of orofacial clefts or asthma, both conditions related to maternal smoking in pregnancy. In another study<sup>37</sup>, the associations between nitric dioxide (NO<sub>2</sub>) exposure at residential addresses during pregnancy and cord blood DNA methylation were meta-analysed in four European and North American birth cohorts. Nitric dioxide exposure during pregnancy was associated with differential offspring DNA methylation in mitochondrial-related genes. Exposure to NO<sub>2</sub> was also associated with differential methylation as well as with differential expression of genes involved in antioxidant defence pathways<sup>52</sup>.

These studies underline the effect of early-life exposure to environmental factors, although whether these methylation changes and expression profiles are causally related to asthma and lung function deterioration or are only markers of exposure is not known.

## Integrative analysis of targeted proteomics

As part of the systems approach in MeDALL it was possible to integrate genetic, epigenetic, protein and phenotype data. Two studies were conducted on the chitinase-like protein YKL-40 and IL-1RL1 respectively. Participants with high YKL-40 levels had increased odds for asthma compared with subjects with low YKL-40 levels. In contrast, neither single nucleotide polymorphisms (SNPs) nor methylation levels at CpG sites in CHI3L1 (the YKL-40 gene) were associated with asthma<sup>25</sup>. The SNPs in IL-1RL1-a strongly regulate IL-1RL1-a methylation, lung messenger (m)RNA expression, and serum IL-1RL1-a levels, although no relation of these (epi)genetic effects was observed in asthmatic patients<sup>26</sup>.

## BEYOND MEDALL

Understanding the relevant gaps and challenges of a large project like MeDALL is also an opportunity to learn for future projects. In this section, we briefly describe and comment on some relevant gaps and challenges of MeDALL.

1. There was insufficient integration among the different work packages (WPs). The ambition of MeDALL led to an unprecedented

level of complexity and multidisciplinarity and the desired degree of integration among the different WPs was not fully achieved. The consequence is that hypothesis and experiments performed in the different WPs did not fully converge in a single integrated framework and the results were somewhat fragmented. Integration of *in vitro* and *in vivo* studies was particularly challenging.

2. The existence of specific shortcomings that limited the experimental plan included the limited number of samples available for the study below 4 years of age and the limited sample size to study extreme but uncommon phenotypes in detail.
3. The limited age span in MeDALL did not allow providing a complete view of allergic diseases during the life cycle. In the future, extending birth cohorts for asthma and allergy in Europe to the complete life cycle should be a priority. This is especially important in the context of the increasing attention to the healthy ageing policies in Europe.
4. Attention to environmental risk and protective factors was limited to few exposures like smoking and air pollution. With growing interest in the exposome in birth cohort studies, future efforts to understand the mechanisms of allergic diseases need to consider environmental factors more comprehensively.
5. As in many other large projects, the duration of four and a half years was clearly insufficient. MeDALL achieved a remarkable disciplinary integration but it took about three years to get to the point of productive cross-communication. With the cohesion achieved it would have been very productive to have time for extending

MeDALL to a fully integrated systems medicine framework.

6. MeDALL developed a knowledge-management platform including a central database of the birth cohort data from the new as well as the historic follow-up assessments. However, the project duration was not enough to achieve a fully optimized and easily accessible platform. This difficulty is especially important in the context of increasing emphasis in open data. Currently, access to MeDALL data can be consulted either through the individual MeDALL partners or at Biomax Informatics (<https://www.biomax.com/project/medall/>).

7. By design, MeDALL was focused on understanding the multimorbidity of allergic diseases. In the future, it is necessary to extend the study of multimorbidity beyond allergy and childhood. The evidence that MeDALL has provided about the multimorbidity of asthma, rhinitis and eczema in children is probably only the tip of the iceberg. There is evidence that these allergic diseases and their multimorbidity in childhood are often associated with further respiratory problems in adult life, such as adult-onset asthma, lower lung function and a higher risk of chronic obstructive pulmonary disease (COPD)<sup>40-42</sup>. Further, some population-based studies have observed that those with allergic diseases have a higher prevalence of other non-communicable diseases (NCDs), specifically cardiovascular and metabolic, both in children<sup>43</sup> and adults<sup>44,45</sup>. In addition, there is consistent evidence from population-based studies of a significant association between asthma and attention deficit hyperactivity disorder (ADHD) both in children and adults<sup>46,47</sup>. Interestingly, also the

allergic multimorbidity has been associated with higher ADHD<sup>48</sup>. Depression and anxiety, the most common mental health disorders, have also been associated with allergic diseases or their multimorbidity both in children and adults at the population level<sup>43,49,50</sup>. Altogether, data suggests that multimorbidity extends across many body systems and that, although fully visible in adult life, it may have been evolving along the early life span.

## CONCLUSIONS

MeDALL was a successful EU-FP7 project conducted by 23 partners that developed an innovative approach to applying the new concepts and methods of systems medicine to the understanding of the mechanisms of allergic diseases (asthma, allergic rhinitis and eczema). MeDALL combined different sources of data including epidemiological, clinical and molecular data using a stepwise, large-scale and integrative approach. Information from children followed in 14 birth cohorts spread across Europe was combined with IgE measurement using microarrays, targeted proteomics, epigenetics and transcriptomics as well as with socio-demographical and environmental data. Multimorbidity of asthma, allergic rhinitis and eczema, in the same child, is more common than expected by chance, suggesting that these diseases share causal mechanisms irrespective of IgE sensitisation. The latter was independently associated with multimorbidity and should be considered differently in mono- and polysensitized individuals. Allergic multimorbidities and IgE polysensitisation are often associated with the persistence or severity of allergic diseases. The type 2 signalling

**TABLE 3.** A Mechanisms of the Development of ALLergy (MeDALL) framework for a systems-based research approach to allergic symptoms: summary of main results

**1. Establishment of the complexity of immunoglobulin (Ig)E associated diseases.**

- A systematic analysis of the literature identified up to 33 different phenotypes of allergic diseases<sup>18</sup>.
- Comorbidity of eczema, rhinitis, and asthma in the same child, including all combinations of the three diseases are more common than expected by chance<sup>27</sup>.
- Multimorbidity of eczema, rhinitis and asthma confirmed using machine-learning methods; population stratified in two groups, the symptomatic one, containing 30% of children between 4 and 8 years and 100% of multimorbidity<sup>23</sup>.
- Multimorbidity was present both in IgE-sensitized and non-sensitized children<sup>23,27</sup>.
- IgE sensitization accounted for 38% of multimorbidity; poly-sensitization increased the risk of developing multimorbidity as compared with mono-sensitization<sup>27</sup>.
- Integration of multimorbidities and polysensitization resulted in a new classification framework of allergic diseases<sup>34</sup>.

**2. Innovative research approaches to the clinical and epidemiologic aspects of the allergic phenotypes**

**2.1 Unsupervised statistical modelling of phenotypes.**

- Use of machine-learning methods including hierarchical cluster analysis, latent class analysis and self-organising maps<sup>23</sup>.
- Computational analysis of the topology of the protein interaction network to characterise the molecular mechanisms of multimorbidity of asthma, eczema and rhinitis<sup>24</sup>.

**2.2 Candidate biomarkers.**

- IgE polysensitization: multimorbidity was significantly more frequent in the paucisensitized and polysensitized groups<sup>29</sup>.
- The adjusted ratio of the mean number of IgE-reactive microarray molecules was higher in those with multimorbidity of asthma and rhinitis<sup>30</sup>.
- Children with higher levels of Bet v 1-specific IgE or increasing numbers of IgE-reactive pathogen-related class 10 proteins at age 4 years had an increased risk of incidence and persistence of allergic rhinitis to birch pollen up to age 16 years<sup>28</sup>.
- Participants with high chitinase-like protein YKL-40 levels had increased odds for asthma compared with subjects with low YKL-40 levels<sup>25</sup>.
- Clara cell secretory protein (CC16): low levels of early CC16 at age 4 years predicted subsequent forced expiratory volume in one second (FEV1) deficits up to age 16<sup>51</sup>.

**2.3 IgE microarrays.**

- A “MeDALL allergen-chip” was developed including a collection of 170 allergen molecules used for the reliable detection of IgE and other isotypes of allergen-specific antibody signatures showing a higher sensitivity than the traditional ImmunoCAP system and skin prick testing<sup>21,22</sup>.
- Studies using the MeDALL microarray have approached several relevant questions<sup>28,30-33</sup>.

**3. Novel approaches to the IgE-associated phenotypes: from the individual mechanisms to the systems.**

**3.1 Epigenetics.**

- An EWAS study in children 4 to 16 years showed 27 seven methylated CpG sites associated with asthma, 14 of them being replicated. Lower methylation levels were observed at all associated loci in participants with asthma, but not in cord blood at birth. CpG sites indicated increased activation of eosinophils, effector and memory CD8 T cells and natural killer cells, and reduced number of naive T cells<sup>37</sup>.

**3.2 Transcriptomics.**

- Transcriptome assays were performed in whole blood samples collected from three participating cohorts in 784 children at 4 and 15-16 years of age. The analysis retrieved a set of 35 genes involved in all three diseases and all three cohorts. These genes are mainly involved in respiratory diseases, inflammatory and immune responses (MeDALL final report; unpublished data).

**3.2 System biology.**

- An *in silico* study identified 15 different pathways involved in the multimorbidity of asthma, eczema and rhinitis, including IL4 signaling and GATA3-related pathways. The study also reported new candidate genes potentially contributing to multimorbidity<sup>24</sup>.
- The effects of CHI3L1 genetic variation on circulating YKL-40 levels are partly mediated by methylation profiles. YKL-40 levels, but not CHI3L1 SNPs or methylation levels associated with childhood asthma<sup>25</sup>.
- SNPs in IL-1RL1-a strongly regulate IL-1RL1-a methylation, lung mRNA expression, and serum IL-1RL1-a levels, although no relation of these (epi)genetic effects were observed in asthmatic patients<sup>26</sup>.

**4. Understanding the population-based Ig-E associated phenotypes in children and adolescents.**

**4.1 Age effect of maturation.**

- Allergic multimorbidity prevalence was higher among boys than girls especially in earlier childhood; these differences decreased as the participants grew older to smaller or to no differences between males and females<sup>36</sup>.

**4.2 In utero and early-life exposures.**

- Maternal smoking during pregnancy but not latter on was associated with persistent asthma and persistent rhino-conjunctivitis<sup>35</sup>.
- A meta-analysis of PACE cohorts showed that maternal smoking was associated to blood DNA methylation of about 6,000 CpGs some of them linked to genes related to asthma<sup>39</sup>.
- A study in four European and North American birth cohorts showed that NO2 exposure at residential addresses during pregnancy was associated with differential offspring DNA methylation in mitochondria-related genes as well as in genes involved in antioxidant defense pathways<sup>37</sup>.

**4.3 Ethics of the new approaches in MeDALL.**

- A bibliographical analysis together with informed consent forms and questionnaires from MeDALL members showed that there is no agreed policy for communication of research results and incidental findings; a policy was proposed<sup>53</sup>.

pathway has been identified as a common pathway to multimorbidity by *in silico* computational analysis. Several loci significantly associated with allergic diseases have been identified using epigenetics. The mechanisms of allergic diseases are the result of a complex interplay of many different pathways. Environmental exposures are relevant to the development of allergy-related diseases. Methods and tools used in systems biology were applied both to facilitate an effective knowledge management strategy and to integrate the findings of the different approaches. The integration of multimorbidities and polysensitization resulted in a new classification framework of allergic diseases that could help to improve the understanding of genetic and epigenetic mechanisms of allergy as well as to better manage allergic diseases. MeDALL resulted in many different studies covering a wide range of topics related to the mechanisms of allergic diseases in children, the main findings of which are summarized in table 3.

## Members of the MeDALL study

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## DISCLOSURES

Dr. Antó has nothing to disclose. Dr. Bousquet reports personal fees and other from Chiesi, Cipla, Hikma, Menarini, Mundipharma, Mylan, Novartis, Sanofi-Aventis, Takeda, Teva,

Uriach and others from KYomed INNOV, outside the submitted work.

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