Project final report

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Abbreviations

AD: atopic dermatitis
AHA: Active and Healthy Ageing
AIRWAYS ICPs: Integrated Care Pathways for Airway Diseases
AMICS-Menorca: Asthma Multicentre Infant Cohort Study
BAMSE: Barn Allergi Milj. Stockholm Epidemiologi Projektet
Bet v1: *Betula verucosa* 1 allergen 1
BIB: Born in Bradford
Can f1: *Canis familiaris* allergen 1
CCAAPS: Cincinnati Childhood Allergy and Air Pollution Study
CD: Cluster Differentiation
CHICOS: Developing a Child Cohort Research Strategy for Europe, FP7
COPD: Chronic Obstructive Pulmonary Disease
DARC: The Danish Allergy Research Centre
DARC: The Danish Allergy Research Centre
DC: Dendritic cell
EAACI: European Association of Allergy and Clinical Immunology
ECA: Environment and Childhood Asthma
ECRHS: European Community Respiratory Health Survey
EDEN: Etude des Déterminants pré et post natals du développement et de la santé de l' Enfant
EGEA: Epidemiological study on the Genetics and Environment of Asthma, bronchial hyperresponsiveness and atopy
EIP on AHA: European Innovation Parternship on Active and Healthy Ageing
ENRIECO: Environmental Health Risks in European Birth Cohorts, FP7
FcεRI: high affinity receptor for IgE
FcγRIIB: low affinity receptor for IgG
Fel d1: *Felix domesticus* 1 allergen 1
FeNO: Exhaled nitric oxide
FOXP3: forkhead box P3
FP6, FP7: Framework Program for Research and Technological Development 6, 7
GA²LEN: Global Allergy and Asthma European Network
GARD: World Health Organisation Global Alliance against Chronic Respiratory Diseases
GINIplus: German Infant Study on the influence of Nutrition Intervention plus environmental and genetic influences on allergy development study
GWAS: Genome wide association study
IFN-γ: Interferon-gamma
Ig: Immunoglobulin A, E, G
IgE: Immunoglobulin E
IL: Interleukin
IL1RL1: Interleukin 1 receptor-like 1 (ST2)
ILC: Innate lymphoid cell
INMA: Infancia y medio ambiente
IoW: Isle of Wight
ISAAC: International Study of Asthma and Allergies in Childhood
Japan Environment and Children's Study JECS
LISAplus: Influences of Lifestyle-Related Factors on the Immune System and the Development of Allergies in Childhood plus Air Pollution and Genetics
MAAS: Manchester Asthma and Allergy Study
mAb: monoclonal antibody
MAS: German Multicenter Allergy Study
MAS: German Multicenter Allergy Study
MeDALL: Mechanisms of the Development of ALLergy
MHC: major histocompatibility complex
MS: manuscript
NHLBI: National Heart, Lung and Blood Institute of
NIAID: National Institute of Allergy and Infectious Diseases
NIH: National Institutes of Health
ORMDL3: Sphingolipid Biosynthesis Regulator 3
PARIS: Pollution and Asthma Risk: an Infant Study
PCDH1: gene encoding Protocadherin-1
PIAMA: Prevention and Incidence of Asthma and Mite Allergy
RHEA: Mother-Child cohort in Crete
ROBIC: Roma-Bologna birth cohort
SNP: Single nucleotide polymorphism
SPT: skin prick test
ST2: Suppression Of Tumorigenicity 2
TH1: T helper cell 1
TH2: T helper cell 2
TJ: Tight junction
TNFAIP3: Tumor necrosis factor, alpha-induced protein 3
TLR: Toll like receptor
Treg: T regulatory cell
WAO: World Allergy Organization

A- Executive summary

Allergic diseases (asthma, rhinitis and atopic dermatitis) are complex and associated with allergen-specific IgE and non-allergic mechanisms that may coexist in the same patient (multimorbidity). MeDALL (Mechanisms of the Development of ALLergy) generated novel knowledge to propose early prediction, diagnosis, prevention and targets for therapy (1, 2). MeDALL linked epidemiological, clinical and basic research (3), using a novel, stepwise, large-scale and integrative approach combining precisely phenotyped children followed in 14 birth cohorts spread across Europe with systems biology (omics, IgE measurement) and environmental data. The main MeDALL achievements include:

1- Innovative methods

a. **Development of the harmonised MeDALL-Core questionnaire** (in 8 languages) (4), **and a database of pooled cohorts** (5): 44,000 children at birth, 22,000 at 4 yrs, 19,000 at 8 yrs, and 13,000 prospectively followed after puberty. Japan Environment and Children's Study (JECS) uses the MeDALL questionnaire. A joint NIH-MeDALL workshop on birth cohorts (NIAID and NHLBI) was held in September 2012 (3).

b. **Integration in the database** of Omics (23,000 historical GWAS, 9,500 epigenetics, 2,000 proteomics, 750 transcriptomics), IgE micro-arrays (4,000 subjects) and individual environmental data (10,000 children) using land use model.

c. **Development of a new allergen microarray technology:** The "MeDALL allergen-chip" (6-8).

2- Novel classification of allergic diseases: The MeDALL hypothesis (9): IgE sensitization should be considered as a quantitative trait. Allergic multimorbidities and IgE polysensitization are associated to the persistence/re-occurrence of foetal Type 2 signalling.

3- Novel findings

a. **Multimorbidity:** Multimorbidity is more common than expected by chance alone suggesting that the diseases share causal mechanisms (hypothesis-driven (10) and unsupervised cluster analyses (11)). IgE sensitisation, independently associated, accounts for 38% of multimorbidity.

b. **Mono and polysensitization:** In the BAMSE cohort (Sweden) the same 825 children were tested at 4, 8 and 16 years. Mono and polysensitization represent two different phenotypes of IgE-associated diseases. These results were confirmed in the MeDALL cohorts (8).

c. **Prediction of persistence of allergic diseases at 4 years:** Polysensitization and multimorbidity at 4 yrs predicts the persistence of allergy later in life.

d. **Prediction of onset of new allergic diseases at 4 years:** Polysensitization at 4 yrs predicts the onset of new allergic diseases later in life.

e. **The Type 2 signalling pathway** has been identified as a common pathway to multimorbidity by *in silico* computational analysis.

f. **Several loci** significantly associated with allergic diseases have been identified using epigenetics . One pathway could be identified using epigenetics and proteomics. An integrative genomics approach identified new asthma pathways related to air pollution exposure.

g. To complement mechanistic experimental studies in children, MeDALL included *in vitro* and animal *in vivo* studies (12-20) and studies in biodiversity (21-25)

4- Clinical impact: Many MeDALL data have been translated into clinical practice. A meeting at the European Parliament was organized by EFA concluded the project (May 27, 2015). MeDALL results improve the stratification of allergic preschool children for diagnosis, prognosis, allergen-specific immunotherapy. Multimorbidity and/or IgE polysensitization are markers of persistence of disease. MeDALL was used as a model of systems medicine (26, 27).

5- Ethics: Relevant ethical and societal issues are being internationally debated within MeDALL. The communication of results and disclosure of incidental findings in longitudinal paediatric research has been another innovative MeDALL initiative (28).

6- Dissemination to the European Parliament (29) and the European Innovation Partnership and Active and Healthy Ageing (30) (Action Plan B3) (31, 32).

B- Summary description of the project context and objectives

B1- Project context

Allergic diseases (asthma, rhinitis and eczema) (33) represent a global health problem increasing in prevalence and severity. An epidemic of IgE-associated allergic diseases has occurred over the past decades in all parts of Europe (34). They are complex and associated with allergen-specific IgE and non-allergic mechanisms that may coexist in the same patient (multimorbidity). IgE-associated allergic diseases are multifactorial disorders, with both genetic and environmental components. These interactions are likely to start *in utero* and during perinatal development, and then develop in infancy and childhood (35). Allergic diseases are not separate diseases but are linked by complex and currently insufficiently-defined inter-relationships which occur during childhood and persist throughout life. A better understanding of these links at the clinical and mechanistic levels was proposed to be of importance.

B2- General objectives proposed for MeDALL

MeDALL (Mechanisms of the Development of ALLergy; EU FP7-CP-IP; Project No: 261357; 2010-2015) was launched to generate novel knowledge on the mechanisms of initiation of allergy from early childhood to young adulthood (1, 2). MeDALL linked epidemiological, clinical and basic research (3). It was based on a novel, stepwise, large-scale and integrative approach led by a network of complementary experts in allergy, epidemiology, allergen biochemistry, immunology, molecular biology, epigenetics, functional genomics, bioinformatics, computational and systems biology. The following steps were proposed to be developed during the project:

1- Identification of "classical" and "novel" phenotypes in existing birth cohorts
2- Building discovery of the relevant mechanisms in IgE-associated allergic diseases in existing longitudinal birth cohorts
3- Validation and redefinition of classical and novel phenotypes of IgE-associated allergic diseases and
4- Translational integration of systems biology outcomes into healthcare, including societal aspects.

MeDALL was proposed to lead to:

- A better understanding of allergic phenotypes, thus expanding the current knowledge of the genomic and environmental determinants of allergic diseases in an integrative way.
- Novel diagnostic tools for the early diagnosis of allergy, targets for the development of novel treatment modalities and prevention of allergic diseases.
- Improving the health of European citizens as well as increasing the competitiveness and boosting the innovative capacity of Europe, whilst addressing global health issues and ethical issues.

B3- Specific objectives

WP2

1. To advance the understanding and identification of the IgE-associated phenotypes (**Task 2.1**).
2. To re-define the different IgE-associated allergic diseases and their phenotype (called classical phenotypes), from birth to adolescence, by consensus among experts (**Task 2.2**).
3. To perform a hypothesis-free phenotypical analysis of allergic diseases on MeDALL birth cohorts using new types of modelling (eg cluster analysis, and scale-free network analysis) (**Task 2.3**).
4. To systematically make literature-based knowledge about allergy available as semantically structured resource applicable to computational analysis and reasoning (**Tasks 2.1 & 2.2**).
5. To design a new standardized examination of the phenotype of allergy-associated diseases to be performed in the follow up of the included birth cohorts (**Tasks 2.3 & 2.4**).
6. To validate the molecular fingerprints obtained in the WPs 3-7 and 9 as markers and predictors of both the classical and the novel phenotypes (**Task 2.4**).

7. To perform an epidemiological validation by comparing the associations between the risk and preventive factors with the classical and the novel phenotypes (**Task 2.4**).

WP3

1. To build a common database of recent ongoing longitudinal birth cohorts in allergy (**Task 3.1**).
2. To use the same protocol to harmonize phenotype definition at follow up (**Task 3.2**).
3. To obtain a large set of cases of early-life allergy in different European environments to assess gene-environment interactions and predictors of high risk groups (**Task 3.3**).
4. To assess protecting or inducing risk factors of early-life allergic phenotypes in infants and young children in order to assess the role of exposures during pregnancy and early life (**Task 3.4**).
5. To provide biological repository material collected during pregnancy and at birth and newly collected material for the genomic and immunologic assays (**Tasks 3.5 & 3.6**).

WP4

1. To perform a common and standardized follow-up assessment with adolescents of the largest and longest-running, high-quality birth cohort studies on asthma and allergies (**Tasks 4.2, 4.3 & 4.4**).
2. To build a common database of these studies with existing and with newly collected data for pooled and meta-analyses for higher statistical power than individual studies analyses (**Tasks 4.1 & 4.5**).
3. To evaluate the influence of peri-natal, early and late childhood environment and gene-environment interactions on the initiation of late-onset and persistent allergic responses (**Task 4.6**).
4. To specifically address the role of gender in the prevalence of allergic diseases by identifying factors that are associated with the sex-switch from a male predominance of allergies in childhood to a female predominance in adolescence and young adulthood (**Task 4.7**).

WP5

1. To include respiratory and food allergens relevant to the call and which are not yet characterized by IgE immunoscreening of cDNA libraries prepared from the allergen sources (**Task 5.1**).
2. To establish an allergen microarray containing the most relevant respiratory and food allergens (170) to measure the levels of specific IgE, IgG subclass (**Task 5.2**).
3. To study if allergen-specific antibody response profiles can be used to predict the development of clinical manifestations of allergic disease or the absence of disease (**Tasks 5.3 & 5.4**).
4. In a subsample of birth cohorts where maternal and cord blood is available, to study if the placental transfer of allergen-specific IgG antibodies may protect from allergic sensitization (**Task 5.3**).
5. To assess how allergen-specific IgE and other isotypes affect basophil degranulation using cultured rat basophils expressing the high affinity receptor for human IgE (**Task 5.5**).
6. To search for and determine factors which affect skin sensitivity to Bet v1 (**Task 5.6**).

WP6

1. To identify methylation sites in a panel of allergy related genes and biomarkers through consultation of currently available annotated databases (see WP 10) (**Task 6.1**).
2. To perform a genome-wide methylation status at birth and age 4 in children with the phenotypes defined in WP2. To assess persistence of methylation patterns during childhood, and find differentially methylated genes and assess environmental interaction (e.g. family history of atopy, smoking, nutrition during pregnancy, smoking air pollution and nutrition in childhood) (**Task 6.2**).
3. To design a custom made methylation array based on the candidate genes (identified in 1) and top hits identified by genome-wide methylation (identified in 2) (**Task 6.3**).
4. To define fingerprints of methylation in all available subjects from Karelia, now living in Finland and Russia (custom made array, designed under 3) (**Task 6.4**).
5. To compare the methylation status of allergy related genes (fingerprints) in a longitudinal way, by comparing methylation status at birth and at age 4 and 16 years in children followed up in birth cohorts according to the different phenotypes defined in WP2, and assess environmental interaction (**Task 6.5**).
6. To assess whether the risk of IgE-associated symptomatic diseases (e.g. eczema, rhinitis and asthma) increases by gene methylation and by subsequent methylation changes in addition to environmental exposures later in childhood (**Task 6.5**).

7. To analyse a set of serum biomarkers (proteins) in the different phenotypes defined in WP2 to find fingerprints and confirm them in birth cohort follow up studies (**Tasks 6.6 & 6.7**).
8. To analyse a set of serum biomarkers (proteins) in relation to methylation of specific genes and perform gene-methylation-biomarker-phenotype interaction (**Task 6.8**).

WP7

1- Establish the knowledge management and bioinformatics infrastructure necessary to achieve MeDALL objectives in relation with U-BIOPRED (**Tasks 7.1 & 7.2**).
2- Perform unbiased discovery of allergy phenotype biomarkers of severe asthma in birth cohorts using transcriptomics fingerprints and integrated network phenotype handprints (**Tasks 7.3, 7.4 7.5**).
3- Validate the fingerprints and handprints in birth cohort follow-up for disease progression, therapeutic interventions, and for improvement of human and animal models (**Task 7.6**).

WP8

1- To get a clear picture on how epigenetic mechanisms interfere with the host-environment interactions at the origin of allergic sensitization (**Task 8.1**).
2- To get a clear picture of the impact of microRNA (miRNA) as a mechanism of gene regulation in HDM driven asthma in both mice and humanized mice. Validation platform (**Task 8.2**).
4- To validate critical environmental pathways that emerge from the other consortium members and that either protect from or promote allergic type inflammation (**Task 8.3**).
5- To validate 2 therapeutically applicable targets in the humanized SCID model of asthma that will be selected based on consortium discoveries (**Task 8.4**).
6- To generate transgenic mice for Cre-inducible overexpression of the two major isoforms of the asthma susceptibility gene encoding Protocadherin-1 (Pcdh1) (**Task 8.5**).

WP9

1- To better understand the immunologic mechanisms in the initiation of allergic diseases, molecular and cellular mechanisms leading to either a healthy or an allergic immune response will be investigated (**Task 9.1**).
2- To investigate the mechanisms of allergen-specific T cell tolerance (**Tasks 9 2 & 9.3**).
3- To define molecular mechanisms of allergen-specific T cell and B cell regulation. More specifically, we will investigate the following focused areas of research: a) the role of functional balance between Th2 cells and T regulatory cells and molecular mechanisms of allergen-specific T cell tolerance; b) demonstration of the role of B regulatory cells and molecular mechanisms in their development, and their role in allergen tolerance (**Tasks 9.3 & 9.4**).
4- To investigate the interaction of epithelium and T cell subsets (**Task 9.3**).

WP10

1- Identify new opportunities for early diagnosis (including biomarkers: threshold levels for disease expression using classical and novel phenotypes) (**Task 10.1**).
2- Characterize risks groups in the population, leading to risk charts for allergic diseases (**Task 10.2**).
3- Define novel approaches for prevention addressing the relevant environmental exposures to lead to national preventive plans (**Task 10.3**).
4- Integrate all gender related effects reported in MeDALL (**Task 10.5**).
5- Identify innovative targets for therapy. In particular this will focus on patients with severe allergic diseases, currently usually insufficiently controlled by medication, but also in terms of possible targets for common presentation of allergic diseases (**Task 10.4**).
6- Estimate the MeDALL research outcomes into societal and economic impacts in the European context (**Task 10.6**).

WP11

1. Disseminate the results of MeDALL and educate health care professionals and patients using the GA^{2}LEN network, in collaboration with EFA and EAACI (**Task 11.2**).
2. Promote integration and exchanges within and outside the MeDALL community (**Task 11.3**).
3. Manage intellectual property issues arising from the project (**Task 11.1**).

4. Help to make policies at the EU level on best care and prevention for allergic diseases (**Task 11.4**).

WP12

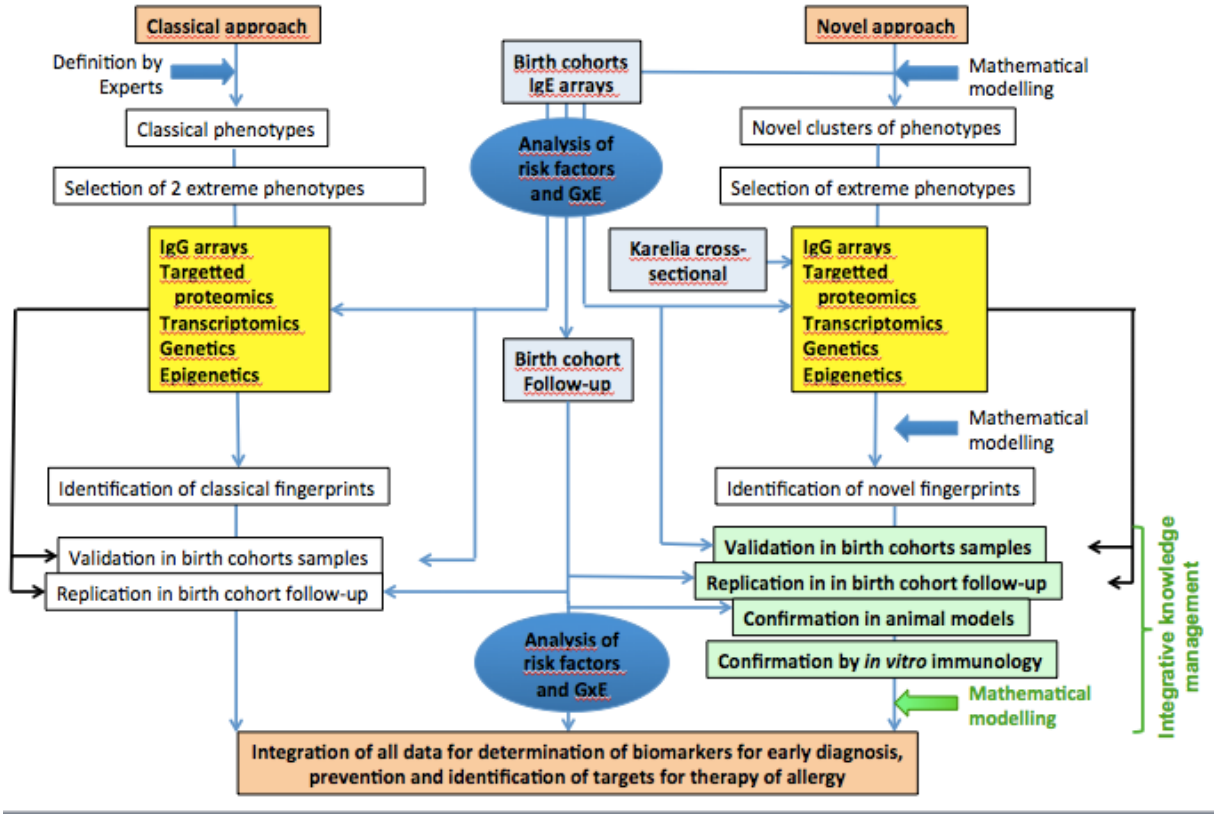
1. Ensure that all ethical aspects of the planned activities are fulfilled (**Tasks 12.1 & 12.2**).
2. Provide the Commission with ethical authorisations prior to begin each part of the project (**Task 12.1**).
3. Coordinate the Ethical Advisory Board meetings and reports (**Task 12.1**).
4. Analyse the new ethical dimensions raised by large scale studies involving genomics, environment and epigenetic studies in cohorts of children, especially regarding the incidental findings, the scope of the consent/assent, the access to results, the privacy and confidentiality issues as personal information accumulates (**Task 12.2**).
5. Analyse the specific issues relevant for participation of children in MeDALL (**Task12.2**).
6. Organise as appropriate internal debates and training session within the Consortium (**Task 12.3**)

B4- Integrative strategy of MeDALL

The strategy of MeDALL was based on information and samples (in repository and obtained during the project) obtained from a large network of existing birth cohorts. Birth cohorts were stratified into younger and older cohorts depending on the length of study. Classical and novel phenotypes of IgE-associated diseases were established. A wide range of mechanistic studies were built up on the samples provided by the birth cohorts. 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 mechanistic approaches (Figure 1).

The identification of classical phenotypes was based on experts' criteria following a review of the literature aiming at the definition of IgE-associated allergic diseases. To identify the novel phenotypes, the children from birth cohorts were analysed using hypothesis-free methods by cluster analysis.

Figure 1: The MeDALL approach proposed for the grant application (from (1))



C- Main S&T results/foregrounds

C1- Knowledge on phenotypes of allergic diseases at the initiation of MeDALL (WP2)

Allergic diseases are defined according to established criteria including allergen symptoms, specific IgE, skin tests to allergens and family history of allergy. In presence of respiratory, nasal, skin, digestive and/or systemic symptoms, these criteria allow the definition of IgE-associated allergic diseases as separate entities. However, the diagnosis and treatment of allergic diseases as different entities is complicated because none of the above criteria are either necessary or sufficient alone (Pinart et al., MS submitted). Although allergic diseases are currently approached as separate entities, each of them involves substantial heterogeneity. The reasons for such heterogeneity are unclear and its understanding is critical to explore novel avenues for diagnosis, prevention and treatment.

1. Review of the literature on the phenotypes and course (from infancy to young adulthood) of IgE-associated allergic diseases (Task 2.1)

There is a large heterogeneity on allergic phenotypes and a systematic review on phenotype classification, multimorbidity and its defining criteria has been conducted (Pinart et al., MS submitted). We searched MEDLINE up to December 2012 to identify relevant original studies published in English in subjects aged 0-18 years. The screening of titles, abstracts and data extraction were conducted independently by two reviewers. From a total of 13,767 citations, 197 met the criteria for inclusion, of which 54% were cohorts.

Allergic diseases were studied as a single entity (55%; 109/197) or as multimorbidity (45%). Asthma accounted for 81.7% of the studies examining single diseases. Overall, up to 33 different phenotypes of allergic diseases were reported, and transient early wheeze, late-onset and persistent wheeze were the most frequently reported phenotypes. Most studies (78%) used questionnaires. Skin prick test was the preferred measurement of sensitisation (64%). Spirometry and bronchial hyperresponsiveness (BHR) were assessed in one third of the studies, whereas peak flow rate was used in 8.6%.

The knowledge on multimorbidity was mostly restricted to links between asthma and rhinitis. ARIA (Allergic Rhinitis and its Impact on Asthma (36, 37)) had proposed and confirmed the links between asthma and rhinitis with a primary disease (usually rhinitis). The definition of co-morbidity was then suggested (Table I).

Table I: Definition of co-morbidity and multimorbidity

- Co-morbidity is the presence of one or more additional diseases co-occurring with a primary disease or the effect of such additional disorders or diseases.
- Multimorbidity is a term which means co-occurring diseases in the same patient without a knowledge of the primary disease.

The Atopic March is unclear and only some of the young children with eczema develop asthma and rhinitis subsequently, whereas others have two or the three diseases at the same time and others have a different response.

Disease severity was assessed in 35% of the studies.

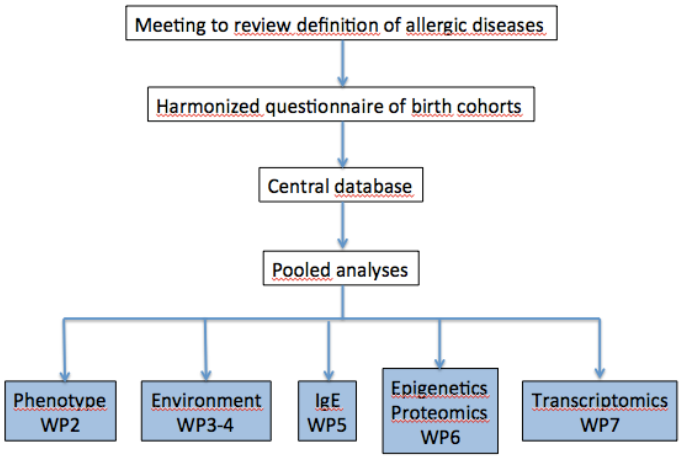
The meta-analysis showed the gaps in our knowledge, in particular for co or multi-morbidities. Studies reporting phenotypes of allergic diseases in children are highly heterogeneous and often lack objective phenotypical measures. A concerted effort to standardize methods and terminology is necessary.

2. Expert meeting for the definition of IgE-associated allergic diseases (Task 2.2)

Definitions of current eczema, current rhinitis and current asthma with questionnaires were agreed upon by a panel of experts - members of MeDALL and invited external participants. This meeting was convened in collaboration with the WHO Collaborating Center on Asthma and Rhinitis (Montpellier). A modified version of the GA^{2}LEN (Global Allergy and Asthma European Network) questionnaire

was proposed to define current asthma (38) and questions of the ISAAC (International Study of Asthma and Allergies in Childhood) to define current rhinitis and current eczema (39). These questions were further used to combine existing birth cohorts (Figure 2).

Figure 2: MeDALL flow



- **There were wide gaps in our knowledge concerning the phenotypes of allergic diseases.**
- **A wide heterogeneity existed for the definition of allergic diseases.**
- **Multimorbidity was insufficiently understood. Only links between rhinitis and asthma were defined but their mechanisms poorly understood.**
- **The definition of asthma, rhinitis and eczema was proposed by an expert meeting. These definitions were used for the harmonization of birth cohort questionnaires and the database.**

C2- Combining data of existing birth cohorts (WP3-4)

Several existing birth cohorts have already collected data on asthma and allergies since birth or even in pregnancy. Using their data in combined analysis is challenging, as data were collected in different languages, at varying age periods, by self-reports or study doctor assessments, with differently worded questions and in varying data formats. The goal of MeDALL was to use the wealth of already existing European birth cohort data (Table II) and facilitate the use of historical and newly-generated data in a harmonized format for future combined analyses and to perform some pooled analyses on gene-environment interactions and on gender (3, 5).

Table II: Birth cohorts included in MeDALL (from Bousquet et al (1))

Acronym of Birth Cohort	Name of Birth Cohort	Country	Recruitment of subjects started in	Number recruited at birth	Age at new examination (in 2012-2013)
AMICS-Menorca** (40)	Asthma Multicentre Infant Cohort Study	Spain	1997-1998	482	15-16 yr
BAMSE** (41)	Children, Allergy, Milieu, Stockholm	Sweden	1994-1996	4,089	16-18 yr
BIB* (42)	Born in Bradford	UK	2007-2009	1,000	4-6 yr
DARC** (43)	Danish Allergy Research Council	Denmark	1998-1999	562	14-15 yr
ECA** (44)	Environment and Childhood Asthma	Norway	1992-1993	3,754	16-17 yr (in 2009; no field work in MeDALL)
EDEN* (45)	Etude des Déterminants pré et post natals du développement et de la santé de l' Enfant	France	2003-2005	1,900	8-10 yr
GINI** (46)	German Infant Nutritional Intervention-Study	Germany	1995-1998	5,991	16-18 yr
INMA* (47)	Infancia y medio ambiente	Spain	2004-2008	2,500	5-8 yr

Acronym of Birth Cohort	Name of Birth Cohort	Country	Recruitment of subjects started in	Number recruited at birth	Age at new examination (in 2012-2013)
LISA** (48)	Lifestyle-related Factors on the Immune System and Development of Allergies. In Childhood	Germany	1997-1998	3,097	15-16 yr
PARIS* (49)	Birth cohort in Paris	France	2005	2,700	8 yr
PIAMA-NHS** (50)	Prevention and Incidence of Asthma and Mite Allergy - Natural History Study	NL	1996-1997	3,291	16-17 yr
RHEA*(51)	Mother-Child cohort in Crete	Greece	2007-2008	1,500	7-8 yr
ROBIC* (52)	Roma-Bologna birth cohort	Italy	2003-2004	1,400	9-10 yr
Total	***: Younger cohorts ****: Older cohorts**			**22,900 21,127**	

1. Harmonized definition and web-based questionnaire across all studies: MeDALL Core Questionnaire (Tasks 3.2, 4.2 and 4.3)

A key part of WP3 and WP4 was the newly developed standardized MeDALL Core Questionnaire that included a number of previously validated and widely used questions (WP2.2). All cohorts agreed on two core questionnaires, one for the participants and one for the parents (4).

Numerous birth cohorts have been initiated in the world over the past 30 years using heterogeneous methods to assess the incidence, course and risk factors of asthma and allergies. The aim of the tasks was (i) to provide the stepwise proceedings of the development and current version of the harmonized MeDALL-Core Questionnaire (MeDALL-CQ), used prospectively in 11 European birth cohorts. The harmonisation of questions was accomplished in 4 steps:

- Collection of variables from 14 birth cohorts.
- Consensus on questionnaire items.
- Translation and back-translation of the harmonized English MeDALL-CQ into 8 other languages (Danish, Dutch, French, German, Greek, Italian, Swedish and Spanish: paper and e-questionnaire) by professional translators and/or cohort members.
- Implementation of the harmonized follow-up.

The MeDALL Core Questionnaires contain eight sections with items on asthma, allergic rhinitis, eczema, food allergy and nutrition, indoor environment (dampness/mould, tobacco smoke exposure, air pollution), lifestyle factors (physical activity, smoking, alcohol and drugs), and puberty development. The questionnaires are available free of charge for other research teams.

Three harmonized MeDALL-CQs (2 for parents of children aged 4-9 and 14-18, 1 for adolescents aged 14-18) were developed and used for the historical assessment of data and a harmonized follow-up assessment of 11 European birth cohorts on asthma and allergies with over 13,000 children. The harmonized MeDALL follow-up produced more comparable data across different cohorts and countries in Europe and offered the possibility to verify the results of former cohort analyses.

Thus, MeDALL can become the starting point to stringently plan, conduct and support future common asthma and allergy research initiatives in Europe and elsewhere. The MeDALL questionnaire was presented during a MeDALL/NIH workshop in Bethesda (3). It is now used in one US cohort (CCAPS) and the Japanese national birth cohort (K Yamamoto, M.D., Division of Allergy, Department of Medical Specialties, Medical Support Center for Japan Environment and Children's Study, National Center for Child Health and Development, Tokyo, Japan, MS in preparation).

2- Central database (Tasks 3.1, 4.1 and 4.5).

Another key part of WP3 and WP4 is the development of a central database for a common storage of the birth cohort data (harmonized questionnaires and biologic samples) from the new as well as the historic follow-up assessments. Seven of the Work Package 4 birth cohorts stored data of up to 20 follow-ups in this database. The pseudo-anomyseddata in the central MeDALL database belongs to

the corresponding birth cohorts, which have to be contacted prior to each analysis and agree to data access and use (Table III).

The central MeDALL database was developed and maintained by BIOMAX. This database is operational and used by the scientists by MeDALL allowing combined analyses with the data of several cohorts. The database includes clinical information from 44,010 participants, IgE-chip data from 3,292 experiments, epigenetic data from 2,173 experiments, biomarker data from 1,427 experiments and transcription data from 723 experiments. In addition sample information about more than 30,000 available blood, plasma, serum, DNA, RNA and Leukocyte samples has been collected. Data has so far been provided to 6 different partners for use in over 10 different analyses.

Table III: Pooled MeDALL database

- Partner 3 (MAS): data from up to 1314 participants of 20 follow-ups
- Partner 2 (AMICS-M): data from up to 482 participants of 12 follow-ups
- Partner 9 (ECA): data from up to 3754 participants of 6 follow-ups
- Partner 12 (BAMSE): data from up to 4089 participants of the 0, 1, 2, 4, 8 and 16-year follow-up for questionnaires and the 4, 8 and 16 year follow-up for clinical investigations
- Partner 13 (LISA): data from 3095 participants of 9 follow-ups
- Partner 13 (GINI): data from 5991 participants of 8 follow-ups
- Partner 14 (PIAMA): data from up to 3963 participants of the birth, 3months, 1, 2, 3, 4, 5, 6, 7, 8, 11, 14 and 16 years follow-up for questionnaires and the 1, 4, 8, 12, and 16 year follow-up for medical exams
- Partner 22 (DARC): data from up to 562 participants of 9 follow-ups

3- Prospective cohort with the MeDALL Core e-questionnaire and SOPs (standard operative procedures) (Tasks 3.2, 4.2 and 4.3)

A prospective cohort has been established in MeDALL to follow children. All cohorts have completed the field work, with the exception of the EDEN birth cohort which provided repository data only (Table IV).

Table IV: MeDALL follow up study

AMICS-M, adolescents aged 14 years
- 264 blood samples (serum, plasma and buffy coat aliquots)
- 312 urine samples
- 327 measures of spirometry and anthropometry and blood pressure
- 347 Adolescent Core Questionnaires sent (292 fully completes and others partially completed)
- 347 Parental Core Questionnaires sent (300 fully completed and others partially completed)

LISAplus, complete cohort, adolescents aged 14-16 years
- 1033 blood samples
- 1471 Adolescent Core Questionnaires
- 1721 Parental Core Questionnaires

GINIplus, complete cohort, adolescents aged 14-16 years
- 1901 serum, plasma, PaXgene, blood
- 2665 Adolescent Core Questionnaires

BAMSE, adolescents aged 16-17 years
- 2605 clinical investigations
- 2549 blood samples
- 2312 spirometry
- 2273 NO
- 3115 Adolescent Core Questionnaires
- 3181 Parental Core Questionnaires

DARC

- 371 spirometry
- 371 clinical investigations
- 380 Adolescent Core Questionnaires
- 380 Parental Core Questionnaires

PIAMA, adolescents aged 14-16 years

- 802 clinical investigations, 753 serum, 745 DNA (age 15-16 years)
- 2531 Adolescent Core Questionnaires age 14-15 years
- 2350 Parental Core Questionnaires age 14-15 years

The MeDALL follow-up assessment included not only questionnaire data, but also physical examinations and clinical tests at the individual study centres with additional (optional) tests: fractional exhaled nitric oxide (FeNO) breath test, spirometry and blood sampling. To ensure standardized assessments across all cohorts, common MeDALL SOPs for clinical investigations were developed in English and then translated into five other languages (Danish, Dutch, German, Swedish, and Spanish).

- **A harmonized e-questionnaire has been developed for inter-operable historical and prospective data.**
- **A central database using the harmonized e-questionnaire and biological data is operational.**
- **Pooled analysis done with harmonized data across cohorts allows a higher statistical power and a greater geographic diversity than individual cohorts.**
- **However, the sample size for some multimorbid subjects with asthma, rhinitis and eczema is small and may not be sufficient for some analyses.**

C3: Phenotypes of allergic diseases demonstrated in MeDALL (WP 2)

1. Hypothesis-driven analysis of the phenotype of allergic diseases in birth cohorts (Task 2.3).

Eczema, rhinitis and asthma often coexist (multimorbidity), but the proportion of multimorbidity attributable to chance or to IgE sensitisation was unknown. In 16,147 children aged 4 years and 11,080 aged 8 years, the classical (hypothesis-driven) approach showed that multimorbidity occurs more often than expected by chance, suggesting that these diseases share causal mechanisms. IgE sensitisation at age 4 years explained 38% of multimorbidity at age 8 years, suggesting that IgE sensitisation is not the dominant causal mechanism of multimorbidity for these diseases at this age (10). The trajectories of allergic diseases depend on multimorbidity (Table V). Children with multimorbidities at 4 years had an increased risk of having persistence of symptoms at 8 years including multimorbidity.

Table V: Risk of multimorbidity at age 8 years according to presence of disease at 4 years by log-linear model (Pinart et al, (10))

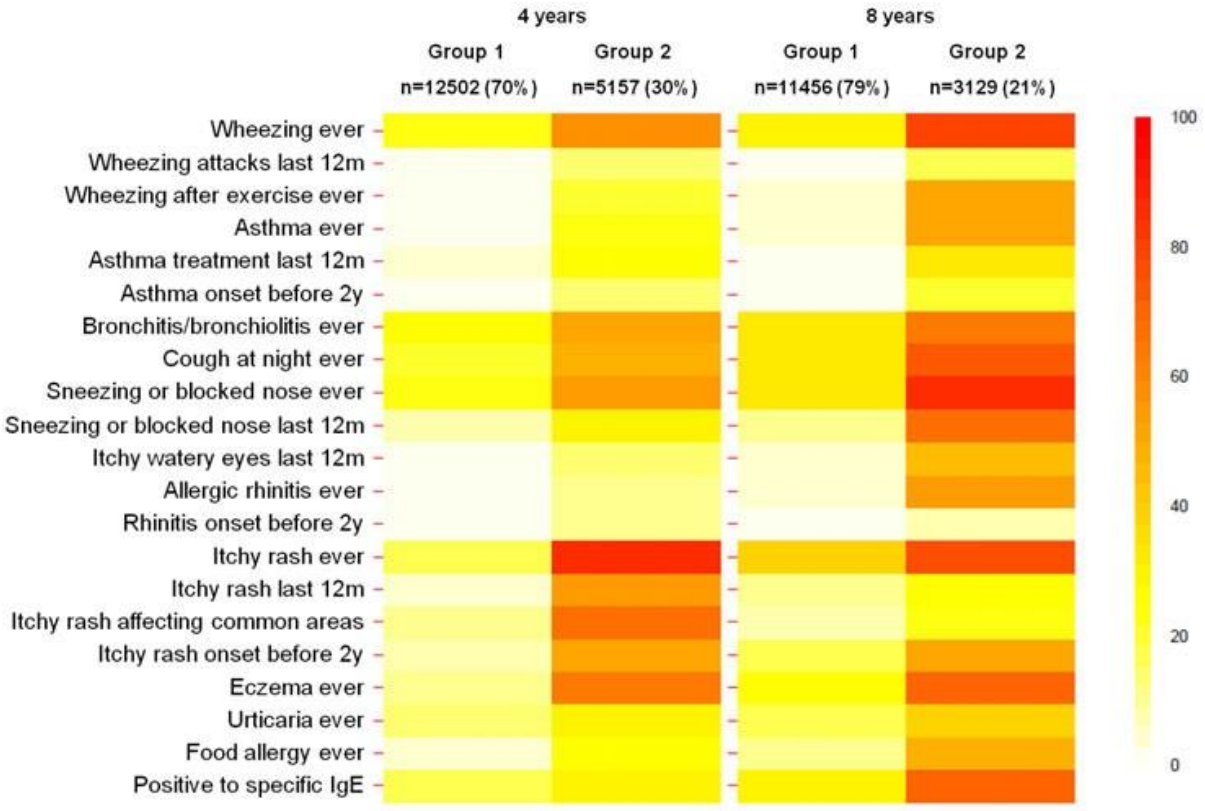
	All children (N=8366)*			Children with IgE sensitisation (N=1587)			Children without IgE sensitisation (N=3423)		
	n at 4 years	n at 8 years (%)	RR (95% CI)	n at 4 years	n at 8 years (%)	RR (95% CI)	n at 4 years	n at 8 years (%)	RR (95% CI)
None	6839	102 (1·5%)	1	1128	60 (5·3%)	1	2910	18 (0·6%)	1
Asthma only	316	59 (18·7%)	12·5 (9·3–16·9)	84	35 (41·7%)	7·8 (5·5–11·2)	133	12 (9·0%)	14·6 (7·2–29·7)
Rhinitis only	110	28 (25·5%)	17·1 (11·7–24·8)	36	16 (44·4%)	8·4 (5·4–13·0)	40	5 (12·5%)	20·2 (7·9–51·8)
Eczema only	859	85 (9·9%)	6·6 (5·0–8·8)	224	46 (20·5%)	3·9 (2·7–5·5)	296	13 (4·4%)	7·1 (3·5–14·4)
Asthma and rhinitis	34	22 (64·7%)	43·4 (31·7–59·4)	15	13 (86·7%)	16·3 (11·9–22·4)	6	2 (33·3%)	53·9 (15·9–182·8)
Asthma and eczema	107	65 (60·8%)	40·7 (31·9–52·1)	45	34 (75·6%)	14·2 (10·6–19·1)	27	10 (37·0%)	59·9 (30·5–117·5)
Rhinitis and eczema	63	34 (54·0%)	36·2 (26·8–48·8)	32	19 (59·4%)	11·2 (7·7–16·3)	8	6 (75·0%)	121·3 (65·9–223·2)
Asthma, rhinitis, and eczema	38	36 (94·7%)	63·5 (51·7–78·1)	23	23 (100%)	19·7 (15·4–25·2)	3	2 (66·7%)	107·8 (42·8–271·3)

RR=relative risk. The log-linear models included children who had two or three diseases and those who had none at age 8 years. Children with one disease at 8 years were excluded from the model. *3356 children had no information available for serum-specific IgE test results.

2. Wide hypothesis-free (data-driven) analysis of the phenotype of allergic diseases in birth cohorts (Task 2.3).

In 17 209 children at 4 years and 14 585 at 8 years, cluster analysis (novel approach) (11) confirmed the results and showed that asthma, rhinitis and eczema are more accurately classified together, as an allergic multimorbidity cluster, than as three independent diseases. Two groups were identified as the optimal way to cluster the data at both age periods and in all sensitivity analyses (**Figure 3**). The first (reference) group at 4 and 8 years (including 70% and 79% of children, respectively) was characterised by a low prevalence of symptoms and sensitisation, whereas the second (symptomatic) group exhibited more frequent symptoms and sensitisation. 99% of the children with multimorbidities were found in the symptomatic group at both ages. The strength of MeDALL is that only pooled data of cohorts made it possible to have a statistical power to analyse the data.

Figure 3: Prevalence* of symptoms of asthma, rhinitis and eczema according to the 2 groups identified in cluster analysis, at 4 and 8 years (Garcia-Aymerich et al (11)).



** Each coloured line represents a variable, whose prevalence ranges from 0% (white colour) to 100% (red colour).*

The results of WP2.3 can be of great value in the design of (i) current predictive models as an opportunity for early diagnosis, (ii) healthcare strategies to manage multimorbidity and (iii) future clinical studies for the discovery of new targets for co-morbid allergic diseases and novel approaches for intervention. Future research including the analysis of phenotypes depending on the MeDALL hypothesis and time-repeated assessments and biological data will help to understand the interrelationships between these diseases. However, these results apply to children at 4 and 8 years and the same study should be done at a later age.

3. Novel analysis of phenotypes assessing the IgE response as a quantitative trait (9)

Using the same MeDALL database we aimed at finding whether IgE sensitization is a quantitative trait with different degrees of response being associated to different phenotypic profiles (9) in 4 and 8-year old children (Bousquet et al, in preparation). We found that polysensitization, as compared to non-sensitization and to monosensitization is associated to:

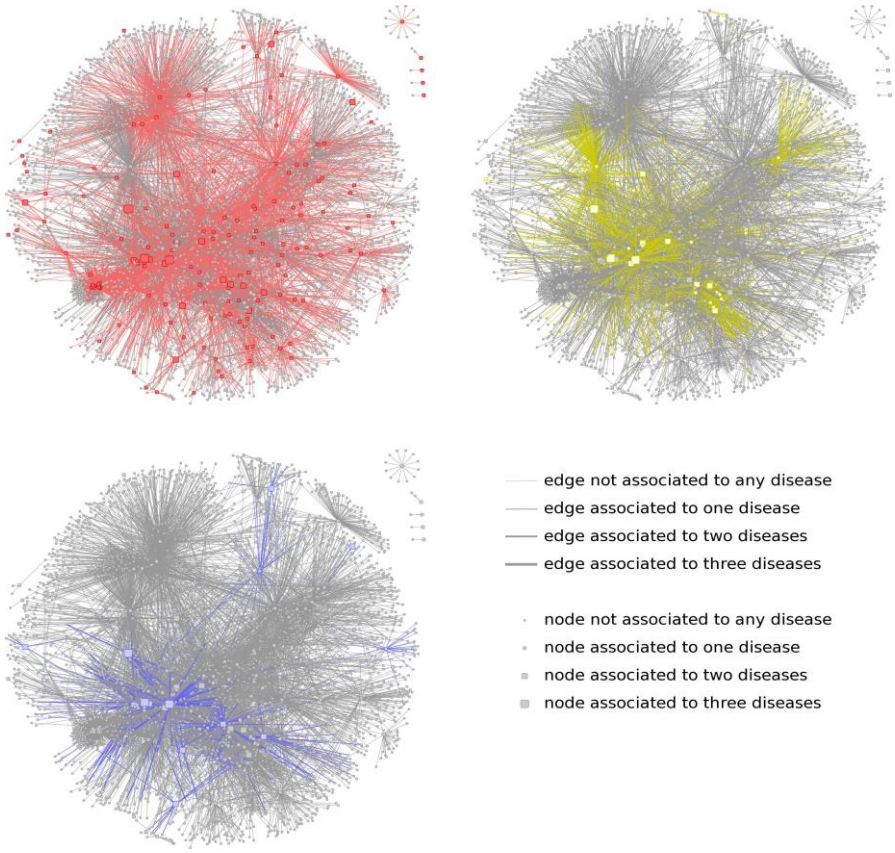
A higher frequency of family history of allergy (asthma and rhinitis). This has been confirmed in the MAS study at 20 years (53).

- A higher prevalence of asthma and rhinitis symptoms.
- A higher prevalence of multimorbidity.
- For those IgE sensitized, a higher level of specific IgE and a higher level of total IgE.
- The persistence of allergic diseases with a lower probability of remission of IgE sensitization and clinical allergy.

4. Epidemiological validation of classical and novel phenotypes (Task 2.4)

To address the biological plausibility of multimorbidity at molecular level, we attempted to identify common proteins and cellular processes associated to the three diseases using computational analysis of the topology of the protein interaction network (Aguilar et al, submitted). We performed a network-based analysis of the molecular mechanisms of asthma, eczema and rhinitis, which allowed us to identify molecular mechanisms and predict disease-associated proteins. Multimorbidity shares a significant number of associated proteins and a significant degree of network connectivity, which cannot be explained solely by their immunity-related nature (**Figure 4**). We identified cellular pathways shared by the 3 diseases, which represent candidate mechanisms for multimorbidity. Finally, we used a network-based algorithm to generate a ranked list of potential comorbidity-associated proteins.

Figure 4: Proteins associated to asthma, eczema and rhinitis in the Functional Interaction Network.



Fraction of the Functional Interaction Network comprising the proteins associated to the diseases under study. Nodes are proteins, edges represent functional interactions between them. Red: asthma-associated proteins and interactions. Yellow: eczema-related proteins and interactions. Blue: rhinitis-related proteins and interactions. The number of diseases associated to a node is represented by the node size. The number of diseases associated to an edge is represented by the edge width

Type 2 pathways are the only ones common to the three diseases (Aguilar, submitted). This network-based analysis represents to our knowledge: (i) the first systematic description of the molecular mechanisms underlying the observed multimorbidity between the three main allergic diseases, and (ii) the first set of predictions of cellular pathways and individual proteins related to the multimorbidity

process. These results confirm the MeDALL hypothesis and are valuable in the design of future clinical studies, as they can lead to the discovery of new targets for co-morbid allergic diseases and novel approaches for intervention.

- **Pooled analyses of birth cohorts are needed to have a sufficient statistical power for the analysis of phenotypes.**
- **The studies suggest that allergic multi-morbidities are more precisely describing the phenotypes than comorbidities since the primary disease is not known. A study at an earlier age may be needed.**
- **Hypothesis driven (classical) and data driven (cluster analysis, novel) approaches showed that allergic multi-morbidities are more common than chance and that IgE sensitization could explain 38% of multimorbidity in children at 8 years.**
- **Multimorbidity is associated with persistence of allergic diseases between 4 and 8 years.**
- **IgE sensitization is a qualitative and a quantitative trait with different mono-sensitization and poly-sensitization phenotypes.**
- ***In silico* analysis suggests that Type 2 signalling pathways are associated with multimorbidity of asthma, rhinitis and eczema.**
- **These studies suggest that multi-morbidity and poly-sensitization represent extreme allergic phenotypes possibly associate with Type 2 signalling pathways.**

C4- Role of the environment in the initiation of early-onset allergy and of persistent allergic diseases (WP 3-4)

1. Gene-environment interactions and predictors of high risk groups (Task 3.3)

The environment during pre-natal and post-natal life has a fundamental role in the development of allergic diseases (2). Several risk and protective factors during these periods have been related with allergy initiation, but results are generally scarce, controversial and often lack statistical power. The inclusion of MeDALL birth cohorts from different environments provides large variability in the exposure levels. The pooling of several cohorts enhances the statistical power to the size adequate for the study.

MeDALL research attempts to assess the role of multiple and complex exposures to risk and protective factors in different environments given different genetic propensities. The proposal was built using six birth cohorts initiated after 2003 and has collected extensive environmental information after week 12 of pregnancy, and covering the whole pregnancy (at weeks 12, 20, 36) and early life (at 6, 12, 24 and 48 months). The risk and protective factors were measured during pregnancy and early life. Data to characterize allergic phenotypes (food allergy, eczema, rhinitis, asthma) have been collected at each of the repeated examinations. The quality of the environmental information is ensured through detailed SOPs, quality controls, use of biomarkers and modelling. In addition, these cohorts are studied using shared protocols and questionnaires, and are all involved in common EU projects such as New-generis (54), ESCAPE (55) and ENRIECO (5).

Four combined data analyses were carried out examining associations of indoor and outdoor environmental exposures (tobacco smoke, mould/dampness, road traffic air pollution) with allergies and asthma in adolescence as well as evaluating gender-specific asthma and allergy prevalence differences. The development of standard tools for five of the cohorts led to harmonised data with sufficient power for the detection of new risks and understanding the natural history of classical and novel asthma allergy-related phenotypes. At birth, there are only two cohorts (EDEN and INMA), and at 4 years there are four (EDEN, INMA, PIAMA and BAMSE). The co-variables were selected based on testing the difference of the correlation p value before and after correction by the Kolmogorov–Smirnov test. The objective of the task was to assess the association between genome-wide DNA methylation signatures and:

1. Maternal smoking during pregnancy

2. Traffic-related air pollution: (traffic-related nitrogen dioxide (NO2). All participating cohorts assessed NO2 exposure at birth and follow-up addresses by means of land-use regression modelling according to a uniform exposure protocol (within the EU ESCAPE project) (Gehring et al, submitted).
3. Breast feeding

2. Evolution of sex switch in allergic diseases during adolescence (Task 4.7)

Five MeDALL cohorts (BAMSE, GINI, LISA, PIAMA, MAS) had suitable data for the planned analyses. We found evidence for a gender switch during puberty in asthma prevalence and to a lesser extent in rhinitis prevalence from male to female predominance. The newly created central MeDALL database will also allow analyses of a possible gender switch in prevalence of allergic multimorbidity including asthma, rhinitis and eczema.

- **MeDALL made it possible to better understand environmental factors acting on allergic disease development in preschool children and older children.**
- **In a large number of subjects, sex switch after puberty was found in asthma, but not in rhinitis**

C5- Characterization of inhalant and food allergens (WP 5)

Allergen microarrays allow the measurement of IgE, IgG_4 and other isotype antibody reactivity profiles against more than 100 allergen molecules with small volumes of serum. These assays are particularly suited to the amount of serum available in birth cohorts. Exposed to a common environment, the IgE-mediated immune response differs among sensitized subjects. Some react towards one or a limited number of allergens (mono or pauci-sensitized) whereas others are sensitized to a wide array of allergens (polysensitized) (56, 57). WP5 has helped to better understand these phenotypes and to investigate better mono and polysensitization phenotypes proposed in WP2.

1- Advances in allergen-microarray technology for the diagnosis and monitoring of allergy: the MeDALL allergen-chip (Tasks 5.1, 5.2)

Allergy diagnosis based on purified allergen molecules provides detailed information regarding the individual sensitization profile of allergic patients. It also enables the monitoring of the development of allergic disease and of the effect of therapies on the immune response to individual allergen molecules. However, the sensitivity of the classical chip is not sufficient for many allergens. MeDALL has made important progress in the field of allergen microarray technology and has introduced the MeDALL allergen-chip that was developed for the specific and sensitive monitoring of IgE and IgG reactivity profiles towards more than 170 allergen molecules (instead of 110) using 100 µl of serum (suited for birth cohorts). The MeDALL-chip is about 5 times more sensitive than the commercialised one (6). These characteristics are unique and allow MEDALL to confirm the hypotheses.

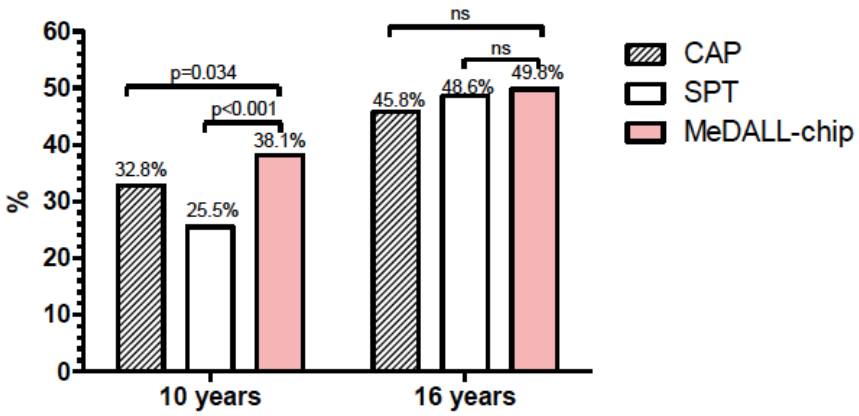
2- Determination of allergen-specific IgE and other antibody classes and subclasses in prospective birth cohorts (Task 5.3)

IgE and IgG reactivity was studied in the case-control study EGEA (Epidemiological study on the Genetics and Environment of Asthma, bronchial hyper-responsiveness and atopy) (58, 59) in order to study a sufficient number of subjects with severe allergic disease. It was found that IgG reactivity will not provide sufficient new information for the purpose of MeDALL and that only IgE will be studied in MeDALL (Siroux, in preparation).

We initially planned to study 2,000 subjects, but the first results were sufficiently interesting to increase the number to over 3,500. Before assessing IgE reactivity in the entire MeDALL cohort, two cohorts were selected (BAMSE, Sweden and ECA, Norway) in order to optimize the analytic methodology in a very large number of samples. In ECA, the same 265 children were sampled at 10 and 16 years. Allergic sensitization at 10 years was more frequently detected using the MeDALL-chip (38.1%) compared to the ImmunoCAP (32.8%) and skin prick test (25.5%), but no significant

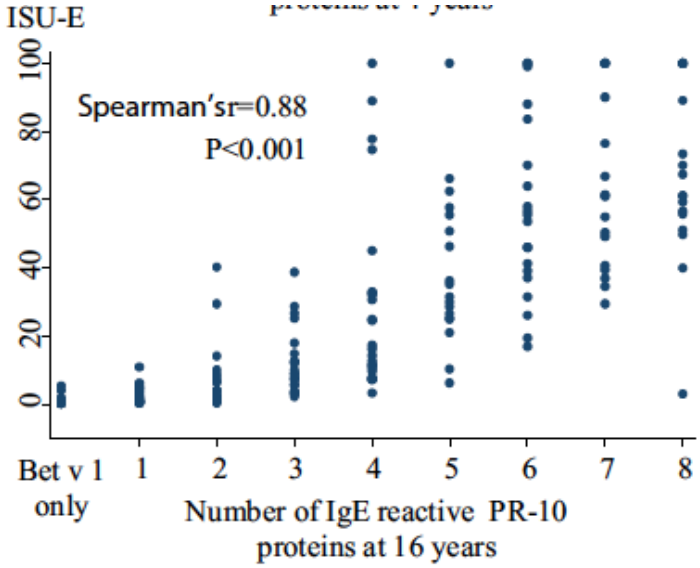
difference was seen at 16 years (MeDALL-chip 49.8%, ImmunoCAP 48.6%, SPT 45.8%) (7) (Figure 5). It is likely that the MEDALL-chip allows the detection of allergic subjects at an earlier stage than the classical methods.

Figure 5: Overall allergic sensitization in ECA (Skrindo et al. (7))



In BAMSE, the same 764 children were sampled at 4, 8 and 16 years. In a first study, early childhood IgE reactivity to pathogenesis-related class 10 proteins (PR-10 proteins) was found to predict allergic symptoms in adolescence (8). The level of Bet v1 increased with the number of PR10 proteins, indicating that the phenotypes of mono and poly-sensitized subjects to birch pollen differs (Figure 6). There was an increased risk of incidence and persistence of allergic rhinitis and asthma during the birch pollen season up to age 16 years, with increasing levels of Bet v 1-specific IgE or increasing numbers of IgE-reactive PR-10 proteins at 4 years. Children with severe disease at age 16 years had higher levels of Bet v 1-specific IgE at age 4 years as compared to children with mild symptoms. In another study in the same subjects (Asarnoj et al., submitted), IgE to Fel d 1 and Can f 1 early in life are useful markers of cat and dog allergy at 16 years. Poly-sensitized individuals more often have symptoms to cat or dog than mono-sensitized individuals, and the presence of many dog or cat allergens at 4 years predicts the onset of symptoms at 8 and 16 years. Thus, the MeDALL chip is valuable in predicting the onset and persistence of allergy in preschool children.

Figure 6: Bet v1 specific IgE levels depending on the number of PR10 proteins (Westman (8))



In another BAMSE study, it was found that specific IgE is strongly associated with eczema and comorbidity throughout childhood and with asthma and rhinitis from age 4 years. However, 23% of

children with IgE sensitization do not develop any disease in childhood (Ballardini, submitted).

- **The MeDALL chip is as sensitive as the gold standard test for IgE reactivity and contains 60 additional allergens by comparison to the commercial test.**
- **The sensitivity of the MeDALL chip will allow to better assess the sensitization of children in MeDALL cohorts.**
- **The MeDALL chip has confirmed the importance of mono- and poly-sensitization phenotypes.**
- **The MeDALL chip is valuable in predicting the onset and persistence of allergy in preschool children.**

C6- Epigenetics (WP6)

Environmental factors, especially early in life, can lead to methylation changes that persist and subsequently relate to the development of asthma and allergy (27). These methylation changes in turn can regulate protein levels, which can either be causally implicated in disease or act as biomarkers.

1- Genome wide methylation array on DNA (Task 6.2, 6.3, 6.4)

In the discovery phase, we studied genome wide methylation patterns using paired samples at birth (DNA from cord blood) and 4 years (whole blood), as well as ages 4 and 8 years (both whole blood DNA) of BAMSE (Sweden), PIAMA (Netherlands), EDEN (France) and INMA (Spain). We identified unique methylation patterns related to asthma and allergy. At the age of 4 one CpG site was significant for asthma. At the age of 8 a set of highly significant CpG sites were related to asthma (106) and allergy (85) (Table VI-A).

Table VI-A: Samples size and cohorts in the discovery phase.

	EDEN	INMA	BAMSE	PIAMA	Total
Asthma	34/113	35/177	99/146	40/174	208/610
Allergic eczema		5/153	30/165	30/181	65/499
Allergic rhinitis		2/156	10/182	6/198	18/536
≥ 1 Allergic disease		38/129	118/136	64/166	220/431

In the replication phase, we have defined 156 DNA methylation sites of interest from 13 bundles and designed the replication study. After quality control, 5594 samples were obtained from 10 birth cohorts. (See table VI-B) In this study, we found methylation changes in important genes that have been previously linked to asthma and allergy in genetic studies or in biological studies of asthma, and were proposed as novel targets for treatment.

We also showed 21 stronger associations between allergic multi-morbidity and methylation of 13 genes as compared to the classical definitions at the age of 8 years.

We also studied if the environment can change DNA methylation across the genome. We found a strong effect of maternal smoking during pregnancy on the DNA methylation of the child, and showed that although the strongest effect was seen at birth, the changes in DNA methylation in a gene called AHRR (aryl-hydrocarbon receptor repressor) were still present at 8 years. The aryl hydrocarbon receptor (AhR) signalling cascade mediates dioxin toxicity and is involved in the regulation of cell growth and differentiation. Weaker effects were observed for traffic-related air pollution; our findings on the relation of air pollution and DNA methylation are currently being studied together with other populations across the world (PACE consortium).

Table VI – B: Samples and cohorts included in the replication phase

Cohort	Total samples	Good quality samples
BAMSE	Age 16: 2438	Age 16: 0 (0%)
BIB	Age 0: 470 Age 4: 627	Age 0: 299 (64%) Age 4: 569 (91%)
ECA	Age 10: 234 Age 16: 248	Age 10: 213 (91%) Age 16: 166 (67%)
INMA-GIP	Age 0: 515 Age 4: 212	Age 0: 460 (89%) Age 4: 127 (60%)
INMA-MEN	Age 4: 400 Age 14: 261	Age 4: 362 (91%) Age 14: 118 (45%)
INMA-VAL	Age 0: 391 Age 7: 223	Age 0: 371 (95%) Age 7: 59 (26%)
Karelia	108	101 (94%)
PIAMA	Age 8: 738 Age 16: 745	Age 8: 738 (100%) Age 16: 685 (92%)
Rhea	Age 4: 749	Age 4: 572 (76%)
Robbic-Bologna	Age 0: 178 Age 8: 179	Age 0: 135 (76%) Age 8: 126 (70%)
Robbic-Rome	Age 0: 371 Age 8: 375	Age 0: 279 (75%) Age 8: 214 (57%)
Total	**9462**	**5594 (59%)**

Since we had the unique opportunity to study DNA from the same children taken at two different time points, we studied how DNA methylation changes with age. There are strong effects of ageing, and in children who develop asthma, we found evidence that certain methylation sites change are stronger compared to healthy controls .

Finally, we know that methylation changes do not act alone and we integrated DNA variation with DNA methylation, protein levels and disease outcome. We show two examples of serum proteins that are regulated by DNA variation and DNA methylation (YLK40 and IL1RL1-a). We also showed how to use this information to infer if a certain protein is the cause or the consequence of asthma and allergic disease. This integrative genomic analysis sheds more light on causal pathways to asthma and allergy.

- **A large data set was obtained from MeDALL cohorts for the discovery and replication phases.**
- **Genes of interest have been observed for individual diseases and multi-morbidity**

C7- Targeted proteomics (WP6)

Consistent with the overarching MeDALL design, protein biomarker studies were completed in two stages and they addressed four main goals:

1. In a targeted proteomic approach, in stage 1 we measured concentration levels of a large panel of proteins via multiplexing and ELISAs. In light of the main focus on integration in MeDALL, we measured biomarkers in serum/plasma samples from the same cohorts and the same children that had been included in epigenetic analyses. Of the four cohorts that were included in stage 1 epigenetic studies, samples for biomarker measurements were available from INMA-Sabadell and BAMSE. In stage 2 (the replication stage), top biomarkers of interest were measured in the additional cohorts of BIB, RHEA, INMA-G, INMA-V, INMA-M, ROBBIC-R and PIAMA. These analyses identified a potential novel biomarker of asthma and the role of systemic inflammation in allergic multi-morbidities in early childhood, both using the classical definition of multi-morbidty (i.e., the presence

of at least two of the three phenotypes of asthma, eczema and rhinitis) and the comorbidity cluster – novel phenotype identified by unsupervised statistical techniques.

2. In comprehensive integrative analyses in which we analyzed protein levels in relation to both genetic variation and methylation profiles in their encoding genes, we found evidence for multiple CpG sites from 25 genes for possible mediation of effects of genetic variation on protein levels. By identifying a set of putatively functional SNPs and CpG methylation sites, these results will be also valuable to provide specific loci to be investigated in association studies.

3. A focused integrated analysis in childhood asthma was completed with genetic-epigenetic-protein data for the asthma biomarker *CHI3L1*/YKL-40. We found methylation levels at several CpG sites (cg13134650, cg07423149, cg17014757, cg14085262 and cg03625911) to be strongly related to both *CHI3L1* genetic variation (mainly, rs10399931 and rs4950928) and circulating YKL-40 levels. YKL-40 levels, but not *CHI3L1* SNPs or CpG sites, were weakly related to asthma at age 4 years. Thus, our findings indicate that *CHI3L1* genetic variation affects circulating YKL-40 by regulating its gene methylation profiles. In addition, these results indicate that *CHI3L1* is unlikely to play a major causal role in early childhood asthma and demonstrate the value of integrated analyses on genetic-epigenetic-protein data to infer causality in biomarker studies.

4. MeDALL data for CC16 (club cell secretory protein) were also included in a large international collaborative study on the effects of this biomarker on lung function maturation in childhood. In BAMSE in adjusted random effects models we found low levels of early CC16 at age 4 years to predict subsequent FEV1 deficits up to age 16 and these data were in line with independent results from the CRS (USA) and MAAS (UK) cohorts. The lowest tertile of early CC16 was associated with a meta-analyzed 68-ml deficit in FEV1 levels up to age 16 years (p=0.0001) across the three cohorts (Guerra et al, *Lancet Respir Med*, in press)(56).

- **A large data set was obtained from MeDALL cohorts for the discovery and replication phases**
- **integrated analyses are of importance on genetic-epigenetic-protein data to infer causality in biomarker studies**

C8- Transcriptomics and systems biology for allergy biomarker discovery (WP 7)

1- Fingerprints of asthma, eczema and rhinitis

Currently only a small number of expression studies with a focus on allergy have been published, are rather fragmented in their experimental approach and mainly based on cell culture or mouse models. Even accounting for the larger number of expression studies published in the context of asthma or inflammation in general, most of these rely on model systems and again experimental approaches vary widely. Therefore, while possibly providing external data for validation, these are in no way sufficient for the development of the types of predictive biomarkers seeked in MeDALL The purpose of WP7-B was to define molecular signatures, or fingerprints, of the three allergy diseases of interest, i.e. asthma, rhinitis and eczema, and multi-morbidities.

Whole blood samples suitable for transcriptome assays were collected from three participating cohorts: BAMSE (Sweden, 16 years), GINI (Germany, 15 years), and INMA (Spain, 4 years). A subset of these was chosen so it enabled transcriptome profiling and offered the best match with other MeDALL molecular studies to enable their integrative analyses. A stepwise approach was implemented to extract the ribonucleic acids, witness of gene expression, and analyse the expression levels with microarray technique. The standard operating procedures were first validated, tested on a reduced number of samples, and utilized for an exhaustive analysis on a large set of participants (n=784). The analysis retrieved a set of 35 genes involved in all three diseases and all three cohorts (asthma: 36 cases and 164 controls at age 4 and 112 cases and 311 controls at age 15-16; rhinitis: 158 cases and 265 controls at age 15-16; eczema: 68 cases and 138 controls at age 4, and 77 cases and 346

controls at age 15-16). These genes are mainly involved in respiratory diseases, inflammatory and immune responses (Ballereau et al, unpublished data).

2- Handprints of asthma, eczema and rhinitis

In systems medicine, molecular profiles of disease status, 'fingerprints', obtained for the same subjects for different types of molecules and tissues may be integrated into a handprint to assess the concordance of fingerprints, identify their specific features and thereby better characterise groups of samples. Combined analysis of complementary datasets using pattern recognition and machine learning algorithms allow to confirm known and reveal novel relationships between elements, and highlight important nodes in networks and pathways that are of relevance for patient classification or disease mechanism identification.

In MeDALL, large scale methylome and transcriptome data sets were obtained for the 119 children from the INMA-Sabadell birth cohort at age 4, and protein levels were measured in 89 of these children (17 with eczema only, 10 with asthma only, 11 with eczema and asthma, 46 controls and excluded 5 outliers; rhinitis was excluded from the analysis due to the small number of cases). Integration of features differentially methylated and/or expressed between children with and without eczema, , asthma or combination thereof using methods to analyse the correlation between the various data sets, build classifiers or using molecular data only to identify features of interest and relationships between features of different type, and subgroups of samples that overlap only partially with the clinical diagnostic. Although these results are preliminary and should be treated with caution due to the small sample size, they enable the identification of molecular features associated with eczema, asthma and their co-occurrence (Ballereau et al, unpublished data).

- **This extensive transcriptome data set, one of the largest to date, was obtained with the latest microarray technology and has already enabled identification of genes of interest.**
- **The initial allergy handprint sheds light on the correlation between methylation, transcription and protein information and is the first step to the study of mechanisms of allergy in children in a systematic manner.**

C9- Animal models confirming the mechanisms of allergy epidemic *in* vivo (WP 8)

In WP8, we have performed important validation tasks of findings of the consortium. (Epi)genetic association studies in cohorts of human individuals always need to be validated using experiments in human cell systems or in animal models of disease. Given the complexity of the human immune system, it is often impossible to get the full impact of a given gene product in the allergy process and we used a refined mouse model system. The animal model was *refined* to mimic asthma (by using house dust mite as the allergen), *reduced* by performing multiple analysis on single animals and *replaced* as much as possible with *in vitro* experiments on mouse co-culture systems to unravel cellular interactions.

1- Study a mouse model of house dust mite-driven asthma (Tasks 8.1, 8.2, 8.3)

We have found an important role of IL-21 producing cells in the development of asthma. This was a project undertaken because HDM was found to induce IL-21 expression and because polymorphisms in the IL-21 or the IL-21 receptor gene are associated with asthma, psoriasis and other inflammatory disorders. Essentially, this work identified a separate subset of allergen-specific IL-21 producing effectors cells that migrate to the lung and induce IL-33R on memory Th2 cells, that thus become much more responsive to epithelial cell derived IL-33.

GWAS studies on asthma have identified SNPs in Ormdl3 in multiple studies across various ethnic groups. Therefore, Ormdl3 was the first gene that we have chosen to validate. We have succeeded in generating Ormdl3 conditional knock-out and conditional transgenic mice. Experiments are currently ongoing in follow-up studies to study the impact of this important gene in the regulation of HDM

asthma, and its role with passive smoking. Again these tools will be unique and a great progress to the field of allergy.

Multiple GWAS studies as well as MeDALL transcriptomics analysis of three cohorts has identified SNPs in the IL1RL1 (a.k.a. IL-33 receptor, also cosing for the decoy sST2 receptor via epigenetic regulation of splicing) and IL-33 gene to be associated with asthma. We have also validated the role of IL-33 pathway in pediatric asthma, making use of a newly developed mouse model to study sensitization and asthma development in the early postnatal period. These studies showed that the IL-33 axis is hyperactive shortly after birth, to contribute to the alveolarization phase of lung development. Sensitization at this age led to strong asthma features. Strikingly, the soluble ST2 mainly affected pediatric onset, and not adult onset asthma in mice.

2- Generation of transgenic mice for Cre-inducible overexpression of the two major isoforms of the asthma susceptibility gene encoding Protocadherin-1 (*PCDH1*) (Task 8.5).

Epithelial fragility is a consistent observation in human asthmatics, and is probably also at the heart of development of eczema. Epithelial fragility is controlled by key genes that determine epithelial cohesiveness, such as protocadherins and cadherins. We have characterized the basic lung function parameters in PCDH1 transgenic mice as well as the response of PCDH1 transgenic mice to HDM-induced allergic airway inflammation. In addition, we have characterized mouse models displaying airway-epithelial loss of PCDH1 for the analysis of PCDH1 function in asthma, and of E-cadherin, for the analysis of the role of airway epithelial integrity in asthma. These studies demonstrate that epithelial leakiness can promote allergic sensitization and thus constitutes a risk factor for development of allergic diseases (Tellez et al, submitted, Nawjin et al, in preparation).

- **Validation of the role of key genes associated with asthma and allergies in human cohort studies, addressing mechanisms of the development of allergy.**
- **This has led to important insights into the role of the IL1RL1 gene frequently found to be associated with**
- **Asthma in many MeDALL cohorts.**
- **We have also validated the role of IL21 and IL21R, a known genetic association with allergy and mechanism of action in a mouse model of asthma.**
- **We have extensively studied the role of fragility of the airway epithelium (which is determined by genetic susceptibility) in causing key features of asthma.**

C10- Novel developments in *in vitro* human immunology (WP9)

In order to better understand the results of the other WPs, we proposed to study some of the new areas of IgE immunology with four objectives:

1. Immunologic mechanisms of the initiation of allergic diseases

In allergic patients the immune profile of the tonsils represents the atopic status of patients, with low expression of the Th1. Human tonsils show very low levels of allergen-induced T-cell proliferation, thus representing a very suitable *in vivo* model to assess mechanisms of breaking allergen-specific T-cell tolerance. Molecular and cellular mechanisms leading to either a healthy or an allergic immune response to allergens were investigated in human tonsils. CD4+FOXP3+ Treg cells in tonsils were 3 times higher than that in blood. They expressed classical Treg-cell surface markers but not the IL-7 receptor α-chain (CD127), which differentiates human regulatory cells from activated T cells. Tonsil pDCs (dendritic cells) stimulated with IL-3, TLR7-L, and TLR9-L induced CD4+FOXP3+ Treg cells with suppressive capacity *in vitro* (12, 13). Triggering of Toll-like receptor (TLR) 4 or TLR8 and the proinflammatory cytokines IL-1β or IL-6 break allergen-specific T-cell tolerance in human tonsils and peripheral blood through a mechanism dependent on the adaptor molecule myeloid differentiation primary response gene 88 (MyD88) and myeloid DCs. Tolerance-breaking conditions induced by different molecular mechanisms were associated with a mixed cytokine profile with a tendency toward

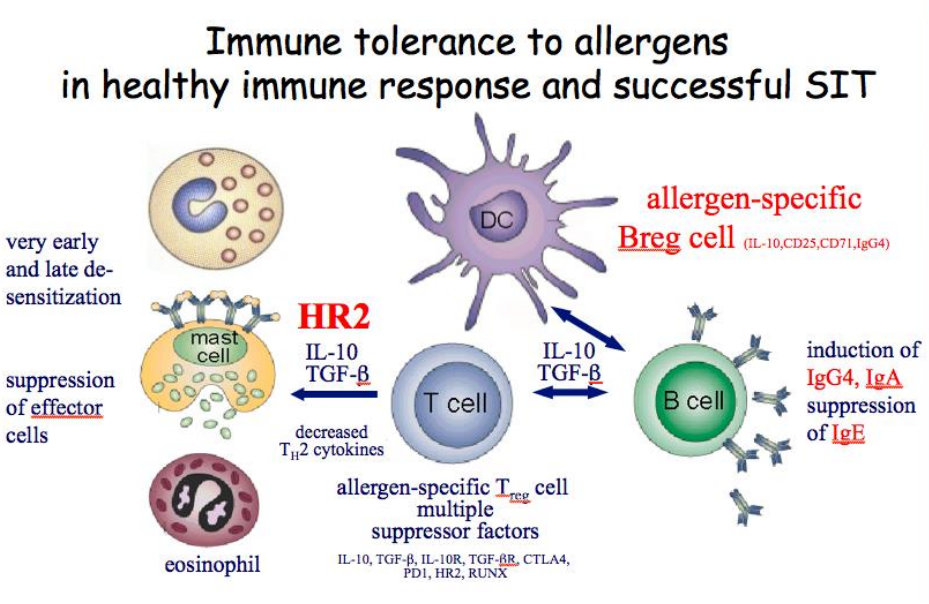
increased levels of IL-13 and IL-17, which are TH2 and TH17 cytokines. We demonstrated that allergen tolerance could be broken by innate immune response stimulating substances such as TLR4 andTLR8 triggers as well as IL-1 and IL-6 (18).

Circulating Treg cells were significantly reduced during asthma exacerbations as compared to subjects with asthma in the well-controlled period. The reduction in frequency of Treg cells was accompanied by changes in the pattern of expression of FOXP3 and Helios, lineage characteristic transcription factors. Both viral and non–viral acute asthma episodes were strongly associated with increased percentage of IL–17A–producing CD3+CD4+ T cells. In conclusion, our data opens a new window for a rapid and on site analysis to distinguish between viral and non–viral asthma exacerbations (19).

2. Mechanisms of allergen-specific T cell tolerance

We studied peripheral blood mononuclear cells from cat allergic individuals before and after Immunotherapy. The mechanisms of allergen-specific peripheral tolerance were studied by only three doses of modular allergen translocation (MAT)-Fel d 1 vaccine injected into lymph nodes in humans. Increased cellular internalization of the allergen, activation of inflammasome and generation of allergen-specific peripheral T cell tolerance were the features of this highly effective vaccine (14) (Figure 7).

Figure 7: Mechanisms of immune tolerance to allergens in healthy people and after successful immunotherapy



3. Molecular mechanisms of allergen-specific B cell regulation

Human IL-10-producing regulatory B cells (BR1) expressed high surface CD25 and CD71 and low CD73 levels. Sorted CD73-CD25+CD71+ B cells produced high levels of IL-10 and suppressed antigen-specific CD4+ T-cell proliferation. IgG4 was selectively confined to human BR1 cells. B cells specific for the major bee venom allergen phospholipase A (PLA) isolated from non-allergic beekeepers show increased expression of IL-10 and IgG4. The frequency of IL-10+ PLA-specific B cells increased in allergic patients receiving immunotherapy (16). IL-10-overexpressing B cells acquired an immunoregulatory profile comprising upregulation of suppressor of cytokine signalling 3, glycoprotein A repetitions predominant, CD25, and programmed cell death 1 ligand 1. These cells showed a significant reduction in the levels of many proinflammatory cytokines, and augmented the production of anti-inflammatory IL-1 receptor antagonist and vascular endothelial growth factor. Therefore, human IL-10–overexpressing B cells represent regulatory B cells with a prominent

immunoregulatory phenotype, capable to exert potent anti-inflammatory functions and modulation of the immune response, contributing to a tolerance-inducing environment (15).

4. Interaction of epithelium and T cell subsets

Epithelial tight junctions (TJs) are involved in different epithelial integrity of the airways and the skin in asthma, inflammatory bowel diseases, and skin disorders. Th2 cells reduce tight junction (TJ) integrity in normal but not asthmatic bronchial epithelial cells. Tregs and TGFβ regulate bronchial epithelial integrity and leakiness. A defective epithelial barrier was found in patients with chronic rhinosinusitis (CRS) with nasal polyps along with a decreased expression of TJ proteins. The disruption of epithelial integrity by IFN-γ and IL-4 in vitro indicates a possible role for these pro-inflammatory cytokines in the pathogenesis of patients with CRS (20). The picture of the junctional apparatus in each cell type and tissue is distinct, and the regulation of epithelial integrity might be more complex than being considered (17).

- **Cellular and molecular mechanisms of tolerance induction to food and aeroallergens occur in human tonsils suggesting they are a suitable lymphatic organ for direct immune interventions.**
- **Tonsils comprise a distinct cellular profile compared with peripheral blood.**
- **In addition to Treg cells, IL-10-producing regulatory B cells have been demonstrated with a potent anti-inflammatory activity.**
- **Epithelial tight junctions (TJs) are involved in epithelial integrity of allergic diseases and can be regulated by cytokines of the allergy pathways.**
- **Treg cells are reduced during asthma exacerbations.**

C11- The Karelia study

The on-going Finnish Allergy Plan 2008-2018 was developed in order to reduce the severity of allergic diseases and in an attempt to prevent them. The plan was based on studies showing that environmental factors may promote or reduce allergy onset. The example of Karelia is noteworthy. In the Russian Karelia, allergy has not increased over the past 50 years, whereas in Finland, it has boomed (24). Although the reasons for the differences are not fully understood, they may be related to biodiversity (22). Along the same lines, greens around homes reduce allergic sensitization (23). There is a greater biodiversity in the Russian Karelia, both at the macro- and micro-level, than in the Finnish part. Studies in microbiome suggest that IgE sensitization is associated with a decrease in the number of microbial abundance and diversity (21). These data propose that environmental biodiversity, human microbiota and allergy are inter-related. Constant exposure to "non-danger" signals (commensals) leads towards making the difference between good and bad (danger vs non-danger, self and non-self), preventing inappropriate inflammatory responses. The causes of allergy appear to be complex, but solutions may be simpler (61)

C12- Planning of Responses of Public Health systems to the future prevalence of atopic diseases (WP 10)

WP10 is an essential MeDALL WP due to the large burden of allergic diseases in Europe and the focus of MeDALL on how to reduce it by integrating new knowledge and translating it into action. The action has been based upon new scientific knowledge brought by the work performed in WP2-9 and by adding a few other analyses.

1- Identify new opportunities for early diagnosis (Task 10.1)

The role of allergic sensitization in the development of allergic diseases has been considered in WP2, WP3, WP4 and WP5. The MeDALL chip, a microarray of 170 components, was included in the analyses of the ECA and BAMSE cohorts from Norway and Sweden (up to 16 years of age) to evaluate the role of low-levels of allergen specific IgE in allergic disease (7-9), the predictive role of sensitization to particular patterns of allergen components in early childhood (8), and to determine how the chip compares to the skin prick test and immunoCAP analyses of allergens (7).

Polysensitization at 4 years predicts the onset and persistence of allergic diseases in the pooled analysis (10, 11). In BAMSE, children with multimorbidities at 4 years with or without IgE sensitisation had higher risks of co-morbidity at 8 years than those with a single disease (8). The stability of allergic diseases was observed in the ECA study through puberty (62). Polysensitization, especially in early childhood, is a major risk for developing allergic diseases (8). The number and level of IgE to the major PR-10 protein Bet v 1 at 4 yrs are related to the prevalence and severity to birch pollen-related allergy at 8 and 16 years (8). These studies have a direct impact on parent counselling as well as the initiation of allergen specific immunotherapy in preschool children.

2- Characterise risk groups in the population, leading to risk charts for allergic diseases (Task 10.2)

The demonstration that (i) the allergic diseases are linked more often than by chance, (ii) complex allergic disease is associated with more severe allergy as well as abnormal lung function from birth suggests a role and need for preventive strategies from early life. A new study has been designed (PreventADALL) in Norway to identify when and which factors may be implicated (Clinicaltrial*NCT02449850)*.

A systematic review and comprehensive assessment of all available "asthma/wheeze scores" has been performed but it was found that available data in the literature are unable to adequately predict future asthma in pre-school children (63).

3- Define novel approaches for prevention addressing the relevant environmental exposures to lead to national preventive plans (Task 10.3)

The first results of "The Finnish Allergy Programme 2008-2018" (64, 65) indicate that allergy burden can be reduced with relatively simple methods. This programme is supported by the Karelia studies partly carried out under the frame of MeDALL on the loss of biodiversity in the epidemic of allergy.

The Finnish Allergy Programme has been endorsed by the Norwegian Allergy Health Programme and both will serve as a platform for other countries (Oslo, November 2014) (66). These activities are currently scaled up at the EU level by AIRWAYS ICPs (Integrated Care Pathways for Airway Diseases (31)) in the frame of the European Innovation Partnership on Active and Healthy Ageing (EIP on AHA (30)) Action Plan B3. In the new National Asthma and Allergy Plan "Allergy Health" in Norway starting in 2015, several actions are being undertaken (Table VII) as outlined in a report from a MeDALL/AIRWAYS ICPs meeting at the Norwegian Department of Health (31).

Table VII: Specific goals of the Norwegian Asthma and Allergy Plan (31)

Diagnosis and treatment – A 20% reduction in subjects with allergic diseases/ hypersensitivity – Improve diagnostic work-up – Optimize treatment of severe allergic reactions and reduce hospitalisations by 40% – Focus on severe allergic reactions to reduce exacerbations and hospitalisations
Prevention – No tobacco exposure to children – Improve preventive advice on the population level – A 50% reduction in children with allergen diets – A 50% reduction in subjects with work-related allergies

4- Identify innovative targets for therapy (Task 10.4)

Novel targets for therapy in respiratory medicine have been assessed using ligand-based approaches to *in silico* pharmacology (67). The study was based on computational methods that can estimate the various pharmacodynamic and pharmacokinetic parameters that characterise the interaction of drugs with biological systems (J Mestres, paper in preparation).

The important findings of MeDALL in mono/polysensitization are of importance for the stratification of patients for allergen immunotherapy since these allergic phenotypes were studied in the 1990s, and the conclusions raised (68, 69) may need to be revised using the databases of existing trials on sublingual immunotherapy (over 6,000 subjects). Moreover, the same conclusions apply for multimorbidities. These studies are urgently needed due to a recent meta-analysis in which the lack of patient stratification in trials leads to a small effect of sublingual immunotherapy (70). Thus, the MeDALL results are likely to help physicians to better stratify patients for an expensive treatment. The integrative use of the novel MeDALL chip to promote more detailed and precise diagnostic work in practice will also be used to improve allergen immunotherapy and, again, to stratify patients. It is likely that some allergens (biomarkers) can predict efficacy and others safety of allergen immunotherapy. The data obtained by MeDALL in the general population are invaluable for these studies (Wickman and Bousquet, paper in preparation). The results of MeDALL are considered in a new trial of sublingual immunotherapy in preschool birch pollen allergic children (Wickman et al.).

MeDALL research on epigenetics, transcriptomics and proteomics may lead to innovative biomarker discovery but more in-depth analysis is needed before any conclusion can be made.

The results of immune-tolerance should lead in the future to a novel administration of allergens for immunotherapy.

5- Estimate economic and societal impacts of MeDALL in the European context (Task 10.5): *see section 3 of Impact*

- **MeDALL allows the identification new opportunities for early diagnosis and prognosis of allergic diseases with multi-morbidity and poly-sensitization.**
- **There are still gaps in our knowledge for the establishment of risks charts for the development of allergic diseases or asthma.**
- **The successful Finnish Allergy Programme has been deployed to Norway and further to the EU using AIRWAYS ICPs.**
- **The MeDALL results have an important impact on the stratification of patients for allergen immunotherapy.**

C13- Ethical issues in birth cohorts

A specific WP was dedicated to ethics in MeDALL to manage ethical issues all along the project, identified ethical issues and informed the consortium about relevant information needed to address problems related to ethical and regulatory issues, and public consultations.

At the beginning of the project, a questionnaire was prepared and sent to all partners. Also a template was designed for the ethics follow up of MeDALL activities, and a procedure to manage ethical issues. This resulted in proposing recommendations based on the analysis of the questionnaire results, in accordance with internal ethics policies that have already been produced.

In addition, WP12 prepared practical information on regulatory issues for exchanging biological samples and attached data for relevant partners of MeDALL and make this available as a practical web based tool, as a complementary development of the site hSERN.eu developed in GA²LEN.

From the beginning of the project, MeDALL have tracked the revision of the data protection Directive. We have analysed the EU Parliament amendments of the proposal of European Commission for new general data protection Regulation. Especially, we have highlighted the potential implications for the research done in Europe. We have also checked the conditions for exchanges of samples and

data regarding a few countries (www.hsern.eu). We integrated the validated information online on the hSERN's website, once validations/responses were received from our collaborators.

Finally WP12 developed the MeDALL core informed consent and ethical policy taking into account general principles, international texts and local rules and experiences. The two models of informed consent forms (prospective informed consent form and the retrospective informed consent forms) established in MeDALL have been updated following the comments of the MeDALL Ethical Advisory Board (EAB) members. The latter met 4 times during the project lifetime and provided recommendations to MeDALL.

Another task of WP12 was to follow up international debates in ethics and societal issues relevant for MeDALL, and report to MeDALL consortium. MeDALL created a list of International, European and National websites in order to track news on clinical research, biomarkers, children's rights, data protection etc. The team have also established a bibliography on the on-going international debates on paediatric research that is regularly updated. The analysis of the literature was performed. The results of this were described in two reports as well as different papers. We have transmitted to the consortium different public consultations launched.

Finally WP12 organised internal debates on ethical aspects at consortium meetings on a specific relevant aspect and write up each time a synthetic report.

Two articles and three chapters of books were published, and several communications and posters were presented. The team also contributed to summer schools on bioethics and health law.

In addition some complementary aspects related to ELSI (ethical, legal and social issues) are relevant for MeDALL: WP12 informed the consortium about the Common Service ELSI of BBMRI-ERIC (Biobank and biomolecular research infrastructure), since the work in MeDALL has contributed to the field in its ELSI dimension.

MeDALL answered the public consultation launched by the Council of Europe in the context of revision of Recommendation (2006)4 on research on biological materials of human origin.

D- Potential impact

D1- Potential impact

1-1- Innovative methods

1- Development of the harmonised MeDALL-Core questionnaire (in 8 languages) (4), **and a database of pooled cohorts** (5) (WP2,3,4).

A major MeDALL effort has consisted in a harmonised follow up of all participating birth cohorts (3, 4) with both historical databases and the harmonised follow up being integrated in a knowledge-based management: 44,000 children at birth, 22,000 at 4 yrs, 19,000 at 8 yrs, and 13,000 prospectively followed after puberty. Japan has decided to use the MeDALL questionnaire. A joint NIH-MeDALL workshop on birth cohorts in allergy and asthma was organised by the National Institute of Allergy and Infectious Diseases (NIAID) and the National Heart, Lung, and Blood Institute (NHLBI) in September 2012 (3). The workshop allowed scientists conducting research on asthma and allergic diseases in birth cohorts to meet in order to address a wide research agenda, including potential new study designs and the harmonisation of existing birth cohort data. Following the workshop, an online database containing information about existing cohorts was created to facilitate collaboration.

The MeDALL Core Questionnaires will be included in future follow-up assessments of the Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS) birth cohort in USA (71). A Japanese version of MeDALL is currently being developed by the national birth cohort study Japan Environment and Children's Study (JECS), which recently completed the recruitment of 100,000 parent-child pairs (www.env.go.jp/en/chemi/hs/jecs/).

The MeDALL project reinforced previous collaborations between most of the largest and oldest ongoing European birth cohorts, enhancing harmonized population-based asthma and allergy research activities in Europe rather than to continue with fragmented individual approaches often lacking sufficient statistical power. The MeDALL project with its new central database is the starting point to conduct future common and sustainable asthma and allergy research initiatives. It can be easily extended by including other population-based observational birth cohort studies.

2- Development of a systems medicine knowledge base integrating disease knowledge with biobanking and clinical, phenotypic and Omics data (WP3,4,7)

The MeDALL knowledge base integrates information from 44010 participants on 398 clinical and phenotypic attributes (harmonised from 7495 individual cohort variables) and 160 different follow-ups at 25 different time-points between pregnancy and age 20 as well as information about available samples (>30 000 blood, plasma, serum, DNA, RNA and leukocyte samples).
Information about 863 genes involved in allergy (283 from a systematic literature review, 580 from automatic text mining) is integrated with data on protein-protein interactions, transcriptional regulation, miRNA regulation and signalling pathways from public databases and directly connected to the omics data generated within MeDALL.
Omics data produced or made available within MeDALL includes 23,000 historical GWAS, 9,500 epigenetics, 2,000 proteomics, 750 transcriptomics, IgE micro-arrays (4,000 subjects) and individual environmental data (10,000 children) using land use model (as part of the ESCAPE project).
The epigenetics, proteomics, transcriptomics and IgE micro-array data are integrated into the MeDALL knowledge base which currently includes 3292 IgE-chip, 2173 DNA-methylation, 1427 biomarker and 723 transcription experiments with the remaining data undergoing the pre-processing and cleaning process.

3- Development of a new allergen microarray technology (WP5)

The “MeDALL allergen-chip” is a collection of 170 allergen molecules for the reliable detection of allergen-specific antibody signatures showing a higher sensitivity than the traditional ImmunoCAP system. This new tool is to be integrated in clinical work in MeDALL and beyond allowing the monitoring of the early evolution of the allergic immune response (6-8).

4- Implications for epidemiologic studies of multimorbidity (WP2,3,4)

MeDALL is an extensive study combining 14 European cohorts (44,000 children at birth) to study the most common chronic disease (allergy). The power of the study is sufficient to assess primary diseases (asthma, rhinitis and eczema) but it is at the limit for multi-morbidity, in particular at the early stages of the disease (4 yrs) and for the discovery of biomarkers. In studies of multi-morbid chronic diseases (e.g. COPD, cardiovascular diseases, diabetes….), the power of cohorts will be sufficient to assess established diseases but cohorts are likely to fail identifying early multi-morbid diseases, their causality and discovery of biomarkers. A new methodology should be proposed to combine the strengths and weaknesses of cohorts possibly enriching the cohorts by patient populations. Thus, the data of MeDALL are generalizable to multi-morbid chronic diseases.

1-2- Novel findings

1- Multimorbidity (WP2)

The term multimorbidity appears to be more appropriate than co-morbidity since the primary allergic disease is poorly known and the allergy march only accounts for few patients (72). Although the multimorbidity of allergic diseases was already known, MeDALL has refined the interactions with diseases using hypothesis-driven (10) and data-driven (unsupervised cluster analyses) approaches (11). MeDALL is unique since it was needed to pool the data of 14 birth cohorts to make conclusions due to the limited percentage of children with all three diseases. The coexistence of eczema, rhinitis, and asthma in the same child is more common than expected by chance alone, suggesting that these diseases share causal mechanisms. Although IgE sensitisation is independently associated with excess multi-morbid eczema, rhinitis and asthma, its presence accounted for only 38% of multimorbidity in children at early school age, suggesting that IgE sensitisation can no longer be considered the dominant causal mechanism of multimorbidity for these diseases in this age group.

2- Mono and poly-sensitisation (WP2, 5)

The concept of mono and poly-sensitization is not new but it could not be tested because of the lack of adequate birth cohorts and methods that allowed to study a wide array of allergens with a small serum sample. This was possible in MeDALL. In the BAMSE cohort (Sweden), the same 825 children were tested at 4, 8 and 16 years. Results show that mono and polysensitisation represent two different phenotypes of IgE-associated diseases. These results were confirmed in the MeDALL cohorts. Data on multi-morbidity and poly-sensitization have significant clinical implications.

3- Effect of maternal smoking and pollutants (WP 3, 4, 6,10)

MeDALL identified remarkable effects of maternal smoking during pregnancy and DNA methylation signatures in cord blood and early childhood. This shows that foetal exposure to smoking may have long lasting effects. Besides, we identified effects of traffic related air pollution on DNA methylation, which are currently being replicated. The key question is whether these methylation changes are mediating the adverse effects of (maternal) exposures on child health.

4- The Type 2 signalling pathway has been suggested as a common pathway to multi-morbidity by *in silico* computational analysis (WP2

5- Several loci significantly associated with allergic diseases have been identified; several loci of cord blood DNA that can predict the development of allergic diseases later in life have been identified as well as CpC loci at age 4 that predict allergic disease at age 8. An integrative genomics approach has identified new asthma pathways related to air pollution exposure indicating that functional genomics analyses in conjunction with environmental exposures may give valuable insights about pathophysiologic mechanisms (unpublished data) (WP 6, 7).

6- To complement mechanistic experimental studies in children, MeDALL includes *in vitro* and animal *in vivo* studies (WP 6,7,8)

Research in WP8 has identified and validated the early life period in being crucial in determining the outcome of allergen encounters and subsequent development of allergic disease. The first year of life is a critical window in which genetic make-up controls development of allergy. Results from this WP8 also show that growing up in protective environment is not enough to prevent the onset of allergies. Genetic polymorphisms predict whether a particular child will still develop allergy, even when growing up in a protective environment.

7- *In vitro* human immunology (WP8)

Existing therapies for allergies and asthma are effective in controlling symptoms, however the major unmet needs including better control of severe forms and long-term curative therapies remain to be identified. Our studies and many others support the role of immune tolerance and immunosuppressive cytokines as a mechanism, by which allergen immunotherapy and healthy immune response to allergens is mediated. Cytokine-producing memory B cell subsets exist in humans and may show different pro-inflammatory, anti-inflammatory as well as immune effector and immune regulatory functions. We identify novel molecules that play a role in B cell regulation and demonstrate whether functional human B cell subsets show *in vivo* clonal expansion during allergen-tolerance, such as AIT and high dose allergen exposure. Our data will lead to the determination of effector and regulatory B cell subsets in addition to T cell subsets for immune monitoring of inflammatory diseases. Moreover, markers if virus-induced asthma exacerbations are of great interest for the development of future biomarkers.

1-3- Novel classification of allergic diseases: The MeDALL hypothesis (9) (WP2, 5, 6, 7, 8, 9)

IgE sensitisation should be considered as a quantitative trait, as important clinical and immunological differences exist between mono or poly-sensitized subjects. Allergic multi-morbidities and IgE poly-sensitization are associated with the persistence or re-occurrence of foetal Type 2 signalling. Asthma, rhinitis and eczema are manifestations of a common systemic immune imbalance (mesodermal origin) with specific patterns of remodelling (ectodermal or endodermal origin).

The integration of comorbidities and poly-sensitization has resulted in a new classification framework of allergic diseases which could help to improve the understanding of genetic and epigenetic mechanisms of allergy as well as better manage allergic diseases (Table VI).

Table VI: Implications of the novel definition of IgE-associated allergic diseases (9)

Subphenotyping of allergic diseases: Phenotyping subtypes can be used to characterize allergic diseases, severity and progression, and may help identify unique targets for prevention and treatment. Clinical practice: An updated definition provides a framework to inform decisions relating to treatment priorities and to indicate need for improvement in health care and delivery through better organisation for prevention, diagnosis and treatment, but also on prediction. The prediction of allergic disease trajectories in preschool children is essential.
Clinical trials: Clarity on definitions is essential for clinical trials, evaluating efficacy and safety. The stratification of patients by sensitization and co-morbidity is essential in allergen immunotherapy (both for treatment and prevention).
Research on mechanisms and genetics: The new definition is likely to change the concepts of the mechanisms of allergic disease and to propose novel mechanisms. Population studies: In longitudinal epidemiological population studies, standardised definitions are required to be able to compare cohorts across time and place and to develop dynamic models capturing risk factors which predict transitions through different stages of health.
Public health planning: For public health purposes, a comprehensive definition is needed (i) to identify the prevalence, burden and costs incurred by all phenotypes; (ii) to improve quality of care and optimise health care planning and policies; and (iii) to model the economic and social benefits of specific interventions to improve or maintain health.

Social welfare planning: For social welfare purposes, a phenotypic definition is also needed to predict the burden and costs at an early age in order to model the individual and collective economic and social benefits of specific interventions.

Applicability to high and low-income countries: A uniform allergy definition should be applicable to the local and geographical conditions of all countries, phenotypes, risk factors, availability and affordability to treatment differing widely around the world. This would help to better understand mechanisms specific to different environments and interactions with parasitic diseases in particular

Development of novel preventive approaches and therapies: Detailed cellular and molecular phenotyping is needed to identify novel primary and secondary prevention strategies, as well as new targets for the development of novel therapies. Ultimately, novel therapies studied in clinical trials should help define IgE-mediated pathways and determine the importance of the intervention in large patient populations or in sub-populations of patients based on the concept of distinct phenotypes. The life course approach of allergic diseases is of great interest since it may lead to health promotion strategies.

1-4- Clinical impact

Ultimately aiming to improve the health of European citizens, MeDALL includes a translational work package to reinforce its impact (WP10) working with all other WPs. Many data of MeDALL have been translated into clinical practice, and a meeting at the European Parliament was organised by EFA (European Federation of Allergy and Airways Diseases patient's association) to conclude the project (May 27, 2015). In particular, MeDALL results improve the stratification of allergic preschool children for diagnosis, prognosis, and allergen-specific immunotherapy. Multi-morbidity and/or IgE poly-sensitisation are markers of persistence of disease. MeDALL was used as a model of systems medicine (26) and has initiated a common language for the assessment of all non-communicable diseases (27). **Among the important clinical findings which can be used immediately** are:

1- Prediction of the persistence of allergic diseases at 4 years (WP2,5,10)

Poly-sensitisation and multi-morbidity at 4 yrs predict the persistence of allergy later in life. This result is of importance for parent counselling: "will my child loose allergy"? is the invariable question raised by the parents. MeDALL results are the first that can easily propose a simple answer to the practicing physician.

2- Prediction of the onset of new allergic diseases at 4 years (WP2,5,10)

Poly-sensitisation in asymptomatic children at 4 yrs predicts the onset of new allergic diseases later in life. This is an important finding but it raises the issue of the communication of results and disclosure of incidental findings. MeDALL has approached this problem in longitudinal paediatric research (28).

3- Stratification of patients for the initiation of allergen specific immunotherapy

Although large randomized controlled trials have been performed in SLIT, a recent controversy was published in the JAMA stating that this treatment administered to hundred of thousands of allergic patients was not recommended due to the lack of patient stratification (70). The methods (cluster analysis) and results of MeDALL(mono-polysensitization, multi-morbidity) as well as recent studies in allergic rhinitis patients (73) and in the EGEA cohort (E Burte et al, paper in favourable revision in Plos one) can make it possible to analyse the pharma database in order to propose a stratification of patients which may allow to answer the criticisms of the JAMA meta-analysis and to optimize the selection of patients for future randomized control trials.

4- Initiation of allergen specific immunotherapy in preschool children

It is important to initiate allergen specific immunotherapy as early as possible to modify the natural course of the allergic disease (74). However, the treatment is usually not recommended before 5 year of age because the natural history of the disease was unclear (75). MeDALL, for the first time, is proposing a scientific answer as it has found that preschool children with multimorbidity will have persisting allergic diseases (vast majority of children). This finding will allow physicians to propose the treatment earlier and with a greater efficacy to modify the course of allergy.

1-5- Ethics

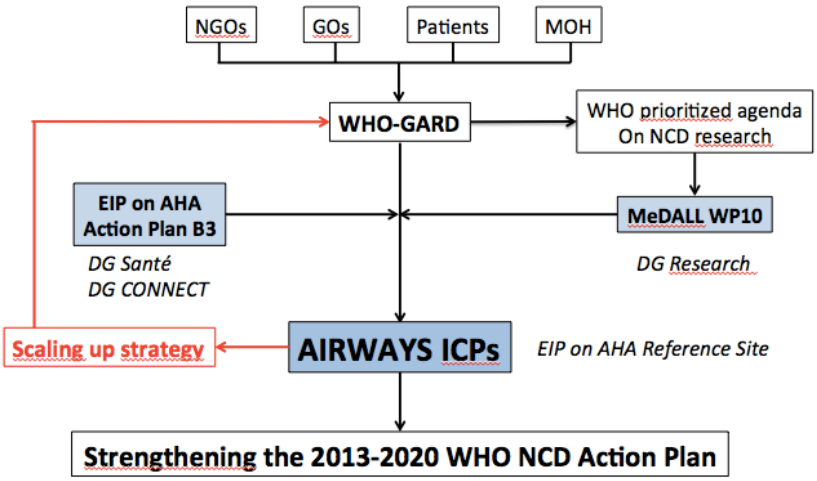
Relevant ethical and societal issues on allergic diseases were being internationally debated within MeDALL. The communication of results and disclosure of incidental findings in longitudinal paediatric research has been another innovative MeDALL initiative (28).

1-6- MeDALL at the cross-road of EU and WHO previous and ongoing initiatives

The WHO research agenda on non-communicable diseases (NCD) indicated that longitudinal birth cohorts were needed for the understanding of the early determinants of chronic respiratory diseases for innovative health promotion strategies (76). MeDALL is in line with this agenda.

The WHO Global Alliance against Chronic Respiratory Diseases action plan (77-79) was the model of AIRWAYS ICPs (31), a new initiative of the EIP on AHA, which was jointly organised by MeDALL WP10, the Reference Site Network of the EIP on AHA (DG Santé and DG Connect) (80) and WHO GARD (Figure 8). The scaling up strategy of AIRWAYS ICPs is likely to be used as a model for the EIP on AHA. Finally, AIRWAYS ICPs is a GARD Research Demonstration Project and will strengthen the WHO NCD 2013-2020 Action Plan.

Figure 8: MeDALL interactions between EU and WHO



GARD: Global Alliance against Chronic Respiratory Diseases, NGO: nongovernmental organisation, GO: Governmental organisation, MOH: Ministry of Health, NCD: noncommunicable disease.

D2- Socio-economic impact and wider societal implications

2-1- Patient empowerment

The goal and rationale of patient involvement in medical decisions is patient empowerment. Empowered patients know their disease. Patient empowerment commences with the initial consultations at the primary care level encompassing discussions about the patients ideas, concerns and expectations coupled with patient education about the specific disease process, what can be done to ameliorate the disease and ultimately self management. Patients have the skills and motivation to take good care in their everyday life, to adjust their treatment and are prepared for new or potentially exacerbating situations. They are able to detect side-effects, contact healthcare professionals when necessary and they adhere to the treatment regime. Many tools support empowerment, shared decision making models and patient education. Patient empowerment should be included in the health care professional's curriculum. For an optimal dissemination of good practices, there is a need for patient involvement and empowerment.

MeDALL has since its inception worked for and with the patients for their empowerment in the project. EFA, one of the MeDALL partners has been at the initiation and termination of the project. Several meetings have been held to implement and discuss the prevention plan on national and international levels in **Brussels at the EU-parliament together with EAACI** in July 16 th 2014 and the meeting concluding MeDALL May 28th 2015 "The MeDALL Project: *Are Europeans developing more allergies? From research to policies"* organized by EFA with a large participation of patient's organisations (De Carlo, in preparation).

2-2- Impact of MeDALL on the EU policies in early diagnosis and management of allergic diseases

The leading priority for the Polish Presidency of the Council of the European Union (2011) was to reduce health inequalities across European societies, and, within its framework, prevention and control of respiratory diseases in children to promote AHA (81, 82). The clinical implications of MeDALL reinforce the priority of the EU and provide important solutions to implement it.

2-3- Impact of MeDALL in the concept of active and healthy ageing

Pre-and peri-natal events play a fundamental role in health, the development of diseases and ageing (Developmental Origins of Health and Disease: DOHaD). Biological ageing is the progressive deterioration of function that occurs in the post-maturity phase, and is developing at the individual, physiological systems and cellular levels intertwined with socio-economic determinants (81-83). The European Union (EU) leads a global effort to understand the early determinants of ageing. The developmental determinants of chronic diseases in ageing were reinforced during the Cyprus Presidency of the EU Council (2012) (29). Several projects of the EU Sixth and Seventh Framework Programme for Research and Technological Development (FP6 and FP7) were funded to understand the mechanisms of pre-natal and early life events on the development of chronic diseases. A meeting was convened by the Reference Site of Languedoc Roussillon (80) on early determinants of active and healthy ageing (NIH, EIP on AHA, MeDALL) (32). These concepts have been considered in the senioral policy of Poland (84).

2-4- Interactions of MeDALL with the European Partnership on Active and Health Ageing

MeDALL has interacted with the EIP on AHA (30, 32) by several actions. Synergies have been achieved between MeDALL and the AIRWAYS ICPs (Integrated care pathways for airway diseases), the model of chronic diseases of Area 5 of the B3 Action Plan of the EIP on AHA (31) due to their burden, mortality and co-morbidities (78) as well as their early development (81, 82). AIRWAYS ICPs is a WHO GARD (World Health Organisation Global Alliance against Chronic Respiratory Diseases) demonstration research project) (77). It was launched by NHS England (Newcastle,

February 2014) (85) and has been endorsed by the EIP on AHA Reference Site Network (80). AIRWAYS ICPs has strategic relevance to the European Union Health Strategy and the WHO NCD (non-communicable disease action plan) Action Plan (2013–2020), adding value to existing public health knowledge. The next meeting will be organized by the EIP on AHA Reference Site Network in Lisbon, Portugal (July 1-2, 2015). The scaling up strategy of AIRWAYS ICPs will be presented during this meeting.

Objectives: AIRWAYS ICPs aims to launch a collaboration to develop practical multisectoral care pathways (ICPs) to reduce CRD burden, mortality and multimorbidity across the life cycle with a focus on old age people (31). It is implemented in European countries and regions, as part of the EIP on AHA and scaled up globally with the WHO GARD (World Health Organisation, Global Alliance against Chronic Respiratory Diseases) (GARD demonstration research project). AIRWAYS-ICPs propose a feasible, achievable and manageable project from science to guidelines and policies using existing networks and stakeholders committed to the Action Plan B3 of the EIP on AHA and GARD.

Action Plan: An action plan has been set up and achievements after 2 years are reported in this paper.

1. Proposing a common framework of care pathways (ICPs) for CRDs to facilitate comparability and trans-national initiatives (86).
2. Proposing plans targeted to all populations according to culture, health systems and income.
3. Developing a strategy based on WHO programmes such as the GARD (87, 88) and WHO PEN and the essential list of drugs for low and middle income countries and settings.
4. Informing cost-effective policy development, in particular strengthening those on smoking and environment exposure, and health promotion strategies.
5. Aiding risk stratification in chronic disease patients with a common strategy (26, 27, 79).
6. Defining important questions on CRDs in the elderly.
7. Developing ICPs for CRDs and their comorbidities, with a specific focus on the elderly.
8. Building a sentinel network for asthma and other allergic diseases (89-94).
9. Tackling chronic diseases across the life cycle (29, 32, 81, 82).
10. Defining AHA (84, 95).
11. Integrating and preventing frailty in CRDs (A3-B3 EIP on AHA Action Plan).
12. Implementing emerging technologies for individualised and predictive medicine, based on systems medical principles, in accordance with the European Commission (https://www.casym.eu) (94).
13. Scaling up strategies in Europe and strengthening the WHO NCD Action Plan (66, 96).
14. Having a significant impact on the health of citizens in the short term (reduction of morbidity, improvement of education and work) and the long-term (AHA) (97).
15. Ultimately reducing the healthcare burden (emergency visits, avoidable hospitalizations, disability, absenteeism, presenteeism and consequently, direct and indirect costs) while improving quality of life and promoting AHA. In the longer term, the incidence of disease may be reduced by innovative prevention strategies.

Conclusions: AIRWAYS ICPs has strategic relevance to the European Union Health Strategy and the WHO NCD (non-communicable disease) Action Plan (2013–2020), adding value to existing public health knowledge.

D3- Main dissemination activities

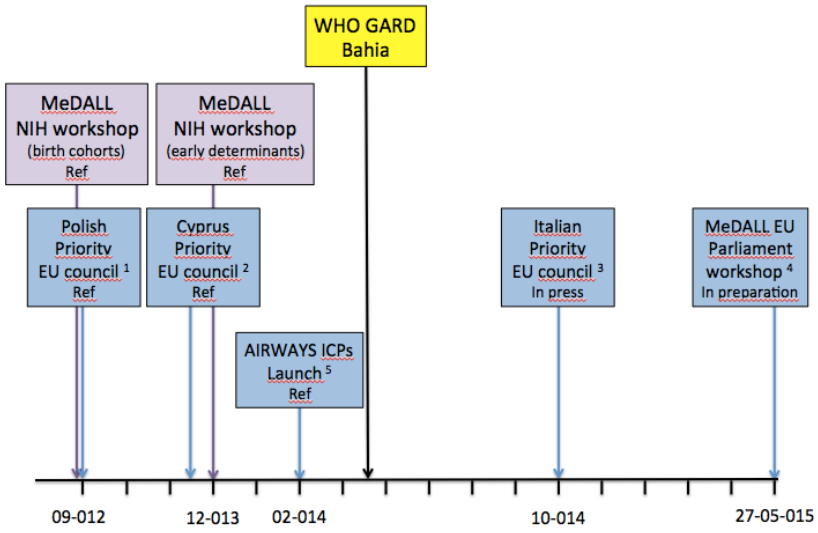
Several meetings have been held to implement and discuss the prevention plan on national and international levels; including in **Brussels with the EU-parliament together with the EAACI and EFA** in July 16th 2014 and May 28th 2015. The May meeting was titled "The MeDALL Project: *Are Europeans developing more allergies? From research to policies".*

3-1- Dissemination at the EU and WHO levels

The dissemination of MeDALL results has been carefully considered. MeDALL has been presented at the European Parliament (Presidency of the Cyprus EU council (29)) and has initiated two activities in the European Innovation Partnership of Active and Healthy Ageing (30) (Action Plan B3): AIRWAYS ICPs and the meeting on early determinants of ageing was co-organised by the Reference Site MACVIA-LR of the EIP on AHA and NIH (NIAID) (31, 32) (Figure 9).

The concepts and results of MeDALL has been presented during the annual GARD meetings in Salvador de Bahia (Brazil, 2014) and Lisbon (2015).

Figure 9: Dissemination of MeDALL at the EU and WHO levels



3-2- Dissemination at national levels

The first results of the "The Finnish Allergy Programme "2008-2018", supported by MeDALL, indicate that allergy burden can be reduced with relatively simple means. This has been endorsed by the Norwegian Allergy Health Program and with the Finnish one will serve as a platform for other countries (Oslo, November 2014) (66)

3-3- Publications

Since we aim at publishing all results, we attempted not to present abstracts for confidentiality reasons. The list of publication is presented below.

1. Bousquet J, Anto J, Auffray C, Akdis M, Cambon-Thomsen A, Keil T, et al. MeDALL (Mechanisms of the Development of ALLergy): an integrated approach from phenotypes to systems medicine. Allergy. 2011;66(5):596-604.
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12. Bousquet J, Anto JM, Berkouk K, Gergen P, Pinto Antunes J, Auge P, et al. Developmental determinants in non-communicable chronic diseases and ageing. Thorax. 2015.
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D4- Exploitation of results

MeDALL delivered the following tools and results:

- **Three harmonized MeDALL Core Questionnaire**s (available to the scientific community)
- A **central database of the birth cohort data** from the new as well as the historic follow-up assessments. The database includes clinical information from 44,010 participants, IgE-chip data from 3,292 experiments, epigenetic data from 2,173 experiments, biomarker data from 1,427 experiments and transcription data from 723 experiments. In addition sample information about more than 30,000 available blood, plasma, serum, DNA, RNA and Leukocyte samples has been collected. The way to exploit and maintain the database is currently being discussed by the consortium. Databases are usually protected by suis generis and copyright. One of the possibilities to sustain the database could be to establish a Public/Private Partnership with industry, with the objective to give access to the database to industrial partners for epidemiological studies. Access rights can be granted through licences.
- A **database integrating Omics**, including 23,000 historical GWAS, 9,500 epigenetics, 2,000 proteomics, 750 transcriptomics, IgE micro-arrays (4,000 subjects) and individual environmental data (10,000 children) using land use model.
- **Standard Operating Procedures** for clinical investigations, available in several languages (available to the scientific community)
- **MeDALL chip**: an important progress in the field of allergen microarray technology with the MeDALL allergen-chip that was developed for the specific and sensitive monitoring of IgE and IgG reactivity profiles, towards more than 170 allergen molecules using 100 µl of serum. The MeDALL-chip is a customised application with unique characteristics designed by the Medical University of Vienna together with Phadia, and is about 5 times more sensitive than the commercialised one.
- **Animal models** (transfer by material transfer agreement) such as
  - Ormdl3 conditional knock-out and conditional transgenic mice
  - TCR transgenic mice specific for Der P 1
- **Fingerprints of asthma, eczema and rhinitis**

  - Evidence for multiple CpG sites from 25 genes for possible mediation of effects of genetic variation on protein levels (proteomics)
  - Identification of molecular features associated with eczema, asthma and their co-occurrence (transcriptomics)
  - Such results can lead to an invention disclosure and to a proof of concept, to be validated in animal models.
- **New targets for co-morbid allergic diseases** and **novel approaches for intervention** which can emerge from the epidemiological validation of classical and novel phenotypes. We identified cellular pathways shared by the 3 diseases, which represent candidate mechanisms for multimorbidity. The new targets could lead to a patent application.
- **Recommendations and guidelines**
  - Risk assessment report (paper to be published summer 2015)
  - Recommendations were integrated in a novel interventional trial of primary prevention of allergic disease PreventADALL, AirwaysICP, National Asthma and Allergy Plan "Allergy Health" in Norway, National plans in Finland

The MeDALL General Assembly will have to make the decision on the business models it elects for the transfer of technology generated by MeDALL. Consideration will be given to the intellectual property generated, and to the identification of potential industrial partners for the technology. Potential partners may be existing companies in the field, or start-ups (created by partners) with the view of exploiting technology generated within the consortium.

D5- SWOT analysis of MeDALL

5-1-Strengths

1- **A systems medicine approach to understand the complexity of allergic diseases.** The systems approach involves a paradigm change in understanding allergic diseases from the result of deterministic causal mechanisms to the result of environmentally disrupted complex regulatory networks.

2- **A multidisciplinary consortium ranging from population health sciences and clinical allegory / immunology to basic molecular sciences.** The magnitude of the multidisciplinary effort has provided an unprecedented opportunity to explore multiple dimensions of allergic diseases.

3- **A life cycle approach.** Starting during pregnancy and early life (retrospective components) and following children up to adolescence (prospective component) providing a unique opportunity to understand allergic diseases from it inception to the pubertal shift.

4- **An innovative project integrating new concepts and technologies.** In addition to the innovative character of systems medicine, other salient innovations have been: the use of classical and novel phenotypes, a harmonized questionnaire, a knowledge management platform for allergic diseases, the integration of different omics in the same set of subjects.

5- **A novel view of allergic diseases.** MeDALL has shown that asthma, eczema and rhinitis should be understood as a allergic multi--morbidity cluster modulated by IgE mono and poly-sensitization and other type 2 signaling pathways in the context a wider, still ill-defined, allergic proteome.

6- **A large translational effort,** integrating experimental, clinical and socio-economic results and developing links with DG Santé, DG Connect and WHO.

5-2- Weaknesses

1- **Insufficient integration among the different WPs.** The ambition of MeDALL led to an unprecedented level of complexity and multidisciplinary and we did not achieve the desirable degree of integration among the different WPs. The consequence is that hypothesis and

experiments performed in the different WPs did not fully converge in a single integrated framework and the results are somewhat fragmented. A robust set of handprints of allergic diseases is not yet available.

2- **Project duration was insufficient.** For a project like MeDALL duration of 10 y conditional to a mid-term full review, should be considered. MeDALL has achieved a remarkable disciplinary integration but this has taken about three years to get 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.

3- **The existence of specific shortcomings has limited the experimental plan.** Among others the following specific limitations should be considered: insufficient samples were available for the study below 4 years of age, the allergy march may not have been considered adequately, statistical power was insufficient for some of the analyses due to the low number of subjects in extreme phenotypes.

4. **A new consortium of birth cohorts of asthma and allergy is not yet build.**

5-3- Opportunities

1- **Continuation of longitudinal studies.** The assessments of birth cohorts in adolescence and young adulthood help to identify risk and protective factors as well as early life-determinants for several public health relevant chronic respiratory and skin diseases. The results will be beneficial for planning intervention trials and eventually to develop better prevention strategies of asthma and allergies. Future follow-up assessments of the cohorts will collect information on other chronic diseases in adulthood, thus also improving our knowledge of non-allergic multi-morbidities of asthma and allergic diseases.

2- **Enrichment of the MeDALL database and experimental analyses by patients with multimorbidities at age 4, 8 and 16 years.** Some case-control studies, cohorts or EU projects (U-BIOPRED) and pharma companies have such data and samples and some have already been analysed. Ethics should be considered carefully.

3- **Extending birth cohorts for asthma and allergy in Europe to the complete life cycle.** The network of birth cohorts for asthma and allergy in Europe that has resulted from a sequence of concerted efforts from GALEN or MeDALL provides the unique opportunity to study the links between allergy and non-communicable diseases to study active and healthy ageing from birth to adulthood across Europe.

5-4- Threats

1- **A new consortium of birth cohorts of asthma and allergy is not yet build.** Discussions are taken place with EU Structural Funds (3S) to get funding to maintain the database. Moreover, a MeDALL consortium has been proposed to continue the activies beyond the EU funding. However, these activities are not established yet.

2- **Funding for extending the research plan is hard to obtain.** With the cohesion achieved and the generated new hypothesis it would have been very productive to have time and funding for extending MeDALL to a fully integrated systems medicine framework.

3- **Fast technological change risk to make some MeDALL developments limited.** MeDALL has made a huge effort to integrated genetics, epigenetics, proteomics and transcriptomics in the same set of children and developed a handprint model. During the years of MeDALL new technologies like complete gene sequencing have evolved rapidly. Applying gene sequencing to multi-morbidity phenotypes is an attractive perspective. An extended time period.

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