HEALS

Health and Environment-wide Associations based on Large population Surveys

FP7-ENV-2013- 603946

http://www.heals-eu.eu/

D1.2

A critical review of published information relating to "critical life events" in defining when and how frequently biological samples should be collected to define the exposome

WP 1 Overview of scientific state of the art

Version 1

Lead beneficiary: UPMC

Date: 8th of September 2014

Nature: R

Dissemination level: PU

TABLE OF CONTENTS

Document Information

Grant Agreement Number	ENV-603946	**Acronym**	HEALS
Full title	Health and Environment-wide Associations based on Large population Surveys		
Project URL	http://www.heals-eu.eu/		
EU Project Officer	Tuomo Karjalainen,- Tuomo.KARJALAINEN@ec.europa.eu		

Deliverable	**Number**	1.2	**Title**	A critical review of published information relating to “critical life events” in defining when and how frequently biological samples should be collected to define the exposome
Work Package	**Number**	1	**Title**	Overview of scientific state of the art

Delivery date	**Contractual**	M6	**Actual**	08/09/2014
Status	Draft □		Final ⊗	
Nature	Demonstrator □ Report ⊗ Prototype □ Other □			
Dissemination level	Confidential □ Public ⊗			

Author (Partners)	Name (Organization) UPMC			
Responsible Author	Isabella Annesi-Maesano		**Email**	isabella.annesi-maesano@inserm.fr
	Partner	**UPMC**	**Phone**	

Document History

Name	Date	Version	Desciption
UPMC-AUTH	05/09/2014	1	Final version of the deliverable

Authors: Isabella Annesi-Maesano (UPMC), Nour Baïz (UPMC), Spyros Karakitsios (AUTH) and Denis Sarigiannis (AUTH) on behalf of the HEALS projects.

A. ABSTRACT

The main specific question which this review wanted to reply is “in which period of life is it important to monitor the exposome?” This question implied one further consideration: “when are data collections of exposure and health outcomes respectively, and in the case of biomarkers of exposure, effect and disease and omics…, is biomonitoring most informative?” Literature shows that the answer depends on the type of health event - namely endotypes, phenotypes and sub-phenotypes - the target organ, the type of agent (diet, pollutant, toxicants…) action, and the individual’s characteristics. Theoretical knowledge on individual development was retrieved for the entire lifespan from the literature and findings from population-based data were investigated using various publications databases (Cochrane Database of Systematic Reviews (CDSR), Current Contents, EMBASE, MEDLINE, Scisearch, Scopus) using specific search criteria. As a main result, ten critical life periods were identified: from early life, pre-conception included, to old age more than 85 years. This will be explored in the transgenerational survey conducted within the European Exposure and Health Examination Survey (EXHES) as part of the HEALS project, where both singletons and twins and their parents and siblings will be recruited.

B. Introduction

The concept of the exposome, representing the totality of exposures received by a person during her or his life from pre-conception onward (Wild, 2005), encompasses three domains, namely:

- external specific exposures that include physical agents like radiation and noise, chemical agents/pollutants, diet/food, pharmaceuticals, drugs, injuries and infections,
- external non-specific exposures, such social economic status, level of education, psychosocial factors, activity levels, behaviour, location and climate, and internal exposures, the internal chemical environment determined by internal processes (metabolic, inflammatory, presence of xenobiotics, gut microbiome, ageing…) assessed through evaluation of gene expression, DNA methylation status, proteins, lipid mediators, xenobiotics and their metabolites. These processes in turn govern the structure, function, and dynamics of an organism. These domains constitute the external exposome and the internal exposome, respectively. Table 1 outlines selected approaches and tools that can be used to measure the exposome. The exposome paradigm may offer scientists a comprehensive approach for investigating the exposures that are responsible of chronic diseases coupling multi-sensor technologies with agnostic and targeted multi-platform analyses supported by advanced bioinformatics and biochemical/kinetic modelling.

The individual exposome has to be intended as dynamic and continually changing. Indeed, all exposures and their determinants and modifiers can vary over the course of a day, not to mention over the weeks, months, and years that make up a lifetime, as our bodies, diets, risk factors and lifestyles change. Because sources and levels of exposure change over time, and because capturing all these changes verges on the impossible in the impracticality of “high-resolution real-time” monitoring of all the exposures for the entire lifetime, the exposome has to be constructed by assessing the exposures at critical life periods through representative snapshots that act as demonstrative measures of these critical periods. In particular, for the comprehension of the mechanisms underlying the development of the diseases, the assessments of the internal chemical environment in biological specimens at critical life stages is mandatory. The major challenge consists then in identifying critical life stages that are informative at most as well as the snapshots reflecting the exposures and the downstream consequences at the individual level.

The main aim of the present review is to provide the state-of-the-art of existing knowledge on critical life stages and events in view of the construction of the exposome and to suggest timings of internal and external exposure assessments. The main specific question, which this review wants to reply, is “In which period of life is it important to monitor the exposome?” This question implies one further question: “When are exposure data collections and in the case of pollutants/toxicants biomonitoring at most informative?” The literature shows that the answer depends on the type of health event, the target organ, the type of stressor action, and individual characteristics but that in general early life, pre-conception included, is already crucial.

Table 1: Some examples of approaches and tools to measure the exposome

Approach	Tools
Biomarkers (omics)	
Untargeted	Genomic sequencing, toxicogenomics (transcriptomics, proteomics, metabolomics), epigenetics
Targeted	Adductomics, lipidomics, immunomics
Sensor technologies (including mobile phones)	Environmental pollutants, physical activity, stress circadian rhythms, location [global positioning systems (GPS)]
Imaging (including mobile phones, video cameras)	Diet, environment, social interactions
Portable computerized devices (including palmtop computers)	Behaviour and experiences (ecological momentary assessment), stress, diet, physical activity
Improved conventional measurements (combined with environmental measures)	Job-exposure matrices, dietary recall (e.g. EPIC-Soft)

In this review, for each disease endotypes (subtype of a condition, which is defined by a distinct functional or pathobiological mechanism), phenotypes (any observable characteristic or trait of a disease, such as morphology, development, biochemical or physiological properties, or behaviour, without any implication of a mechanism) and sub-phenotypes were considered.
This review does not want to be systematic; its purpose is to provide rather essential messages than detailed information. Of note, the effects of acute exposure to toxicants, responsible for acute intoxication for example, were not considered as of interest as punctual and thus not characteristic of the exposome.

C. Methods

Approaches to identify clinical life stages life stages were built on: 1) scientific knowledge on timelines of development of organs and systems in early life and childhood and 2) data and results from population and other studies in the entire lifespan. They were based on the search methods indicated below.

1. Search methods

Search terms and databases to be searched have been defined as follows:

- Search terms: ["critical events" or "critical stages" or "periods of susceptibility" or "periods of vulnerability"] and ["environmental exposure" or "exposure" or "exposome", "epigenetics" …..] and

["health" or "immunity" or "hormonal changes" or "organ development" or "obesity" or "psychomotor and neurodevelopment"] and ["prenatal" or "postnatal" or "childhood" or "adolescence" or "adulthood" or "elderly"]. Meta-analyses were considered as bringing more convincing proof compared to other study designs. For this, meta-analysis was added as filter.

- Databases: Cochrane Database of Systematic Reviews (CDSR), Current Contents, EMBASE, MEDLINE, Scisearch, Scopus.

2. Evidence criteria to accept associations and findings

The judgement of the strength of evidence of associations and findings from population studies has been based on the number of available studies, the study design and its level of proof (Table 2a).

Table 2a: Level of proof of study design and evidence of the associations and findings

Type of study/publication	Level of proof	Strength of proof of the studies
Meta-analysis of randomised, controlled intervention studies	Ia	Convincing
Randomised controlled intervention studies	Ib	Probable
Non-randomised/non-controlled intervention studies (if well-designed, otherwise level IV)	Ic	Possible
Meta-analysis of cohort studies	IIa	Convincing[a]
Cohort studies	IIb	Probable[b]/possible[c]/insufficient[d]
Meta-analysis of case–control studies	IIIa	Probable[b]/
Case–control studies	IIIb	Possible[c]/
Non-analytic; (Cross-sectional studies, case reports etc.); Reports/opinions of expert committees or consensus conferences, which did not determine the strength of the evidence, and/or clinical experience of respected authorities	IV	Possible[c]/insufficient [d]

In total, four categories of strength of evidence have been established *a priori* for judging the relevance of associations and findings: convincing, probable, possible, and insufficient (Table 2b). We propose these criteria as judgement criteria, although these criteria could not be entirely applied in this review because of lack of data.

Table 2b: Level of evidence of associations and findings

Strength of evidence of associations and findings	
Convincing	A finding or association is judged as "convincing" if at least 2 studies of highest quality (level of proof I) including prospective observational studies and, wherever possible, randomised controlled intervention studies of sufficient size, duration and quality with consistent results.
Probable	If at least 4 studies show fairly consistent relations between factor and disease (level of proof I and/or level of proof II), but there are noticeable weaknesses regarding the evidence or there is evidence of an opposite relation, which does not allow a definite judgement
Possible	The strength of the association is judged as "possible", if at least 3 epidemiological studies (proof up to III) showed consistent results. There may exist a few other studies without any statistical relationships.
Insufficient	The strength of the association is judged as "insufficient" if data were lacking because the relation has not yet or only rarely been investigated in the studies. Further criteria were inconsistent results with a majority of studies without association and nearly equally as strong opposite results. However, despite the assignment of the proof of evidence to each study and the strict specification of the strength of evidence, the database has not been shown to be always clear. Thus, in addition to the proof of evidence and the number of studies, both the assessment of the study quality and the current estimation of the studies' importance based on its design and size were considered as well.

D. Defining critical life stages and events and windows of exposure

Critical life stages, critical life events and critical window of exposure

There exist several definitions of critical life stages and events according to the type of domain in which they have been used. For the purpose of the HEALS project:

- Critical life stages were defined as the periods of time in an individual's lifespan in which critical life events occur characterized by changes of the organism status, because some quality, property or phenomenon suffers a definitive modification. Examples of critical life events include, for instance, fetus development according to the stage of growth, immune system maturation, organs development, puberty, menopause…. These changes can be normal or abnormal as result of either reshaping of the ordinary pattern (for instance: anticipation of puberty) or modification of the event (for instance: event amplification, increase in severity…) (Figure 1), the latter because of external or internal influences. Vice versa, changes in exposures may be present as a consequence of developmental changes or altered patterns of behaviour.
- Windows of exposures are the periods of time in an organism's lifespan in which the organism is the most susceptible or vulnerable (see the definitions below) to the adverse effects caused by exposure to stressors including toxicants at the origin of abnormal and pathophysiological changes.

It is important to underline here that a same critical life event can be observed at different life stages.

E. Defining susceptibility and vulnerability

Individuals are not all equal in the respect of environment impacts depending on personal characteristics and situations. The terms of susceptibility and vulnerability are used to describe these differences *(http://www.integrated-assessment.eu/guidebook/vulnerable_and_susceptible_groups)*.

- Susceptibility refers to the degree to which individuals or groups may respond to a given exposure to a hazard. Susceptibility can be subdivided into innate and acquired susceptibility. Innate susceptibility is to a large extent due to genetic predisposition or to incomplete development of normal (adult) physiological functions. For example, a young child may be susceptible to a given pollutant because detoxification processes are not yet fully developed. Such susceptibility is transient and disappears with age and growth. Acquired susceptibility may be due to disease or age.
- Vulnerability refers to the variations in exposure between individuals or groups - and thus to the potential for health effects. This is likely to be due to variations in the hazards themselves as well as to the fact that exposure is also a function of where people live, how (and where) they spend their time, and their more general lifestyle. In the case of pesticides, living close to areas where crops are sprayed or eating foodstuffs that have been heavily treated during production, storage or processing likewise acts to increase exposure, and thus vulnerability.

Susceptibility and vulnerability change according to the age and social factors respectively. Susceptibility is increased in young children and in elderly. Vulnerable groups refer to specific populations within a country that have been excluded from financial and social services for a variety of reasons that increase their risk of exposures and adverse health outcomes.

F. Results

* Critical life stages in human beings

At the end of the review process, ten critical stages have been identified as of interest for the exposome of the major health outcomes considered in HEALS, namely asthma and allergies, overweight and diabetes and neurodevelopmental troubles (Figure 2):

1. Pre-conception
2. Pregnancy
3. Birth and perinatal period
4. Infancy before 3 years of age
5. Childhood (school children)
6. Teen-ageing and adolescence
7. Adulthood before 40 years
8. Adulthood before 65 years (in the 50s)
9. Adulthood before 80-5 years (according to the gender, 85 years in women)
10. Adulthood after 80-5 years

These stages correspond to phases of development in humans (Table 3).

Preconception life is crucial for the development of oocytes and spermatozoa that is important for the transmission of susceptibility even before fertilization. Gestation is important for embryonic development, organogenesis, cell migration and growth of the fetus.

Perinatal life is relevant for the development of the immune system, among others through lactation. Infancy is characterized by the continuation of the development of the acquired immune system through infections and other exposures. Thymic involution marks the middle childhood. Hormonal influences feature the adolescence. Adulthood is characterized by maturation, menopause, ageing and senescence. Old age is characterized by both normal and pathological ageing and underlying phenomena. Ageing is a complex multifactorial process, reflecting the progression of all degenerative pathways within an organism. Due to the increase of life expectancy, in recent years, there is a pressing need to identify early-life events and risk factors that determine health outcomes in later life. Genetic variation only explains ~20-25 % of the variability of human survival to age 80+. This clearly implies that other factors (environmental, epigenetic and lifestyle) contribute to lifespan and the rate of healthy ageing within an individual. Thereafter defining the exposome in the elderly is of great interest.

In these critical stages, various processes can be considered: metabolism, endogenous circulating hormones, body morphology, gut microflora, inflammation…

Of note, data up to adolescence allow to avoid reverse causation (*a posteriori* data do not allow to disentangle whether the effect has preceded the cause without any mechanistic consideration to guide the analysis). However, adulthood and ageing have also to be considered as important life periods for chronic diseases taking into account that important clinical and subclinical phenomena start with organ maturity.

Table 3: Major stages of human development

1) Preconceptual: that is, oocyte and spermatozoa development, particularly important for oocyte development as susceptibility may be prolonged for many years before fertilization
2) Gestational: Embryonic ➔ mid-gestation, late fetal (organogenesis, cell migration, growth, education)
3) Perinatal: early postnatal (lactation)
4) Infancy: early childhood – infectious exposures
5) Middle childhood – thymic involution
6) Adolescence – hormonal influences
7) 'Maturity'
8) Senescence

Source : West LJ, 2002

Twin studies in the past two decades proved to be a very powerful tool to discriminate the genetic from the environmental component. Metabolomics analysis in twins is currently under investigation. It appears to be possible to discriminate between monochorionic and dichorionic twins. Important metabolites appear to be galactitol, N-acetylcysteine, N-acetylglutamate, N-acetyltyrosine, methylguanidine, N-dimethylformamide and 5-hydroxyindol-3- acetate.

1. From pre-conception to adolescence

The information obtained for early childhood will be applied in the EXHES: European Exposure and Health Examination Survey planned in HEALS (www.heals-eu.eu) to recruit birth cohorts of children to be followed-up from pre-conception to at least 3 years.

Normal life events that occur in the periods of life going from preconception to adolescence data are well-known on the theoretical level. The first trimester is the most critical period for fetal development:

- The start of the third week after fertilization marks the beginning of the embryonic period : the period most crucial to organ and structural development
- The fetal period is usually considered to start by the eighth week after fertilization. Development from this period consists of the growth and maturation of structures that were formed during the embryonic period. According to Williams Obstetrics, a primary textbook in the obstetrical field, the milestones in fetal development can be marked every four weeks of the fetus's menstrual/gestational age.

- The twenty-fourth week represents a major milestone as hospitals with high-tech (level 3) neonatal intensive care nurseries and neonatal specialists consider fetuses at this age to be viable.
- The thirty-sixth week represents another important milestone.

One example is the response to stress and affective state that are progressively altered in pregnant women, suggesting that timing of stress exposure during gestation may be critical in determining its impact (first trimester stress has greater effect on the child's health than third trimester stress). Reversely, data from population studies on abnormal life events are sparse for these periods so that evidence of findings is at maximum probable with convincing evidence only rarely.

Susceptibility and vulnerability in early life and childhood

Ways in which early life impacts in children on susceptibility and vulnerability to adverse environmental exposures include:

- *Dynamic developmental physiology* - Due to their dynamic developmental physiology children are often subjected to higher exposures to toxicants found in air, water and food. These exposures may be handled quite differently by an immature set of systems (respiratory, metabolic, endocrine, neurodevelopmental…). Furthermore, the developmental component of a child's physiology is changing; maturing, differentiating and growing in phases known as "developmental windows". These "critical windows" create unique risks for children exposed to hazards that can alter normal function and structure. The timing of these windows during development is important for understanding downstream health consequences of environmental exposures.
- *Different and unique exposures*- children often have different, and sometimes unique, exposures to environmental hazards from those of adults. Young children exhibit exploratory behaviour, exemplified by hand-to-mouth activity, which is most prevalent in children between 1 and 3 years of age. Children learn by putting things in their mouths and can ingest significant quantities of contaminated soil, dust and dirt at early ages.

1.1. Based on scientific knowledge and timelines of development

Preconceptual life has been identified as of risk for the offspring. A growing body of research across numerous disciplines has identified an important link between individuals' health at birth, in infancy, and in early childhood and their later-life health, educational attainment, and overall wellbeing (Almond D, 2011). In particular, studies of siblings and twins in several countries, including the United States, show that low birth weight (less than 2,500 grams) children have greater infant mortality rates, lower cognitive test scores, more behavioural problems throughout childhood, and lower IQ scores. There is also a wealth of medical evidence that links preterm births (children born at less than 37 weeks of gestation) to increased mortality rates. Other complications related to adverse events in *in utero* life include respiratory distress syndrome, asthma, chronic lung disease, injury to intestines,

compromised immune system, obesity, diabetes, cardiovascular disorders, hearing and vision problems, psychiatric conditions, and neurological insults (Behrman R, 2007).

Exposures to various risk factors during childhood have been related to asthma and allergies, overweight and diabetes, and neurodevelopmental troubles.

Lastly, adolescence through hormones changes is significant for asthma and allergies development, weight gain or loss and behaviour.

One crucial factor that influences to what degree health may be affected is the time at which the developing and/or changing organism is exposed to a specific risk. Some factors cause damage only during specific days or weeks early in pregnancy, when a particular part of the body is being formed. Others can be harmful at any time, but how severe the damage is, depends on when the exposure occurred. The time of greatest susceptibility is called the critical window or period. Each body structure has its own critical period. A gestation trimester in particular or the entire prenatal period can be considered as a critical period of development.

In the context of the period from early life, including preconception, to adolescence, one broad life course model could be suggested to highlight the importance of timing and duration of exposures on later health and disease risk. The critical period model is defined as a model when an exposure in earlier life has lifelong effects on structure or function that are not modified by later experience. This critical period usually corresponds to a period of rapid change when there are rapid and generally irreversible intrinsic changes towards greater complexity taking place.

Rappaport and Smith (Rappaport SM, 2010) proposed a number of key stages of life (snapshot approach) where cross-sectional measures of the exposome could be made, including gestation, early childhood, puberty and the reproductive years.

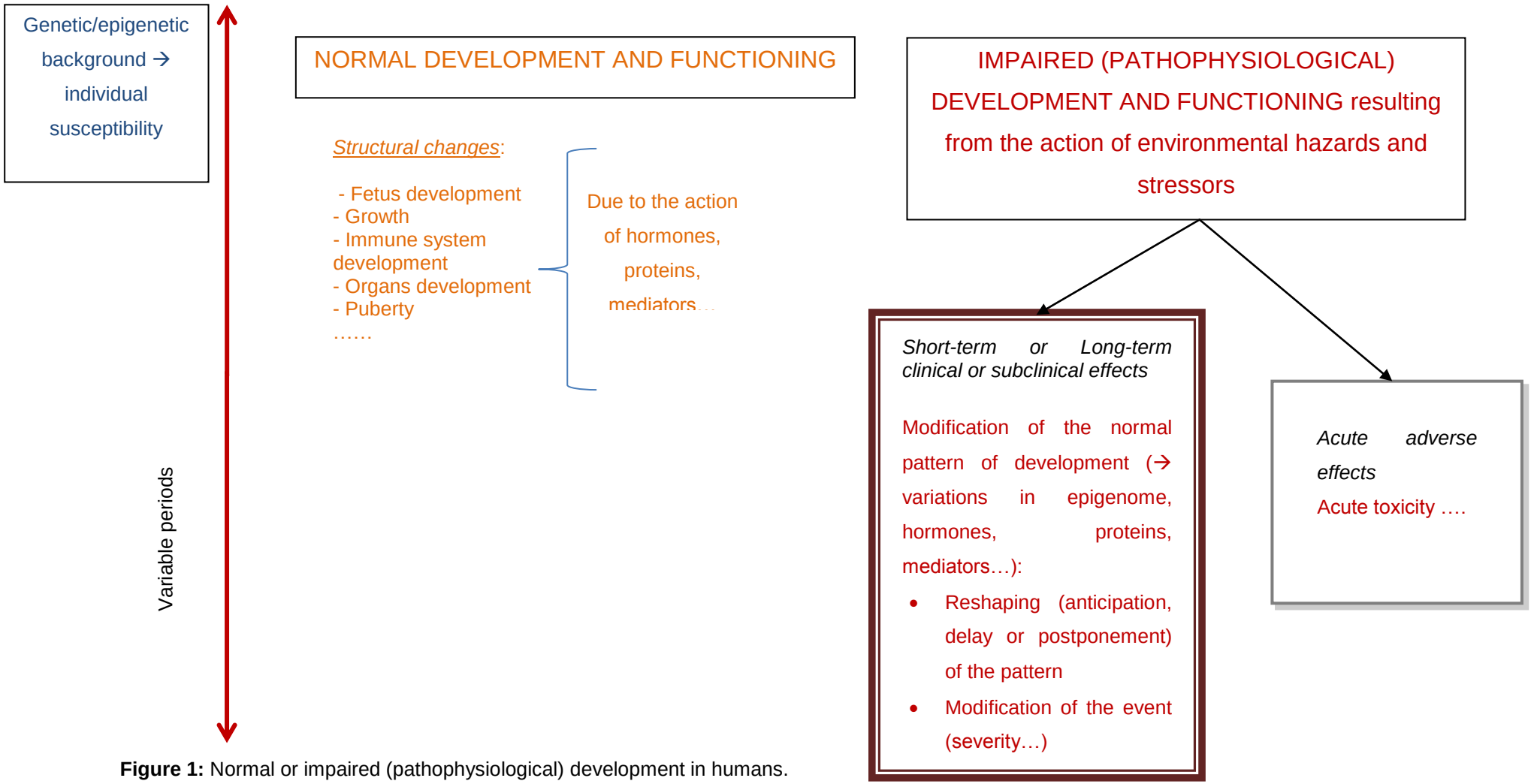


Figure 1: Normal or impaired (pathophysiological) development in humans.

1.1.1.Immunity, respiratory and allergic diseases

The first step in identifying critical windows for the development of diseases involving the immune system, such as asthma and allergies, is defining general stages of development during which exposures may be toxic to the immune system. Human developmental stages can be characterized as shown in Table 3 (West LJ, 2002). The developmental stage of the individual must then be integrated with other fundamental factors that may predispose the developing immune system to risk, and which may vary significantly between individuals. These factors include biologic parameters of the fetus, infant, child, and adolescent, such as differences in metabolism, in physiology and in other characteristics.

Identifying critical windows in the immune system development is crucial for the determination of either safety or vulnerability to exposure to specific agents during rapidly changing phases of ontogeny. In human beings, these phases range from post-conception and early gestation to adolescence. A detailed understanding of these windows will facilitate avoidance of environmental pollutants as well as allow improved planning for unavoidable exposures. Critical windows of immune development are influenced by concomitant development, maturation and growth of other organ systems, thus the influence of potentially toxic exposures must be determined within a coordinated multisystem and multidisciplinary approach. Several timelines for human immune system development have been suggested and have highlighted the existence of different critical windows. Figure 2 shows a timeline for human development including conception, prenatal development, birth and neonatal maturation to 2 years of age.

Above the timeline, four categories of environmental risk factors are shown that have been reported to contribute to both postnatal immune dysfunction and paediatric allergic disease. Below the timeline, four developmental windows of immune maturation are illustrated that have particular relevance to allergic disease and asthma (AD-A). Disruption of maturation among any of the four windows is likely to impact the risk of childhood AD-A. Among the designations, BALT (bronchus-associated lymphoid tissue) and GALT (gut-associated lymphoid tissue) are specialized immunological tissues that represent part of the mucosal immune tissue.

❖ *T-cells development stages during gestation and acquisition of immune competence in childhood and adolescence*

T lymphocytes cells are essential for the development of the immune response involved in asthma and allergies. Table 4 present the stages of T-cell development during pregnancy. After birth, there is continued acquisition of immune competence concomitant with increased antigen exposure until the age of 2 years. During this time and after,

Table 4: Comparison of T-cells development stages between mouse and human

Function	Mouse	Human
Length of gestation	21 days	40 weeks
Lymphohematopoietic cells colonize primordial thymus	Days 11-12	Week 9
Morphologic division of thymus into cortex and medulla	Days 13-14	Weeks 11-14
Expression of γδ TCR then αβ TCR	Days 14-16	Weeks 11-13
Proliferative response demonstrable in MLR	Days 16-18	Week 12 (thymus) Week 19 (spleen) (weak until week 23)
Mitogen responsiveness	Day 18 (thymus) (to some mitogens only after birth)	Weeks 13-14 (thymus) Weeks 16-18 (spleen, peripheral blood)
Cytotoxic response demonstrable (CML)	Weak until postnatal day 7	Beginning from about weeks 20-23 (thymus)

Source : West LJ, 2002

Legend: γδ TCR: gamma delta T-Cells Receptors; *αβ* TCR: alpha beta T-Cells Receptors; MLR: Mixed-Lymphocyte Reaction; CML: chronic myelogenous leukemia

❖ Environmental/epigenetic and genetic programming of a respiratory disease such asthma

Some papers have highlighted different epigenetic and genetic effects of perinatal environments and parental genetic backgrounds on the perinatal programming of asthma (Yang KD, 2002). These factors play different role at distinct crucial period of development. As summarized from these articles and shown in Figure 3, maternal prenatal environments have a strong impact on the programming of childhood asthma in which folic acid supplement, parental smoking, oxidative stress, and cold and herb medication are risk factors for childhood asthma. In new-born and infant stage, prematurity, vacuum delivery, skewed (high or low) birth weight, and bottle feeding are risk factors for childhood asthma. In toddler stage, early day-care placement, antibiotic uses, and parental smoking are also associated with childhood asthma. The environmental programming of asthma in perinatal stage is likely mediated via epigenetic modification or immune differentiation toward a higher T-cell type 2 (Th2) response or a lower T-cell regulatory type (Treg) differentiation.

.

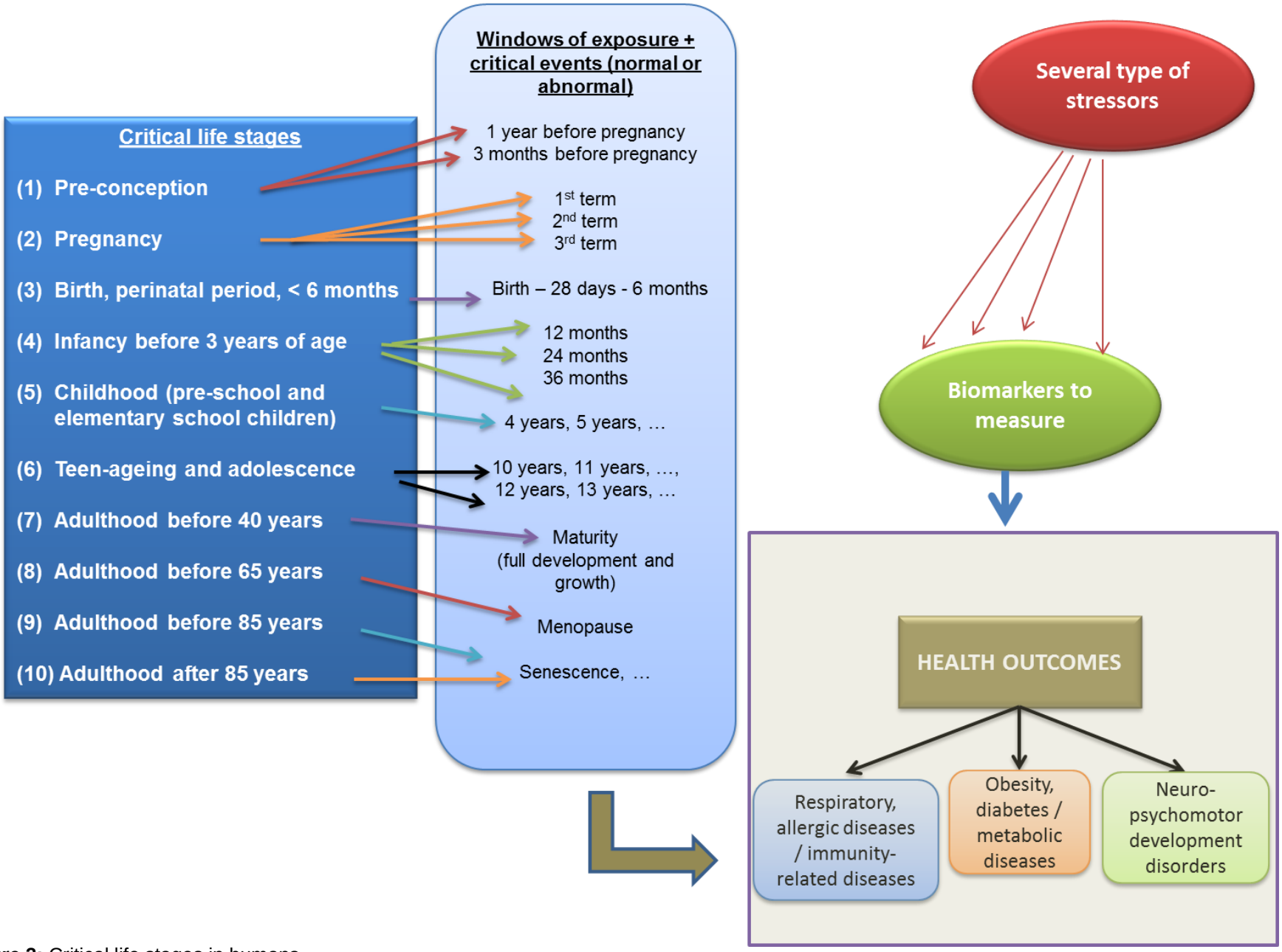
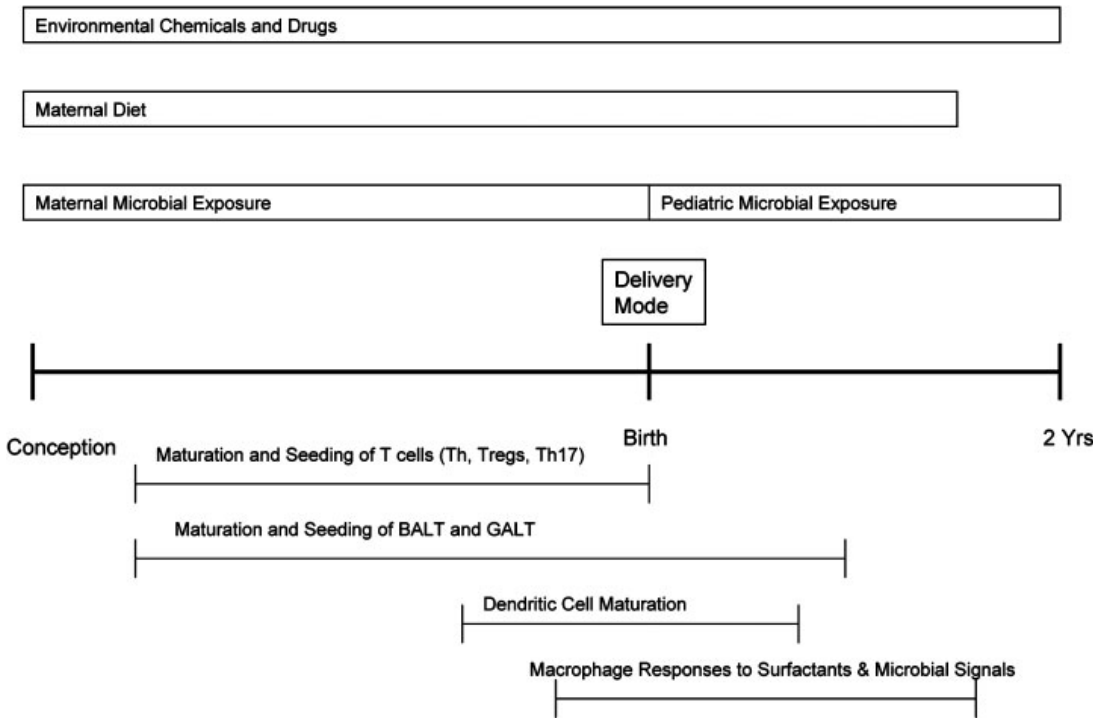


Figure 2: Critical life stages in humans



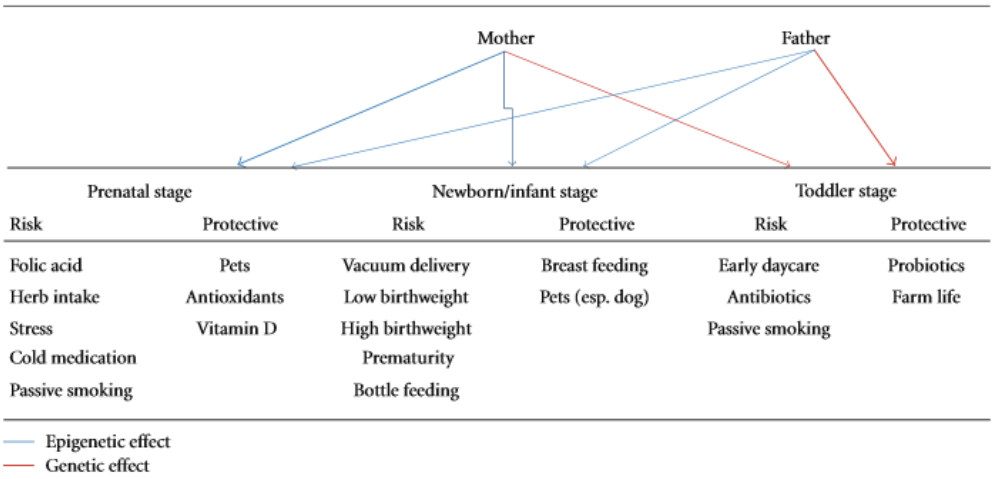
Source: Dietert RR, 2008

Figure 3: Timeline for human immune system development from conception to 2 years. Legend: Th = T helper cell lymphocyte population (such as Th1 and Th2 cell populations); Treg = T regulatory cells; Th17 are a specialized population of T helper cells that produce interleukin-17.

On the other hand, paternal influence on the development of asthma occurs in later childhood, suggesting genetic effects on the development of allergic sensitization are also important in children exposing to aeroallergens, pollution, and complementary foods beyond infancy. Fortunately, not all perinatal factors are risk to the development of childhood asthma; certain perinatal factors may contribute to protection from the development of asthma. Prenatal exposure to pets, vitamin D supplement, or antioxidants such as Mediterranean diet is shown to be protective from the development of asthma. In postnatal stage, breastfeeding, intake of probiotics, and growing up in farms are associated with less risk to allergy.

- Timeline of critical windows of exposure for immune system development

Each critical life stage is different from another according to the period of life. For example, several developmental toxicants and pollutants are more toxic to children than to adults, when considering their effects on the immune and respiratory systems. Differential windows of vulnerability during development can be identified. Specific approaches have been provided to directly investigate differential windows of vulnerability (Dietert RR, 2000). These approaches are based on fundamental developmental biology and the existence of discrete developmental processes within the immune and respiratory systems.



Source : Yang KD, 2012

Figure 4: Summary of environmental (epigenetic) and genetic programming of asthma

The processes are likely to influence differential developmental susceptibility to toxicants and other pollutants, resulting in lifelong toxicological changes. Although there are many examples of developmental immunotoxicity, the information to date tends to be based on rather broad categories of development (e.g., exposure throughout gestation or exposure during gestation and lactation vs. exposure of the adult). Given the relative scarcity of information regarding chemicals and differential risk during more narrow and discrete windows of embryonic and early postnatal development, the first challenge is one of defining logical developmental windows for future comparison. Fortunately, this can be based on known developmental changes occurring within the immune system as well as on selected immunotoxicity data. Clearly, a standardized approach to compare specific periods of embryonic and juvenile periods of development for relative immunotoxic risk would facilitate the comprehensive risk assessment process. We have attempted to identify discrete windows of immune development where differential immunotoxic risk is likely to exist and through which comparative assessment can be pursued (Figure 3). A parallel approach for the respiratory system is shown in Figure 4.

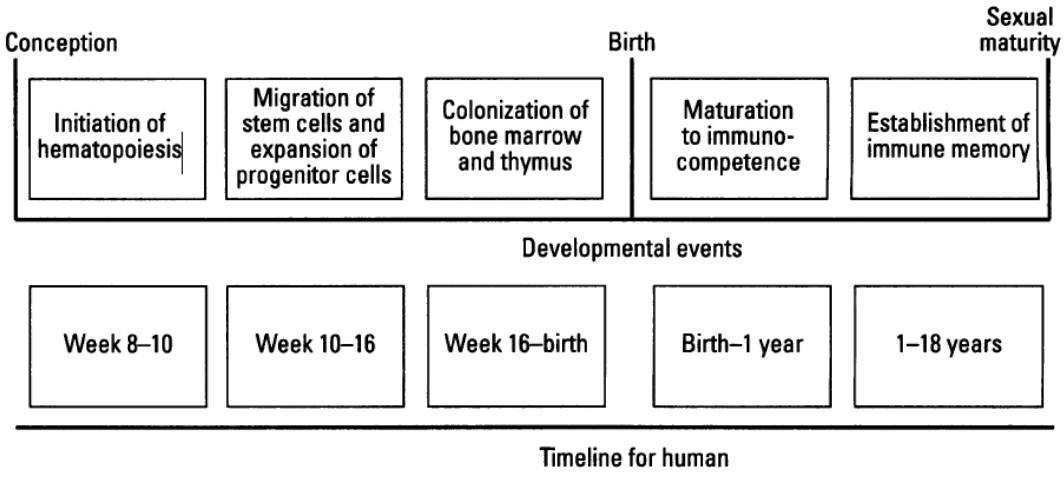


Figure 5: Timeline of critical windows of exposure for immune system development.

In the case of the immune system development, the windows represent discrete steps in the formation of the mature immune system and periods in which differential vulnerabilities to immunotoxicants might be expected (Figure 5). Thymus and immune-competence maturation occurs in the first year of life followed-up by the establishment of the immune memory up to adolescence.

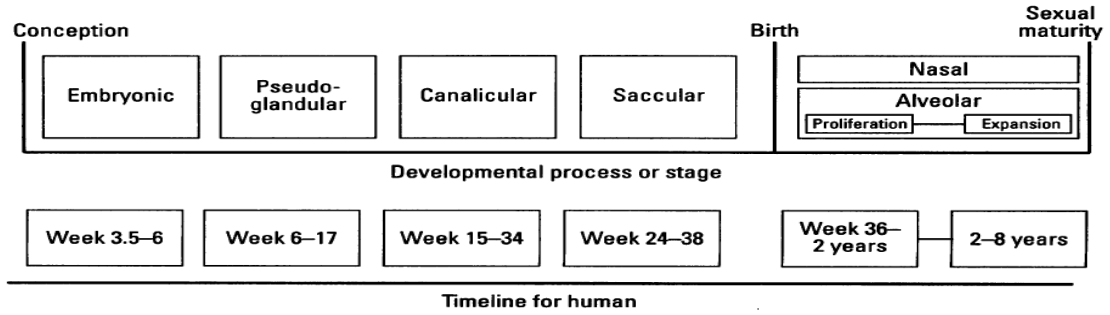


Figure 6: Timeline of critical windows of exposure for respiratory system development, illustrated as discrete maturational windows during pre- and postnatal development.

In the respiratory system development, the maturational stages are periods in which differential vulnerabilities to respiratory toxicants/pollutants would likely occur and depend on morphology (Figure 6). The structure of the respiratory system develops until 8 years but the lung function grows until 20-25 years.

1.1.2. Obesity, diabetes and other metabolic diseases

Metabolic diseases result from complex interactions of many factors, including genetic, physiological, behavioural and environmental influences. The recent rate at which these diseases have increased suggests that environmental and behavioural influences, rather than genetic causes, are fuelling the present epidemic. In this context, the developmental origins of health and disease hypothesis has highlighted the link between the peri-conceptual, fetal and early infant phases of life and the subsequent development of adult obesity and the metabolic syndrome.

Adolescence may be the most critical period of metabolic disease development. This period is characteristic of changes in body composition (location and quantity of body fat), physical fitness and decreased insulin sensitivity during puberty. This period of growth and maturation is also marked with behavioural changes in diet, physical activity, sedentary behaviour and psychological health. Physical activity and sport participation decline during adolescence especially in teenage girls, while sedentary behaviour, risk for depression and body esteem issues increase during the teenage years. These physiological and behavioural changes during adolescence warrant the attention of health practitioners to prevent the onset and continuation of obesity throughout the lifespan. The adolescent phase is the period between childhood and adulthood (13–19 years), marked by changes in body composition, insulin sensitivity and growth during pubertal maturation. Obesity is caused by an energy imbalance between energy intake (carbohydrates, fat and protein) and energy expenditure (resting metabolic rate, thermic effect of food and physical activity), and is continuously influenced by the obesogenic environment that we live in. There are several behavioural changes in dietary habits,

physical and sedentary activity patterns that take place during adolescence that may influence the development and persistence of obesity.

1.1.3. Neuro/psychomotor development disorders

Neurodevelopment

The first major neurodevelopmental event critical to normal brain development is formation of the neural tube, a process event known as neurulation. In humans, this occurs by 3–4 weeks of gestation (Workman AD, 2013), following which cortical neurogenesis occurs predominantly during *in utero* gestation, but can continue up to 2.5 years of age (Workman AD, 2013), outside the neurogenic niches that persist in defined areas of the postnatal brain. Besides, hippocampal neurogenesis peaks around 8–9 weeks and this can persist well into the postnatal period (Workman AD, 2013). Gliogenesis also begins during the *in utero* period and mature astrocytes are present in the brain by 15 weeks of gestation. Although these vary in density in different anatomical locations, they continue to differentiate throughout the fetal stage and well into the postnatal period (Roessmann U, 1986). This peak period of gliogenesis coincides with a large increase in neuronal complexity through elaboration of the dendritic fields (Wise SP, 1976), coupled with a robust increase in synaptogenesis only after astrocytes appear within the brain (Barker AJ, 2010), suggesting mechanisms exist to ensure that appropriate neuronal–glial interactions are established.

The majority of organ and tissue development occurs during embryogenesis, and postnatal changes are primarily concerned with growth. In addition, considerable amount of morphological development, cell differentiation, and acquisition of function takes place during postnatal development. Synaptogenesis begins in earnest in the human brain after approximately post-birth (after the appearance of astrocytes) and synaptic density increases rapidly after birth to reach maximum levels by approximately 2 years of age, at which point there are 50% more synapses than are found in the adult brain (Petanjek Z, 2011). After this stage, the brain undergoes a process of synaptic refinement and elimination to reduce the number of synapses in a region-specific manner to adult levels by mid-adolescence (Glantz LA, 2007).

Brain maturation undergoes a crucial developmental phase during childhood and adolescence. The adolescence period is considered the most critical for development and onset of various brain disorders (Figure 5) (Paus T, 2008). Early adolescence is a key stage during neurodevelopment with various structural, neurochemical, and molecular changes occurring in response to genetic and environmental signals (Paus T, 2008). These include synaptic pruning, where the elimination of the extra synapses occurs, resulting in decreased levels of cortical gray matter as the brain matures. Coinciding with this is the formation of new neuronal connections producing a phase of high plasticity throughout much of the brain.

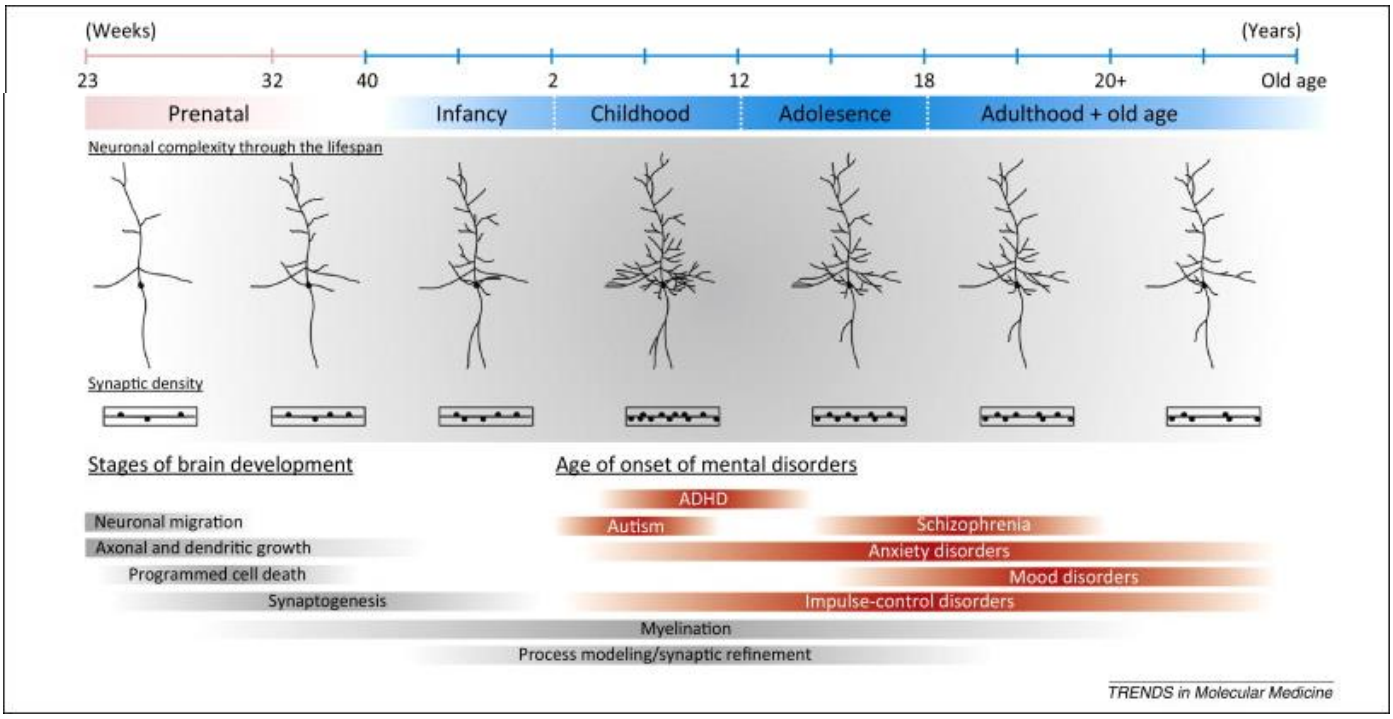


Figure 7: Temporal profile of neurodevelopmental sequences in relation to the age of onset of mental disorders throughout life. During critical stages, the organism is vulnerable to external stressors, which may result in mental disorders. ADHD, attention deficit hyperactivity disorder.

Psychomotor development

Postnatally, during the early life period, most infants develop motor abilities in the same order and at approximately the same age. In this sense, most agree that these abilities are genetically pre-programmed within all infants. The environment does play a role in the development, with an enriched environment often reducing the learning time and an impoverished one doing the opposite.

The following chart delineates the development of infants in sequential order. The ages shown are averages and it is normal for these to vary by a month or two in either direction.

2 months – able to lift head up on his own

3 months – can roll over

4 months – can sit propped up without falling over

6 months – is able to sit up without support

7 months – begins to stand while holding on to things for support

9 months – can begin to walk, still using support

10 months – is able to momentarily stand on her own, without support

11 months – can stand alone with more confidence

12 months – begin walking alone without support

14 months – can walk backward without support

17 months – can walk up steps with little or no support

18 months – able to manipulate objects with feet while walking, such as kicking a ball

These new motor skills are the most dramatic and visible changes in the first few years of life, frequently noted and commented on.

Other critical stages have been revealed in regards to cognitive and psychosocial development. Probably the most cited theory in the cognitive development in children is Jean Piaget. As with all stage theories, Piaget's Theory of Cognitive Development maintains that children go through specific stages as their intellect and ability to see relationships matures. These stages are completed in a fixed order with all children, even those in other countries. The age range, however can vary from child to child. Piaget described different stages: (1) the sensorimotor stage: this stage occurs between the ages of birth and two years of age, as infants begin to understand the information entering their sense and their ability to interact with the world. The major achievement during this stage is that of object permanency, or the ability to understand that these objects do in fact continue to exist. This includes his ability to understand that when mom leaves the room, she will eventually return, resulting in an increased sense of safety and security. Object Permanency occurs during the end of this stage and represents the child's ability to maintain a mental image of the object (or person) without the actual perception; (2) the preoperational stage: the second stage begins after object permanency is achieved and occurs between the ages of two to seven years of age. During this stage, the development of language occurs at a rapid pace. Children learn how to interact with their environment in a more complex manner through the use of words and images. A second important factor in this stage is that of conservation, which is the ability to understand that quantity does not change if the shape changes; (3) the concrete operations stage: occurring between ages 7 and about 12, the third stage of cognitive development is marked by a gradual decrease in centrist thought and the increased ability to focus on more than one aspect of a stimulus. They can only apply this new understanding to concrete objects (those they have actually experienced); (4) the formal operations stage: in the final stage of cognitive development (from age 12 and beyond), children begin to develop a more abstract view of the world. By the teenage years, they are able to develop their own theories about the world. This stage is achieved by most children, although failure to do so has been associated with lower intelligence.

Like Piaget, Erik Erikson maintained that children develop in a predetermined order. Instead of focusing on cognitive development, however, he was interested in how children socialize and how this affects their sense of self. Erikson's Theory of Psychosocial Development has eight distinct stage, each with two possible outcomes. According to the theory, successful completion of each stage results in a healthy personality and successful interactions with others. Failure to successfully complete a stage can result in a reduced ability to complete further stages and therefore a more unhealthy personality and sense of self. These stages, however, can be resolved successfully at a later time.

(1) trust versus mistrust: from ages birth to one year, children begin to learn the ability to trust others based upon the consistency of their caregiver(s). If trust develops successfully, the child gains confidence and security in the world around him and is able to feel secure even when threatened. Unsuccessful completion of this stage can result in an inability to trust, and therefore an sense of fear about the inconsistent world. It may result in anxiety, heightened insecurities, and an over feeling of mistrust in the world around them; (2) autonomy vs. shame and doubt: between the ages of one and three, children begin to assert their independence, by walking away from their mother, picking which toy to play with, and making choices about what they like to wear, to eat, etc. If children in this stage are encouraged and supported in their increased independence, they become more confident and

secure in their own ability to survive in the world. If children are criticized, overly controlled, or not given the opportunity to assert themselves, they begin to feel inadequate in their ability to survive, and may then become overly dependent upon others, lack self-esteem, and feel a sense of shame or doubt in their own abilities; (3) initiative vs. guilt: around age three and continuing to age six, children assert themselves more frequently. They begin to plan activities, make up games, and initiate activities with others. If given this opportunity, children develop a sense of initiative, and feel secure in their ability to lead others and make decisions. Conversely, if this tendency is squelched, either through criticism or control, children develop a sense of guilt. They may feel like a nuisance to others and will therefore remain followers, lacking in self-initiative; (4) industry vs. inferiority: from age six years to puberty, children begin to develop a sense of pride in their accomplishments. They initiate projects, see them through to completion, and feel good about what they have achieved. During this time, teachers play an increased role in the child's development. If children are encouraged and reinforced for their initiative, they begin to feel industrious and feel confident in their ability to achieve goals. If this initiative is not encouraged, if it is restricted by parents or teacher, then the child begins to feel inferior, doubting his own abilities and therefore may not reach his potential; (5) identity vs. role confusion: during adolescence, the transition from childhood to adulthood is most important. Children are becoming more independent, and begin to look at the future in terms of career, relationships, families, housing, etc. During this period, they explore possibilities and begin to form their own identity based upon the outcome of their explorations.

Role of epigenetics

Epigenetic processes have profound influence on gene translation and play a key role in embryonic development and tissue type specification. Recent advances in our understanding of epigenetics have pointed out that epigenetic alterations also play an important role in neurodevelopment and may increase the risk to psychiatric disorders. In addition to genetic regulation of these processes, compelling evidence suggests that environmental conditions produce persistent changes in development through epigenetic mechanisms. Adverse environmental influences in early life such as maternal care, alcohol exposure and prenatal nutrition interact with epigenetic factors and may induce neurodevelopmental disturbances that are related to psychiatric disorders (Kofink, 2013).
The EXHES study in which it is planned to dispose of various members of the same family affords the opportunity to make transgenerational epigenetics. Transgenerational epigenetics provides a comprehensive analysis of the inheritance of epigenetic phenomena between generations. Recent research points to the existence of biological phenomena that are controlled not through gene mutations, but rather through reversible and heritable epigenetic processes. Epidemiological studies have suggested that environmental factors may be heritable. In fact, environmental factors often play a role in transgenerational epigenetics, which may have selective or adverse effects on the offspring. This epigenetic information can be transferred through a number of mechanisms including DNA methylation, histone modifications or RNA and the effects can persist for multiple generations.

1.2. Based on observational studies

Few population studies met the inclusion criteria and were included in the review. There was heterogeneity across studies in terms of methodology, including the assessment of exposures, biological assessments and health outcomes and results. Table 5 shows a summary of reviewed studies in order to determine the differents period of time under study and the nature of the internal and external exposure assessments, from early life to adolescence. Such data are sparse for these periods so that evidence of findings is mostly probable.

Population study confirms Rappaport's and Smith's snapshot approach (Rappaport, 2010) proposing a number of key stages of life where cross-sectional measures of the exposome could be made, including gestation, early childhood, puberty. These represent rational selections likely to exhibit significant differences in exposure patterns for a given individual. Notably, study populations show increasing evidence that early life and *in utero* exposures or those during adolescence, including factors as diverse as birth weight, diet including probiotics, air pollution, chemicals, moulds, pets, hormones infections and maternal psychosocial stress, are important to chronic disease risk later in life. However, population studies also show that pre-conceptional life is of relevance as a crucial period for making change to the lifestyle and diet that can both help increase the chances of getting pregnant and birthing a healthy baby. Recent data have identified additional factors of risk and shown that both parents may play a risk for offspring's health. Pre-conceptional maternal and paternal concentrations of several POPs were associated with statistically significant differences in birth size among offspring (Robledo CA, 2014). Preconception has then to be considered as another snapshot.

Of note, monozygous twins share a common genotype. However, most monozygotic twin pairs are not identical; several types of phenotypic discordance may be observed, such as differences in susceptibilities to disease and a wide range of anthropomorphic features. There are several possible explanations for these observations, but one is the existence of epigenetic differences. Twins will be considered in HEALS.

Table 5: Life critical events according to observational studies in childhood

Type of study **Country (If possible)** **Author**	**n**	**Age** **Period**	**Health event (Q or Dg)**	**Health assessment (clinical tests, spirometry, scales…)**	**Biosample (blood, urins, saliva…°**	**Biological assessment (eosinophilia, omics, DNA, RNA…)**	**Environmental stressor (questionnaire)**	**Environmental stressor (objective assessments)**	**Main results and <u>period of assessment</u>**	**Evidence***
Cohort USA Zhumin Z	285	Birth-8 yrs	Asthma	Spirometry, height, weight	none	none	none	none	Being <u>overweight</u> at age <u>1 year</u> was associated with a decreased risk of asthma at age 6 and 8 yrs and better lung function. Children who were overweight at age 5 but not at age 1 year had increased risk of asthma at age 6 yrs	IIb
Population-based prospective cohort The Netherlands Sonnenschein-van der Voort A	5125	Birth- 4 yrs	Asthma symptoms, eczema (Q)	-Fetal:Crown-rump length, head circumference, abdominal circumference, femur length. - Infant:length, weight, head circumference	none	none	none	none	Weight gain acceleration was associated with increased risks of asthma symptoms in preschool children, independent of fetal growth. Early infancy = critical period for the development of asthma	IIb
Randomized, double-blind, placebo-controlled trial. Finland Kukkonen K	1223	2 yrs	Food allergy, eczema, asthma, and allergic rhinitis (Dg)	IgE sensitization (skin prick test)	Serum	Antigen-specific IgE	none	4 probiotic bacterial strains along with prebiotic galacto-oligosaccharides 2 to 4 weeks before delivery, and postnatally for 6 m to their infants	No effect of probiotic on the incidence of all allergic diseases by age <u>2 yrs</u> but significantly prevented eczema and especially atopic eczema.	Ib
Double-blind, randomized, placebo-controlled trial Sweden Abrahamsson TR	232	birth until 2 yrs	IgE associated eczema (Dg)	IgE sensitization (skin prick test)	Serum	Antigen-specific IgE	none	L reuteri ATCC 55730 (1 x 10(8) colony forming units) daily from gestational week 36 until delivery. Postnatally for 12 m to their infants	Although a preventive effect of probiotics on infant eczema was not confirmed, the treated infants had less IgE-associated eczema at 2 y and therefore possibly run a reduced risk to develop later respiratory allergic disease.	Ib
Randomized controlled trial Australia Boyle RJ	250	First yr	Eczema (Dg)	IgE sensitization (skin prick test)	Serum Cord blood Breastmilk	Antigen-specific IgE Cord blood: dendritic cells, Tregs, TGFβ, IL-10, IL-12, IL-13, IFN-γ and TNFα Breastmilk : total IgA, soluble CD14 and TGFβ	none	Probiotic supplementation (LGG 1.8 × 10(10) cfu/day) from 36 weeks gestation until delivery	Prenatal probiotic treatment was not associated with reduced risk of eczema	Ib

Type of study **Country (If possible)** **Author**	**n**	**Age Period**	**Health event (Q or Dg)**	**Health assessment (clinical tests, spirometry, scales…)**	**Biosample (blood, urins, saliva…°**	**Biological assessment (eosinophilia, omics, DNA, RNA…)**	**Environmental stressor (questionnaire)**	**Environmental stressor (objective assessments)**	**Main results and <u>period of assessment</u>**	**Evidence***
Randomized, controlled trial Australia Dunstan JA	98	1 yr	Atopic dermatitis (Dg)	Skin prick test	Erythrocyte membranes Cord blood	At birth: PUFAs, IL-5, IL-13, IL-10, and IFN-gamma	none	Fish oil (3.7 g n-3 PUFAs per day) from 20 weeks gestation until delivery	Potential reduction in subsequent infant allergy after maternal PUFA supplementation.	Ib
Cross-sectional Austria,Germany, the Netherlands, Sweden, and Switzerland Ege MJ	(n = 8263) (n = 2086) (n = 322)	5-13yrs	Atopic sensitization, asthma (Q)	none	Serum	IgE, gene expression of Toll-like receptors (TLR2, TLR4), CD4	Maternal exposure to farm environment	Endotoxin + fungal extracellular polysaccharide (EPS) measured in mattress dust samples	Maternal exposure to farm environment: protect against development of atopic sensitization + lead to upregulation of receptors of innate immune system.	IV
Cross-sectional New-Zealand Douwes J	1,333	5-17 yrs	Asthma, hay fever, eczema (Q)	none	none	none	Current, early and prenatal farm-related exposures	none	Prenatal exposure to farm environment: lower prevalence of asthma, hay fever and eczema in farmers' children, but continued exposure may be required to maintain optimal protection.	IV
Meta-analysis European Tischer CG	31 742	First 2 yrs	Asthma, allergies (Q)	none	none	none	Exposure to mould, dampness	none	A mouldy home environment in early life is associated with an increased risk of asthma particularly in young children and allergic rhinitis symptoms in school-age children	IIa
Population-based prospective cohort The Netherlands Guxens M	4848	1- 4 yrs , 6yrs	Asthma (Dg), wheezing (Q)	Global index, 2 symptoms scales (depression and anxiety)	none	none	Parental psychological distress at 20 weeks of gestation and at 3 yrs after delivery, maternal psychological distress at 2 and 6 m aft delivery	none	Maternal psychological distress during pregnancy is associated with increased risk of wheezing during the first 6 yrs of life	IIb
Cross-sectional Italy Roberto de Marco	3854	3–14 yrs	Asthma, allergic rhinitis and atopic dermatitis (Q)	none	none	none	Maternal stressful life events during pregnancy (SLEP)	none	Children of mothers who had experienced SLEP were at a moderately increased risk of having wheezing, asthma, eczema and allergic rhinitis during their childhood. SLEP might enhance the expression of asthma and atopic phenotypes in children.	

Type of study Country (If possible) Author	n	Age Period	Health event (Q or Dg)	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urins, saliva…°	Biological assessment (eosinophilia, omics, DNA, RNA…)	Environmental stressor (questionnaire)	Environmental stressor (objective assessments)	Main results and <u>period of assessment</u>	Evidence*
Population-based prospective cohort The Netherlands Leermakers E	4656	1- 4 yrs	Wheezing (Q)	Maternal pre-pregnancy weight, gestational weight gain	none	none	none	none	Maternal pre-pregnancy obesity was associated with an increased risk of wheezing in the child at 4y, Higher gestational weight gain = higher risks of wheezing at 1 yr and overall (1-4y)	IIb
Population-based prospective cohort The Netherlands Sonnenschein-van der Voort A	4634	1-3 yrs	Wheezing (Q)	none	none	none	Parental tobacco smoking	Average annual PM_{10} and NO_2 (dispersion modeling, home addresses)	Long term exposure to traffic-related air pollutants is associated with increased risks of wheezing in children exposed to tobacco smoke in fetal life and infancy	IIb
Population-based prospective cohort Germany Sausenthaler S	2641	2 yrs	Atopic dermatitis, allergic sensitization (Q)	none	none	none	Maternal diet during the last 4 weeks of pregnancy	none	Intake of allergenic foods and foods rich in n-6 polyunsaturated fatty acids during pregnancy may increase and foods rich in n-3 polyunsaturated fatty acids may decrease the risk of allergic diseases at 2 years of age.	IIb
Population-based prospective cohort France Baïz N	370	Birth	Immune system related to asthma/allergic diseases	Lymphocyte phenotypes (Treg, CD8+, Natual killer cells (NK), ...)	Cord blood	CD3+, CD4+, CD8+, CD4CD25+, NK cells, CD19+		Exposure to background particulate matter less than 10 µm in diameter (PM_{10}) and nitrogen dioxide (NO_2), personal exposure to four volatile organic compounds (BTEX)	Maternal exposure to air pollution before and during pregnancy may alter the immune competence in offspring thus increasing the child's risk of developing health conditions later in life, including asthma and allergies.	IIb

Type of study Country (If possible) Author	n	Age Period	Health event (Q or Dg)	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urins, saliva…°	Biological assessment (eosinophilia, omics, DNA, RNA…)	Environmental stressor (questionnaire)	Environmental stressor (objective assessments)	Main results and <u>period of assessment</u>	Evidence*
Population-based prospective cohort Sweden Goksör E	3487	8 years	Asthma (Q)	none	none	none	Administration of antibiotics **in the first week after delivery**		Neonatal antibiotic treatment influences the risk of asthma into school age (8 years)	IIb
Meta-analysis Jaakkola JJ	27 studies	Children	Asthma, allergies (Q)	none	none	none	PVC surface materials in the home		Studies in children showed an association between PVC surface materials in the home and the risk of asthma [fixed-effects model: summary odds ratio (OR), 1.55; 95% confidence interval (CI), 1.18-2.05; four studies] and allergies (OR, 1.32; 95% CI, 1.09-1.60; three studies).	IIa
Meta-analysis Elazab N		Children	Asthma, wheezing, atopy (Q)	Skin prick tests	Blood	IgE	Administration of probiotics during pregnancy (maternal) and in early life (infant)		Prenatal and/or early-life probiotic administration reduces the risk of atopic sensitization and decreases the total IgE level in children but may not reduce the risk of asthma/wheeze	IIa
Meta-analysis Bager P	26 studies	Children	Food allergy/food atopy, inhalant atopy, eczema/atopic dermatitis, allergic rhinitis, asthma				C-section delivery (Q)		Delivery by c-section is associated with a moderate risk increase for allergic rhinitis, asthma, hospitalization for asthma, and perhaps food allergy/food atopy, but not with inhalant atopy or atopic dermatitis.	IIa
Longitudinal cohort USA Rehkopf DH	2150	9-19 yrs	Overweight, obesity (Q)	none	none	none	Forty-one baseline predictors	none	Family socio-economic position and emotion regulation appeared as the top predictors of both BMI change and onset of overweight and obesity.	IIb
Cohort Italy Savino F	**89**	8.8 yrs	Obesity (BMI)	Height, weight	Serum	Leptin	Breastfeeding, formula feeding	none	A higher leptin level in infancy may be inversely associated with BMI in childhood, suggesting that this hormone in infancy is a potential predictor of obesity in later life	IIb
Cohort USA Boeke CE	**540**	birth, 3 yrs and 7 yrs	Obesity (adiposity)	Height, weight, waist circumference, skinfold thickness, dual X-ray absorptiometry body fat.	Cord blood serum	Leptin	Breastfeeding, formula feeding	none	Higher perinatal leptin was associated with lower 3-yr adiposity, whereas higher age 3-yr leptin was associated with greater weight gain and adiposity by 7 yrs.	IIb
Meta-analysis USA Williams AJ	21 studies	4 - 11 yrs	Obesity	none	none	none	Physical activity, diet	none	The evidence from this review suggests that, when implemented alone, school diet and physical activity	IIa

									related policies appear insufficient to prevent or treat overweight or obesity in children, however, they do appear to have an effect when developed and implemented as part of a more extensive intervention programme.	
Type of study **Country (If possible)** **Author**	**n**	**Age Period**	**Health event (Q or Dg)**	**Health assessment (clinical tests, spirometry, scales…)**	**Biosample (blood, urins, saliva…°**	**Biological assessment (eosinophilia, omics, DNA, RNA…)**	**Environmental stressor (questionnaire)**	**Environmental stressor (objective assessments)**	**Main results and <u>period of assessment</u>**	**Evidence***
Meta-analysis Weng SF	30 studies	0 – 2 yrs	Overweight	none	none	none	Maternal pre-pregnancy overweight, high infant birth weight and rapid weight gain during the first year of life, breasfeeding, maternal smoking during pregnancy, introduction of solid foods	none	Breastfeeding is protective against childhood overweight. Maternal smoking during pregnancy is a risk factor for childhood overweight. There was some evidence associating early introduction of solid foods and childhood overweight. There was conflicting evidence for duration of breastfeeding, socioeconomic status at birth, parity and maternal marital status at birth. No association with childhood overweight was found for maternal age or education at birth, maternal depression or infant ethnicity. There was inconclusive evidence for delivery type, gestational weight gain, maternal postpartum weight loss and 'fussy' infant temperament due to the limited number of studies.	IV
Cohort study on children of women Norway Veiby G	n = 44147, n = 61351, n = 78744	6 m, 18 m, 36 m	Psychomotor development (Q)	Motor and social skills, language, and behavior using items from standardized screening tools	none	none	Antiepileptic drugs Breastfeeding	none	At 36 m, prenatal antiepileptic drug exposure=risk factor of adverse development regardless of breastfeeding status during the first y. Continuous breastfeeding in children of women using antiepileptic drugs : protector against impaired development at 6 + 18 m	IIb
Mothers and Children's Environmental Health (MOCEH) study South Korea Kim E	520	birth to 24 months of age	Neurodevelopment and psychomotor development (Q)	Mental and psychomotor development indexes from a Bayley (BSID II) test	none	none	Average exposure levels to PM_{10} and NO_2 during the entire pregnancy, estimated using the inverse distance weighting method	None	Exposure to air pollution may result in delayed neurodevelopment in early childhood	IIb

Q: questionnaire; Dg: diagnosis; *: according to evidence criteria

1.2.1. Immunity and respiratory and allergic diseases

Predisposition toward asthma and allergic disease (A/AD) is among the constellation of adverse outcomes following developmental immunotoxicity (DIT; problematic exposure of the developing immune system to xenobiotics and physical environmental factors). Because novel immune maturation events occur in early life, and the pregnancy state itself imposes certain restrictions on immune functional development, the **period from mid-gestation until 2 years after birth** is one of particular concern relative to DIT and A/AD. Several prenatal-perinatal risk factors have been identified as contributing to a DIT-mediated immune dysfunction and increased risk of A/AD. These include parental smoking (Burke H, 2012), diesel exhaust and traffic-related particles (Takenoue Y, 2012; Gasana J, 2012), heavy metals (Dietert RR, 2008), antibiotics (Penders J, 2011), environmental oestrogens and other endocrine disruptors such phthalates (Jaakkola JJ, 2008; Bornehag CG, 2010), exposure to mould (Tischer CG), maternal stress (Cantani A, 1999; Guxens MS, Roberto de Marco) and birth events such as the caesarean section delivery (Bager P, 2008). Diet and microbial exposure also significantly influence immune maturation and risk of allergy (Dietert RR, 2008; Halken S, 2004). Early-life events occurring during critical windows of immune vulnerability can have long-term impact on immune development. The maternal dietary and microbial environment during pregnancy may programme the immune development of the child. Prenatal environmental exposures may alter gene expression via epigenetic mechanisms, aiming to induce physiological adaptations to the anticipated postnatal environment, but potentially also increasing disease susceptibility in the offspring if exposures are mismatched (Jenmalm, 2013). Early life sensitivity to early life immune insults, including DIT, results from the heightened vulnerability of the developing immune system to disruption and the serious nature of the adverse outcomes arising after disruption of one-time immune maturational events. The resulting health risks extend beyond infectious diseases, cancer, allergy, and autoimmunity to include pathologies of the neurological, reproductive, and endocrine systems (Dietert RR, 2008).

Several factors produce early-**life**-induced immune dysfunction in offspring:

Microbial burden, including farm environment, pet exposure, infections – hygiene hypothesis

A number of epidemiological studies have suggested that the increase in the prevalence of allergic disorders that has occurred over the past few decades is attributable to a reduced microbial burden during childhood, as a consequence of Westernized lifestyle (the ‘hygiene hypothesis’). However, the mechanisms by which the reduced exposure of children to pathogenic and non-pathogenic microbes results in enhanced responses of Th2 cells are still controversial. The initial interpretation proposed a missing immune deviation of allergen-specific responses from a Th2 (humoral response) to a type 1 Th (Th1) (cellular response) profile, as a result of the reduced production of interleukin-12 and interferons by natural immunity cells which are stimulated by bacterial products via their Toll-like receptors. More recently, the role of reduced activity of T regulatory cells (Treg) has been emphasized. The experimental evidence available and the epidemiological findings so far suggest that both mechanisms may be involved. Genetic studies of CD14 and toll-like receptors 4 (TLR4) polymorphism

allow, at least in part, to understand the role of family size, structure changes, rejection of anthroposophic lifestyle, living in a farm in the increased prevalence of allergy. Recently, the hygiene hypothesis has been extended to include the concept of “gut flora”. Having a cat or growing up on a farm, exposure to livestock and consumption of unheated farm milk confers significant protection against the atopy (Ege MJ, Douwes J). Exposure to various infectious agents or their products however may have bidirectional effects on allergy and asthma, and relates to the timing of the exposure. Prenatal (maternal) or early life contact with cats, microbes of cattle sheds, haylofts and farm animals prevents allergy (Ege MJ, Douwes J) while later in life the same microbial exposure to livestock or poultry may exacerbate existing atopic disorders (Douwes J, 2002). In addition, key studies supporting the concept that farming exposure protects children from asthma and atopy based on studies performed largely in European paediatric cohorts. Various types of farming in certain regions appear to have a greater effect on asthma protection, as does the consumption of unpasteurized milk. In the United State, where concentrated animal feeding operations (CAFOs) are more common, asthma is increased in children exposed especially to swine CAFOs; whereas, rates of atopy and allergy are lower in these children (Wells AD, 2014). The importance of microbes in farming environments and the contribution of various components of the innate immune system including toll-like receptors to the underlying mechanisms have been shown in this context.

Of note, the “hygiene” hypothesis does not fully explain the allergy epidemic in the case of helminthic infections, which promote a Th2 immune response but do not reduce the risk of allergic disease, in the case of high asthma prevalence in poor urban environments where children who have asthma are also at risk of infections. Therefore, in the past 20 years new hypotheses have focused on the negative changes in human diet and noxious air pollution as environmental factors responsible potentially for an increase in allergic disorders in the developed countries (Baïz N, 2012).

Diet, including breastfeeding

Maternal (during pregnancy) and neonatal diet is a significant factor influencing immune maturation and risk of allergic disease. Several reviews (Devereux, 2007; Prescott & Dunstan, 2007; Bjorksten, 2008, Halken S, 2004) have addressed this topic. Maternal diet can influence specific immune maturation events (Langley-Evans & Carrington, 2006; Prescott and Dunstan, 2007), allergic sensitization (Sausenthaler et al., 2007), and the incidence of childhood allergic disease (Moore et al., 2006;Warner, 2007). A recent meta-analysis on the effect of early administration of probiotics on asthma and atopy (Elazab N, 2013) showed that prenatal and/or early life probiotic administration reduces the risk of atopic sensitization and decreases the total IgE level in children but may not reduce the risk of asthma/wheeze. Another meta-analysis provided evidence in support of a moderate role of probiotics in the prevention of atopic dermatitis and IgE-associated atopic dermatitis in infants (Pelucchi C, 2012). Concerning breastfeeding, studies show a paradoxical effect of breastfeeding on the prevention of asthma, with an apparent protective effect against early wheezing illness in the first years of life yet an increased risk of asthma in later life; however, these findings must be interpreted with caution. Existing studies fail to adequately adjust for confounders, including the critical issues of protection against early life respiratory illnesses and reverse causation. Therefore, it is possible that

the effect of breastfeeding on early wheezing illness reflects protection against respiratory infection, the predominant trigger of wheezing in early childhood, rather than a true reduction in risk of asthma (Halken S, 2004; Matheson MC, 2012). **Prenatal** and **neonatal periods** are crucial periods regarding the effect of diet on the development of asthma and allergic diseases in early childhood.

Antibiotics

Neonatal treatment with antibiotics has been reported as a risk factor for childhood wheezing (Alm B, 2008) and for asthma in school aged children (Goksör E, 2013).

Heavy metals

Prenatal and early postnatal exposure to heavy metals such as lead have been reported to skew immune responses toward a Th2 bias and elevate production of IgE. Therefore, some metals may have the potential to affect risk of AD-A. Lead exposure and blood concentration have been shown to directly relate to IgE levels in cord blood (Annesi-Maesano I, 2003). In a meta-analysis on the role of exposure to phthalates in the development of asthma and allergies (Jaakkola JJ, 2008), studies in children showed an association between exposure to phthalates and the risk of asthma and allergies.

Parental smoking and environmental tobacco smoke

Airway and lung diseases of later life can be greatly influenced by environmental exposures occurring during critical windows of development for the immune and respiratory systems (Dietert et al., 2000). Tobacco smoke is one of the major concerns. A meta-analysis on the effect of smoking by parents or household members on the risk of wheeze and asthma at different stages of childhood was conducted using 79 prospective studies (Burke H, 2012) and found that exposure to **pre- or postnatal passive smoke exposure** was associated with increased risk of incident wheezing (strongest effect from postnatal maternal smoking on wheeze in children aged ≤2 years), and an increase in incident asthma (strongest effect from prenatal maternal smoking on asthma in children aged ≤2 years).

Indoor air pollutants, including moulds

Indoor air pollution has shown to play a significant role in the development of AD/A at 1 and 2 years of age and later in childhood, especially for an exposure from birth to age 2 years (Mendell MJ, 2007; Sharma HP, 2007). In a meta-analysis of 31 742 children, it was demonstrated that mouldy home environment in the first 2 years of life is associated with an increased risk of asthma particularly in young children and allergic rhinitis symptoms in school-age children (Tischer CG, 2011).

Outdoor air pollution

Outdoor air pollutants, including traffic-associated pollutants, represent an important component of the risk for childhood immune dysfunction and AD/A (Takenoue Y, 2012; Gasana J, 2012, Baïz N, 2011). In the meta-analysis by Tajenoue et al., it was demonstrated that exposure to NO_2 in the air significantly influences the development of childhood asthma (Takenoue Y, 2012). In general, two

major effects have been described. First, among allergic children there is the capacity of diesel exhaust to act as an adjuvant enhancing the potency of allergens during exposure (Kim J, 2011). A second effect is to alter the early-life immune system of the child, and that even before and during pregnancy (Baïz N, 2011). It was found that maternal exposure to ambient air pollution **three months before the beginning of pregnancy** and **during gestation** altered the relative distribution of cord blood lymphocyte phenotypes of the newborn. In particular, the effect of air pollution during the **second trimester of pregnancy** was stronger (higher decrease in Treg cells). In addition, Treg cells decreased while maternal exposure to benzene during the second trimester of pregnancy increased.

Maternal stress

Several studies showed that maternal psychological distress **during pregnancy** is associated with increased risk of wheezing during the first 6 years of life (Guxens MS, …) and with increased risk of having asthma, eczema and allergic rhinitis during their childhood (Roberto de Marco, …). Stressful life events during pregnancy might enhance the expression of asthma and atopic phenotypes in children.

Birth events

Caesarean section delivery has been linked to a moderate risk increase for allergic rhinitis, **asthma**, hospitalization for **asthma**, and perhaps food allergy/food atopy (Bager P, 2008). **Birth** can be considered as a critical life event that will have an important effect on the future health of the child.

Epigenetics

Although existing studies have to be interpreted with caution, it seems that methylation is affected by environmental stimuli such as prenatal smoke exposure and farming environments and asthma and allergies are associated with change in methylation in early childhood. The exact impact of these epigenetic mechanisms on disease development needs to be elucidated further.

Overall, early life is crucial in the development of asthma and allergies. However, the exact upper limit of the window of exposure has not been identified. We suggest then 1 and 2 years of age.

1.2.2. Obesity

Epidemiological, prospective clinical studies and experimental research have clearly shown that the propensity to develop the metabolic syndrome in later life is increased when early life development has been adversely affected. The pathogenesis is not based on genetic defects but on altered genetic expression as a consequence of an adaptation to environmental changes during early life development. However, little is known about the interaction between the pre- and postnatal nutritional environment on either amplification or resolution of the programming phenotype depending on the degree of nutritional match/mismatch.

Maternal nutrition during pregnancy

The importance of maternal nutrition and, in particular, the effect of poor nutrition on birth weight and development of adult disease was addressed in studies of famine exposure. The most widely reported of these being the Dutch Hunger Winter of 1944-1945 where the timing of the exposure was a major determinant in phenotypic outcomes. Whereas famine exposure during early gestation was associated with adult hypertension, reduced maternal caloric intake in late gestation was associated with an in- creased adult adiposity and glucose intolerance. Famine exposure **in late gestation** led to a greater impairment of glucose tolerance than during early or mid-gestation. The rate of obesity was higher in men exposed in the **first half of gestation** and lower in men exposed in the **last trimester of gestation** as compared to non-exposed men (Figure 8). Thus, while fetal exposure to a substrate limited environment at most stages of development appears to lead to adult dysregulation of metabolism, the precise mechanisms responsible may vary with the timing of exposure.

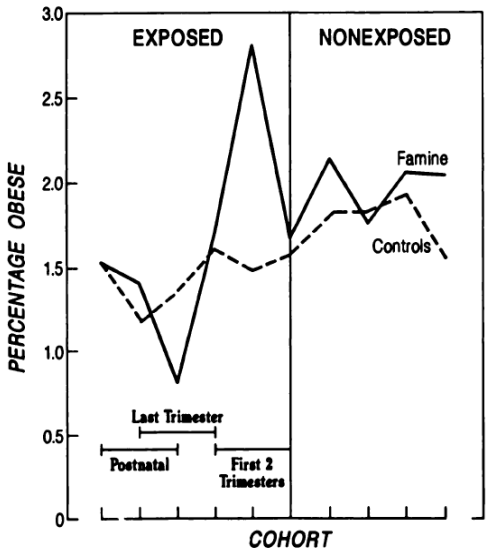


Figure 8: Prevalence of obesity in 19 years old men exposed to famine either *in utero* or during early postnatal life. Exposure to famine in the last trimester of pregnancy or early infancy is associated with a reduced prevalence of obesity at age 19 years, whereas exposure early in pregnancy is associated with an increased prevalence of obesity at age 19 years.

Breastfeeding and child's diet

Breastfeeding was found to be protective against childhood overweight (at 2 years of age) (Weng SF, 2012), with however conflicting evidence for duration of breastfeeding. There was some evidence associating early introduction of solid foods and childhood overweight (Weng SF, 2012).

Maternal smoking

From a toxicological perspective, the linkages between maternal smoking **during pregnancy** and childhood overweight/obesity provide proof-of-concept of how early-life exposure to an environmental toxicant can be a risk factor for childhood obesity (Behl, 2013; Weng SF, 2012).

Physical activity and child's diet

A meta-analysis of 21 studies (Williams AJ, 2013) evaluating the effects of policies related to diet and **physical** activity in schools, either alone, or as part of an intervention programme on the weight status of children aged 4 to 11 years, found that when implemented alone, school diet and **physical** activity related policies appear insufficient to prevent or treat overweight or **obesity** in children, however, they do appear to have an effect when developed and implemented as part of a more extensive intervention programme.

Other factors

A meta-analysis on the risk factors for childhood overweight identifiable during infancy (Weng SF, 2012) found no association with childhood overweight for maternal age or education at birth, maternal depression or infant ethnicity. There was inconclusive evidence for delivery type, gestational weight gain, maternal postpartum weight loss and 'fussy' infant temperament due to the limited number of studies.

Epigenetics

The phenotypic effects of epigenetic modifications during development may not manifest until later in life, especially if they affect genes modulating responses to later environmental challenges, such as dietary challenges with a high-fat diet. The timing of the developmental windows and the induction of epigenetic changes in key physiological systems are not well characterized, but it appears to extend from the peri-conceptional period into postnatal life.

Overall, early life is crucial in the development of obesity. The critical windows that we could suggest are early and late gestation, 1 and 2 years of age, as well as adolescence.

1.2.3.Neuro/psychomotor development disorders

Neurodevelopment

The **prenatal and postnatal periods** in human development are critical developmental windows that are characterized by rapid changes in neuronal and microbial organization. During these periods environmental factors could have a long-term impact on brain and behaviour, resulting in brain disorders. Brain development requires a delicate and complex balance of genetic and environmental factors both during prenatal and postnatal periods. Disruption of these elements can alter developmental trajectories and may lead to the onset of neurodevelopmental and other brain disorders later in life (Ben-Ari Y, 2013). One theory of the basis of the critical periods in brain development: is that they correspond to a period of synaptic excess in the brain: between infancy and the early grade school years, the brain actually over-produces connections--some 50 percent more than will be preserved in adulthood. During the critical period, a child's experience--sensory, motor, emotional, and

intellectual--determines which of these synapses will be preserved, through pruning of the least useful connections.

Importantly, the developing brain has been shown to be susceptible to both internal and external environmental factors during prenatal life. Maternal diet, infection, prenatal stress, and microbial pathogen infections **during the prenatal period** have been associated with neurodevelopmental disorders such as autism, attention deficit hyperactivity disorder (ADHD), and schizophrenia (Marques H, 2013). Maternal health plays a key role in microbiota development and neurodevelopment (Donnet-Hughes A, 2010), therefore characterizing the composition of the microbiota during pregnancy and its contribution to the development of the newborn's microbiota, and potentially brain development is an important step in developing microbiota-modulating interventions. In addition, exposure to air pollution **during pregnancy** was associated with delayed neurodevelopment in early childhood (2 years) (Kim E, 2014).

The **postnatal period** is critical for brain development. In particular, the third year of life is a critical period because the brain, until the age of three is as soft as clay, and once it is "pushed" upon, it does not change for life. Perhaps the "sensitive" period and the "critical period" are in synchronization with the theory of the plasticity of the brain. The critical period for language-learning begins to close around five years of age and ends around puberty.

Brain development is most sensitive to a baby's nutrition between mid-gestation and two years of age.

During **adolescence**, a consequence of the major neuronal rewiring occurring during this period, is a high level of vulnerability to pathological insults ranging from stress to drugs, to abuse, and to dietary deficiencies (Paus T, 2008). This developmental period is also the peak time for the onset of numerous psychiatric disorders including schizophrenia, substance abuse, and mood disorders (Paus T, 2008).

Psychomotor development

Prenatal antiepileptic drug exposure was found to be a risk factor of adverse development (at 36 months) regardless of breastfeeding status during the first year of life (Veiby G, 2013). Continuous breastfeeding in children of women using antiepileptic drugs was protective against impaired development at 6 and 18 months.

Several tests have been developed to test psychomotor development at different moment in life, in particularly in early childhood. Table 6 presents a summary of the neuropsychological tests used in reviewed studies, categorized based on age range and the areas (mental, motor and behavioural development) and/or specific functions evaluated (González-Alzaga B, 2013). Several early ages have been considered up to late adolescence. They are all important.

Table 6: Summary of tests applied in reviewed studies (González-Alzaga B, 2013) to assess functions of mental development (MD), psychomotor development (PD) and behavior (B) according to life stage.

Age	Test	MD	PD	B	Reference
Since birth	VABS	Communication	Fine and gross motor	Daily living skills, socialization, maladaptive behavior	Ruckart et al., 2004
Newborn-2-months	BNBAS			Habituation, orientation, motor performance, range of state, regulation of state, autonomic stability and reflexes	Engel et al., 2007; Young et al., 2005
1 month-12 years	PSI			Adaptability, acceptability, demandingness, mood, distractibility / hyperactivity and reinforces parent	Ruckart et al., 2004
2 months-6 years	GDS	Adaptive behavior, language, personal and social behavior	Motor behavior (locomotion, reaching, balance, comprehension, drawing and hand control)		Guodong et al., 2012
3 months-5 years	A&S Q	Communication, problem solving and personal-social	Gross motor and fine motor		Handal et al., 2008
6 months-3 years	BSID	Language (receptive and expressive) and cognitive development	Fine motor and gross motor		Engel et al., 2011; Eskenazi et al., 2007; Rauh et al., 2006
2-7 years	WPPSI-III	Verbal comprehension, perceptual organization and processing speed abilities			Engel et al., 2011
2-18 years	CBCL			Social withdrawal, somatic complaints, anxiety and depression, destructive behavior, social problems, thought problems, attention problems, aggressive behavior and delinquent behaviors	Eskenazi et al., 2007; Lizardi et al., 2008; Marks et al., 2010; Rauh et al., 2006
3-16 years	NEPSY	Attention, executive functions, language, communication, learning, memory and social perception	Sensor-motor and Visuospatial	Attention	Kofman et al., 2006; Marks et al., 2010
4-5 years	K-CPT			ADHD	Harari et al., 2010; Marks et al., 2010

Age	Test	MD	PD	B	Reference
>4 years	BARS K-Bit	Attention, memory, visual memory, learning, motivation, response speed General intelligence, verbal ability and nonverbal reasoning	Coordination	Sustained attention	Rohlman et al., 2005 Ruckart et al., 2004
>5 years	PP RCPM M&L Test	 General intelligence Memory	Visual-motor coordination, manual dexterity and motor speed		Ruckart et al., 2004 Harari et al., 2010 Ruckart et al., 2004
5-19 years	PIC	Cognitive impairment		Family Dysfunction and Phychological Discomfort, Impulsivity and Distractibility, Reality Distortion, Social Withdrawal, Delinquency, Somatic Concern and Social Skill Deficits.	Ruckart et al., 2004
6-16 years	WISC-III WISC-IV WISC-R	Verbal comprehension, perceptual organization, processing speed and freedom from distractibility Verbal comprehension, perceptive reasoning, working memory and processing speed Verbal comprehension, perceptual organization and freedom from distractibility			Lizardi et al., 2008 Bouchard et al., 2011; Engel et al., 2011; Horton et al., 2012; Rauh et al., 2011 Grandjean et al., 2006; Kofman et al., 2006; Harati et al., 2010
6-18 years	DISC-IV			ADHD	Bouchard et al., 2010
6-8 years	Santa Ana Form Board		Motor coordination		Grandjean et al., 2006; Harari et al., 2010

1.2.4. Omics

"Omics", appear to be a promising tool in early life. However studies with omics are rare in children. In neonatal life (Fanos, 2013), the monitoring of postnatal metabolic maturation, the identification of biomarkers as early predictors of exposure and health outcomes, the diagnosis and monitoring of various diseases and the "tailored" management of young of neonatal disorders are the most promising application of metabolomics. Among the liquids that can be used, urine ("a window on the organisms") which can be collected easily is particularly suitable for metabolomics. Existing literature data support the possibility of making a prompt and sensitive diagnosis of pneumonia (including information about etiology and therapy monitoring), asthma and bronchiolitis (Atzei, 2011). Metabolomics allows studying not just a single metabolic defect but consequences and alterations in an integrated way body-wide. Some investigators applied untargeted mass spectrometry-based metabolomics to methylmalonic acidemia (MMA) and propionic acidemia (PA). Plasma propionyl carnitine was easily identified as the best biomarker of disease. Moreover, two acylcarnitine metabolites and several unidentified species differentiated MMA and PA. In addition, metabolomics represents a promising tool for studying issues related to nutrition through maternal milk and health of preterm infants (Cesare-Marincola, 2013). Lastly, metabolomics is informative for perinatal programming. Urinary metabolomics detected differences between healthy adults born preterm with extremely low birth weight (be- low 1000 g), and healthy adults born at term, who served as controls. Alterations in the metabolism of arginine, proline, purine, pyrimidine, histidine and beta-alanine, as well as in the urea cycle were observed. It was possible to associate this condition of apparent health with an increase in markers

2. Later life critical windows of exposure

In the natural sciences a critical period of development refers to a time window when intrinsic changes in the organisation of living systems or sub-systems towards increasing complexity, greater adaptivity and more efficient functioning occurs rapidly and may be most easily modified in a favourable or unfavourable direction (Scott 1986). In life course epidemiology the relevance of changes during a critical period is in respect of their long term effects on disease risk many years later. Thus, we define a critical period as a limited time window in which an exposure can have adverse or protective effects on the development and subsequent outcome of disease. Outside this time window there is no excess disease risk associated with exposure.

In this chapter of the report, critical windows of exposure of later life are investigated. In this context, although human physiology is continuously being modified through life, critical windows of exposure might be considered periods such as the menopause for women and the age beyond 60-65 for both genders.

Susceptibility and vulnerability to environmental toxicants

A major risk factor for the elderly population is the reduced ability to detoxify and transport chemicals out of the body with age. This could result in increased susceptibility to some classes of chemicals in the aging population. Although this conclusion is derived from toxicological studies in rats (Lee et al. 2008), it is considered to be valid also for humans, considering the similarities between human and rat toxicokinetics. Similarly, the lack of induction of phase II enzymes in aging mice was suggested as a factor of susceptibility of aging population to air pollution (Zhang et al. 2012). Aging tissues have lower homeostatic capabilities, resulting in lower recovery from potential environmental insults exerting oxidative stress. Finally, the effect of DNA hypermethylation from environmental insults seems to be more evident in older populations, as indicated by the Lind et al. (2013) study, which is the first to confirm an age-dependent association between dioxin exposure and DNA hypermethylation.

Elderly constitutes a vulnerable group because due to their lifestyle they are potentially more exposed to hazards (reduced activities and immobility may lead to increased exposure to exposed to indoor air pollution…).

2.1. Neurodegenerative disorders

Aging brings a lot of physiological changes, usually accompanied by deterioration in cognitive function as a result of neurological degeneration (Stein et al. 2008). However, this process does not follow a standardized course for all individuals, (9) as a result of multiple factors.

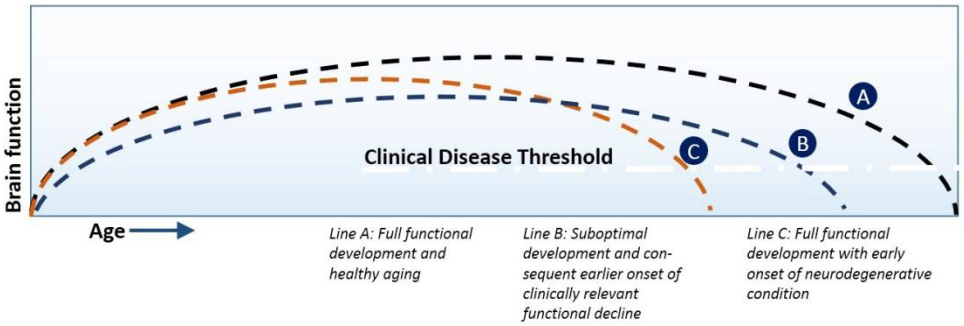


Figure 9: Arc of brain development and function (adapted by Stein et al. (2008))

These factors include genetic susceptibility, exposure to environmental chemicals, lifestyle aspects such as poor nutrition, excessive alcohol ingestion, lack of exercise and stress, as well as health status (e.g. hypertension or diabetes). In addition, age-dependent hormonal changes during normal aging contribute to the overall declining process.

A major mechanism resulting in this degenerative process is the one related to oxidative stress and inflammation. Although these processes consist of distinct biochemical cascades, there are common nodes in the respective pathways of disease, especially regarding brain function, which is highly susceptible to oxidative stress (10). This is evidenced by the concurrent presence of oxidative stress markers found in brain specimens such as ROS and the respective markers of effect, and inflammation markers, such as cytokines and other inflammatory mediators or activated immune cells.

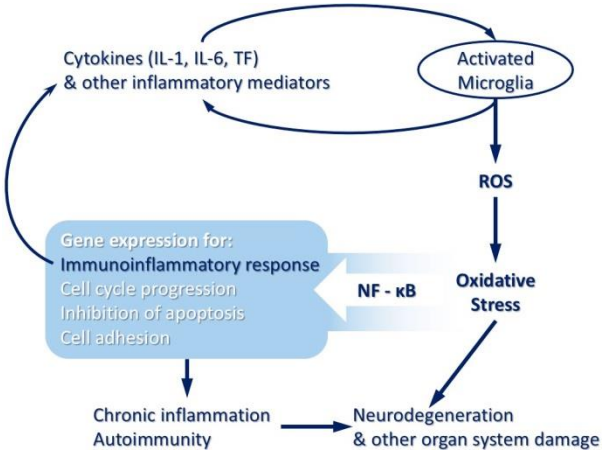


Figure 10: Oxidative stress and inflammation interactions (adapted by Stein et al. (2008))

Although diet is considered as a major exogenous factor, up-regulation of the inflammatory metabolic pathway and in many cases the oxidative stress pathway might be triggered by other exogenous exposures such as exposure to man-made chemicals (e.g. industrial chemicals and pesticides), as well as lifestyle parameters. The linkage among these various parameters is graphically illustrated in 11.

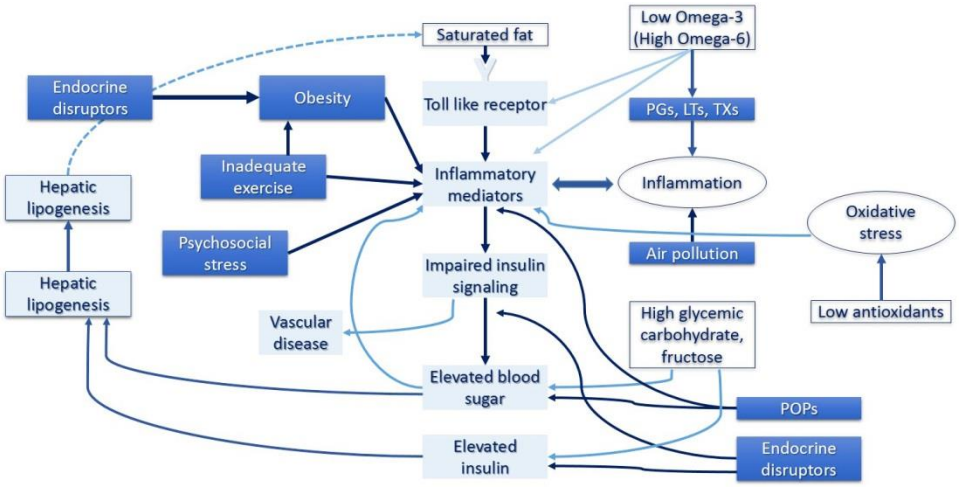


Figure 11: Multiple drivers of inflammation: aberrant metabolism, novel nutrients, toxicants, inadequate exercise, obesity, stress (adapted by Stein et al. (2008))

At the moment there is epidemiological evidence suggesting that health status might predispose for Alzheimer's disease/dementia and cognitive decline through oxidative stress pathways, as illustrated in 12.

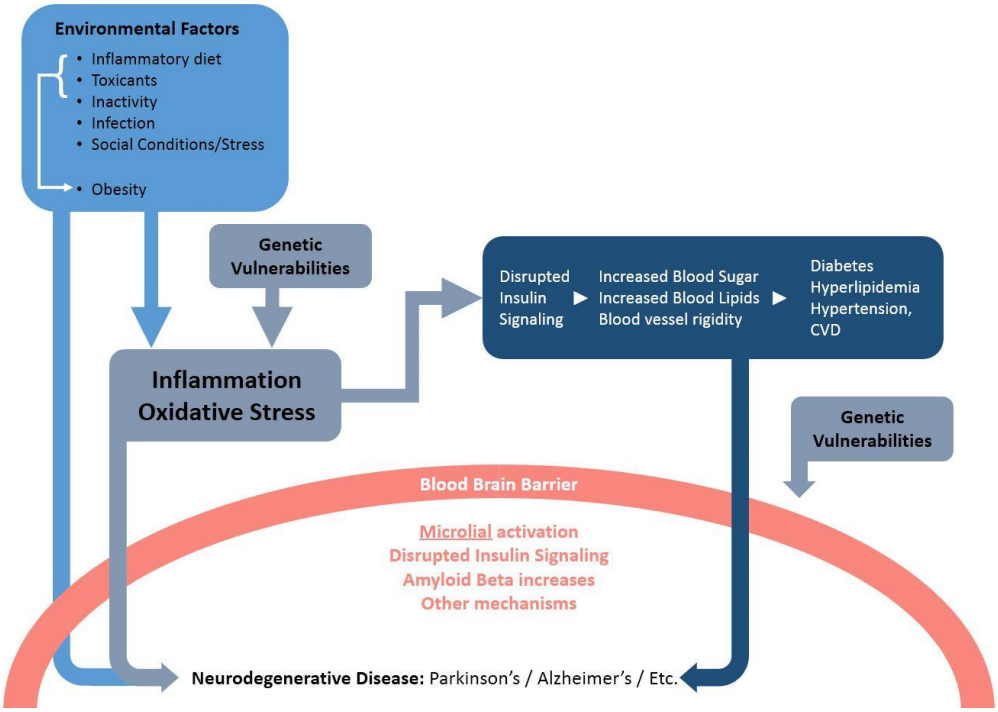


Figure 12: Environmental factors drive diseases such as diabetes, CVD and neurodegeneration (adapted by Stein et al. (2008))

Although most of the evidence is associated with diabetes, other pathological conditions such as mid-life hypertension, obesity and elevated total cholesterol substantially contribute to the onset and the progress of these neurodegenerative disorders. Despite the inconsistencies among the studies reviewed, there is an emerging awareness of the association these parameters have with neurodegenerative disorders. An interesting finding of the studies done so far is that mid-life obesity, high total cholesterol, and elevated systolic blood pressure contribute in an additive manner, each one increasing the risk for dementia by approximately two-fold, while a 6.2-fold increase of risk is observed when all three factors were present (Kivipelto et al. 2005).

Alzheimer's disease (AD)

Age dependent susceptibility

Alzheimer's disease (AD) is accompanied by significant changes in the expression of many genes with up-regulation of genes involved in inflammation and in transcription regulation and down-regulation of genes involved in neuronal functions (Avramopoulos et al. 2011). The overlap between the biological processes of normal aging and susceptibility to AD suggests that age-related gene expression changes might increase the risk of developing AD. Some significant novel genes and other variants for various

biological processes have been reported by Panigrahi and Singh (2013) as being associated with AD, ageing (AG), and other diseases. Ten major classes for TFs (transcription factors) have been identified in the data, where hundreds of TFBS patterns are being found associated with AD, and other diseases. Unique miRNA (micro RNA) targets have been identified as regulatory markers for AD. A major vascular susceptibility factor gene is apolipoprotein E (Amouyel 2002), found to be associated with sporadic late-onset AD cases (Rocchi et al. 2009). Another interesting vascular susceptibility gene is angiotensin converting enzyme. Other possible genes include VLDL-R, LRP, NOS3, CST3, OLR1, MTHFR, PON1 and VEGF, but many of the related studies have shown conflicting results. It has also been found that genetic variation modifies the association between AD biomarkers and neurodegeneration (Hohman et al. 2014). Genes that regulate the molecular response in the brain to oxidative stress may be particularly relevant to neural vulnerability to the damaging effects of amyloid-β.

Studies on differences in gene regulation related to AD onset have addressed the timing and nature of triggers that lead to AD, introducing the Latent Early-life Associated Regulation (LEARn) model which postulates latent expression of specific genes triggered at the developmental stages of life (Lahiri et al. 2009). This model integrates both the neuropathological features (e.g., amyloid-loaded plaques and tau-laden tangles) and environmental conditions (e.g., diet, metal exposure, and hormones) associated with AD. The LEARn model combines genetic and environmental risk factors to explain the etiology of the most common, sporadic, form of AD. While control cohorts exhibited no significant difference between physiological and chronological ages, frontotemporal lobar degeneration (FTLD) and AD exhibited prematurely aged expression profiles. Accelerated expression profiles associated with AD and FTLD suggest some common mechanisms underlying the risk of developing these diseases (Cao et al. 2010).

Beyond gene expression regulation, accumulating evidence suggests that late onset AD not only results from the combined effects of variation in a number of genes and environmental factors, but also from epigenetic abnormalities such as histone modifications or DNA methylation (Bihaqi et al. 2012). In comparison to monogenic diseases, late onset AD exhibits numerous anomalies that suggest an epigenetic component in disease etiology. Onset of different forms of AD has been identified to be affected by environmental exposures, especially for individuals having the APOE4/4, where an earlier age of onset was noticed (Schmechel et al. 2006) in persons with history of toxic exposure (62 ± 2.9 years compared 68 ± 1.5 years).

Additional perturbations, such as the impaired brain iron homeostasis (Crespo et al. 2014), have been associated to AD in the elderly. Intracellular iron accumulation would lead to a rise in oxidative damage, contributing to AD pathophysiology. Considering the reduced counteractive response of elderly to oxidative stress, this mechanism is an additional risk factor related to the underlying susceptibility. The activity of ROS on newly generated neuronal cells in the adult brain may contribute to the pathogenesis of AD (2010). Antioxidants may be used to reduce the deleterious activity of ROS, particularly on newly generated neuronal cells of the adult brain, potentially delaying the development of AD and promoting its regenerative capacity.

Menopause dependent susceptibility

The precipitous loss of sex hormones either through menopause or normal aging can increase susceptibility to AD pathogenesis. One of the most promising translational tools thus far may be the development of selective estrogen and androgen receptor modulators (Carroll and Rosario 2012). In addition, a wide range of beneficial neural actions of sex steroid hormones may contribute to their hypothesized protective roles against AD (Vest and Pike 2013), since both estrogens and androgens exert general neuroprotective actions. However, cognitive and mood outcomes have been dependent to the extent of variations or polymorphisms in the estrogen receptor α gene (ESR1) (Sundermann et al. 2010). The relationships between ESR1 and cognitive impairment tend to be specific to or driven by women and restricted to risk for Alzheimer disease rather than other dementia causes. A strong relationship between ESR1 variants and cognitive outcomes has been observed and preliminary evidence suggests a role of the ESR1 gene in certain mood outcomes. It is also found that longer duration of estrogen exposure may have a protective effect against AD risk; for every additional month with estrogen, women might experience on average a 0.5% decrease in AD risk ($N = 89$, $p = 0.02$), as found by Fox et al. (2013). More menstrual cycles may also have a protective effect against AD risk, although this result was of borderline statistical significance ($p < 0.10$). Overall, AD is likely to occur through interactions between heritable causal and susceptibility genes, environmental exposures, mid-life health status, and lifestyle choices. In addition, mounting evidence suggests that the neuropathological processes characteristic of AD can be detected several years before the onset of clinical symptoms (Reichman and Rose 2012). Under this view, the Androgen Receptor gene (AR) was investigated in a clinical cohort of male and female AD patients and normal control subjects by sequencing all coding exons and evaluating the length and distribution of the CAG repeat in exon 1 (Ferrari et al. 2013). Ferrari et al. (2013) could not establish a correlation between the repeat length, sex, and the disease status, nor did they identify possible pathogenic variants. AR is located on the X chromosome; to assess its role in AD, X-inactivation patterns will need to be studied to directly correlate the actual expressed repeat length to a possible sex-specific phenotypic effect.

Parkinson disease (PD)

Age dependent susceptibility

The nicotine dependence (ND) risk variant, rs588765, has been found to exert a protective effect in PD, and is associated with later age at onset (AAO), but only when the individual was previously exposed to nicotine (Greenbaum et al. 2013). Nicotine has a neuroprotective effect on dopaminergic cells, as shown in cultured cells and animal models. The 15q24 cluster SNPs are associated not only with ND but also with quantitative cigarettes consumption measurements (e.g. cigarettes per day), supporting the notion that delayed AAO is mediated by the beneficial effect of increased nicotine exposure. If greater nicotine consumption is associated with delayed PD AAO, those patients who carry a less severe ND phenotype, which is associated with the 'T' allele of the rs588765 SNP, would develop PD earlier, compared to those

who manifest a more severe ND phenotype, which is associated with the 'C' allele of this SNP. This means that onset of PD is the result of multifactorial interactions, enhancing age dependent susceptibility by genotypic susceptibility factors and environmental exposure factors. An additional parameter that may increase the risk of PD is glutathione transferase polymorphisms, since GSTP1-1, which is expressed in the blood-brain barrier, may influence response to neurotoxins and explain the susceptibility of some people to the parkinsonism-inducing effects of pesticides (Menegon et al. 1998). Moreover, the age of PD onset also differs with regard to being a GSTT1 gene carrier or not, where a median age of 49 is identified among GSTT1 as null compared to a median age of 64 among GSTT1 gene carriers (Ahmadi et al. 2000).

Menopause dependent susceptibility

There is a large literature suggesting that sex steroid hormones may modify the risk for Parkinson's disease (Cereda et al. 2013). Age at PD onset has been positively associated with:

i) age at menarche and at menopause,

ii) length of fertile life and

iii) duration of estrogen exposure.

These observations support the concept that hormonal exposure of the nigro-striatal network during life may influence its susceptibility to degenerative stimuli in later life, but the association does not seem to be unidirectional. In particular, increased severity of PD signs correlates with shorter duration of estrogen exposure. Overall, women may be more susceptible to brain neurodegenerative disorders. The onset of PD is delayed in women with higher number of pregnancies, longer fertile life and longer cumulative length of pregnancies (Yadav et al. 2012). Additional risk parameter for the development of PD has been found the use of oral contraceptives (Nicoletti et al. 2011).

Other neurodegenerative disorders

Age dependent susceptibility

Brain aging is associated with inflammatory changes. The specific changes in the arachidonic acid cascade in the hippocampus may alter phospholipids homeostasis and possibly increase the susceptibility of the aging brain to neuroinflammation (Aïd and Bosetti 2007). Analysis of human aging brain expression datasets from three frontal cortex regions (Cao et al. 2013) showed that different pathways undergo transitions at different ages (13), and the distribution of pathways and age thresholds varies across brain regions. Age-correlated expression changes at particular age points allows one to estimate the age of an individual with better accuracy than previously published methods. In addition, for each age-informative gene the algorithm identifies the age threshold with the most drastic change in expression level, which allows us to associate genes with particular age periods.

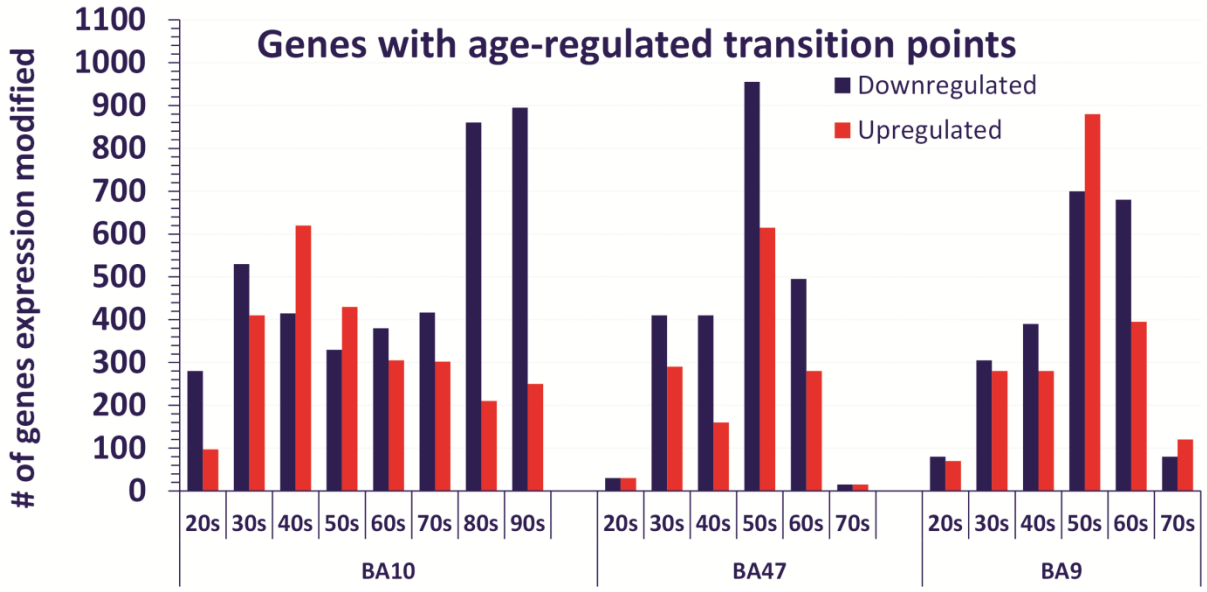


Figure 13: Age-correlated expression changes at particular age points (adapted from Cao et al. (2013))

Many neurodegenerative diseases share oxidative stress and nitrosative stress as common terminal processes. According to the free radical theory of aging, an elevation in reactive oxygen species (ROS) and reactive nitrogen species (RNS) damages neural membranes and induces oxidative and nitrosative stress. The increase in oxidative and nitrosative stress is accompanied by the concomitant decline in cognitive and motor performance in the elderly population, even in the absence of neurodegenerative diseases (Farooqui and Farooqui 2009). Markedly increased rates of oxidative and nitrosative stress are the major factors associated with the pathogenesis of neurodegenerative diseases.

Environmental factors may contribute for neurodegeneration through induction of epigenetic modifications, such as DNA methylation, and chromatin remodeling, which may induce alterations in gene expression programs. Since aging is the most important risk factor for idiopathic AD and PD, it is expected that epigenetic alterations on DNA and/or chromatin structure may also accumulate in neurodegeneration, accounting at least in part to the etiology of these disorders (Marques et al. 2011).

White matter was observed to remain relatively stable until the age range of 60–70 years (Risher et al. 2010). Brain MRIs of subjects ranging in age from 19 months to 80 years revealed that brain volume and intracranial space grew from early childhood through adolescence, and then decreased (Courchesne et al. 2000). The volume of gray matter continued to increase slowly to a plateau in the fourth decade. However, the gray matter-to-white matter ratio in healthy subjects declined after the fourth decade of life, (Courchesne et al. 2000), resulting in brain volume decreases to levels similar to those that exist in young children in the age range 71–80. With aging, the loss of neurons and a decreased capacity for sending nerve impulses to and from the brain results in the diminished processing of sensory information, as a result of the reduction in conduction velocity, that begins to be manifested around age 60 (Dorfman and Bosley 1979).

Menopause dependent susceptibility

The fertile period of women's life is associated with a lower incidence of degenerative inflammatory diseases compared to menopause. In brain, estrogens ameliorate brain performance and have positive effects on selected neural pathologies characterized by a strong inflammatory component, suggesting that inflammatory response is a target of estrogen action (Pozzi et al. 2006). By lacking the protective effect of estrogens, premature menopause results in detrimental neurological outcomes (Scott et al. 2014).

Although the response of neuroinflammation to estrogen hormones is biphasic, overall, the presence of estrogens seems to act protectively, thus menopause is an interesting time – window for capturing the late onset multiple sclerosis (MS) (Bove and Chitnis 2013). For men, the protective effect of testosterone starts to decline at a later age, of about 65. Estrone produced by adipocytes may represent an important source of inflammatory signaling in both males and females, while there may also be a potential interaction between obesity and vitamin D status in mediating MS is females. In terms of genetic susceptibility, the presence of HLA-DRB1*1501 allele seems to be a risk factor (Bove and Chitnis 2014).

The lack of the protective effect of estradiol against neurodegenerative disorders is eventually enhanced by exposure to environmental insults mediating oxidative stress such as ozone (Guevara-Guzman et al. 2009). The fact that these effects can be prevented by estradiol treatment suggests that increased susceptibility to neurodegenerative disorders in aging women may be contributed to reduced post-menopausal estrogen levels.

2.2. Asthma and Allergies

Age dependent susceptibility

There is strong, consistent evidence that the elderly experience higher risk of particular matter–associated hospitalization and death, as identified by the meta-analysis of estimated mortality associations by age and sex (Bell et al. 2013). Because authors used different age categorizations, we considered “older” populations as the oldest age group selected from each study (e.g., ≥65, ≥66, ≥75, ≥76, ≥80, or ≥85 years). The increasing number of population-based and epidemiologic associations between oxidant pollutant exposures and cardiopulmonary disease exacerbation, decrements in pulmonary function, and mortality underscores the important detrimental effects of oxidants on public health (Ciencewicki et al. 2008). Any change in the respiratory tract lining fluid (RTFL) antioxidant defense network in the elderly may explain their apparent increased susceptibility to air pollution (Kelly et al. 2003) (Figure 14). This may occur as a consequence of decreased vitamin intakes, increased basal oxidative stress (hence greater antioxidant demand), and probably most importantly, a decrease in their adaptive capacity.

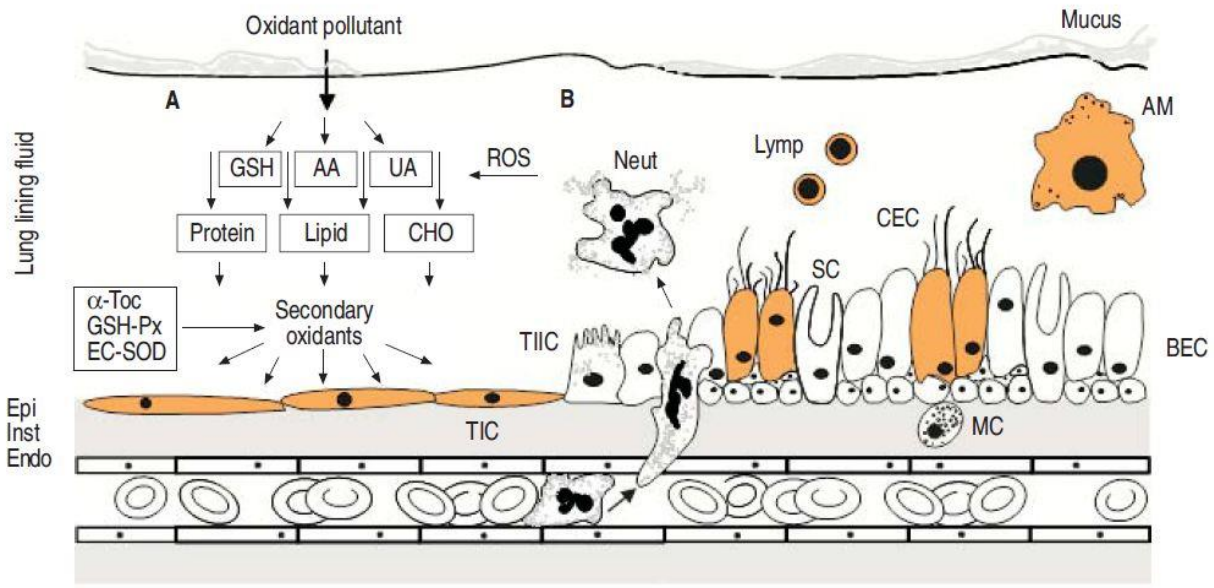


Figure 14: Proposed mechanism of oxidant pollution toxicity in the lung. A: biochemical events; B: cellular events. AA: ascorbate: UA: urate; GSH: glutathione (reduced); CHO: carbohydrate; ROS: reactive oxygen species; a-Toc: a-tocopherol; GSH-Px: glutathione peroxidase; EC-SOD: extracellular superoxide dismutase; epi: epithelial cell; inst: interstitium; endo: endothelial cell; neut: neutrophil; lymp: lymphocyte; AM: alveolar macrophage; MC: mast cell; TIC: type 1 epithelial cell; TIIC: type II epithelial cell; CEC: ciliated epithelial cell; BEC: bronchial epithelial cell; SC: secretory cell (from Kelly et al. (2003))

Significant associations have been found between UFPs and respiratory outpatient visits by Diaz-Robles et al. (2014) with the elderly (population ≥ 65 years) being the group that ran the greatest risk. More in detail, an interquartile increase of 4.73 μg/m^3 in UFPs (lag 5 days) was associated with respiratory outpatient visits with a relative risk (RR) of 1.1458 [95% CI (1.0497-1.2507)] for the elderly. Moreover, subchronic exposure (3 to 28 days accumulated) to traffic-related pollutants (black carbon, carbon monoxide and nitrogen dioxide) has been associated with significantly reduced lung function in the elderly, while non-traffic pollutants (particles, ozone) had weaker associations (2014). Innate immunity responses, and particularly TLRs, are implied in pulmonary inflammation. TLRs recognize damage-associated molecular patterns and activate nuclear factor kappa–light-chain enhancer of activated B cells (NF-κB), which initiate the production of numerous cytokines and host-defense molecules. However, effects of NO_2, total PM2.5, and non-traffic PM2.5 on lung function varied significantly according to the methylation status in the promoter region of TLR2.

Asthma in women

Women with asthma often notice changes to their asthma around puberty, menstruation (periods), pregnancy, and the menopause. This is because of changes to levels of the female hormones, oestrogen and progesterone, in the body. However, there is discrepancy between clinical and epidemiological data. A recent meta-analysis found no significant association of menopause with asthma prevalence or

incidence except for women reporting use of menopausal hormone therapy (MHT) (Zemp, 2012). However, these findings result from a small number of studies, including only 1 large cohort with incidence rates for pre- as well as post-menopause. Further studies are needed addressing more closely subgroup analyses and a possible modification of the association of menopause and asthma by MHT. When stratifying by use of MHT, the association between menopause and asthma rates was increased in women reporting use of MHT (Relative Risk 1.32, 95%CI 1.01-1.74) but not in women not using MHT. Perimenstrual aggravation of asthma (PMA) with an increase in symptoms and a significant decline in peak flow (>20%) has been reported in 30–40% of women (Van den Berge, 2009). Importantly, PMA has been reported to be independent of the presence or absence of allergy. Furthermore, females have a higher risk of developing non-allergic asthma. The latter suggests that hormone-related events play an important role in the development and severity of adult-onset non-allergic asthma. A possible interaction between asthmatic inflammation and obesity and insulin resistance through sex hormones can be hypothesized.

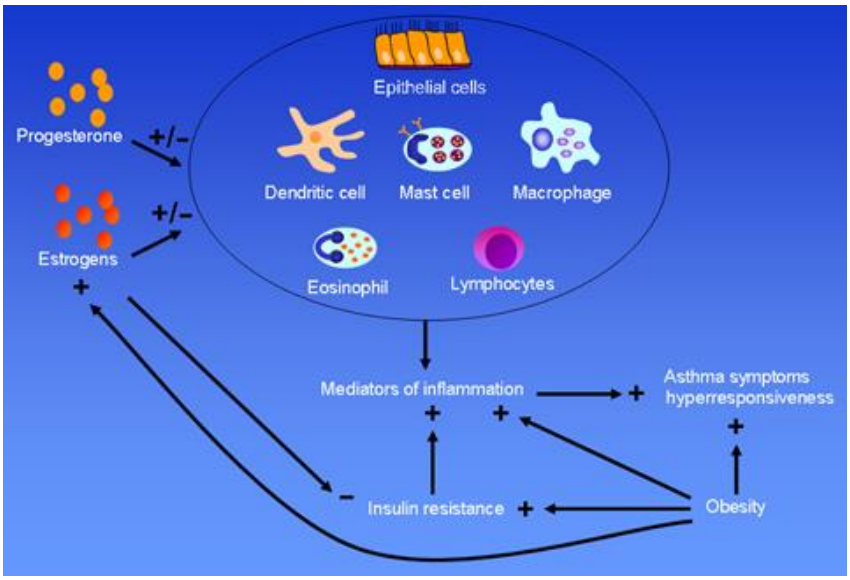


Figure 15: Influence of female sex hormones on asthmatic inflammation and asthma symptoms. Possible interactions with obesity and insulin resistance (from Van den Berge, 2009).

2.3. Metabolic disorders

Age dependent susceptibility

Genetic and environmental factors contribute to age-dependent susceptibility to type 2 diabetes. This age-dependent decrease in muscle gene expression was partially heritable and influenced by the PGC-1α Gly482Ser polymorphism, since skeletal muscle PGC-1α and PGC-1β expression are stimulated by insulin and reduced by aging (Ling et al. 2004). The data also suggest different regulatory functions for PGC-1α and PGC-1β on glucose and fat oxidation in muscle cells. The finding that the age-dependent decrease in the expression of these key genes regulating oxidative phosphorylation is under genetic

control could provide an explanation by which an environmental trigger (age) modifies genetic susceptibility to type 2 diabetes. These conclusions were further strengthened by Olsson et al. (2011), who identified that type 2 diabetes patients exhibit a reduction in oxidative muscle fibres and an increase in glycolytic muscle fibres. The expression levels of the MHC genes are associated with age and both PGC-1α and PGC-1β and indicate that the MHC genes may to some extent be used to determine fibre-type composition in human skeletal muscle.

The synergistic effect of aging as a natural process with increased susceptibility to ubiquitous environmental contaminants has been recently identified to result in metabolic disorders in the elderly. Exposure to PM10, O_3, and NO_2 may increase insulin resistance in the elderly, and GSTM1-null, GSTT1-null, and GSTP1 AG or GG genotypes may increase susceptibility to potential effects of ambient air pollutants on insulin resistance (Kim and Hong 2012). Benzene at levels currently observed in the urban elderly population has also been found to increase insulin resistance (IR) in the elderly (aged ≥60 years), independently of traditional risk factors (Choi et al. 2014). With regard to perfluorinated compounds (PFCs), it has been found that perfluorononanoic acid (PFNA) is related to prevalent diabetes in a non-monotonic fashion, supporting the view that this perfluoroalkyl substances might influence glucose metabolism in humans at the level of exposure seen in the general elderly population (Lind et al. 2014). Similar results have been found regarding elderly population exposure to phthalates (Lind et al. 2012), where high levels of the phthalate metabolites monomethyl phthalate (MMP) ($P < 0.01$), monoisobutyl phthalate (MiBP) ($P < 0.05$), and monoethyl phthalate (MEP) ($P < 0.05$), but not mono(2-ethylhexyl) phthalate, were associated with an increased prevalence of diabetes. MiBP was mainly related to poor insulin secretion, whereas MEP and MMP mainly were related to insulin resistance. Elevated levels of C-reactive protein and plasma fibrinogen have been found in smokers, suggesting that tobacco smoke might activate inflammation leading to Type 2 diabetes (Kowall et al. 2010). Both passive and active smoking cause oxidative stress, which has been implicated to be responsible for the observed systematic inflammatory responses.

Pregnancy dependent susceptibility

Significant changes occur in the metabolism of maternal glucose, as a response to the increased energy needs related to fetus development. Susceptibility genes that predispose for Type 2 diabetes have been also associated to gestational diabetes mellitus, suggesting similarities in both disease mechanisms. Meta-analyses of studies associating Type 2 diabetes to gestational diabetes have demonstrated association of variants within eight different genetic loci, TCF7L2, GCK, KCNJ11, KCNQ1, CDKAL1, IGF2BP2, MTNR1B and IRS1, with an increased risk of gestational diabetes (Lowe and Karban 2014). However, most recent analysis using a candidate gene approach, have identified that LEPR, IL8, TNFa, IL6, ADIPOR2 and RETN of inflammatory pathway genes are associated with maternal metabolic traits. It has been also found that although the inflammatory pathway is unlikely to have a strong impact on maternal metabolic phenotypes in pregnancy, variation in individual members of the pathway (e.g. RETN,

IL8, ADIPOR2, LEPR, IL6, and TNF alpha,) may contribute to metabolic phenotypes in pregnant women (Urbanek et al. 2012). It has been also found that in pregnant women, the G allele for the rs8111699 variant in STK11 is associated with a more favorable metabolic phenotype and may protect against the development of gestational diabetes, particularly in heavier women (Bassols et al. 2013). It has also been found that variation in the copy of the fetal IGF2 gene inherited from the father, but not that from the mother, is associated with maternal glucose levels. This could potentially alter the risk of gestational diabetes in the mother (2011).

In terms of exposure to environmental compounds, arsenic exposure was associated with increased risk of impaired glucose tolerance test at 24-28 weeks gestation, suggesting an association to increased risk of gestational diabetes (Ettinger et al. 2009). Other studies related exposure to organic compounds such as chlordecone (Saunders et al. 2014) or bisphenol A (Robledo et al. 2013), found no statistically significant associations.

An additional parameter that has to be investigated is that diabetes mellitus alters pharmacokinetics and pharmacodynamics of drugs and environmental compounds, by affecting the ADME process through several mechanisms (Dostalek et al. 2012). This results in increased internal exposure levels for the pregnant woman, as well as for the fetus. This loop is of particular interest regarding HEALS and the individual exposome to be investigated.

Menopause dependent susceptibility

Although weight gain per se cannot be attributed to the menopause transition, the change in the hormonal milieu at menopause is associated with an increase in total body fat and an increase in abdominal fat (Davis et al. 2012). There is a mechanistic linkage related to estrogen signaling in metabolic disorders involving glucose sensing tissues, such as liver, pancreatic β cells, adipose tissue, and skeletal muscle (Faulds et al. 2012). The impact on metabolic processes of impaired estrogen signaling and knock out of each ER subtype are graphically illustrated in 16 and 17.

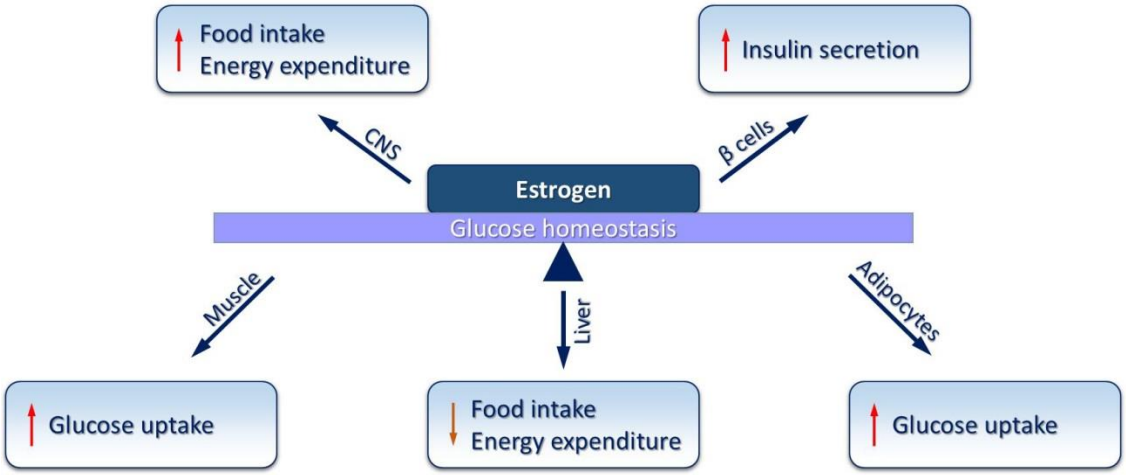


Figure 16: Model showing estrogenic control of glucose homeostasis by regulatory actions in CNS, b cells, muscles, liver, and adipocytes (adapted from Faulds et al. (2012))

Moreover, a low-grade inflammatory state has been related to impaired glucose tolerance and type 2 diabetes, since cytokines derived from adipocytes contribute to insulin resistance by impairing insulin signalling pathways. Postmenopausal women have increased circulating markers of inflammation compared with premenopausal women, resulting in increased risk of type 2 diabetes.

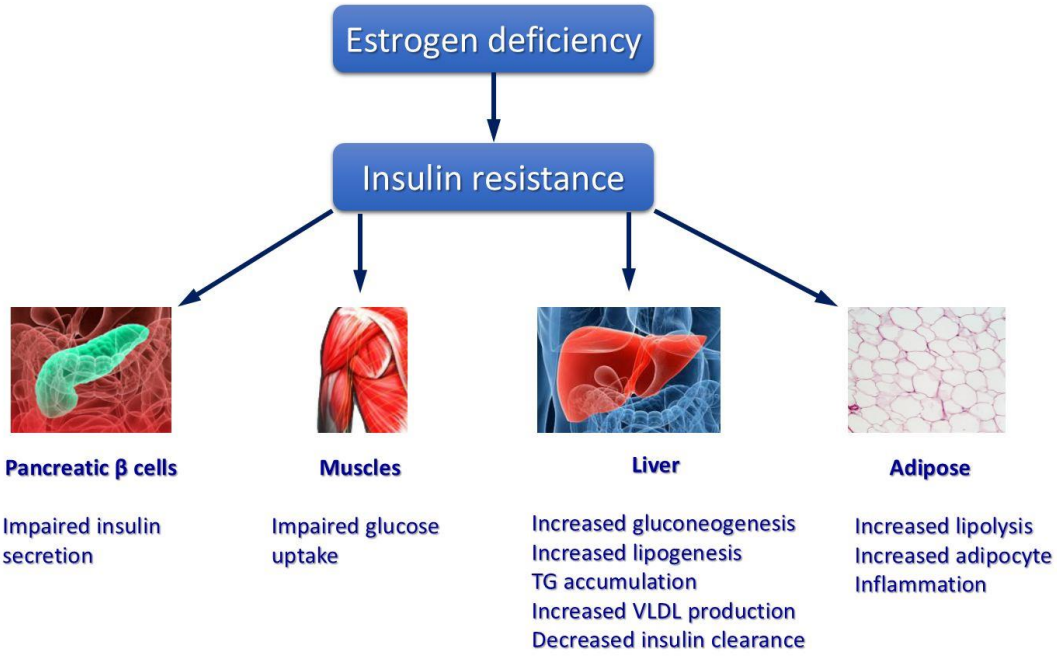


Figure 17: Overview of IR induced by estrogen deficiency and subsequent disturbances in metabolic tissues (adapted from Faulds et al. (2012))

Significant interactions of menopause status and BMI provide additional evidence that obesity influences hormones during the menopause transition (2005). It has also been found that that in post-menopausal women, metabolic syndrome prevalence exceeded that in men by more than twice (60.0% vs. 19.0%). In addition, irregular menses have been associated with significantly higher rate of hypertriglyceridemia and abdominal obesity (2010).

However, the effect of menopause in metabolic disorders is modified by several factors, such as the number of children given birth, as well as the levels of physical exercise. The prevalence of overweight and abdominal obesity were higher for postmenopausal women who had three or more children. Age over 65 years was also a risk factor for abdominal obesity and no use of hormonal replacement therapy was a risk factor for overweight (Gravena et al. 2013). However, regular physical activity may help to mitigate the tendency for weight gain and adverse changes in body composition and fat distribution that accompany aging and the menopausal transition (2005).

Table 7: Life critical events according to observational studies in adulthood

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2000	Case - Control Study - Sweden - Ahmadi et al.	35 male PD Patients and a male control group including 283 subjects.	30–80 years old	Parkinson's Disease (PD)	Statistical analysis were performed using nonparametric method; Wilcoxon rank sum test. Relative risk was expressed as Odds Ratio (OR) and 95% Confident Intervals (CI) were calculated for the ORs.	Blood	Two amino acid polymorphisms in the mEPHX gene were determined in genomic DNA in a PCR-RFLP assay. The GSTM1 and GSTT1 null genotypes were assessed in a multiplex PCR-reaction with b-globin as an internal control gene for a successful PCR amplification. Isolation of DNA and RNA were performed by extracation with TRIzol (GibcoBRL, Grand Island, NY) from normal tissue of SN. Expression of GSTM1, GSTT1 and mEPHX genes were analysed with exonic primers spanning an intervening intron by reverse transcriptase (RT)-PCR of isolated total RNA from SN.			Homozygosity of the histidine (H) 113 isoform of mEPHX was significantly increased in PD patients (odds ratio=3.8 CI 95% 1.2–11.8) and analysis of allele frequencies displayed an increased frequency of the H-allele among PD patients (odds ratio=1.9 CI 95% 1.1–3.3). However, a significantly elevated median age for the onset of PD was found among GSTM1 gene carriers (median age=68 years) compared to PD patients being GSTM1 null genotypes (median age 5 57 years). Our observations suggest that (H) 113 isoform of mEPHX, which has been suggested as a low activity isoform is overrepresented in PD patients and that inherited carriers of the GSTM1 gene postpone the onset of PD.	IIIb	GSTM1, GSTT1 and mEPHX
2007	Prospective cohort - USA - Aïd and Bosetti	Male Fischer-344 rats,	4-, 12-, 24- and 27–30-month-old rats	neuroinflammation		The levels of gene expression of COX-1, COX-2, cPLA2, iPLA2, and glial fibrillary acidic protein (GFAP) were measured by real-time quantitative RT-PCR	phospholipase A2 (PLA2)/COX-mediated AA metabolic pathway in the hippocampus and cerebral cortex of 4-, 12-, 24- and 30-month-old rats.		arachidonic acid (AA) metabolism	age related genes expression changes might increase the risk of developing AD	IIb	
2002	general article - like a review or non analytic study - Amouyel	non analytic study	non analytic study	Alzheimer's and other ageing diseases (lower levels of LDL-cholesterol, myocardial infarction, diabetes or high blood pressure)			genetic polymorphism		overfeeding, lack of physical activity	The approach to pathologies through gene–environment interactions allows us to define new management ways.	IV	the apolipoprotein E: APOE2, APOE3 and APOE4

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2011	Case–control study - USA - Avramopouls et al.	RNA extracted from the temporal lobe of 22 late onset AD and 23 control brain donors.	All the details pertaining to the sets are shown on supplementary Table 1	Alzheimer's disease			3-mm punch biopsies from the superior temporal lobe (Brodman area 22) of 22 deceased patients with confirmed AD pathology and 23 controls with no brain pathology. 1030 genes			enrichment for genes in the interleukin signaling pathway, immunity and defense, and specifically macrophage mediated immunity among the genes with higher expression in AD (Table 1) which supports links between inflammation and AD. enrichment in transcription factors and other genes involved in transcription, likely reflecting the induction of cellular responses by the disease process. Among the genes with lower expression in the AD brain, we found enrichments in genes involved in synaptic transmission, ion channels, and generally genes involved in neuronal activities	IIIb	
2013	Case–control study - Israel - Barak et al	wild-type (WT) male mice (for immunofluorescence: n = 6 EE group, n = 12 control group, for miRNA/ protein measurements: n = 10 EE group, n = 10 control group).	three-week-old	Alzheimer's disease			Three-week-old wild-type (WT) male C57BL/6J mice for immunofluorescence, for miRNA/protein measurements, for RNA extraction.		microRNAs (miRNAs)	It is found for the first time miRNAs that were inversely regulated in AD and EE, and may affect synaptic proteins and modulators, molecular factors associated with AD pathology, and survival and neuroprotective factors.	IIIb	Snap25, Stx6, Vamp1, Vamp2, Calm1, Hspa1, Gja1, Bcl2, Cplx2, Vamp2, Syt1, CaMKII, Syt, NMDA-R, Vamp, Bace1, Bace2, Mapt, APP, COX-2, Stxbp5l, Calm1, Hspa1, NMDA-R, Synb, Syt13, Bdnf, TNFa, CTSB, TACE, Gria2, Gabra5, Gabra6, Cplx2, Snap29, Snapin, Unc13c, APBA2, Unc13c, Synj, Rims1, Syt2, APPBP2, Syntaxin12, Vapa / Luciferase, TAU, Tomosyn2, β-synuclein

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2013	Cross - sectional Study - Spain - Bassols et al.	561 pregnant women: 318 without and 243 with GDM.	30-33 years old	Gestational diabetes mellitus (GDM)	protocolized clinical exams, ultrasonograms, and laboratory tests. Fasting glucose, insulin (homeostatic model assessment of insulin resistance and b-cell function [HOMA-IR and -b]) and C-peptide assessed at 24–28 weeks' gestation. Statistical analyses were performed with the use of the SPSS statistical package version 12.0 (SPSS).	Urine, Blood	rs8111699 variant in STK11 (Taqman technology).			In non-GDM women, the G allele in rs8111699 was associated with lower HOMA-IR (CC: 1.3 ± 0.1 mIU/L; GG: 0.9 ± 0.1mIU/L) and HOMA-b (CC: 165 ± 20 mIU/L; GG: 118 10 mIU/L). In GDM women, the G allele was related to lower body mass index (BMI; CC: 27.9 ± 1.0 kg/m2; GG: 24.5 ± 0.6 kg/m2) and C-peptide (CC: 2.3 ± 0.1 ng/mL; GG: 1.6 ± 0.1 ng/mL). The GG genotype was less frequently observed in GDM women (18% vs. 26%), particularly in heavier GDM women (BMI > median: 14% vs. 28%).	IV	rs8111699 variant in STK11
2013	abstract only	Review of epi studies		Asthma hospitalization (among others)					PM10	higher risk of death of 0.64% (95% confidence interval (CI): 0.50, 0.78) for older populations compared with 0.34% (95% CI: 0.25, 0.42) for younger populations per 10 μg/m3 increase of particulate matter	abstract only	
2012	Review of epi studies - USA - Bihaqi et al.	Review	Review	Alzheimer's disease			Review		as histone modifications or DNA methylation (it's not a stressor - access to abstract only)	The Latent Early-life Associated Regulation (LEARn) model attempts to present the evidence that support the role of epigenetics in the development of AD and explore the potential pathways and mechanisms that may be involved	IIa	(access to abstract only)

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2008	non analytic study - USA - Brunton and Russell	Not available Review study	Not available Review study							In the brain, these pregnancy signals upregulate oxytocin receptors, which are important for mater - nal behaviour; alter intracellular signalling in appe - tite-regulating neurons; suppress the activity of the TIDA neurons that regulate prolactin; and inhibit the responses of oxytocin and CRH neurons to stimulation. Induction of central inhibitory-opioid mechanisms, perhaps by allopregnanolone, provides enhanced restraint of noradrenergic, oxytocin, CRH and TIDA neurons and the maternal behaviour cir - cuitry. The withdrawal of the actions of pregnancy hormones on the brain just before birth enables partu - rition, maternal behaviour and lactation; concomitants are maternal aggression in rodents and, commonly, low mood in women.	IIb	
2007	7-year period study - Chile- Cakmak, Dales et al.	general population sample	all ages - theyt obtained the daily number of deaths in metropolitan Santiago for all nonaccidental cardiovascular and respiratory causes	mortality caused by air pollution	examination of the association between pollution and mortality during the period 1997–2003 in seven Chilean cities: Daily time-series analyses tested the association between daily air pollution and daily mortality in seven Chilean urban centers during 1997-2003.				ozone, sulfur dioxide, PM10, and CO	The percentage increases in nonaccidental mortality associated with an increase in PM10 equivalent to its mean were 4.53 (t-ratio 1.52) for those < 65 years and 14.03 (3.87) for those > 85 years. Respective values were 4.96 (1.17) and 8.56 (2.02) for O3; 4.77 (2.50) and 7.92 (3.23) for SO2; and 4.10 (2.52) and 8.58 (4.45) for CO. Results suggest that the very elderly are particularly susceptible to dying from air pollution.	IIb	
2010	Case–control study - USA - Cao et al.	34 normal human brain samples	subjects in each dataset were between 20 and 95 years old, with individuals in every decade.	rate of aging and neurodegeneration						accelerated expression profiles associated with AD and FTLD suggest some common mechanisms underlying the risk of developing these diseases	IIIb	

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2013	age estimation model study - USA - Cao et al.			rate of aging and neurodegeneration	developed a naïve Bayes (NB) model for assessing the capacity of genes undergoing relatively large transitions in expression at particular age points to provide an estimate of an individual's age.	none				reveals age-correlated expression changes at particular age points and allows one to estimate the age of an individual with better accuracy	IIb	gene expression profiles from three different areas of the frontal cortex: the rostral cortex (Dataset H1 for the Brodman Area 10 (BA10) region, see Methods section), the dorsolateral prefrontal cortex (H2 for the BA9 brain region), and the orbital prefrontal cortex (H3, BA47)
2012	Review - Carroll and Rosario	Review	Review	Alzheimer's Disease	Experimental in vivo and vitro studies					There are numerous neuroprotective actions of estrogens, progestogens and androgens that have direct relevance to AD pathogenesis and compelling potential to prevent and possibly treat the disease. Both estrogens and androgens can regulate indices of neuron viability, Aβ and tau hyperphosphorylation. While less is known about the progestogens, they are emerging as important regulators of these processes. There are numerous neuroprotective actions of estrogens, progestogens and androgens that have direct relevance to AD pathogenesis and compelling potential to prevent and possibly treat the disease. Both estrogens and androgens can regulate indices of neuron viability, Aβ and tau hyperphosphorylation. While less is known about the progestogens, they are emerging as important regulators of these processes. Table 2, p. 11	IIa	Estrogens, Progestogens, Testosterone

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2011	cohort study - Spain - Casas, Fernández et al.	120 pregnant women			evaluate the extent of exposure to several phthalates and phenols in a sample of Spanish pregnant women – according to their individual characteristics (age, social class, education, and body mass index) – and children who participated in the INMA project	urine	Three metabolites of di(2-ethylhexyl) phthalate, mono-2-ethyl-5-carboxypentyl phthalate, mono-2-ethyl-5-hydroxyhexyl phthalate, and mono-2-ethyl-5-oxohexyl phthalate; two metabolites of dibutyl phthalates, mono-isobutyl phthalate and mono-n-butyl phthalate; monoethyl phthalate (MEP), the main metabolite of diethyl phthalate; and two phenols, methyl paraben (M-PB) and 2,5-dichlorophenol were detected in the urine samples of all women.		Phthalate and phenol exposure	Phthalate and phenol exposures are prevalent in a group of pregnant women and young children, two susceptible populations, and these exposures might be positively related to social class.	IIb	

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2013	Cross-sectional study - Milan (Italy) - Cereda, E., et al.	579 patients, 497 of them reported menopause before PD onset	At inclusion: 68.5 ± 9.9, at onset of disease: 62 ± 10.1	Parkinson's Disease	data collected at the first visit. Attention was focused on: age at onset (age at which one of the cardinal signs was first noted) levodopa therapy (in mg/day and mg/kg/day; including equivalent dose of other antiparkinsonian medications), clinical rating scales (Hoehn & Yahr and the motor score of the United PD ications), Rating Scale [UPDRS; in the "ON" phase]) and symptoms at onset (the first noted). Multivariable linear regression models adjusted for diabetes, hypertension, NSAID use, pollutants exposure, positive family history of PD, previous or current smoking, education, sedentary lifestyle, birth cohort and regular menses. Analysis was adjusted (the inclusion in single models is specified where appropriate) also for: age at onset of PD, disease duration, total daily levodopa equivalent dose (mg/kg) and body mass index.			age at menarche, number and duration of pregnancies, number of miscarriages/ abortions, regularity of menses, age at menopause, type of menopause (natural vs. surgical [bilateral oophorectomy]), use of contraceptives and HRT (>6 months).		The observations support the concept that hormonal exposure of the nigro-striatal network during life may influence its susceptibility to degenerative stimuli in later life, but the association does not seem to be unique? unidirectional. In particular, increased severity of PD signs correlates with shorter duration of estrogen exposure. The magnitude of the associations found (e.g. a delay of 5 months in age at onset for 1-year increase in age at menarche) was such to suggest looking at reproductive factors more as contributing factors rather than main determinants of PD.	IIb	

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2014	Cohort study - Seoul - Choi et al.	505 adults	>60	Insulin Resistance	Samples were then analyzed by gas chromatography with a mass selective detector. Quality assurance and quality control (QA/QC) procedures. Linear mixed-effect models and marginal logistic models were used to evaluate associations of t,t-MA concentration with HOMA-IR score and elevated IR, defined as HOMA-IR ≥2.6.	Urine, respiratory area		demographic characteristics, health behavior, and medical history	Benzene	After adjustment for sociodemographic and behavioral factors, environmental co-exposures, and metabolic conditions, quartile levels of urinary t,t-MA demonstrated a dose-dependent association with elevated IR (p-trend< 0.001) and the level of oxidative stress estimated by urinary malondialdehyde (p-trend<0.001). As compared to the lowest quartile, the upper quartiles of t,t-MA (t,t-MA concentration>0.017 mg/g CR) were associated with elevated IR [odds ratio= Q2: 2.00 (95% confidence interval (CI): 1.16–3.46); Q3: 3.33 (95% CI: 1.90–5.84); Q4: 2.07 (95% CI: 1.02–4.22)].	IIb	
2008	review - USA - Ciencewicki et al.	Review	Review	Asthma, chronic obstructive pulmonary disease, and acute respiratory distress syndrome			in vivo, in vitro studies		ozone [O3], SO2, NO2, PM2.5, PM10, ROSs	See page 8 of 13 for Elderly. Mechanisms of oxidant stress in the lung, the role of oxidants in lung disease pathogenesis and exacerbation (eg, asthma, chronic obstructive pulmonary disease, and acute respiratory distress syndrome), and the potential risk factors (eg, age, genetics) for enhanced susceptibility to oxidant-induced disease.	IIa	Enzymic genes

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2000	Cohort Study - California - Courchesne et al.	116 volunteers	19 months to 80 years old	normal brain development and aging	Magnetic resonance (MR) images of 116 volunteers were analyzed with semiautomated procedures validated by means of comparison with manual tracings. Volumes measured included intracranial space, whole brain, gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF). Statistical Analysis.					Whole brain and intracranial space grew by 25%–27% between early childhood (mean age, 26 months; age range, 19–33 months) and adolescence (mean age, 14 years; age range, 12–15 years); thereafter, whole-brain volume decreased such that volunteers (age range, 71–80 years) had volumes similar to those of young children. GM increased 13% from early to later (6–9 years) childhood. Thereafter, GM increased more slowly and reached a plateau in the 4th decade; it decreased by 13% in the oldest volunteers. The GM-WM ratio decreased exponentially from early childhood through the 4th decade; thereafter, it gradually declined. In vivo patterns of change in the intracranial space, whole brain, and GM-WM ratio agreed with published postmortem data.	IIb	
2014	iron metabolism in the periphery, at both genotypic and phenotypic levels . case - control study - Portugal - Crespo et al	a sample of 116 patients with AD and 89 healthy control subjects.	patients and selected controls ≥60 years	Alzheimer's disease	The expression of several iron metabolism-related genes was measured in PBMCs to assess a different level of iron metabolism regulation in patients with AD	blood	nucleotide polymorphisms		serum iron, ferritin, and transferrin concentrations	the alterations on systemic iron status observed in patients could reflect an iron homeostasis dysregulation, particularly in cellular iron efflux.	IIIb	TF, TFR2, ACO1, and SLC40A1

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2012	Review - Davis, Castelo-Branco et al.	Review	Review	Obesity, Overweight						Weight gain is a major health concern for women at midlife. Weight gain *per se* does not appear to be affected by the hormonal changes of the menopause. The fall in estrogen at menopause favors central abdominal fat accumulation. Other factors that may contribute to obesity in women include a low level of activity, parity, lower level of education, a family history of obesity, use of psychotropic drugs and chemotherapy. In addition to the adverse physical consequences of obesity, weight excess is a major risk factor for psychological distress, low self-esteem, depression and sexual dysfunction. Obesity is an independent risk factor for more severe menopausal symptoms. Estrogen-only or estrogen – progestin therapy does not adversely affect body weight and may ameliorate accumulation of abdominal fat. Methods of weight loss must include increased exercise and calorific control although this can be enhanced by surgery, drug therapy and non-medical means. Metformin is a useful drug for selected overweight individuals who have diabetes or are at high risk for diabetes. Successful maintenance of weight loss involves lifestyle change.	IIa	

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2014	Case Study - Chile - Diaz-Robles et al.		Three age groups, those under and equal to 5 years old (Group 1; G1), older than 5 years old and younger and equal to 64 years old (Group 2; G2), and older than 64 years old (Group 3; G3).	Respiratory illness	The daily health data was collected from three health care municipal centers. The daily ultrafine particles were sampled with a Micro-Orifice Uniform-Deposit Impactor (MOUDI), 100-NR model, made by MSP. The meteorological data included temperature, relative humidity, and wind speed. The data was fitted using a multivariate semi-parametric Poisson regression model, controlling for trends, seasonality, and confounders, using generalized additive model (GAM) parameterization on R-Project.				ultrafine particles (UFP), with an aerodynamic diameter ≤0.1 μm (PM10)	Results of the statistical analyses show significant associations between UFP and respiratory outpatient visits, with the elderly (population ≥ 65 years), being the group that presented the greatest risk. An interquartile increase of 4.73 μg/m3 in UFP (lag 5 days) was associated with respiratory outpatient visits with a relative risk (RR) of 1.1458 [95% CI (1.0497–1.2507)] for the elderly.	IIIb	
1979	Case – control Study (Abstract only) – Dorfman et al.	15 elderly normal subjects and 15 younger normal adults.	Mean age: Elderly: 74.1 years old Younger adults: 31.6 years old.	Spinal cord conduction velocities	Somatosensory evoked potential (SEP) latencies, motor and sensory nerve conduction velocities (CVs), and F-wave latencies were measured. Nerve conduction characteristics of all 30 subjects were analyzed with respect to age.					Peripheral motor and sensory CVs slowed progressively, and the onset latencies of F waves and SEPs increased gradually with advancing age. Spinal cord CVs showed little change until approximately age 60, and declined sharply thereafter. In addition, the latencies of F-waves and SEPs were positively associated with height.	IIIb	
2012	Review - Dostalek et al.	Review	Review	Diabetes mellitus						The effect of diabetes on pharmacokinetics and pharmacodynamics remains unclear and further clinical studies are required to understand the clinical significance of the effects.	IIa	

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2010	Cohort Study - Ebrahimpour, Fakhrzadeh et al.	607 men and 966 women	25-64 years old	Obesity, Overweight	Blood pressure was measured by a mercury sphygmomanometer. Serum FBS, TG, cholesterol were measured through automated colorimetric assays using Hitachi 902 autoanalyser (Boehringer, Manneheim, Germany). Serum HDL and LDL were double checked directly through commercial immunoturbidometric assays (Pars Azmoon Inc., Iran) using the same device.			menstrual history of the participants	waist circumference, fasting serum triglycerides, high-density lipoprotein-cholesterol, blood pressure and fasting plasma glucose	The prevalence was high in women especially after menopause. In post-menopausal women, prevalence exceeded that in men by more than twice (60.0% vs. 19.0%, $p < 0.0001$). The mean levels of metabolic syndrome-related risk factors were significantly higher in post-menopausal women. Even irregular menses was associated with significantly higher rate of hypertriglyceridemia ($p = 0.011$) and abdominal obesity ($p = 0.044$). Although, previous studies have shown that women are protected against cardiovascular disorders, some factors have changed this pattern. So that prevalence of metabolic syndrome in women is now even higher than in men. This process exacerbates with the decrease in estrogen levels through menopause. Consideration of early prevention and therapy in this specific group is of great importance.	IIb	
2009	Birth Cohort Study - Oklahoma - Ettinger et al.	532 women	24.5 ± 5.4 years old	Gestational/ type 2 diabetes	Blood glucose was measured between 24 and 28 weeks gestation after a 1-hr oral glucose tolerance test (GTT) as part of routine prenatal care. Blood and hair were collected at delivery and analyzed for arsenic using inductively coupled plasma mass spectrometry with dynamic reaction cell. Logistic Regression.	Blood, Hair			Arsenic	Arsenic concentrations ranged from 0.2 to 24.1 µg/L (ppb) (mean ± SD, 1.7 ±1.5) and 1.1 to 724.4 ng/g (ppb) (mean ± SD, 27.4 ± 61.6) in blood and hair, respectively. One-hour glucose levels ranged from 40 to 284 mg/dL (mean ± SD, 108.7 ± 29.5); impaired glucose tolerance was observed in 11.9% of women when using standard screening criterion (> 140 mg/dL). Adjusting for age, Native-American race, prepregnancy body mass index, Medicaid use, and marital status, women in the highest quartile of blood arsenic exposure had 2.8 higher odds of impaired GTT than women in the lowest quartile of exposure (95% confidence interval, 1.1–6.9) (p-trend = 0.008).	IIb	

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2009	review - USA - Farooqui and Farooqui	Review	Review	Alzheimer's disease and Parkinson					oxidative and nitrosative stress, diet, Generation of high levels of ROS and downregulation of anti-oxidant mechanisms	An unbalanced overproduction of ROS and RNS may give rise to oxidative and nitrosylative stresses, which can induce neuronal damage resulting in neuronal death by apoptosis or necrosis.	IIa	Thus, ONOO− reacts with lipid, proteins, and DNA. It is also reported that ONOO− interferes with key enzymes of the tricarboxylic acid cycle, the mitochondrial respiratory chain, and mitochondrial Ca2+ metabolism
2012	Review - Faulds, Zhao et al.	Review	Review	Obesity	Experimental in vivo and vitro studies		Polymorphisms			Describtion of the key effects of estrogen signaling in metabolic and glucose sensing tissues, including the liver, pancreatic β cells, adipose tissue, and skeletal muscle. The impact on metabolic processes of impaired estrogen signaling and knock out of each ER subtype will also be discussed. The role of estrogen and its receptors in different tissues involved in metabolic processes: Fig. 1.	IIa	Estrogen

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2013	Cohort Study - Ferrari, Dawoodi et al.	The cohort included 696 individuals subdivided into 241 female AD patients,164 male AD patients,198 female neurologically normal control subjects and 93 male neurologically normal control subjects enrolled by the Texas Alzheimer's Research and Care Consortium; in addition,131 DNA samples of neurologically normal control subjects (n=68 female and n=63 male), obtained from Coriell Institute.		Alzheimer's Disease			DNA sequencing and CAG repeat genotyping, Allelic distribution profile and statistical analysis			A correlation between the repeat length, sex, and the disease status could not be established, nor did identify possible pathogenic variants. AR is located on the X chromosome; to assess its role in AD, X-inactivation patterns will need to be studied to directly correlate the actual expressed repeat length to a possible sex-specific phenotypic effect.	IIb	Androgen Receptor gene (*AR*)

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2013	Cohort Study - Fox, Berzuini et al.		70-100 years old	Alzheimer's Disease	Clinical Dementia Rating (CDR) scale, Cox proportional hazard models			memory, orientation, judgement, prblem solving, home and hobbies, community affairs and personal care		Longer duration of estrogen exposure may have a protective effect against AD risk, such that for every additional month with estrogen, women experienced on average a 0.5% decrease in AD risk (N = 89, p = 0.02). More menstrual cycles may also have a protective effect against AD risk, although this result was of borderline statistical significance (p < 0.10). These results build upon previous methodologies by taking into account a variety of parameters including oral contraceptive use, breastfeeding, post-partum anovulation, abortions, and miscarriages. Additionally, Cox models revealed that longer reproductive span, age > 21 at first birth, and more months in lifetime spent pregnant had protective effects against AD risk.	IIb	Estrogens
2005	Cohort Study - Penn Ovarian Aging Study - Gracia, Freeman et al.	436 women	35-47 years old	Obesity		Blood	Dimeric inhibin B was measured in serum using a sensitive, two-site nonisotopic immunoassay (Serotec).	Menstrual cycle dates, reproductive history, general health status and behaviors, symptom measures, and demographics		Although BMI is a significant independent predictor of inhibin B levels, the relationship between BMI and inhibin B changes with advancing menopause stage. These data provide additional evidence that obesity influences hormones during the menopause transition.	IIb	Inhibit A and Inhibit B

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2013	Population-based Study - Brazil - Gravena, Brischiliari et al.	456 women	45-69 years old	Obesity	Determination of body mass index (BMI) and waist circumference (WC). Based on logistic regression analysis, the factors that were most closely associated with overweight were: having three or more children (odds ratio (OR): 1.78; 95% confidence interval (CI): 1.06-3.00); and not taking hormone replacement therapy (OR: 1.69; 95% CI: 1.06-2.63). The prevalence of abdominal obesity was positively associated with greater parity (OR: 1.34, 95% CI: 1.05-1.72) and age older than 65 years (OR: 1.50; 95% CI: 1.03-2.19).			Behavioral, economic, and sociodemographic data		This study found that the prevalences of overweight and abdominal obesity were higher for postmenopausal women who had three or more children. Age over 65 years was also a risk factor for abdominal obesity and no use of hormonal replacement therapy was a risk factor for overweight.	IIb	

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2013	Cohort Study - Italy - Greenhaum et al.	677 Italian PD patients: 438 had never smoked (NS), and 239 were current or past smokers (ever-smokers, ES).	34-76 years old	Parkinson's Disease (PD)	Using PLINK, the association of the three SNPs of interest with smoking status by logistic regression was studied, controlling for gender. Then, the SPSS 16 univariate GLM procedure was used to analyze the association between gender, smoking status, the three CHRNA3-CHRNA5 SNPs (independent variables) and AAO (dependent variable). Three models were constructed, one for each SNPs. In each model gender (M/F), smoking status (NS/ES) and the relevant SNP served as main effects with smoking status by relevant SNP serving as the interaction effect. Each model was constructed, analyzed and interpreted under a three-level additive model of the studied SNP. The models were tested under a one-tailed analysis due to a clear hypothesized direction of the results.		SNP genotyping was performed with the Sequenom MassARRAY system. Quality control measures were implemented.	Data regarding previous and current smoking status, years of smoking prior to PD onset and average cigarette consumption per day (CPD) was available. Demographic and clinical data was also available.	Nicotine	An interaction between the rs588765 SNP and smoking status (NS vs. ES) was nominally significant in its effect on PD AAO (p=0.04). The rs588765 ND risk allele 'C' was associated with delayed AAO among ES (even when smoking intensity variables are accounted for), but had no significant effect among NS. In the ES group, a dominant model of inheritance was observed: carriers of the 'CC' genotype presented delayed AAO compared to carriers of the 'CT' or 'TT' genotypes.	IIb	CHRNA5-CHRNA3-CHRNB4 gene cluster (rs588765, rs16969968, rs578776)

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2009	Case-control Study - Guevara-Guzman, Arriaga et al.	Adult virgin female Wistar rats: 180 juveniles: 60	Adults and juveniles from 20 to 22 days old	Brain neurodegenerative disorders / Parkinson's and Alzheimer's diseases	Social recognition memory and olfactory perception tests, Lipid Peroxidation was measured using the K-Assay test (Kamiya Biomedical, Co.), Immunohistochemistry for α and β ERs and dopamine-β-hydroxylase, Western blot for α and β Ers, Behavioral data obtained during the three test exposures to juveniles were expressed as means±SEM.	Brain			Ozone O3	The results provide further confirmation of the effects of ozone exposure typical of those occurring in some urban environments on cognitive and sensory processes and possibly the olfactory system may be particularly sensitive. They also show that circulating estrogen levels are important protective agents against such changes suggesting that women with low levels, because of menopause or hysterectomy, may be particularly at risk from ozone exposure or other causes of neurodegenerative decline.	IIb	α and β estrogen receptors and dopamine β-hydroxylase

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2014	Cohort Study - Hohman et al.	Normal Control: 194 Caucasian people Mild Cognitive impairment: 382 Caucasian people Alzheimer's Disease: 114 Caucasian people	Mean Baseline Age: Normal Control: 75 years old Mild Cognitive impairment: 73 years old Alzheimer's Disease: 75 years old	Neurodegeneration / Alzheimer's Disease (AD)	Quantification of ventricular dilation, biomarker groups, statistical analyses: biomarker groups in relation to brain volume , statistical analyses: genetic interaction with biomarker group, post-hoc analyses: hierarchical linear regression.	Cerebrospinal fluid (CSF)	Genotyping was performed using the Illumina InfiniumHuman-610-Quad BeadChip and the Illumina OmniQuad array. Quality control (QC) and statistical analyses were performed using PLINK software.			One intergenic SNP (rs4866650) and one SNP within the SPTLC1 gene (rs7849530) modified the association between amyloid positivity and neurodegeneration. A transcript variant of WDR11-AS1 gene (rs12261764) modified the association between tau positivity and neurodegeneration. These effects were consistent across the two subdatasets and explained approximately 3% of variance in ventricular dilation. One additional SNP (rs6887649) modified the association between amyloid positivity and baseline ventricular volume, but was not observed consistently across thesub-datasets.	IIb	SPTLC1gene(rs7849530), WDR11-AS1gene(rs12261764)
2003	review of toxicological studies	n.a.	n.a.	Respiratory diseases	n.a.	n.a.				Any change in the respiratory tract lining fluid (RTFL) antioxidant defence network in the elderly may explain their apparent increased susceptibility to air pollution. This may occur as a consequence of decreased vitamin intakes, increased basal oxidative stress (hence greater antioxidant demand), and probably most importantly, a decrease in their adaptive capacity.		
2012	longitudinal panel study - The Korean Elderly Environmental Panel (KEEP) Seoul, Korea - Kim et al.	560 persons of whom 146 (26.1%) were male and 414 (73.9%) were female	≥ 60 years of age	Insulin Resistance / impaired glucose tolerance	Estimate the effects of air pollutants on fasting blood levels of glucose and insulin and the homeostatic model assessment (HOMA) index of IR, and evaluate effect modification by GSTM1, GSTT1, and GSTP1 genotypes	blood	Genomic DNA from peripheral blood lymphocytes, genetic polymorphisms of GSTM1, GSTT1, and GSTP1 were determined using a multiplex polymerase chain reaction method	structured questionnaire including demographics, lifestyle habits, and medical history.	PM10, O3, and NO2	GSTM1-null, GSTT1-null, and GSTP1 AG or GG genotypes may increase susceptibility to potential effects of ambient air pollutants on insulin resistance	IIa	GSTM1, GSTT1, and GSTP1

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2005	Meta-analysis of cohort studies – Finland – Kivipelto et al.	1449 individuals	65 – 79 years old	Dementia / Alzheimer's Disease (AD)	A venous blood specimen was taken to determine serum total cholesterol level. Systolic (SBP) and diastolic (DBP) blood pressure were measured from the right arm of the subjects after they had been seated for 5 minutes. Height and weight were measured, and BMI was calculated as weight in kilograms divided by the square of height in meters. Statistical Analyses.	Blood	The apolipoproteinE (ApoE) genotypes were analyzed. Cognitive status was assessed using a 3-step protocol for the diagnosis of dementia: a screening, a clinical, and a differential diagnostic phase.	health behavior, health status, and medical history		Obesity at midlife (body mass index>30 kg/m^2) was associated with the risk of dementia and AD even after adjusting for sociodemographic variables (odds ratio [OR], 2.4 [95% confidence interval (CI), 1.2-5.1]). The association was somewhat modified by further adjusting for midlife blood pressure, total cholesterol level, and smoking (OR, 2.1 [95% CI, 1.0-4.6]) and also for apolipoprotein E genotype and history of vascular disorders (OR, 1.9 [95% CI, 0.8-4.6]). Midlife obesity, high total cholesterol level, and high systolic blood pressure were all significant risk factors for dementia with ORs of around 2 for each factor, and they increased the risk additively (OR, 6.2 for the combination).	IIa	apolipoprotein E (ApoE)

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2010	Cohort Study - Southern Germany - Kowall et al.	1,223 subjects at baseline in 1999-2001, 887 subjects at follow-up)	55–74 years old	Type 2 diabetes mellitus	Incident diabetes was identified by oral glucose tolerance tests or by validated physician diagnoses. Anthropometric and laboratory measurements. Statistical analyses: Multivariate logistic regression models			The baseline questionnaire included the smoking status (regular/occasional/past/never smoker), the number of cigarettes smoked daily (for regular smokers only), the largest number of cigarettes ever smoked daily for a whole year (for current and past smokers), and the year of beginning and (in case of past smokers) of stopping smoking. ETS was characterized by questions whether, and if so, how much other persons smoked in the workplace or in the household of the participants at baseline (very much/much/hardly/not at all). Information about sociodemographic variables, medical history, alcohol consumption and physical activity was gathered in a structured interview. Socioeconomic status (SES) was assessed as previously described, based on income, educational level and occupational status. Dietary intake was assessed with a short 27 item qualitative food frequency list (FFL).	tobacco smoke (ETS)	Among never smokers, subjects exposed to ETS had an increased diabetes risk in the total sample (odds ratio (OR) = 2.5; 95% confidence interval (CI): 1.1, 5.6) and in a subgroup of subjects having prediabetes at baseline (OR = 4.4; 95% CI: 1.5, 13.4) after adjusting for age, sex, parental diabetes, socioeconomic status, and lifestyle factors. Active smoking also had a statistically significant effect on diabetes incidence in the total sample (OR = 2.8; 95% CI: 1.3, 6.1) and in prediabetic subjects (OR = 7.8; 95% CI: 2.4, 25.7).	IIb	
2009	book chapter - review - Lahiri et al.	Review	Review	Alzheimer's disease			polymorphisms		Oxidative stress and metals in the brain and inflammatory factors	The LEARn model combines genetic and environmental risk factors to explain the etiology of the most common, sporadic, form of AD.	IIa	POE and AβPP gene

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2008	trospective nested-cohort study based upon the results of AREDS - USA - Lee et al.	876 patients	no significant differences in age, smoking status, BMI, education	susceptibility to environmental toxins		blood	characterized the expression of xenobiotic metabolizing enzymes (XMEs) from the livers of male F344 and Brown Norway (BN) rats across the adult lifespan			The design and statistics used in this paper were appropriate and sufficient to examine the existence of a pharmacogenetic interaction between zinc/antioxidant therapies and geno-types on the progression of AMD.	IIa	1435 related genes
2014	cohort study - USA - Lepeule et al.	776 elderly men	Participants were mainly white, well educated, and former smokers. 88% of them were ≥65 years old	Lung Function in Elderly Men	We measured forced vital capacity (FVC), forced expiratory volume in 1 sec (FEV 1), and blood DNA methylation one to four times between 1999 and 2009	blood	DNA methylation (<or ≥median) at 26 individual CpG sites in nine candidate genes. We used linear mixed-effects models to estimate the main effects of air pollutants and effect modification by DNA methylation.		black carbon, total and nontraffic particles with aerodynamic diameter ≤2.5μm (PM2.5), carbon monoxide, and nitrogen dioxide	Subchronic exposure to traffic-related pollutants was associated with significantly reduced lung function in the elderly; nontraffic pollutants (particles, ozone) had weaker associations. Epigenetic mechanisms related to inflammation and immunity may influence these associations.	IIb	
2014	cross-sectional study - Sweden - Lind et al.	1,016 men and women aged	70 years old	diabetes	Seven PFAS were detected in almost all participant sera by ultra-high performance liquid chromatograph/tandem mass spectrometry.				perfluoroalkyl substances (PFAS)	PFNA was related to prevalent diabetes in a non-monotonic fashion in this cross-sectional study, supporting the view that this perfluoroalkyl substance might influence glucose metabolism in humans at the level of exposure seen in the general elderly population.	IIb	

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2013	Cohort Study - Sweden - Lind et al.	524 subjects	70 years old	Global DNA methylation	POPs were measured in stored serum samples collected at enrolment. Analyses of POPs were performed using a Micromass Autospec Ultima HRGC/HRMS. Statistical analyses: regression models using Stata.	Serum	A total of 21 SNPs in the Ah receptor-gene were genotyped by minisequencing using the Illumina Golden Gate assay.	Medical history, life style and regular medication.	Twenty-three different POPs, including 16 PCBs, five pesticides, one dioxin (OCDD) and one brominated flame retardant (BDE47).	High levels of toxic equivalency (TEQ) for PCBs and dioxinwere associatedwith DNA hypermethylation (p = 0.030). This was mainly attributed to coplanar non-ortho PCBs. While no significant associations were found between DNA methylation and SNPs in the Ah-receptor, an interaction was found between the SNP rs2237297 and TEQ so that TEQ was associated with hypermethylation (p = 0.009) only in subjects with one G-allele (n = 103). Also high levels of the PCB126 congener, the OCDD, and the pesticide metabolite p,p′-DDE were related to DNA hypermethylation (p = 0.01, 0.03 and 0.003, respectively). In conclusion, in a sample of elderly subjects, high TEQ including PCBs and the dioxin OCDD and high serum levels of PCB126, OCDD, and p,p′-DDE were related to global DNA hypermethylation in a cross-sectional analysis.	IIb	Ah-receptor SNPs

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2012	Cross Sectional Study - Sweden - Lind et al.	1,016 subjects	70 years old	Type 2 diabetes	Human serum (0.5 mL) was analyzed for levels of 10 phthalate metabolites using an isotope liquid chromatograph/tandem mass spectrometer (API 4000LC-MS/MS). Plasma proinsulin and insulin concentrations were determined using the Proinsulin ELISA and the Insulin ELISA immunoassays on a Bio-Rad Coda automated EIA analyzer. Relationships between phthalate metabolites and prevalent diabetes were evaluated by logistic regression models, first using the phthalate metabolites as continuous variables and thereafter following division of the phthalate metabolites into quintiles.	Serum		Sex, BMI, serum cholesterol and triglycerides, educational level, and smoking and exercise habits.	10 phthalate metabolites (MEHP, MEP, MiBP, MMP)	High levels of the phthalate metabolites monomethyl phthalate (MMP) (P<0.01), monoisobutyl phthalate (MiBP) (P<0.05), and monoethyl phthalate (MEP) (P<0.05), but not mono(2-ethylhexyl) phthalate, were associated with an increased prevalence of diabetes. Using the fasting pro insulin–to–insulin ratio as a marker of insulin secretion and the homeostasis model assessment-insulin resistance index as a marker of insulin resistance, MiBP was mainly related to poor insulin secretion, whereas MEP and MMP mainly were related to insulin resistance.	IIb	
2004	cohort study - Denmark - Ling et al.	A total of 98 twin pairs (33 young MZ; 22 young DZ; 21 elderly MZ; 22 elderly DZ) were enrolled in the clinical examination.	A random sample of young and elderly same-sex MZ and DZ twin pairs born in Funen County during 1966–1975 (22–31 years) and 1931–1940 (57–66 years).	Type 2 Diabetes			Measurements of mRNA levels, oral glucose tolerance test (OGTT), Genotyping		environmental factors, obesity, reduced physical activity, age, and intrauterine environment as reflected by birth weight	The finding that the age-dependent decrease in the expression of these key genes regulating oxidative phosphorylation is under genetic control could provide an explanation by which an environmental trigger (age) modifies genetic susceptibility to type 2 diabetes.	IIb	PGC-1α, PGC-1β

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2014	Review - Lowe and Karban	Review	Review	Maternal glucose metabolism/ Gestational/ type 2 diabetes						Maternal metabolism during pregnancy is a major determinant of the intrauterine environment and fetal outcomes. Differences in metabolism in the gravid and non-gravid state and the profound insulin resistance that occurs during pregnancy have long been recognized. While progress has been made in defining the mechanisms underlying these changes and their impact on fetal outcome, much remains to be learned. New technologies are becoming available to better define the mechanisms.	IIa	TCF7L2, GCK, KCNJ11, KCNQ1, CDKAL1, IGF2BP2, MTNR1B and IRS1, FTSJD1/ CALB2 and LBXCOR1 etc
2011	Review - Portugal - Marques et al.	Review	Review	Alzheimer's disease and Parkinson					Aluminum, zinc, copper, iron, mercury, dietary factors	It is expected that epigenetic alterations on DNA and/or chromatin structure may also accumulate in neurodegeneration, accounting at least in part to the etiology of AD and PD // it is possible that HDAC inhibitors, or even modulators of DNA methylation might constitute effective therapeutics for neurodegeneration	IIa	APP, PSEN1, PSEN2, APOE, GAB2, A2m, GALP, SCNA, Parkin, PINK-1, UCHL-1, LRRK2, FBXO7, HTRA2, MAPT
1998	Case – control Study – Australia – Menegon et al.	95 PD patients and 95 controls.	Pathients: 72 years old Controls: 67 years old	Parkinson's Disease (PD)	Analysis of age, sex, family history of Parkinson's disease, and pesticide exposure with logistic regression.	Blood	Genotyping by PCR polymorphisms in four GST classes (GSTM1, GSTT1, GSTP1, and GSTZ1).	Age, sex, family history, pesticide exposure	Pesticides	The distribution of the GSTP1 genotypes differed significantly between patients and controls who had been exposed to pesticides (controls vs patients: AA 14 [54%] of 26 vs seven [18%] of 39; AB 11 [42%] of 26 vs 22 [56%] of 39; BB 1 [4%] of 26 vs six [15%] of 39; AC 0 vs four [10%] of 39, p=0·009). No association was found with any of the other GST polymorphisms. Pesticide exposure and a positive family history were risk factors for Parkinson's disease.	IIIb	GSTM1, GSTT1, GSTP1, GSTZ1

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2011	Case - control Study - Nicoletti et al.	Two hundred PD women (mean age, 68.0 ± 9.5 years) and 299 control women (mean age, 61.8 ± 9.9 years)	PD women: mean age, 68.0 ± 9.5 years and control women: mean age, 61.8 ± 9.9 years	Parkinson's Disease				demographic, epidemiological, and clinical data, cigarette smoking, coffee		Age at menarche, age at menopause, fertile life duration, cumulative duration of pregnancies, hormone replacement therapy, and surgical menopause were not significantly associated with PD. Multivariate analysis showed a significant positive association between use of oral contraceptives and PD, with an adjusted OR of 3.27 (95% CI, 1.24-8.59; P = .01). The data suggest that oral contraceptives could increase the risk of PD.	IIIb	
2011	measuring mRNA study - Sweden and Denmark - Olsson et al.	98 Danish twin pairs without known diabetes 50 sedentary, but otherwise healthy, younger Swedish men	(33 younger MZ, 22 younger DZ, 21 elderly MZ and 22 elderly DZ)	Type 2 Diabetes		blood and urine	oral glucose tolerance test (OGTT), myofibrillar ATPase histochemistry, Genotyping, Real-time PCR, Microarray gene expression			The expression levels of the MHC genes are associated with age and both PGC-1α and PGC-1β indicate that the MHC genes may to some extent be used to determine fibre-type composition in human skeletal muscle.	IIb	MHC7, MHCIIa and MHCIIx/d genes

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2013	Meta-analysis study - Panigrahi and Singh	Three separate microarray data sets: a) 31 individuals, comprising nine controls, seven with incipient AD, eight with moderate AD, and seven with severe AD, b) 30 microarrays representing a study of the effects of aging on frontal lobe gene expression of individuals who died of natural causes between the ages of 26 and 106 and c) 14 normal controls and 14 AD affected samples		Alzheimer's disease	Objective of this study was to estimate the impact of AG factor on the AD disease		PCA revealed the relationship between each modules of AG and AD gene set (total 89 samples from three experiments) and their phenotypic assessments.			Ten major classes for TFs (transcription factors) have been identified in the data, where hundreds of TFBS patterns are being found associated with AD, and other disease	Ia	

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2011	meta analysis of two separate cohorts - Petry et al.	1,160 mother/partner/offspring trios from the prospective Cambridge Baby Growth Study (n = 845 trios) and the retrospective Cambridge Wellbeing Study (n = 315 trios) (3,480 samples in total).	around 30-40	gestational diabetes		blood	paternally transmitted fetal IGF2 polymorphisms were associated with maternal glucose concentrations; specifically, paternally transmitted fetal rs6578987 (P = 0.006), rs680 (P = 0.01), rs10770125 (P = 0.0002), and rs7924316 (P = 0.01) alleles were associated with increased maternal glucose concentrations in the third trimester of pregnancy and placental IGF-II contents at birth (P = 0.03).		glucose concentrations in the third trimester of pregnancy	we report for the first time association between polymorphic variation in a paternally transmitted fetal gene, namely, IGF2, and maternal glucose concentrations in pregnancy. // Polymorphic variation in paternally transmitted fetal IGF2 is associated with increased maternal glucose concentrations in pregnancy and could potentially alter the risk of gestational diabetes in the mother. The association may be at least partially mediated by changes in placental IGF2 expression.	IIIa	IGF2 gene

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2006	Conference Paper - Pozzi, S., et al.			Neurodegenerative disorders / Alzheimer's Disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis, Multiple Sclerosis, Schizophrenia, Depression, Cerebral Ischemia	Experimental in vivo and vitro studies					The data indicate that E2 strongly influences the onset and course of selected brain disorders. Our studies on inflammatory model systems, in agreement with published data, underline the relevant anti-inflammatory role of E2 in brain, which is specifically mediated by ER-α. Considering the relevance of inflammation in brain diseases, more studies are certainly needed to improve our understanding of the role of inflammatory cell activation in neurodegenerative diseases and to identify valid targets for chronic therapeutic settings. The availability of selective ER-α and ER-β ligands (SERMs) allows testing the possibility of selectively modulating inflammation and fostering our future commitment toward the identification of appropriate SERMs as drug candidates in the prevention of neuroinflammatory diseases.	IV	Estrogens - 17β estradiol (E2)

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2012	Article (like a Review) - Reichman and Rose	Article	Article	Alzheimer's Disease	Cerebrospinal fluid biomarkers, volumetric neuroimaging, functional neuroimaging, and cognitive stress tests			cognitive training, physical exercise, dietary choices, social engagement, and psychological stress reduction.		AD is likely to be multidetermined through interactions between heritable causal and susceptibility genes, environmental exposures, midlife health status, and lifestyle choices.	IIa	

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2012	cross-sectional study - Italy - Rentschler et al	Elderly (N=255) and adolescents (N=311) from Northern Italy	Elderly: 63–80 years. Adolescents: 11–14 years	Parkinson			Exposure to Mn in blood and urine by atomic absorption spectroscopy and in soil by a portable instrument based on X-Ray fluorescence technology. Polymorphisms in the Parkinson-related gene ATPase type 13A2 and in the secretory pathway Ca2+/Mn2+ ATPase isoform 1 gene by TaqMan probes.		Manganese (Mn) in the air, soil and water	For both adolescents and elderly, negative correlations between Mn in soil and motor coordination (Rs=-0.20, p<0.001; Rs=-0.13, p=0.05, respectively) were demonstrated. Also among adolescents, negative correlations were seen between Mn in soil with odor identification (Rs=-0.17, p<0.01). No associations were seen for Mn in blood or urine. ATP13A2 polymorphisms rs4920608 and rs2871776 significantly modified the effects of Mn exposure on impaired motor coordination in elderly (p for interaction=0.029, p=0.041, respectively), also after adjustments for age and gender. The rs2871776 altered a binding site for transcription factor insulinoma-associated 1. ATP13A2 variation may be a risk marker for neurotoxic effects of Mn in humans.	Ib	ATPase type 13A2 (ATP13A2, also called PARK9: rs3738815, rs2076602, rs4920608, rs2871776 and rs2076600), Ca2+/Mn2+ ATPase isoform 1 gene (SPCA1: rs218498, rs3773814 and rs2669858)

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2010	book chapter - review - Risher, Todd et al.	interesting: page 36-37 of 63 > review of cases	interesting: page 36-37 of 63 > review of cases	general						..the adverse effects of long-term, low-level exposure to environmental substances will have a longer time to be manifested in a physiologically weakened elderly population. When such exposures are coupled with concurrent exposure to prescription medications, the effects could be devastating.	Ia	
2013	case-control study - Robledo et al.	22 cases of GDM and 72 controls were analyzed (self-reported their race as Hispanic, Non-Hispanic White or Non-Hispanic Black.)		blood glucose levels or diagnosis of gestational diabetes	This pilot study examined the association between BPA exposure, fasting blood glucose levels (FBG) and GDM diagnosis during pregnancy. // Logistic regression models controlling for race/ethnicity	blood and urine samples		A questionnaire administered at the time of enrollment collected information on covariates of interest including demographic factors such as age, race/ethnicity, educational level, and annual household income, parity, previous diagnosis of GDM and family history of type 2 diabetes mellitus.		In this small pilot study it was not possible to demonstrate an association between BPA exposure and development of GDM. Larger studies are needed to assess whether environmental contaminants such as BPA play a role in the etiology of GDM.	IIIb	

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2009	review - Italy - Rocchi et al.	Review	Review	Alzheimer's disease						A relationship between genetic and acquired vascular factors and AD has been hypothesized. Many vascular risk factors for AD, such as atherosclerosis, stroke and cardiac disease in the aging individual, could result in cerebrovascular dysfunction and trigger AD pathology. A major vascular susceptibility factor gene is the apolipoprotein E gene, found to be associated with sporadic late-onset AD cases. Another interesting vascular susceptibility gene is angiotensin converting enzyme. Other possible genes include VLDL-R, LRP, NOS3, CST3, OLR1, MTHFR, PON1 and VEGF, but many of the related studies have shown conflicting results.	IIa	apolipoprotein E, angiotensin converting enzyme, VLDL-R, LRP, NOS3, CST3, OLR1, MTHFR, PON1 and VEGF

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2014	a population-based cohort study - Saunders et al.	779 pregnant women	The mean age of the 779 women included in the analysis was 31 years (range 12.4 to 49.6);	explored the consequences of environmental exposure to organochlorine pesticides for gestational hypertension	Chlordecone exposure was determined with maternal plasma samples, providing an accurate reflection of the load of this compound in the body. Total cholesterol and triglyceride concentrations in plasma were determined by standard enzymatic procedures (DiaSys Diagnostic Systems GmbH, Holzheim, Germany) Total lipid concentrations were also calculated.	maternal blood samples and urine samples			exposure to organochlorine pesticides for gestational hypertension (GH), preeclampsia (PE) and gestational diabetes mellitus (GDM).	No significant associationswere observed between the chlordecone exposure and the risk of PE or GDM. This study suggests an inverse association between chlordecone exposure during pregnancy and GH. Further studies are required to determine the underlyingmechanism, or the potential unknown confounding factors, resulting in this association.	IIb	

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2006	Cohort Study - USA – Schmechel et al.	1136 consecutive patients	> 40 years old	Neurodegenerative disorders	Clinical evaluation was performed using practice guidelines for MCI and dementia syndromes, in a two-visit model with diagnosis confirmed at second visit after medical history, full physical and neurological/psychiatric examination, and appropriate neuropsychometric screens. Additional genetic testing and other blood work. Primary neuropsychiatric diagnoses: normal, cognitively impaired non-demented (CIND), mild cognitive impairment (MCI), possible or probable Alzheimer disease (AD), AD with Parkinsonism (ADPD), mixed etiology AD with presumptive vascular disease (ADVD), frontal lobe dementia, frontotemporal dementia (FTD, often with motor findings such as Parkinsonism), Lewy body dementia (LBD), primary progressive aphasia (PPA), progressive supranuclear palsy (PSP), vascular dementia(VD), and other minor categories. Common secondary neuropsychiatric diagnoses included various sleep disorders (30% of all cases) such as obstructive sleep apnea (OSA), nocturnal myoclonus or restless leg syndrome (PLMS), and REM disinhibition behavior disorder (RBD). Statistical Analysis.	Blood	All testing except lipids was on non-fasted specimens of opportunity at time of visit with APOE genotype, alpha-1-antitrypsin phenotype, Hemochromatosis genotype and other routine laboratory tests		Pesticides, herbicides and volatile organic compounds	The effects of APOE, Hfe, and AAT on glucose, lipid, iron and trace mineral homeostasis may affect normal development and aging of the nervous system in addition to their effects on outcome of toxic environmental and occupational exposures and susceptibility and outcome of neurodegenerative illnesses.	IIb	apolipoprotein E (APOE), hemochromatosis gene (Hfe) and alpha-1-antitrypsin (AAT)

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2014	Review - Scott, Zhang et al.	Review	Review	Alzheimer's Disease						Premature loss of ovarian estradiol enhances the risk of neurological disease. Premature menopause may cause brain hypersensitivity to pathologic stressors. Timely estrogen therapy may avert these negative neurological sequelae. Basic scientists and clinicians should collaborate to address these crucial issues. Surgical menopause cohort studies and neurological outcomes. Average age at surgery and length of follow-up are shown in years. Note that cohort studies finding an enhanced risk of neurological disease and mortality in surgically menopausal women had significantly longer length of follow-up: Table 1, p. 3	IIa	Estrogens - 17β-Estradiol (E2)

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2005	Cross-sectional study - Study of Women's Health Across the Nation (SWAN) - Sternfeld, Bhat et al.	248 white and Chinese women	47-57 years old	Obesity	Body composition (lean mass, percent body fat) was assessed with dual energy x-ray absorptiometry, and central adiposity was determined by waist circumference. Physical activity was assessed from 7 d of accelerometer recordings.			Menopausal status was based on self-reported bleeding patterns.		For both the whites and the Chinese, late peri- and postmenopausal status was associated with lower lean mass, and among the Chinese, tended to be associated with higher percent body fat. These findings suggest that regular physical activity may help to mitigate the tendency for weight gain and adverse changes in body composition and fat distribution that accompany aging and the menopausal transition.	IIb	

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2010	Review - Case-control studies, Cohort Studies - Sundermann, Maki et al.	Review	Review	Alzheimer's Disease			ESR1 polymorphisms			Among studies investigating ESR1 in relation to cognition, 11 of 14 case-control studies reported an association between ESR1 polymorphisms and risk for developing dementia. Three of four prospective cohort studies reported an association between ESR1 polymorphisms and significant cognitive decline. There are inconsistencies between case-control and cohort studies regarding whether specific ESR1 alleles increase or decrease the risk for cognitive dysfunction. The relationships between ESR1 and cognitive impairment tend to be specific to or driven by women and restricted to risk for Alzheimer's disease rather than other dementia causes. Three of five studies examining ESR1 polymorphisms in relation to anxiety or depressive symptoms found significant associations. Significant associations have also been reported between ESR1 polymorphisms and childhood-onset mood disorder and premenstrual dysphoric disorder. A strong relationship between ESR1 variants and cognitive outcomes is evident, and preliminary evidence suggests a role of the ESR1 gene in certain mood outcomes. Insights into the discordant results will come from future studies that include haplotype analyses, analyses within specific ethnic/racial populations, and sex-stratified analyses.	IIa, IIIa	ERα gene (ESR1)

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2010	Review - Taupin	Review	Review	Alzheimer's disease			newly generated neuronal cells of the adult brain		neuroinflammation and oxidative stress, deleterious activity of ROS,	it is stated that the activity of ROS on newly generated neuronal cells in the adult brain may contribute to the pathogenesis of AD. Antioxidant may be used to reduce the deleterious activity of ROS, particularly on newly generated neuronal cells of the adult brain, potentially delaying the development of AD and promoting the regenerative capacity of the adult brain.	IIa	(access to abstract only)
2012	Meta-analysis of cohort studies - Urbanek et al.	We genotyped 508 SNPs mapping to 31 inflammatory genes in **6218** pregnant women of the HAPO cohort	around 30-35 years old	the impact of the inflammatory gene pathway on glycemia during pregnancy	oral glucose tolerance test at 24-32 weeks gestation // We tested for association between 458 SNPs mapping to 31 genes in the inflammatory pathway and metabolic phenotypes in 3836 European ancestry and 1713 Thai pregnant women.	A blood sample for DNA extraction was collected				Based on the genes surveyed in this study the inflammatory pathway is unlikely to have a strong impact on maternal metabolic phenotypes in pregnancy although variation in individual members of the pathway may contribute to metabolic phenotypes in pregnant women.	IIa	RETN, IL8, ADIPOR2, LEPR, IL6, and TNF alpha,

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2013	Review - Vest and Pike	Review	Review	Alzheimer's Disease						Both estrogens and androgens exert general neuroprotective actions relevant to a several neurodegenerative conditions, some in a sex-specific manner, including protection from neuron death and promotion of select aspects of neural plasticity. In addition, estrogens and androgens regulate key processes implicated in AD pathogenesis, in particular the accumulation of β-amyloid protein. Age-related estrogen depletion in women is a risk factor for Alzheimer's disease. Age-related androgen depletion in men is a risk factor for Alzheimer's disease. Relationships between hormones and Alzheimer's are often sex-specific. Estrogens and androgens reduce β-amyloid protein to reduce Alzheimer risk.	IIa	Estrogens, androgens

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2012	Case - control Study - Yadav, Shukla et al.	81 consecutive women with PD and age matched healthy women controls.	Mean age at interview was 55.89 ± 10.07 years for women with PD, 55.05 ± 10.53 years for controls.	Parkinson's Disease				demographic characteristics, history of onset of PD, side of onset, type of PD, response to levodopa, alcohol intake, tobacco, head injury, any family history of neurodegenerative disorder and reproductive history (age at menarche, age at final menstrual period, type of menopause (natural vs. surgical), history of oral contraceptive use, type and duration of contraceptive use, status at reference date, postmenopausal estrogens use, total duration of oestrogen use, parity, abortions, still births, age at first childbirth, age at last childbirth and length of different pregnancies).		Significant positive correlation was observed with cumulative length of pregnancy (r=0.32; p=0.003), age at menopause (r=0.55; p=0.001) and length of fertile life with age of onset of PD (r=0.27; p=0.02). Gravidity (r=0.26; p=0.02) and parity (r=0.35; p=0.001) also correlated positively with age at onset.The onset of PD is delayed in women with higher number of pregnancies, longer fertile life and longer cumulative length of pregnancies. This could also explain the epidemiological observations of lower incidence of PD in women and the protective role of estrogens.	IIIb	Estrogens

Year	Type of study Country Author	N	Age Period	Health endpoint	Health assessment (clinical tests, spirometry, scales…)	Biosample (blood, urine, saliva…)	Biological assessment (eosinophilia, omics, DNA, RNA…)	Stressor (questionnaire)	Stressor (objective assessment)	Main results	Evidence	Genes involved
2012	Zhang et al.	male mice for 150 h over 10 weeks	3 and 18 months old	susceptibility to environmental toxins			basal expression of representative Nrf2-regulated phase II detoxifying enzymes, GCL, NQO1, and HO-1, and their induction by chronic exposure to nPM in the cerebellum, liver, and lung (mice)		nPM	The lack of induction of phase II enzymes in aging mice may be a model for the vulnerability of elderly to air pollution	IIb	Nrf2-regulated phase II detoxifying enzymes, GCL, NQO1, and HO-1

G. Conclusions

In spite of the heterogeneity of existing findings that precludes comparisons and the lack of convincing evidence from population data, the following facts emerge:

- Onset of age-dependent diseases such as asthma, diabetes, PD and AD and of subclinical phenotypes as allergic sensitization, overweight and neurodevelopmental troubles is strongly dependent on multifactorial interactions such as:
  - Presence of specific susceptibility genes/alleles
  - Vulnerability increasing the exposure
  - Methylation status
  - Environmental insults
  - And among adults, pre-existing health status and comorbidities
- Preconception is a crucial period for making change to the lifestyle and diet that can both help increase the chances of getting pregnant and birthing a healthy baby.
- Early-life influences, beginning with the intrauterine environment and continuing through the first few years of life, shape the trajectory of the various organs throughout the life course and are responsible for health outcomes. Prenatal and early-life environmental insults ranging from malnutrition to toxic exposures can tilt the odds toward development of adverse health effects decades later. These effects likely occur, at least in part, through alterations in an individual's genetic potential to thrive in the environment in which he or she will live. These early challenges set the bar for what's "normal," and the fetus and infant adapt for a less-than-optimal environment in ways that may contribute to adult-onset disease. Vulnerability to chemical toxicity after birth may be highest during the first 6 months and continue for years before maturation.
- Both puberty and menopause and the pre-existing periods constitute essential steps in asthma, weight and behaviour changes and downstream health conditions.
- Old age relates to a multidimensional process of physical, psychological and social change. From existing data (The GERIE study, not published) two old ages deserve to be considered, before and after 80 years in men and 85 years in women.

Several mechanisms of actions might be triggered; however oxidative stress seems to be the starting point related to many different endpoints.

Defence against oxidative stress, especially in the lungs requires Phase II metabolic enzymes. Overall, the maturation state of these enzymes is likely to mean that infants have a lower capacity to handle oxidative stress than adults. However, the reduced capacity of elderly to maintain homeostasis under oxidative stress is a major risk factor for onset and progress of several outcomes.

The significant changes in endocrine system related to menopause, negatively affect asthmatic inflammation, neuroinflammation protection (increasing susceptibility to neurodegenerative disorders), as well as to regular metabolism (resulting in metabolic disorders).

Elderly are more susceptible to all kind of environmental insults due to reduced detoxification capacity, lower homeostatic capabilities, as well as the cumulative methylation. This results in statistically

significant associations of ubiquitous compounds at environmentally relevant levels, to several health outcomes.

Middle age lifestyle parameters (nutrition, exercise, smoking), health status (hypertension, diabetes) and use of drugs have been found to be determinant for the onset of neurodegenerative diseases. These parameters comprise basic elements for the individual exposome and have to be monitored.

There are common nodes in the mechanism of actions among the major health outcomes investigated in HEALS, where elderly population seems to be especially vulnerable. Due to these common nodes, and the inherent reduced homeostasis capacity, it seems that onset of specific health problems (e.g. type 2 diabetes), might contribute to the development of another disorder (e.g. Alzheimer disease).

Beyond the fact that compounds inducing oxidative stress contribute to the potential onset of all these disorders, additional loops have to be taken into account, considering the cascade of following events (e.g. reduced metabolic capacity of xenobiotics due to type 2 diabetes).

Twins can be highly informative in terms of omics.

H. Recommendations on critical windows related to the investigated endpoints in HEALS

HEALS targets both existing datasets and forthcoming dataset in the frame of EXHES, where children (both singleton and twins) are recruited since *in utero life*, with their siblings, parents and other relatives, thus constituting a transgenerational study including almost the entire lifespan. Therefore, critical life stages and the feature identified by this review of interest for HEALS are:

- **Preconception** that has to be target in terms of lifestyle and diet.

-The three trimesters of pregnancy have to be monitored according to the event of interest. During **pregnancy**, there is increased risk of modified immunity, gestational diabetes, which shares several common mechanism of action nodes related to diabetes Type 2. Thus, susceptibility factors and compounds associated to Type 2 diabetes are of particular interest to be investigated during pregnancy. In addition, the occurrence of gestational diabetes alters the outcome of internal dosimetry to mother and fetus as well, posing both of them into higher susceptibility to other environmental insults. Before and after 3 years of age constitute also important periods.

Puberty with hormones changes is crucial for asthma, weight and behaviour variations.

Middle age lifestyle parameters (nutrition, exercise, smoking…), health status (hypertension, diabetes) and use of drugs are determinant for the onset and the progress of neurodegenerative diseases. Change in lifestyle choices after the age of 30 years introduces new conditions that increase the risk of metabolic disorders that may eventually lead to obesity and type 2 diabetes. These changes are related to professional advancement for most individuals, altering significantly their daily time-activity patterns leaning towards relatively higher caloric intake, more sedentary life spent indoors and thus exposure to xenobiotics that have been associated with endocrine disruption such as phthalates and brominated flame retardants or plasticizers. Gene-environment interactions worsen asthma and allergies, overall in women due to changes in hormonal factors. Stress and obesity can also be at the origin of asthma in adulthood.

There is sufficient evidence that **menopause** in women (between the age of **45** years and **55 years**) is a period of significant changes in the hormonal system, related to a cascade of effects, asthma, increasing susceptibility metabolic disorders, as well as to neuroinflammation. At the age of **50** years significant changes in gene expression related to brain related function seem to be determinant for the onset of neurodegenerative disorders.
After 65 years both males and females are more susceptible to environmental insults, due to reduced detoxification capacity, as well as reduced capacity of maintaining homeostasis.
At 80 and 85 years, normal ageing is accompanied by pathological ageing.

Data on exposures and health outcomes will be drawn from questionnaires and clinical tests. However, because of ethical considerations, biological specimens will be collected in children recruited in EXHES only twice, at birth and at 2-3 years of age. Biological specimens will also be collected from the respective parents (and eventually grandparents) to address epigenetic considerations. Questionnaires will be administrated at the other periods.

I. References

Abrahamsson TR, Jakobsson T, Böttcher MF, Fredrikson M, Jenmalm MC, Björkstén B, Oldaeus G.

Ahmadi A, Fredrikson M, Jerregård H, Åkerbäck A, Fall PA, Rannug A, Axelson O and Söderkvist P (2000). GSTM1 and mEPHX Polymorphisms in Parkinson's Disease and Age of Onset. *Biochemical and Biophysical Research Communications*, 269(3):676-680.

Aïd S and Bosetti F (2007). Gene expression of cyclooxygenase-1 and Ca2+-independent phospholipase A2 is altered in rat hippocampus during normal aging. *Brain Research Bulletin*, 73(1-3):108-113.

Alberga AS, Sigal RJ, Goldfield G, Prud'homme D, Kenny GP. Overweight and obese teenagers: why is adolescence a critical period? Pediatr Obes. 2012 Aug;7(4):261-73.

Alm B, Erdes L, Mollborg P, Pettersson R, Norvensius SG, Aberg N, Wennergren G. 2008. Neonatal antibiotic treatment is a risk factor for early wheezing. Pediatrics 121:697–702.

Almond D., Currie J. (2011). Killing me softly: The fetal origins hypothesis. *The Journal of Economics Perspectives* 25(3): 153-172.

Amouyel P (2002). Genetic susceptibility to ageing-associated diseases. *Comptes Rendus - Biologies*, 325(6):741-745.

Annesi-Maesano I, Pollitt R, King G, Bousquet J, Hellier G, Sahuquillo J, Huel G. In utero exposure to lead and cord blood total IgE. Is there a connection? Allergy. 2003 Jul;58(7):589-94.

Atzei A, Atzori L, Moretti C, Barberini L, Noto A, Ottonello G, et al. Metabolomics in paediatric respiratory diseases and bronchiolitis. *J Matern Fetal Neonatal Med* 2011;24(Suppl 2):59–62.

Avramopoulos D, Szymanski M, Wang R and Bassett S (2011). Gene expression reveals overlap between normal aging and Alzheimer's disease genes. *Neurobiology of Aging*, 32(12):2319.e2327-2319.e2334.

Bager P, Wohlfahrt J, Westergaard T. Caesarean delivery and risk of atopy and allergic disease: meta-analyses. Clin Exp Allergy. 2008 Apr;38(4):634-42.

Baïz N, Annesi-Maesano I.Is the asthma epidemic still ascending? Clin Chest Med. 2012 Sep;33(3):419-29.

Barker A.J., E.M. Ullian. Astrocytes and synaptic plasticity. Neuroscientist, 16 (2010), pp. 40–50.

Bassols J, Megia A, Soriano-Rodriguez P, Diaz M, Prats-Puig A, Gifre M, Simon-Muela I, Torrent S, Borrell AC, Riera-Socasau JC, Vendrell J, de Zegher F, Ibanez L and Lopez-Bermejo A (2013). A common gene variant in STK11 is associated with metabolic risk markers and diabetes during gestation. *Fertil Steril*, 100(3):788-792.

Behrman R., Butler A.S. (Eds) (2007). *Preterm Birth: Causes, Consequences, and Prevention*. Washington, DC: The National Academies Press.

Bell ML, Zanobetti A and Dominici F (2013). Evidence on vulnerability and susceptibility to health risks associated with short-term exposure to particulate matter: a systematic review and meta-analysis. *American Journal of Epidemiology*, 178(6):865-876.

Ben-Ari Y. Neuropaediatric and neuroarchaeology: understanding development to correct brain disorders. Acta Paediatr., 102 (2013), pp. 331–334.

Beyerlein A, Wehweck F, Ziegler AG, Pflueger M. Respiratory infections in early life and the development of islet autoimmunity in children at increased type 1 diabetes risk: evidence from the BABYDIET study. JAMA Pediatr. 2013 Sep;167(9):800-7.

Bihaqi SW, Schumacher A, Maloney B, Lahiri DK and Zawia NH (2012). Do epigenetic pathways initiate late onset alzheimer disease (LOAD): Towards a new paradigm. *Current Alzheimer Research*, 9(5):574-588.

Bjorksten B. 2008. Environmental influences on the development of the immune system: consequences for disease outcome. Nestle Nutr Workshop Ser Pediatr Program 61:243–254.

Bonassi S, Au WW. 2002. Biomarkers in molecular epidemiology studies for health risk prediction. Mutat Res 511(1):73-86.

Bove R and Chitnis T (2013). Sexual disparities in the incidence and course of MS. *Clinical Immunology*, 149(2):201-210.
Bove R and Chitnis T (2014). The role of gender and sex hormones in determining the onset and outcome of multiple sclerosis. *Multiple Sclerosis*, 20(5):520-526.
Boyle RJ, Ismail IH, Kivivuori S, Licciardi PV, Robins-Browne RM, Mah LJ, Axelrad C, Moore S, Donath S, Carlin JB, Lahtinen SJ, Tang ML. Lactobacillus GG treatment during pregnancy for the prevention of eczema: a randomized controlled trial.Allergy. 2011 Apr;66(4):509-16.
Burdge GC, Lillycrop KA, Jackson AA. Nutrition in early life, and risk of cancer and metabolic disease: alternative endings in an epigenetic tale? Br J Nutr 2009; 101:619–30.
Cao K, Chen-Plotkin AS, Plotkin JB and Wang LS (2010). Age-correlated gene expression in normal and neurodegenerative human brain tissues. *PloS one*, 5(9).
Cao K, Ryvkin P, Hwang YC, Johnson FB and Wang LS (2013). Analysis of Nonlinear Gene Expression Progression Reveals Extensive Pathway and Age-Specific Transitions in Aging Human Brains. *PloS one*, 8(10).
Carroll JC and Rosario ER (2012). The potential use of hormone-based therapeutics for the treatment of Alzheimer's disease. *Current Alzheimer Research*, 9(1):18-34.
Centers for Disease Control and Prevention. 2009. Fourth National Report on Human Exposure to Environmental Chemicals. Atlanta, GA: National Center for Environmental Health, Centers for Disease Control and Prevention.
Cereda E, Barichella M, Cassani E, Caccialanza R and Pezzoli G (2013). Reproductive factors and clinical features of Parkinson's disease. *Parkinsonism & Related Disorders*, 19(12):1094-1099.
Cesare Marincola F, Noto A, Caboni P, A Reali, L. Barberini, M. Lussu, et al. A metabolomic study of preterm human and formula milk by high resolution NMR and GC/MS analysis: preliminary results. *J Matern Fetal Neonatal Med* 2012 Oct;25(Suppl 5):62–7.
Choi YH, Kim JH, Lee BE and Hong YC (2014). Urinary benzene metabolite and insulin resistance in elderly adults. *Sci Total Environ*, 482-483:260-268.
Ciencewicki J, Trivedi S and Kleeberger SR (2008). Oxidants and the pathogenesis of lung diseases. *Journal of Allergy and Clinical Immunology*, 122(3):456-468.
Cohen Hubal EA, Richard AM, Shah I, Gallagher J, Kavlock R, Blancato J, et al. 2010. Exposure science and the U.S. EPA National Center for Computational Toxicology. J Expo Sci Environ Epidemiol 20(3):231-236.
Courchesne E, Chisum HJ, Townsend J, Cowles A, Covington J, Egaas B, Harwood M, Hinds S and Press GA (2000). Normal brain development and aging: quantitative analysis at in vivo MR imaging in healthy volunteers. *Radiology*, 216(3):672-682.
Crespo TC, Silva B, Marques L, Marcelino E, Maruta C, Costa S, Timóteo T, Vilares A, Couto FS, Faustino P, Correia AP, Verdelho A, Porto G, Guerreiro M, Herrero A, Costa C, de Mendonça A, Costa L and Martins M (2014). Genetic and biochemical markers in patients with Alzheimer's disease support a concerted systemic iron homeostasis dysregulation. *Neurobiology of Aging*, 35(4):777-785.
Davis SR, Castelo-Branco C, Chedraui P, Lumsden MA, Nappi RE, Shah D and Villaseca P (2012). Understanding weight gain at menopause. *Climacteric*, 15(5):419-429.
Devereux G. 2007. Early life events in asthma–diet. Pediatr Pulmonol 42:663–673.
Diaz-Robles LA, Fu JS, Vergara-Fernandez A, Etcharren P, Schiappacasse LN, Reed GD and Silva MP (2014). Health risks caused by short term exposure to ultrafine particles generated by residential wood combustion: a case study of Temuco, Chile. *Environ Int*, 66:174-181.
Dietert RR, Dietert JM. Potential for early-life immune insult including developmental immunotoxicity in autism and autism spectrum disorders: focus on critical windows of immune vulnerability. J Toxicol Environ Health B Crit Rev. 2008 Oct;11(8):660-80.
Dietert RR, Etzel RA, Chen D, Halonen M, Holladay SD, Jarabek AM, Landreth K, Peden DB, Pinkerton K, Smialowicz RJ, Zoetis T. Workshop to Identify Critical Windows of Exposure for Children's Health: Immune and Respiratory Systems Work Group Summary. Environ Health Perspect. 2000 Jun;108 Suppl 3:483-9
Dietert RR, Etzel RA, Chen D, Halonen M, Holladay SD, Jarabek AM, Landreth K, Peden D, Pinkerton K, Smialowicz RJ, & Zoetis T. 2000. Windows of vulnerability for the immune and respiratory systems. Environ Health Perspect 108:483–490.

Dietert RR, Zelikoff JT. Early-life environment, developmental immunotoxicology, and the risk of pediatric allergic disease including asthma. Birth Defects Res B Dev Reprod Toxicol. 2008 Dec;83(6):547-60.
Dietz WH. Critical periods in childhood for the development of obesity. Am J Clin Nutr. 1994 May;59(5):955-9.
Donnet-Hughes A., *et al.* Potential role of the intestinal microbiota of the mother in neonatal immune education. Proc. Nutr. Soc., 69 (2010), pp. 407–415
Dorfman LJ and Bosley TM (1979). Age-related changes in peripheral and central nerve conduction in man. *Neurology*, 29(1):38-44.
Dostalek M, Akhlaghi F and Puzanovova M (2012). Effect of diabetes mellitus on pharmacokinetic and pharmacodynamic properties of drugs. *Clin Pharmacokinet*, 51(8):481-499.
Douwes J, Pearce N, Heederik D. Does environmental endotoxin exposure prevent asthma? Thorax. 2002 Jan;57(1):86-90.
Douwes J, Cheng S, Travier N, Cohet C, Niesink A, McKenzie J, Cunningham C, Le Gros G, von Mutius E, Pearce N. Farm exposure in utero may protect against asthma, hay fever and eczema. Eur Respir J. 2008 Sep;32(3):603-11.
Dunstan JA, Mori TA, Barden A, Beilin LJ, Taylor AL, Holt PG, Prescott SL. Fish oil supplementation in pregnancy modifies neonatal allergen specific immune responses and clinicaloutcomes in infants at high risk of atopy: randomized, controlled trial. J Allergy Clin Immunol. 2003 Dec;112(6):1178-84.
Ebrahimpour P, Fakhrzadeh H, Heshmat R, Ghodsi M, Bandarian F and Larijani B (2010). Metabolic syndrome and menopause: A population-based study. *Diabetes and Metabolic Syndrome: Clinical Research and Reviews*, 4(1):5-9.
Ege MJ, Bieli C, Frei R, van Strien RT, Riedler J, Ublagger E, Schram-Bijkerk D, Brunekreef B, van Hage M, Scheynius A, Pershagen G, Benz MR, Lauener R,von Mutius E, Braun-Fahrländer C; team. Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensitization in school-age children. J Allergy Clin Immunol. 2006 Apr;117(4):817-23.
Entringer S, Epel ES, Kumsta R et al. Stress exposure in intrauterine life is associated with shorter telomere length in young adulthood. Proc Natl Acad Sci USA 2011; 108:E513–18.
Esther Thelen. Motor development as foundation and future of developmental psychology. International Journal of Behavioral Development, 2000, 24 (4), 385–397.
Ettinger AS, Zota AR, Amarasiriwardena CJ, Hopkins MR, Schwartz J, Hu H and Wright RO (2009). Maternal arsenic exposure and impaired glucose tolerance during pregnancy. *Environ Health Perspect*, 117(7):1059-1064.
Fagundes CP, Glaser R, Kiecolt-Glaser JK. Stressful early life experiences and immune dysregulation across the lifespan. Brain Behav Immun. 2013 Jan;27(1):8-12.
Fanos V, Iacovidou N, Puddu M, Ottonello G, Noto A, Atzori L. Metabolomics in neonatal **life**. *Early Hum Dev*. 2013 Jun;89 Suppl 1:S7-10.
Farooqui T and Farooqui AA (2009). Aging: An important factor for the pathogenesis of neurodegenerative diseases. *Mechanisms of Ageing and Development*, 130(4):203-215.
Faulds MH, Zhao C, Dahlman-Wright K and Gustafsson JÅ (2012). The diversity of sex steroid action: Regulation of metabolism by estrogen signaling. *Journal of Endocrinology*, 212(1):3-12.
Ferrari R, Dawoodi S, Raju M, Thumma A, Hynan LS, Maasumi SH, Reisch JS, O'Bryant S, Jenkins M, Barber R and Momeni P (2013). Androgen receptor gene and sex-specific Alzheimer's disease. *Neurobiology of Aging*, 34(8):2077.e2019-2077.e2020.
Forns J, Vegas O, Julvez J, Garcia-Esteban R, Rivera M, Lertxundi N, Guxens M, Fano E, Ferrer M, Grellier J, Ibarluzea J, Sunyer J. Association between Child Cortisol Levels in Saliva and Neuropsychological Development during the Second Year of Life. Stress Health. 2013 Jul 1.
Fox M, Berzuini C and Knapp LA (2013). Cumulative estrogen exposure, number of menstrual cycles, and Alzheimer's risk in a cohort of British women. *Psychoneuroendocrinology*, 38(12):2973-2982.
Franco G. 1999. Ramazzini and workers' health. Lancet 354(9180:858-861.

Georgopoulos PG, Lioy PJ. 2006. From a theoretics/ framework of human exposure and dose assessment to computational system implementatino: the Modeling Environment for Total Risk Studies (MENTOR). J Toxicol Environ Health B Crit Rev 9(6):457-483.
Georgopoulos PG, Sasso AF, Isukapalli SS, Lioy PJ, Vallero DA, Okino M, et al. 2009. Reconstructing population exposures to environmental chemicals from biomarkers: challenges and opportunities. J Expo Sci Environ Epidemiol 19(21:149-171.
Giles LC, Whitrow MJ, Rumbold AR, Davies CE, de Stavola B, Pitcher JB, Davies MJ, Moore VM Growth in early life and the development of obesity by age 9 years: are there critical periods and a role for an early life stressor? Int J Obes (Lond). 2013 Apr;37(4):513-9.
Glantz L.A., *et al.* Synaptophysin and postsynaptic density protein 95 in the human prefrontal cortex from mid-gestation into early adulthood. Neuroscience, 149 (2007), pp. 582–591.
Goksör E, Alm B, Pettersson R, Möllborg P, Erdes L, Åberg N, and Wennergren G. Early fish introduction and neonatal antibiotics affect the risk of asthma into school age. Pediatr Allergy Immunol. Jun 2013; 24(4): 339–344.
González-Alzaga B, Lacasaña M, Aguilar-Garduño C, Rodríguez-Barranco M, Ballester F, Rebagliato M, Hernández AF. A systematic review of neurodevelopmental effects of prenatal and postnatal organophosphate pesticide exposure. Toxicol Lett. 2013 Nov 26. pii: S0378-4274(13)01425-2
Gracia CR, Freeman EW, Sammel MD, Lin H and Nelson DB (2005). The relationship between obesity and race on inhibin B during the menopause transition. *Menopause*, 12(5):559-566.
Gravena AAF, Brischiliari SCR, Lopes TCR, Agnolo CMD, Carvalho MDB and Pelloso SM (2013). Excess weight and abdominal obesity in postmenopausal Brazilian women: A population-based study. *BMC Women's Health*, 13(1).
Greenbaum L, Rigbi A, Lipshtat N, Cilia R, Tesei S, Asselta R, Djaldetti R, Goldwurm S and Lerer B (2013). Association of nicotine dependence susceptibility gene, CHRNA5, with Parkinson's disease age at onset: Gene and smoking status interaction. *Parkinsonism & Related Disorders*, 19(1):72-76.
Guevara-Guzman R, Arriaga V, Kendrick KM, Bernal C, Vega X, Mercado-Gomez OF and Rivas-Arancibia S (2009). Estradiol prevents ozone-induced increases in brain lipid peroxidation and impaired social recognition memory in female rats. *Neuroscience*, 159(3):940-950.
Haschke F, Haiden N, Detzel P, Yarnoff B, Allaire B, Haschke-Becher E. Feeding patterns during the first 2 years and health outcome. Ann Nutr Metab. 2013;62 Suppl 3:16-25.
Hindorff LA, Sethupathy P, Junkins HA, Ramos EM, Mehta JP, Collins FS, et al. 2009. Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. Proc Natl Acad Sci USA 106(23):9362-9367.
Hoffmann K, Becker K, Friedrich C, Helm D, Krause C, Seifert B. 2000. The German Environmental Survey 1990/1992 (GerES II): cadmium in blood, urine and hair of adults and children. J Expo Anal Environ Epidemiol 10(2):126-135.
Hohman TJ, Koran ME and Thornton-Wells TA (2014). Genetic variation modifies risk for neurodegeneration based on biomarker status. *Front Aging Neurosci*, 6:183.
Holmes E, Loo RL, Stamler J, Bictash M, Yap IK, Chan Q, et al. 2008. Human metabolic phenotype diversity and its association with diet and blood pressure. Nature 453(7193):396-400.
Jenmalm MC, Duchén K. Timing of allergy-preventive and immunomodulatory dietary interventions - are prenatal, perinatal or postnatal strategies optimal? Clin Exp Allergy. 2013 Mar;43(3):273-8.
Kalliomäki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. Lancet. 2001 Apr 7;357(9262):1076-9.
Kelly FJ, Dunster C and Mudway I (2003). Air pollution and the elderly: oxidant/antioxidant issues worth consideration. *European Respiratory Journal*, 21(40 suppl):70s-75s.
Kim E, Park H, Hong YC, Ha M, Kim Y, Kim BN, Kim Y, Roh YM, Lee BE, Ryu JM, Kim BM, Ha EH. Prenatal exposure to PM10 and NO2 and children's neurodevelopment from birth to 24months of age: Mothers and Children's Environmental Health (MOCEH) study. Sci Total Environ. 2014 Mar 10;481C:439-445.

Kim J, Natarajan S, Vaickus LJ, Bouchard JC, Beal D, Cruikshank WW, Remick DG.Diesel exhaust particulates exacerbate asthma-like inflammation by increasing CXC chemokines. Am J Pathol. 2011 Dec;179(6):2730-9.
Kivipelto M, Ngandu T, Fratiglioni L, Viitanen M, Kareholt I, Winblad B, Helkala EL, Tuomilehto J, Soininen H and Nissinen A (2005). Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease. *Arch Neurol*, 62(10):1556-1560.
Kofink D, Boks MP, Timmers HT, Kas MJ. Epigenetic dynamics in psychiatric disorders: environmental programming of neurodevelopmental processes. Neurosci Biobehav Rev. 2013 Jun;37(5):831-45.
Kowall B, Rathmann W, Strassburger K, Heier M, Holle R, Thorand B, Giani G, Peters A and Meisinger C (2010). Association of passive and active smoking with incident type 2 diabetes mellitus in the elderly population: the KORA S4/F4 cohort study. *Eur J Epidemiol*, 25(6):393-402.
Kukkonen K, Savilahti E, Haahtela T, Juntunen-Backman K, Korpela R, Poussa T, Tuure T, Kuitunen M. Probiotics and prebiotic galacto-oligosaccharides in the prevention of allergic diseases: a randomized, double-blind, placebo-controlled trial. J Allergy Clin Immunol. 2007 Jan;119(1):192-8.
Lagiou P, Tamimi RM, Mucci LA, Adami HO, Hsieh CC, Trichopoulos D. Diet during pregnancy in relation to maternal weight gain and birth size. Eur J Clin Nutr. 2004 Feb;58(2):231-7.
Lahiri DK, Maloney B and Zawia NH. 2009. "How biochemical pathways for disease may be triggered by early-life events." In Current Hypotheses and Research Milestones in Alzheimer's Disease.
Landrigan PJ, Trasande L, Thorpe LE, Gwynn C, Lioy PJ, D'Alton ME, et al. 2006. The National Children's Study: a 21-year prospective study of 100,000 American children. Pediatrics 118(51:2173-2186.
Langley-Evans SC, & Carrington LJ. 2006. Diet and the developing immune system. Lupus 15:746–752.
Lee JS, Ward WO, Wolf DC, Allen JW, Mills C, Devito MJ and Corton JC (2008). Coordinated changes in xenobiotic metabolizing enzyme gene expression in aging male rats. *Toxicological Sciences*, 106(1):263-283.
Leibowitz KL, Moore RH, Ahima RS, Stunkard AJ, Stallings VA, Berkowitz RI, Chittams JL, Faith MS, Stettler N. Maternal obesity associated with inflammation in their children. World J Pediatr. 2012 Feb;8(1):76-9.
Lepeule J, Bind MA, Baccarelli AA, Koutrakis P, Tarantini L, Litonjua A, Sparrow D, Vokonas P and Schwartz JD (2014). Epigenetic influences on associations between air pollutants and lung function in elderly men: the normative aging study. *Environ Health Perspect*, 122(6):566-572.
Lind L, Penell J, Luttropp K, Nordfors L, Syvanen AC, Axelsson T, Salihovic S, van Bavel B, Fall T, Ingelsson E and Lind PM (2013). Global DNA hypermethylation is associated with high serum levels of persistent organic pollutants in an elderly population. *Environ Int*, 59:456-461.
Lind L, Zethelius B, Salihovic S, van Bavel B and Lind PM (2014). Circulating levels of perfluoroalkyl substances and prevalent diabetes in the elderly. *Diabetologia*, 57(3):473-479.
Lind PM, Zethelius B and Lind L (2012). Circulating levels of phthalate metabolites are associated with prevalent diabetes in the elderly. *Diabetes Care*, 35(7):1519-1524.
Ling C, Poulsen P, Carlsson E, Ridderstråle M, Almgren P, Wojtaszewski J, Beck-Nielsen H, Groop L and Vaag A (2004). Multiple environmental and genetic factors influence skeletal muscle PGC-1α and PGC-1β gene expression in twins. *Journal of Clinical Investigation*, 114(10):1518-1526.
Lioy PJ.2010. Exposure science: a view of the past and milestones for the future. Environ Health Perspect 118:1081-1090.
Lioy, PJ and S. M. Rappaport (2011). "Exposure science and the exposome: an opportunity for coherence in the environmental health sciences." Environmental health perspectives 119(11): A466-467.
Lowe WL, Jr. and Karban J (2014). Genetics, genomics and metabolomics: new insights into maternal metabolism during pregnancy. *Diabet Med*, 31(3):254-262.
Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, et al. 2009. Finding the missing heritability of complex diseases. Nature 461(72651:747-753.

Marques H., *et al.* The influence of maternal prenatal and early childhood nutrition and maternal prenatal stress on offspring immune system development and neurodevelopmental disorders. Front. Neurosci., 7 (2013), p. 120.
Marques SCF, Oliveira CR, Pereira CMF and Outeiro TF (2011). Epigenetics in neurodegeneration: A new layer of complexity. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 35(2):348-355.
Matheson MC, Allen KJ, Tang ML. Understanding the evidence for and against the role of breastfeeding in allergy prevention. Clin Exp Allergy. 2012 Jun;42(6):827-51.
McMillen IC, Muhlhausler BS, Duffield JA, Yuen BS.Prenatal programming of postnatal obesity: fetal nutrition and the regulation of leptin synthesis and secretion before birth. Proc Nutr Soc. 2004 Aug;63(3):405-12.
McMillen IC, Rattanatray L, Duffield JA, Morrison JL, MacLaughlin SM, Gentili S, Muhlhausler BS. Adv Exp Med Biol. The early origins of later obesity: pathways and mechanisms. Adv Exp Med Biol. 2009;646:71-81. doi: 10.1007/978-1-4020-9173-5_8.
Mendell MJ. 2007. Indoor residential chemical emissions as risk factors for respiratory and allergic effects in children: a review. Indoor Air 17:259–277.
Menegon A, Board PG, Blackburn AC, Mellick GD and Le Couteur DG (1998). Parkinson's disease, pesticides, and glutathione transferase polymorphisms. *Lancet*, 352(9137):1344-1346.
Merlo DF, Wild CP, Kogevinas M, Kyrtopoulos S, Kleinjans J. NewGeneris: a European study on maternal diet during pregnancy and, child health. Cancer Epidemiol Biomarkers Prev 2009; 18:5–10.
Moore DC, Elsas PX, Maximiano ES, & Elsas MI. 2006. Impact of diet on the immunological microenvironment of the pregnant uterus and its relationship to allergic disease in the offspring--a review of the recent literature. San Paolo Med J 124:298–303.
Mühlhäusler BS, Adam CL, McMillen IC. Maternal nutrition and the programming of obesity: The brain. Organogenesis. 2008 Jul;4(3):144-52.
Muhlhausler BS, Ong ZY.The fetal origins of obesity: early origins of altered food intake. Endocr Metab Immune Disord Drug Targets. 2011 Sep 1;11(3):189-97.
Nicholas LM, Rattanatray L, MacLaughlin SM, Ozanne SE, Kleemann DO, Walker SK, Morrison JL, Zhang S, Muhlhäusler BS, Martin-Gronert MS, McMillen IC. Differential effects of maternal obesity and weight loss in the periconceptional period on the epigenetic regulation of hepatic insulin-signaling pathways in the offspring. FASEB J. 2013 Sep;27(9):3786-96.
Nicholson JK. 2006. Global systems biology, personalized medicine and molecular epidemiology [Editorial]. Mol Syst Biol 2:52; doi:10.1038/msh4100095 [3 October 2006].
Nicoletti A, Nicoletti G, Arabia G, Annesi G, De Mari M, Lamberti P, Grasso L, Marconi R, Epifanio A, Morgante L, Cozzolino A, Barone P, Quattrone A and Zappia M (2011). Reproductive fa
Olsson AH, Rönn T, Elgzyri T, Hansson O, Eriksson KF, Groop L, Vaag A, Poulsen P and Ling C (2011). The expression of myosin heavy chain (MHC) genes in human skeletal muscle is related to metabolic characteristics involved in the pathogenesis of type 2 diabetes. *Molecular Genetics and Metabolism*, 103(3):275-281.
Ott WR. 1990. Total human exposure: basic concepts, EPA field studies, and future research needs. J Air Waste Manage Assoc 40(7):966-975.
Ott WR. 1995. Human exposure assessment: the birth of a new science. J Expo Anal Environ Epidemiol 5(4):449-472.
Panigrahi PP and Singh TR (2013). Computational studies on Alzheimer's disease associated pathways and regulatory patterns using microarray gene expression and network data: Revealed association with aging and other diseases. *Journal of Theoretical Biology*, 334:109-121.
Patel CJ, Bhattacharya J, Butte AJ. 2010. An Environment-Wide Association study (EWAS) on type 2 diabetes mellitus. PLoS One 5(5:e10 rnal.pone.746; doi:10.l371/jou0010746 [Online 20 May 2010].
Paus T., *et al.* Why do many psychiatric disorders emerge during adolescence? Nat. Rev. Neurosci., 9 (2008), pp. 947–957.
Petry CJ, Seear RV, Wingate DL, Manico L, Acerini CL, Ong KK, Hughes IA and Dunger DB (2011). Associations between paternally transmitted fetal IGF2 variants and maternal circulating glucose concentrations in pregnancy. *Diabetes*, 60(11):3090-3096.

Pico C, Palou A. Perinatal programming of obesity: an introduction to the topic. Front Physiol. 2013 Sep 17;4:255.
Pozzi S, Benedusi V, Maggi A and Vegeto E. 2006. "Estrogen action in neuroprotection and brain inflammation." In Annals of the New York Academy of Sciences.
Prescott SL, & Dunstan JA. 2007. Prenatal fatty acid status and immune development: the pathways and the evidence. Lipids 42:801–810.
Rappaport SM, Smith MT. Epidemiology. Environment and disease risks. Science 2010;330:460-61.
Rappaport SM. 2011. Implications of the exposome for exposure science. J Expo Sci Environ Epidemiol 21(1):5-9.
Reichman WE and Rose NS (2012). History and experience: The direction of Alzheimer's disease. *Menopause*, 19(7):724-734.
Risher JF, Todd GD, Meyer D and Zunker CL (2010). The elderly as a sensitive population in environmental exposures: making the case. *Rev Environ Contam Toxicol*, 207:95-157.
Robledo C, Peck JD, Stoner JA, Carabin H, Cowan L, Koch HM and Goodman JR (2013). Is bisphenol-A exposure during pregnancy associated with blood glucose levels or diagnosis of gestational diabetes? *J Toxicol Environ Health A*, 76(14):865-873.
Robledo CA, Yeung E, Mendola P, Sundaram R, Maisog J, Sweeney AM, Barr DB, Buck Louis GM. Preconception Maternal and Paternal Exposure to Persistent Organic Pollutants and Birth Size: The LIFE Study. Environ Health Perspect. 2014 Aug 5.
Rocchi A, Orsucci D, Tognoni G, Ceravolo R and Siciliano G (2009). The role of vascular factors in late-onset sporadic Alzheimer's disease. Genetic and molecular aspects. *Current Alzheimer Research*, 6(3):224-237.
Roessmann U., P. Gambetti. Astrocytes in the developing human brain, An immunohistochemical study. Acta Neuropathol., 70 (1986), pp. 308–313.
Saunders L, Kadhel P, Costet N, Rouget F, Monfort C, Thome JP, Guldner L, Cordier S and Multigner L (2014). Hypertensive disorders of pregnancy and gestational diabetes mellitus among French Caribbean women chronically exposed to chlordecone. *Environ Int*, 68:171-176.
Sausenthaler S, Koletzko S, Schaaf B, Lehmann I, Borte M, Herbarth O, von Berg A, Wichmann HE, Henirich J, & the LISA Study Group. 2007. Maternal diet during pregnancy in relation to eczema and allergic sensitization in the offspring at 2 y of age. Am J Clin Nutr 85:530–537.
Schmechel DE, Browndyke J and Ghio A (2006). Strategies for dissecting genetic-environmental interactions in neurodegenerative disorders. *NeuroToxicology*, 27(5):637-657.
Schmidt Morgen C, Andersen AM, Due P, Neelon SB, Gamborg M, Sørensen TI. Timing of motor milestones achievement and development of overweight in childhood: a study within the Danish National Birth Cohort. Pediatr obes.2013 jun 4.
Scott EL, Zhang QG, Vadlamudi RK and Brann DW (2014). Premature menopause and risk of neurological disease: Basic mechanisms and clinical implications. *Molecular and Cellular Endocrinology*, 389(1-2):2-6.
Scott JP. 1986. "Critical Periods in Organizational Processes." In Human Growth, eds. Frank Falkner and J. M. Tanner: Springer US.
Sexton K, Kleffman DE, Callahan MA. 1995. An introduction to the National Human Exposure Assessment Survey (NHEXAS) and related phase I field studies. J Expo Anal Environ Epidemiol 5(3):229-232.
Sharma HP, Hansel NN, Matsui E, Diette GB, Eggleston P, & Breysse P. 2007. Indoor environmental influences on children's asthma. Pediatr Clin North Am 54:103–120.
Stein J, Responsibility GBPfS, Science and Network EH. 2008. Environmental Threats to Healthy Aging: With a Closer Look at Alzheimer's & Parkinson's Diseases: Greater Boston Physicians for Social Responsibilty.
Sternfeld B, Bhat AK, Wang H, Sharp T and Quesenberry Jr CP (2005). Menopause, physical activity, and body composition/fat distribution in midlife women. *Medicine and Science in Sports and Exercise*, 37(7):1195-1202.
Sundermann EE, Maki PM and Bishop JR (2010). A review of estrogen receptor α gene (ESR1) polymorphisms, mood, and cognition. *Menopause*, 17(4):874-886.

Symonds ME, Mendez MA, Meltzer HM, Koletzko B, Godfrey K, Forsyth S, van der Beek EM. Early life nutritional programming of obesity: mother-child cohort studies. Ann Nutr Metab. 2013;62(2):137-45.
Symonds ME, Sebert S, Budge H. The obesity epidemic: from the environment to epigenetics - not simply a response to dietary manipulation in a thermoneutral environment. Front Genet. 2011 May 31;2:24.
Taupin P (2010). A dual activity of ROS and oxidative stress on adult neurogenesis and Alzheimer's disease. *Central Nervous System Agents in Medicinal Chemistry*, 10(1):16-21.
Téllez-Rojo MM, Cantoral A, Cantonwine DE, Schnaas L, Peterson K, Hu H, Meeker JD. Prenatal urinary phthalate metabolites levels and neurodevelopment in children at two and three years of age. Sci Total Environ. 2013 Sep 1;461-462:386-90
Tischer CG, Hohmann C, Thiering E, Herbarth O, Müller A, Henderson J, Granell R, Fantini MP, Luciano L, Bergström A, Kull I, Link E, von Berg A, Kuehni CE,Strippoli MP, Gehring U, Wijga A, Eller E, Bindslev-Jensen C, Keil T, Heinrich J; ENRIECO consortium. Meta-analysis of mould and dampness exposure on asthma and allergy in eight European birth cohorts: an ENRIECO initiative.
Urbanek M, Hayes MG, Lee H, Freathy RM, Lowe LP, Ackerman C, Jafari N, Dyer AR, Cox NJ, Dunger DB, Hattersley AT, Metzger BE and Lowe WL, Jr. (2012). The role of inflammatory pathway genetic variation on maternal metabolic phenotypes during pregnancy. *PloS one*, 7(3):e32958.
Veiby G, Engelsen BA, Gilhus NE. Early child development and exposure to antiepileptic drugs prenatally and through breastfeeding: a prospective cohort study on children of women with epilepsy. JAMA Neurol. 2013 Nov;70(11):1367-74.
Vest RS and Pike CJ (2013). Gender, sex steroid hormones, and Alzheimer's disease. *Hormones and Behavior*, 63(2):301-307.
Vickers MH. Developmental programming of the metabolic syndrome - critical windows for intervention. World J Diabetes. 2011 Sep 15;2(9):137-48.
Wallace LA, Pellizzari ED, Hartwell TD, Sparacino CM, Sheldon LS, Zelon H. 1985. Personal exposures, indoor-outdoor relationships, and breath levels of toxic air pollutants measured for 355 persons in New Jersey. Atmos Environ 19(10):1651-1661.
Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, et al. 2011. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 472(73411:57-63.
Warner JO. 2007. Early life nutrition and allergy. Early Hum Dev 83:777–783.
Wells AD, Poole JA, Romberger DJ. Influence of farming exposure on the development of asthma and asthma-like symptoms. Int Immunopharmacol. 2014 Jul 31. pii: S1567-5769(14)00278-1.
Weng SF, Redsell SA, Swift JA, Yang M, Glazebrook CP. Systematic review and meta-analyses of risk factors for childhood overweight identifiable during infancy. Arch Dis Child. 2012 Dec;97(12):1019-26.
West LJ. Defining critical windows in the development of the human immune system. Hum Exp Toxicol. 2002 Sep-Oct;21(9-10):499-505.
Wild CP. 2005. Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology. Cancer Epidemiol Biomarkers Prev 14(8):1847-1850.
Wild CP. How much of a contribution do exposures experienced between conception and adolescence make to the burden of cancer in adults? Cancer Epidemiol Biomarkers Prev 2011; 20:580–81.
Williams AJ, Henley WE, Williams CA, Hurst AJ, Logan S, Wyatt KM. Systematic review and meta-analysis of the association between childhood overweight and obesity and primary school diet and physical activity policies. Int J Behav Nutr Phys Act. 2013 Aug 22;10:101. doi: 10.1186/1479-5868-10-101.
Wise S.P., E.G. Jones. The organization and postnatal development of the commissural projection of the rat somatic sensory cortex. J. Comp. Neurol., 168 (1976), pp. 313–343.
Workman A.D., *et al.* Modeling transformations of neurodevelopmental sequences across mammalian species. J. Neurosci., 33 (2013), pp. 7368–7383.

Yadav R, Shukla G, Goyal V, Singh S and Behari M (2012). A case control study of women with Parkinson's disease and their fertility characteristics. *Journal of the Neurological Sciences*, 319(1–2):135-138.
Yang KD. Perinatal programming of childhood asthma. Clin Dev Immunol. 2012;2012:438572.
Z. Petanjek, *et al.* Extraordinary neoteny of synaptic spines in the human prefrontal cortex. Proc. Natl. Acad. Sci. U.S.A., 108 (2011), pp. 13281–13286.
Zhang H, Liu H, Davies KJA, Sioutas C, Finch CE, Morgan TE and Forman HJ (2012). Nrf2-regulated phase II enzymes are induced by chronic ambient nanoparticle exposure in young mice with age-related impairments. *Free Radical Biology and Medicine*, 52(9):2038-2046.