

Gene Environment Interactions in Respiratory Diseases

The GEIRD Study Group

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Preface

This publication contains the protocol and standard operating procedures of the GEIRD (*Gene Environment Interaction in Respiratory Diseases*) study. It is the result of three years of project work and discussion by Italian researchers involved in this study whose aim was to investigate the role that environmental factors, oxidative stress and genes play on the occurrence and persistence of respiratory diseases.

The GEIRD Study Group would like to thank Marco Braggion for his enthusiasm, commitment and patience in collecting and rearranging all the documents that have been written in the frame of the GEIRD project, and for making this publication possible.

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1 The GEIRD Project

1.1 Overview

Asthma, allergic rhinitis and chronic obstructive pulmonary disease (COPD) are airway inflammatory diseases that contribute substantially to morbidity and mortality in adults living in industrialized countries. Oxidative stress causing epithelial damage and increasing bronchial responsiveness is a common trait of all the three conditions. It occurs not only as a result of endogenous inflammation but also upon environmental exposure to pollutants and tobacco smoking. Genetic susceptibility, the intake of anti-oxidants from diet and medication use may interfere with the inflammation process, increasing or decreasing the damage. The extent to which genetic and environmental factors explain the occurrence, persistence and severity of these conditions is largely unknown, as well as the effects of their interactions with treatment on the severity and control of these diseases. This is mainly due to the lack of large scale epidemiological/genetic studies aimed at simultaneously investigating the three respiratory conditions and providing accurate estimates of their associations with environmental exposures and genes.

The Genes Environment Interaction on Respiratory Diseases (*GEIRD*) project is a multidisciplinary, concerted action aimed to collect information on biomarkers of oxidative stress, individual and ecological oxidative exposures (outdoor and indoor air pollutants), diet, early life factors and smoking habits, genetic traits in large and accurately defined, population-based series of asthma, rhinitis and COPD patients. Its scientific aim is to elucidate and to quantify the role that modifiable and genetic factors play in the natural history of the inflammatory diseases of the airways, to better understand their pathogenesis, to better prevent their occurrence and to improve their treatment.

As regards the study design, the GEIRD project is a multicentre, nested, (multi)case-control study, aimed at identifying allergic rhinitis, asthma and COPD phenotypes (plus a control group) in well-defined and population-based cohorts of subjects.

It is designed to produce multiple and historical data bases of clinical traits, environmental risk factors, life-style and dietary risk factors, drug utilization, DNA and biological data banks to be used for epidemiological, pharmacogenetic and clinical studies.

The GEIRD project is based on the harmonization and follow-up of already existing large population-based cohorts. These studies used the same or similar standardized methods and sampling schemes to assess the prevalence/incidence of the studied diseases in a wide age range, i.e.: the Italian branch of ECRHS I (and ECRHS II) (de Marco et al. [1998]), the ISAYA (Cerveri et al. [2003], de Marco et al. [2003, 2002a], Zanolin et al. [2004]) and the GEIRD new cross-sectional study. It involves more than 40,000 subjects, randomly chosen in twelve Italian centres.

A brief overview of the GEIRD project has been recently published (de Marco et al. [2010]).

1.2 Background

The GEIRD project is the first attempt to simultaneously investigate asthma, allergic rhinitis and COPD, using large series of cases and controls from the general population. Several scientific reasons justify the project.

1. **Public health relevance.** Allergic rhinitis and asthma affect a wide proportion of children and adults (ECRHS [1996], ISAAC Committee [1998]) all over the world. While the prevalence of asthma seems to have reached a plateau, the prevalence of rhinitis is still increasing (Chinn et al. [2004], Verlato et al. [2003]). COPD is the fourth leading cause of mortality in the US, is projected to rank fifth in burden of disease worldwide in 2020 (GOLD [2006]) and it is a substantial cause of morbidity also in young adults (Cerveri et al. [2003], de Marco et al. [2004]). Asthma, rhinitis and COPD affect about 25% of young/middle-aged Italian adults.
2. **The strong epidemiological link between the three diseases.** Rhinitis, bronchial asthma, chronic bronchitis and COPD are characterised by systemic and local chronic inflammation of the upper or lower airways. Epidemiological studies have consistently reported strong evidence for the association between rhinitis and asthma both in atopic and non-atopic subjects (Corren [1998], Grossman [1997], Guerra et al. [2002], Leynaert et al. [2000]). There is a strong epidemiologic and clinical link between asthma and COPD: asthmatics have a more than ten-fold higher risk of developing COPD than non asthmatic subjects (Guerra et al. [2005]) and, in a substantial percentage of patients, asthma and COPD coexist. Other recent studies suggest that rhinitis frequently occurs in association with the common bronchial diseases, chronic bronchitis and emphysema (Montnemery et al. [2001]). Accordingly, epidemiological evidence suggests that associations between upper and lower airway symptoms are common in both asthma and chronic bronchitis. The nature of these associations is open to interpretation and needs to be elucidated by more specific studies covering the whole range of these diseases and allowing their natural history to be better understood.
3. **A common underlying mechanism involving oxidative stress.** There is ample evidence that asthma, rhinitis and COPD are mediated by oxidative stress that plays an important role in the injurious and inflammatory responses by causing epithelial damage and increasing bronchial responsiveness. Excessive exposure to reactive oxygen and nitrogen species (ROS) is the hallmark of oxidative stress and leads to damage of proteins, lipids, and DNA. Oxidative stress occurs not only as a result of inflammation but also from environmental exposure to air pollution and cigarette smoke (Bowler et al. [2002], MacNee [2001]). Furthermore, it has been shown that smoking, acute exacerbations of COPD and asthma are associated with a marked oxidant/antioxidant imbalance in the blood (Rahman [1996]).

Under normal conditions, local and systemic protective mechanisms such as the upregulation of protective antioxidant genes occur and limit the degree of airway inflammation. These endogenous defence mechanisms may be down regulated or impaired by particular exposures (as a low intake of antioxidants with the diet) or genetic traits. In conclusion, further research on the role of oxidative exposure in asthma, rhinitis and COPD, on the identification of susceptible subgroups and on the interaction between oxidant/anti-oxidant exposures and genes is needed.

4. **Open questions about common and differential risk factors.** Environmental factors and early life exposures play a key role in asthma, rhinitis and COPD, as triggers and/or by increasing (or decreasing) the occurrence of the diseases. Despite two decades of intense research, many questions remain unanswered about the role of environment and pollution in the occurrence and persistence of asthma and rhinitis (Anto [2004]). If the major role that smoking plays in the incidence of COPD is uncontroversial, the role of smoking, as well as the role of pollutants and allergens, on the incidence and even the prevalence of asthma is unclear. Furthermore, only few studies allow the concurrent evaluation of the association of potential risk factors with all the three diseases. Different risk profiles can not only highlight different patho-physiological mechanisms for the main phenotypes, but may also identify different intermediate phenotypes, as in the case of the coexistence of asthma and allergic rhinitis (Bousquet et al. [2004], Bugiani et al. [2005]). The differential role of environmental exposures to oxidative agents and to aeroallergens in the occurrence, persistence and severity of asthma, rhinitis and COPD has to be elucidated, as well as the impact of diet on the incidence and evolution of asthma and COPD. For these purposes large and concurrent studies of the three diseases are needed.
5. **Open questions about genes involved in asthma and COPD and about the gene-environment interaction.** It has been known for a long time that asthma and other atopic disorders (rhinitis and allergic dermatitis) are familial diseases and that many respiratory disorders are the result of gene-environment interaction. However, only the last two decades have seen a marked increase in research aimed to prove a genetic contribution to the pathogenesis of the respiratory disorders and to identify candidate loci or genes (Haagerup et al. [2002], Sanford et al. [2000]). Numerous candidate loci and genes have been associated with asthma and some of them have also been found to be associated with COPD (Meyers et al. [2004]). However, only few of the reported associations have been replicated (Ioannidis et al. [2001]). Failure to replicate has been attributed to several factors, such as the insufficient power of the different studies, poorly matched control groups, unwarranted candidate genes, genetic heterogeneity and poor definition of the phenotypes (Colhoun et al. [2003]). A precise definition of phenotypes is becoming more and more important, not only for a better understanding of patho-physiologic mechanisms, but in particular to ascertain the specific genes associated with phenotypes (Bel [2004]). For the past two decades, the dominant study design

for investigating the genetic basis of inherited diseases has been the linkage analysis in families. Linkage analysis is optimal for identifying a rare high-risk allele in single-gene mendelian diseases such as cystic fibrosis, but association analysis is expected to be more powerful for the detection of a common modest-risk allele which accounts for a substantial population attributable fraction in common diseases (Carlson [2004]). Large scale case-control studies, where cases are based on an accurate characterization of the three basic phenotypes (rhinitis, asthma and COPD), are needed to make it possible to study the role of the genetic component in these common diseases and the role of the gene-environment interaction (Hoh [2004]).

6. **Open questions about treatment for asthma, rhinitis and COPD and potential gene-treatment interaction.** Inhaled corticosteroids (ICSs) are the first line treatment for asthma according to all the international guidelines. The effects of ICSs in asthma include the reduced severity of symptoms, improved pulmonary function, diminished bronchial hyperresponsiveness (BHR), prevention of exacerbations, and possible prevention of airway wall remodelling (Suissa et al. [2000]). However, a subgroup of severe asthmatics seems not to respond to ICSs and the long-term effectiveness of ICSs may be modified by exposures (like smoking) or individual susceptibility (Wenzel [2003]). Corticosteroids are also the most effective drugs for the treatment of rhinitis when used topically in the nose. Intranasal treatment of rhinitis seems also to improve asthma in some but not all studies (Bousquet et al. [2003]). Furthermore long-acting β_2 -agonists (LABAs) combined with ICSs produce greater improvements than does therapy with ICSs even at higher doses. The therapeutic value of ICSs in COPD is not as clear as it is asthma. While clinical trials in patients with mild COPD have not shown a reduction in decline in FEV₁ over time, other studies have shown that ICS therapy reduces exacerbations in patients with more severe COPD. Combination therapy with both ICSs and LABAs has recently been shown to be effective in COPD, where some studies have documented additive improvement in FEV₁. Overall, the same therapeutic approaches show clinical effectiveness in subgroups of patients affected by both asthma and COPD. This supports the hypothesis that there are some similarities in these obstructive airway diseases that may be due to common genes regulating the response to treatment. Future approaches should further define phenotypes, based in part on pharmacogenetic factors that will guide anti-inflammatory therapy in asthma and COPD (Larj et al. [2004], Pignatti [2004]).

In conclusion, the existing epidemiological evidence and the most recent advance in genetic epidemiology indicate that, to improve the understanding, the prevention and the treatment of asthma, rhinitis and COPD, an integrated and multidisciplinary approach considering the global spectrum of airway inflammation and of the factors able to modify it is required. Large scale epidemiological/genetic studies and accurate characterization of the possible combination of the three basic phenotypes are needed.

The GEIRD project moves along these lines. It is expected to provide one of the largest and most accurate collections of unselected population based cases of asthma,

rhinitis and COPD and controls to be used for epidemiological/genetic/clinical studies and for future follow-up.

GEIRD is a two-stages study. In the first stage, a respiratory screening questionnaire will be administered to large samples from the general population. In the second stage, subjects who reported respiratory symptoms on the screening questionnaire and a random sample of those who did not, will be invited to the clinical centres. All these subjects will be phenotyped through a clinical examination during which lung function tests, FeNO measurement, skin prick test, measurement of biomarkers of oxidative stress in the plasma and the urine, and DNA extraction from blood sample will be performed. Accurately defined phenotypes (asthma, COPD, allergic rhinitis) will be used in the multi-case control design to study the associations between genes, oxidative stress damage, risk factors and the three respiratory diseases in different age groups.

1.3 Objectives

1.3.1 Operational Objectives

Screening Phase

- To develop a brief and simple screening questionnaire to identify probable allergic rhinitis/asthma/COPD phenotypes (probable cases) in the general population, and probable controls;
- To develop a strategy to standardize and harmonize retrospective information of the selected cohorts;
- To centralize and harmonize 'baseline' databases from different studies;
- To identify subjects eligible for the GEIRD clinical protocol (probable cases and controls);
- To plan and execute a multicentre cohort study;
- To update the centralized database with the GEIRD data;
- To test the communication system among centres and revise it when needed;
- To test the data entry and management system and refine them when needed;
- To implement quality control procedures and revise them when needed;
- To develop a protocol for the multiplex genotyping of several SNPs (single nucleotide polymorphism);
- To collect ecological information by centre (pollutant concentrations, allergen concentrations);
- To generate prospective estimates of incidence/changes in self-reported allergic rhinitis, asthma and symptoms of chronic cough and phlegm;

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- To generate prospective estimates for the association between self-reported asthma, allergic rhinitis and chronic symptoms of cough and phlegm.

Case-control Phase

- To identify and standardize instruments and laboratory tests to be used in epidemiology aimed at accurately characterizing the three basic phenotypes of asthma, allergic rhinitis and COPD and their combinations;
- To develop a standardized protocol and instruments for the quantitative assessment of environmental exposures relevant to respiratory diseases;
- To plan and execute clinical assessment, laboratory tests, blood samples and urine collection on probable cases and controls;
- To plan and execute individual environmental assessment (drug use included) on probable cases and controls;
- To identify phenotypes of asthma, allergic rhinitis and COPD and their combinations, based on clinical and laboratory information and on previous medical history;
- To develop a standardized genetic protocol for DNA extraction and conservation;
- To establish multiple national databases of clinical traits, environmental risk factors, dietary risk factors, drug utilisation and DNA and biological data banks, for probable and confirmed cases and controls;
- To genotype all cases and controls for a number of candidate gene polymorphisms;
- To genotype a selected sample of cases and controls for human DNA markers, and to construct haplotypes;
- To build up clinical, environmental and genetic databases, available to the researchers;
- To make gene-gene, gene-environment, gene-disease, and gene-drug response interaction analyses.

1.3.2 Scientific Objectives

Primary Objectives

- To assess the common and differential associations of environmental oxidative exposures and diet with asthma, allergic rhinitis and COPD and their interaction with known risk factors;
- To assess the common and differential associations of candidate genes and loci with asthma, allergic rhinitis and COPD and their potential interactions;

1.3 Objectives

- To assess the interactions between candidate genes and environmental, dietary and early life factors in asthma, allergic rhinitis and COPD;
- To assess the interactions between treatment and genes, treatment and exposures, treatment/genes/exposures on the control of asthma, COPD and allergic rhinitis;
- To assess prospectively whether self-reported symptoms and conditions of upper and lower airways inflammation are associated;
- To assess the interaction between environmental, oxidative stress, diet and candidate genes and asthma and allergic rhinitis and COPD.
- The GEIRD follow-up stage will provide information on the natural history of the three diseases and will make it possible to assess:
 - the time variations in self-reported asthma, allergic rhinitis and chronic bronchitis in a large cohort of young and middle-aged Italian adults from 1991 to 2006;
 - the influence of pollutants and aeroallergens on incidence and prevalence of respiratory disorders.

Secondary Objectives

- To compare the life impairment and the quality of life in people affected by asthma, allergic rhinitis and COPD to the control group;
- To assess the marginal excess cost due to asthma, allergic rhinitis and COPD with respect to the control group;
- To assess the common and differential associations of early-life exposures with asthma, allergic rhinitis and COPD;
- To assess the percentage of undiagnosed asthma, allergic rhinitis and COPD and its variation among centres;
- To assess the percentage of undertreated asthma, allergic rhinitis and COPD and its variation among centres;
- To compare self-reported period prevalence of asthma, allergic rhinitis and chronic cough and phlegm among centres/countries;
- To compare anthropometrical, demographic and lung function measurements between the different phenotypes and the control group.

1.4 Work Packages

The GEIRD project consists of nine work packages (WPs). In particular, WP1, WP2 and WP4 will belong to the screening phase, while the other work packages will pertain to the case-control phase (see figure 1.1).

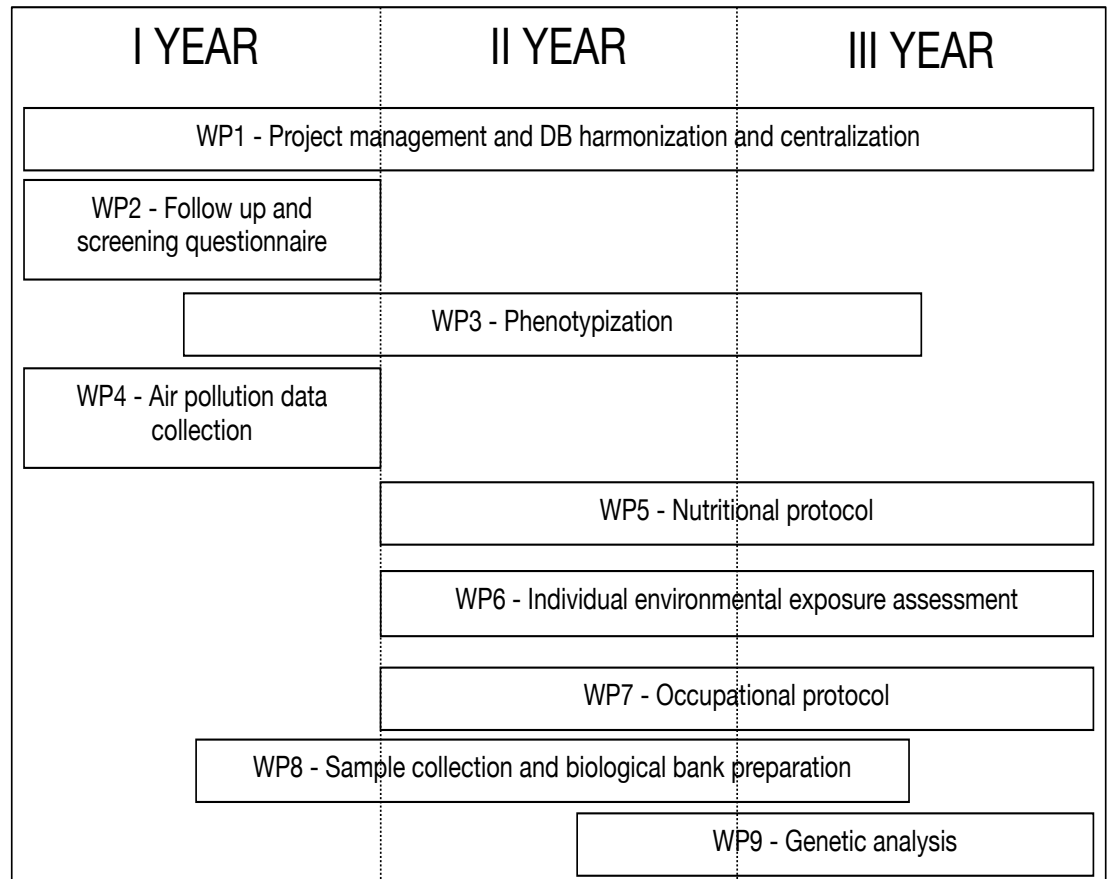


Figure 1.1: Chart of work packages in the GEIRD project.

Work package 1: project management and data bases harmonization and centralization

- Finalizing the final research protocol and manuals, by collecting all standardized

methods to be used in the study: a screening questionnaire, a clinical questionnaire, lung function and challenge test protocols, methods for blood sampling and biological samples collection, environmental samples/exposure measurements, etc.

- Establishing a network of centers participating in the project and identifying a coordinating centre.
- Harmonization of databases, centralization and linkage of the data from historical databases.
- Monitoring the progress of GEIRD project and producing a yearly report.
- Coordination of the GEIRD project.

Work package 2: selection of new random samples, follow-up of historical cohorts and postal screening

All the subjects eligible for the GEIRD project ($> 40,000$) will be administered a common screening questionnaire aimed at identifying people with a high likelihood of being affected by asthma and/or allergic rhinitis and/or COPD (stages I-IV according to the GOLD classification (GOLD [2006])). When available, the information collected in previous surveys will also be considered to identify probable cases.

Work package 3: phenotypization

Based on the reporting of selected symptoms in the screening questionnaire, all the subjects with a high probability ('probable cases') of having asthma or COPD, a random sample of subjects with a high probability of having allergic rhinitis and a random sample of subjects with a low probability ('probable controls') of belonging to one of the three phenotypes under study (asthma, rhinitis and COPD) will undergo an extensive clinical evaluation and laboratory tests in order to define the clinical phenotypes and controls.

Moreover, blood samples for the genetic analyses, urine samples for the measurement of 8-hydroxy-2'-deoxyguanosine (8-OHdG) and 8-isoprostane levels (markers of oxidative exposure) will be collected, and detailed information on some relevant exposures (early life exposures, active and passive smoking, etc.), on the use of medications and on the level of control achieved will be collected by means of a standardized clinical interview. Lung function measurements (FEV_1 , FVC, FEV_1/FVC), reversibility test, methacholine challenge test, measurement of height, weight and blood pressure, 6-Minute Walking Test (6MWT) and skin prick test will be performed. In addition, the asthma control test (ACT) will be administered to asthma cases, the MRC scale on dyspnoea and the St. George's Respiratory Questionnaire to COPD cases, and the Rhinasthma Questionnaire to both asthma and allergic rhinitis cases. A questionnaire aimed at assessing the quality of life (the SF-36 questionnaire) will be administered to all subjects. The number of controls will be chosen to match the number of cases with the most frequent pathology (asthma).

Work package 4: historical air pollution data collection

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Historical air pollution data on concentrations of NO₂, SO₂, O₃, CO, Total Suspended Particle (TSP), and —if available— PM₁₀, will be collected from all monitoring stations in the centres involved in the study.

Work package 5: nutritional protocol

Cases and controls, who consent, will be visited at home by trained fieldworkers, who will administer a standardized questionnaire on diet (the EPIC food frequency questionnaire).

Work package 6: individual environmental exposure assessment

Personal passive samplers will be used to measure individual 7-day exposure to formaldehyde and ozone. 24-hour personal exposure to PM_{2.5} will be assessed by personal active samplers.

Work package 7: occupational protocol

Standardized occupational exposure modules will be administered during the clinical visit ¹, to assess the occupational exposure of cases and controls.

Work package 8: sample collection and biological bank preparation

A biological bank from well defined subjects, including asthma, allergic rhinitis and COPD patients and controls will be created.

Cases and controls will be recruited among the general population. During the clinical visit, all the subjects belonging to phenotypes 1, 2, 3 (asthmatics), 4 (affected by rhinitis) and 7 (controls) will be considered eligibles for the blood sampling, following the order of their arrival in the clinic. The probable COPD cases (probable phenotypes 5 and 6) will be invited to the clinic in a second time, in order to undergo a specific sub-protocol, including additional exams (DLCO and high resolution CT), with the purpose to characterize the disease sub-phenotypes (pink puffer or blue boater). For these subjects, blood samples will be collected in this occasion.

A blood sample of about 18 ml (three 6ml tubes) will be collected from each subject. DNA will be extracted by a salting out standard technique and stored at -20°C. Additional blood samples, for the RNA (two 2.5mL tubes) and protein (one 6mL tube) analysis, will be collected from a subsample of about one hundred subjects. Total RNA and proteins will be purified from the blood samples using specific commercial kits and stored at -80°C.

Work package 9: genetic analysis

A case-control association analysis of candidate gene polymorphisms will be performed in an accurately defined and unselected series of asthma, COPD and allergic rhinitis patients and in a control group, in order to identify susceptibility genes for asthma, COPD and allergic rhinitis, and susceptibility genes that may be common to the three diseases.

¹Some centers will administer the occupational modules during the home visit.

Cases and controls will be genotyped for a series of candidate gene polymorphisms by the use of high-degree multiplexed genotyping techniques. The genes to be investigated will be selected among genes involved in pathways possibly related to the studied diseases, as inflammation, innate immunity and immunoregulation, oxidative stress and metabolism of xenobiotics, regulation of the protease-antiprotease equilibrium, and tissue remodelling. Genetic association studies between the analysed candidate gene polymorphisms and phenotypes will be performed using multivariate statistical methods.

During the clinical visit the subjects that belong to phenotypes 1, 2, 3 (asthmatics), 4 (affected by rhinitis) and 7 (controls) will be considered eligibles for the blood sampling following the order in which they will arrive in clinic. The probable COPD cases (probable phenotypes 5 and 6) will be invited to go to the clinic a second time in order to undergo a specific sub-protocol, that includes additional exams (DLCO and high resolution CT), with the purpose to characterize the disease sub-phenotypes (pink puffer or blue boater). For these subjects, the blood sample for the RNA analysis will be taken in this occasion.

1.5 Study Design

1.5.1 Screening Phase (Cohort Samples)

The GEIRD project is a (multi)case-control study nested in pre-existing cohorts from the general population, identified between 1991 and 2002 in the frame of different surveys. Each cohort was initially selected by a random sampling scheme with a male/female ratio=1 (see Figure 1.2).

In addition, the GEIRD project will also involve new random samples that will be identified in the same target areas of the pre-existing cohorts, in the frame of a multi-centre cross-sectional study. This study is coordinated by the Unit of Epidemiology and Medical Statistics of the University of Verona and has started in 2006.

The cohorts involved in the GEIRD project are:

- **The Italian Branch of ECRHS:** This cohort was enrolled in 1991 by withdrawing random samples from the general population aged 20-44 years in three Italian centres (Pavia, Torino, and Verona). 6,031 subjects were surveyed through a screening questionnaire on asthma and rhinitis in two occasions (1991 and 2001). In 2001 a question on chronic cough and phlegm was also added. A random subsample of 589 subjects underwent extensive clinical and laboratory examinations on both occasions.
- **The ISAYA Cohort:** This cohort was recruited in 1998 and was made up of random samples of people aged 20-44 in 9 Italian centres. Six centres (Ferrara, Pavia, Sassuolo, Torino, Udine, Verona) were located in the Po Valley in Northern Italy, one centre (Pisa) in Tuscany, Central Italy, and two centres in the two major Italian islands (Sassari in Sardinia, Siracusa in Sicily). Eighteen thousand eight

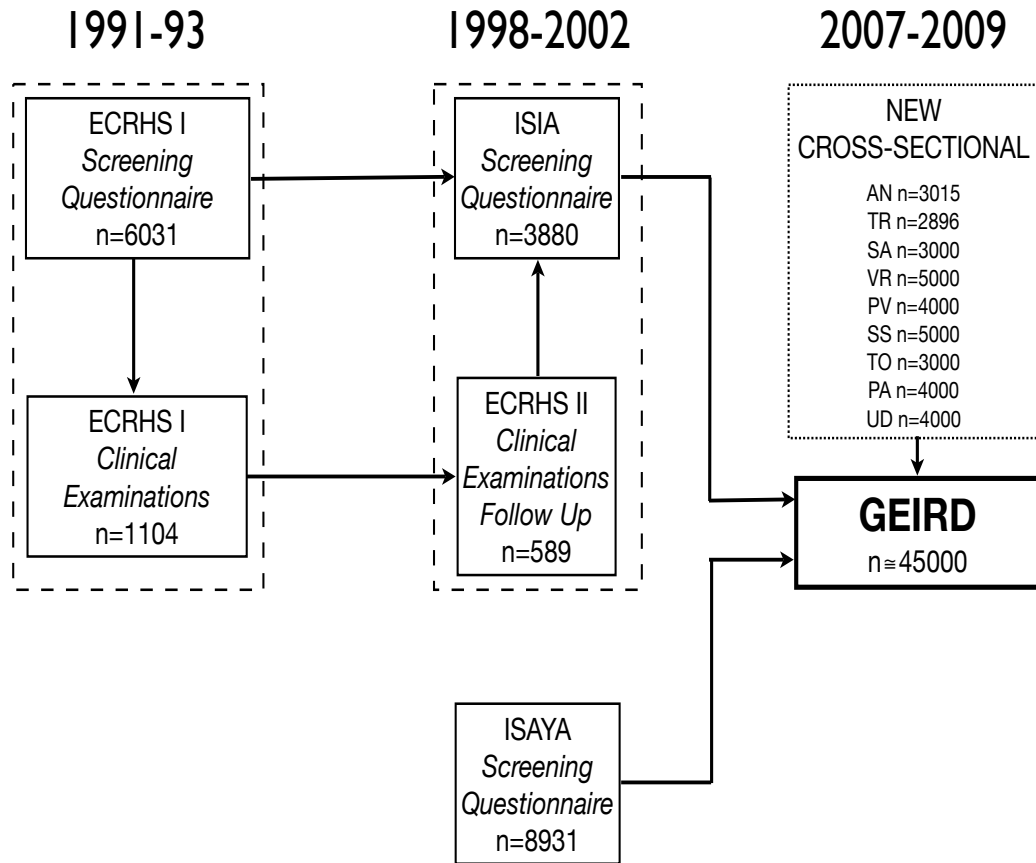


Figure 1.2: Random samples selected in 1991 and 1998 were aged 20-44 years. New random samples selected in 2007 were mainly aged 20-44 years; samples aged 45-64 and 65-84 were also selected in six and three centers, respectively. The centers are ordered by enrollment date. AN stands for Ancona, TR for Terni, SA for Salerno, VR for Verona, PV for Pavia, SS for Sassari, TO for Torino, PA for Palermo and UD for Udine. For a full description see Table 1.1.

hundred and seventy-three subjects replied to a screening questionnaire investigating asthma, rhinitis and symptoms of chronic cough and phlegm and socio-economic costs due to asthma. Four hundred current asthmatics identified in seven centres underwent a telephone interview in 2000: it concerned the frequency of asthma symptoms and medication use in the last 3 months. Only four centers of the ISAYA cohort were included in the GEIRD Study: Pavia (n=2,444), Sassari (n=2,055), Torino (n=2,266) and Verona (n=2,166). The total number of subjects

| | 20-44 years | 45-64 years | 65-84 years |
|--------------|-------------|-------------|-------------|
| Center | | | |
| Ancona (AN) | 3,000 | | |
| Terni (TR) | 3,000 | | |
| Salerno (SA) | 3,000 | | |
| Verona (VR) | 3,000 | 1,000 | 1,000 |
| Pavia (PV) | 3,000 | 1,000 | |
| Sassari (SS) | 3,000 | 1,000 | 1,000 |
| Torino (TO) | 2,000 | 1,000 | |
| Palermo (PA) | | 1,000 | 3,000 |
| Udine (UD) | 3,000 | 1,000 | |

Table 1.1: Distribution of sample size according to age in different centers of the GEIRD new cross-sectional phase.

from these centers is 8,931.

- **The new cross-sectional sub-cohort:** This study was launched in 2006, and it involves 9 centres (Ancona, Palermo, Pavia, Salerno, Sassari, Terni, Torino, Udine, Verona), some of which (seven centres) will contribute cases and controls for the GEIRD project. The new cross-sectional sub-cohort will use the same methodology as the ISAYA. In particular, the same screening questionnaire used in GEIRD will be administered to a random sample of 3,000 subjects from the general population aged 20-44².

All the previous studies have or will have a common set of information collected with the same instruments (see Table 1.2).

Overall, the eligible subjects for the GEIRD study are going to reach an amount of about 41,000 people with an expected age in 2010 ranging from 20 to 84. The number of the follow-up contacts varies from 1 (the new cross-sectional samples) to 4 (ECRHS cohorts). In the first step of the study, a screening questionnaire will be administered by mail to all the participants. In the second step, all the subjects answering affirmatively to the ‘key questions’—identifying all probable cases of asthma and COPD, and a random sample of probable cases of allergic rhinitis and of probable controls—will be invited to undergo the clinical, the environmental, the occupational and the nutritional protocol (see figure 1.3 and 1.4 for a summary of the GEIRD stages).

1.5.2 Case-control Selection and Phenotypization

The ideal epidemiological investigation on genes-environment interactions in respiratory diseases should be a very large study of ‘unselected’ specific (sub)phenotypes that are

²Each centre can optionally recruit 1,000 additional people aged 45-64 and/or 1,000 additional people aged 65-84 in order to gather more information on COPD.

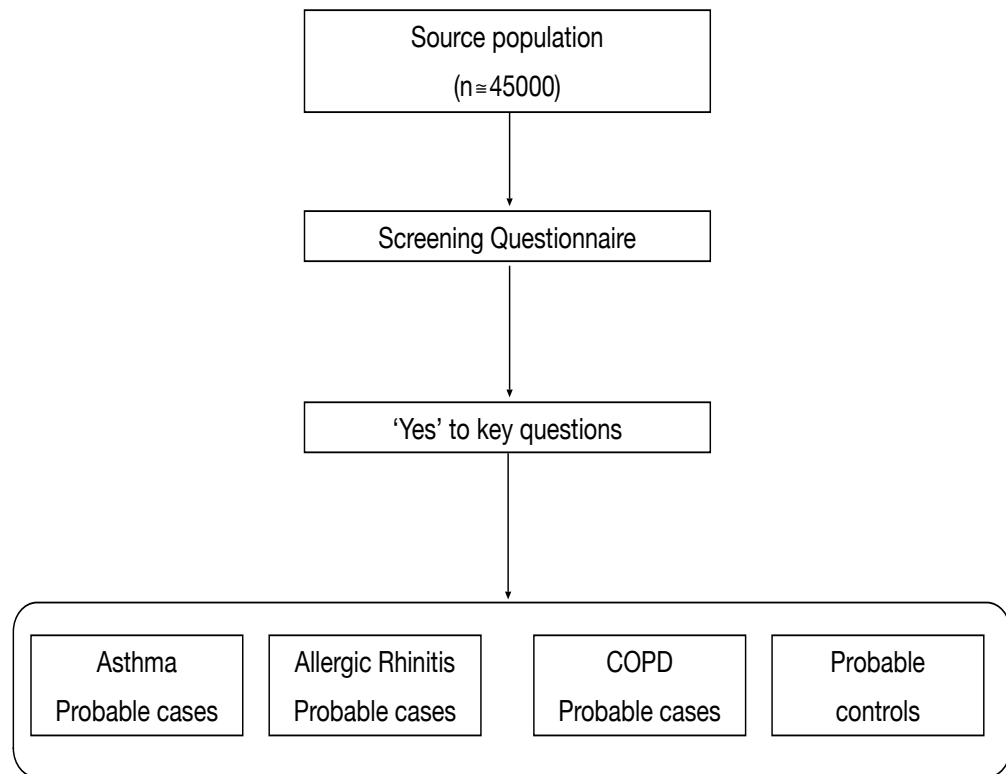


Figure 1.3: The GEIRD screening phase.

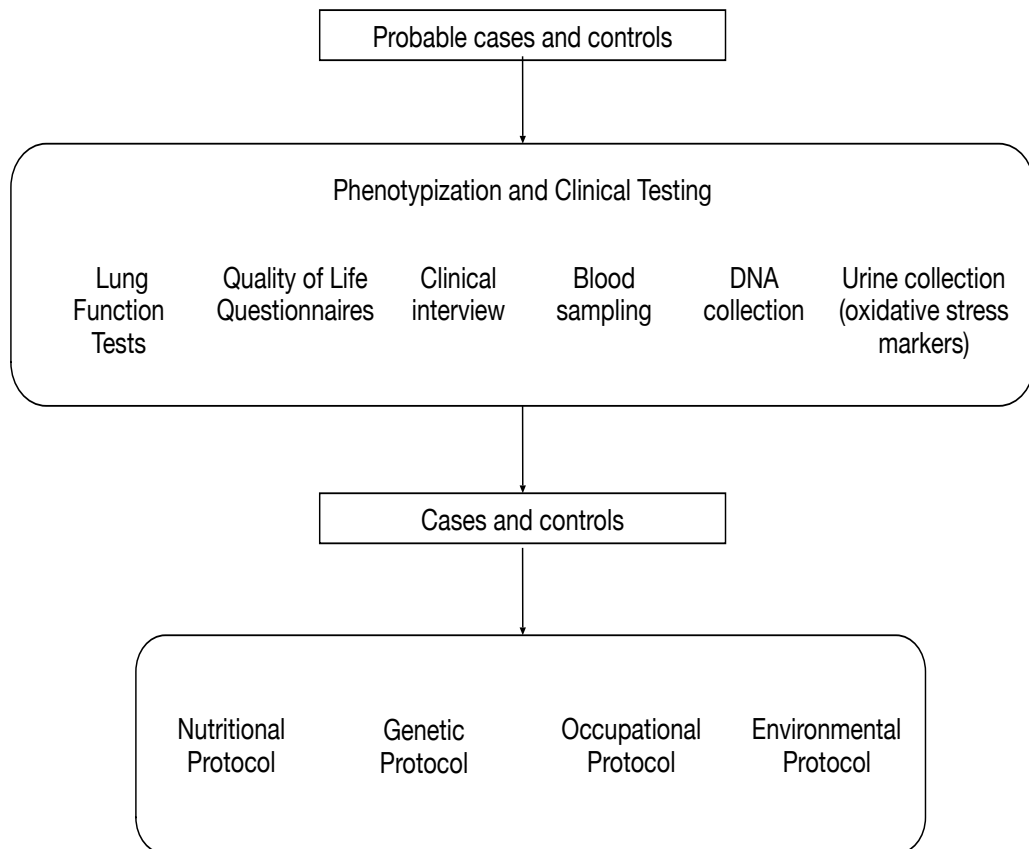


Figure 1.4: The GEIRD case-control phase.

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well characterised by the time-history of their diseases, exposures and treatment and by the use of objective measurements of inflammatory markers. No single research project or single study design would be able to simultaneously satisfy all these features, unless a huge quantity of resources, researchers and time was available.

For this reason, the GEIRD project will use pre-existing or ongoing based surveys carried out with the same or similar two stages protocols (respiratory screening questionnaire and clinical examination), in order to produce an independent, public domain database of cases and controls, in the next few years. The database will be freely accessible to all scientists worldwide, who are willing to share their cases and controls, in exchange for the possibility to address their scientific questions with increased power.

The multi case-control design guarantees the possibility to simultaneously study all the inflammatory diseases of the airways that have common genetic and environmental factors, and to assess the associations of each factor with all the three diseases. A similar design has already been used in a genome-wide association study of seven common diseases (Wellcome Trust Case Control Consortium [2007]).

Although there are several other types of study that can be used in genetic epidemiology (cohorts, linkage studies, etc), the population-based case-control study is considered to be the most powerful design to investigate genetic associations (Caporaso et al. [1999], Clayton et al. [2001]) for complex diseases, as it is efficient and allows to characterise outcomes and phenotypes more precisely than other studies. Other designs,

| | ECRHS I (1991-1993) | ISAYA (1998-2000) | ECRHS II (1999-2002) |
|---------------------------------------------------|-----------------------------------------------------|-----------------------------|---------------------------------------------|
| Questionnaire/Test | n=6031 (1104) aged 20-44 years from 3 centres | n=18873 from 9 centres | n=633 aged 28-52 years from 3 centres |
| Screening questionnaire | Yes | Yes | Yes |
| Clinical interview | Yes | No | Yes |
| SF-36 ('Quality of life') | No | No | Yes |
| Asthma quality of life questionnaire | No | No | Yes (No Verona) |
| Occupational modules | No | No | Yes |
| Cost of asthma | No | Yes | No |
| Spirometry | Yes | No | Yes |
| Methacholine challenge | Yes | No | Yes |
| Height and weight assessment | Yes | No | Yes |
| IgE blood | Yes | No | Yes |
| Skin prick test | Yes | No | No |
| Outdoor exposure to air pollutants* | Yes | No | Yes |
| In/Outdoor individual exposure to NO ₂ | No | No | Yes |
| Indoor questionnaire | No | No | Yes |
| Cats questionnaire | No | No | Yes |

Table 1.2: Common information collected in ECRHS I, ECRHS II and ISAYA (*PM10, PM2.5, SO₂, NO₂, O₃).

especially tailored to study gene-environment interactions, have been proposed, such as case-only or partial case-control studies (Goodman et al. [2007]). However, the estimation of the main effects of environmental and genetic factors, which is one of the aims of this project, is not possible in these studies.

The two-stage strategy to select cases and controls from well-defined cohorts or from new random samples from the general population, guarantees that case and controls come from the same source population (Miettinen [1985]) and that they have the same degree of phenotypic and genotypic assessment (Weiss [2001]).

Finally, the potential bias due to the ‘dilution effect’ in the relative risk estimates (i.e. the misclassification bias due to the presence of ‘diseased subjects’ among controls) is less likely in the multi case-control study than in cross-sectional or classic case-control studies. Indeed in the multi-case control design, subjects with one phenotype are compared to controls who are free from all the other interrelated diseases under investigation: for instance in the GEIRD project asthmatic subjects are compared with controls with neither asthma, nor allergic rhinitis, nor COPD, nor chronic bronchitis. Instead in the cross-sectional or classic case-control design, subjects affected by a specific disease are contrasted with subjects free of that disease. When the controls are affected by correlated diseases that share some environmental and genetic determinants (such as allergic rhinitis and COPD in the case of asthma), a dilution bias occurs.

This effect is documented in Table 1.3: the association estimates obtained using a multi-case control approach are compared with those obtained in a cross-sectional approach, where each disease is considered separately (the estimates were obtained in a simulation study using the database of the European Community Respiratory Health Survey-ECRHS (ECRHS [1996])). When considering known risk factors for a certain disease, the association estimates obtained in the cross-sectional design are underestimated (diluted) with respect to the multi-case control design. As an example, the association between smoking and COPD (or chronic cough and phlegm) is strongly underestimated by the cross-sectional study.

1.5.3 Selection and Identification of Cases and Controls

Because cases and controls have to be identified in pre-existing population-based cohorts or in cohorts whose recruitment is in progress, GEIRD involves a screening phase. Eligible subjects are administered a postal screening questionnaire, which is a modified version of a previous standardized screening questionnaire (de Marco et al. [1999], ECRHS [1996]) and investigates the presence of symptoms of asthma, allergic rhinitis and COPD.

According to their responses to the screening questionnaire (see GEIRD Scientific Committee [2007]), subjects are classified as follows:

- *a probable case of asthma*, in the case of an affirmative answer to at least one of the following questions:
 - Have you had an attack of asthma in the last 12 months?
 - In the last 12 months have you used any medicines (including inhalers, aerosols or tablets) for asthma?

| | Asthma [†] | COPD [‡] | Allergic rhinitis [§] | Chronic cough and/or phlegm [*] |
|----------------------------------------------------------------------------------------|-----------------------------------------------------|----------------------------------------------------|----------------------------------------------------|----------------------------------------------------|
| Parental asthma <i>Cross-sectional</i> <i>Case-control</i> | 2.93 (2.50-3.43) 4.60 (3.89-5.44) | 1.99 (1.53-2.59) 2.37 (1.74-3.20) | 1.75 (1.54-2.00) 1.85 (1.55-2.20) | 1.61 (1.36-1.90) 2.02 (1.60-2.54) |
| Respiratory infection in childhood <i>Cross-sectional</i> <i>Case-control</i> | 2.86 (2.41-3.40) 3.85 (3.21-4.62) | 1.26 (0.91-1.74) 2.15 (1.55-2.96) | 1.26 (1.08-1.41) 1.51 (1.24-1.82) | 1.61 (1.34-1.93) 1.98 (1.55-2.50) |
| BMI \geq 25 <i>Cross-sectional</i> <i>Case-control</i> | 0.91 (0.78-1.05) 1.03 (0.91-1.17) | 1.21 (0.98-1.50) 1.35 (1.10-1.66) | 0.83 (0.74-0.95) 0.94 (0.83-1.04) | 1.38 (1.21-1.51) 1.52 (1.31-1.77) |
| Past smoker (vs. non smoker) <i>Cross-sectional</i> <i>Case-control</i> | 0.93 (0.78-1.09) 0.91 (0.78-1.05) | 1.49 (1.12-1.98) 1.61 (1.20-2.17) | 0.92 (0.82-1.04) 0.99 (0.87-1.13) | 0.97 (0.80-1.81) 1.18 (0.95-1.50) |
| Current smoker (vs. non smoker) <i>Cross-sectional</i> <i>Case-control</i> | 0.73 (0.63-0.84) 1.11 (0.97-1.26) | 1.67 (1.32-2.12) 3.34 (2.63-4.26) | 0.61 (0.54-0.67) 1.06 (0.94-1.19) | 2.43 (2.12-2.79) 4.96 (4.17-5.92) |
| Occupational exposure <i>Cross-sectional</i> <i>Case-control</i> | 1.12 (0.98-1.27) 1.33 (1.18-1.49) | 1.32 (1.07-1.63) 1.52 (1.24-1.86) | 1.12 (1.02-1.22) 1.25 (1.12-1.38) | 1.59 (1.41-1.79) 1.93 (1.67-2.22) |
| Bronchial hyperreactivity <i>Cross-sectional</i> <i>Case-control</i> | 9.22 (7.80-10.90) 26.2 (21.3-32.2) | 5.42 (4.14-7.09) 11.2 (8.30-15.0) | 3.02 (2.63-3.47) 4.01 (3.26-4.94) | 1.98 (1.66-2.35) 3.54 (2.71-4.62) |
| IgE sensitisation <i>Cross-sectional</i> <i>Case-control</i> | 4.85 (4.15-5.65) 9.58 (8.31-11.04) | 2.03 (1.63-2.54) 2.23 (1.76-2.85) | 5.94 (5.31-6.65) 6.19 (5.48-7.00) | 1.23 (1.06-1.41) 1.25 (1.03-1.58) |

Table 1.3: Univariate Association Estimates (*Odd Ratios* for the cross-sectional design and *Relative Risk Ratios* for the case-control design) between potential determinants and asthma, COPD, allergic rhinitis and chronic cough and/or phlegm. Data were obtained from the ECRHS I data base. For the cross-sectional design, a random sample of 9381 subjects was used to estimate the ORs by a logistic model. For the case-control design, all the cases of asthma (n=1965), COPD without asthma (n=424), allergic rhinitis without asthma and COPD (n=2845), chronic cough and/or phlegm without the above diseases (n=981), and all the controls (without any respiratory symptoms and any use of medicine to help breathing in the last year) (n=3166) were considered to estimate the RRRs (a total of 9381 cases and controls) by a multinomial logistic model. Estimates that were significantly different from the unit are highlighted in bold. Estimates are reported in red when the confidence intervals obtained through the two different designs do not overlap or when one estimate is statistically significant and the other is not.[†] Self-reporting of ever asthma; [‡] FEV₁/FVC<70%; [§] Self-reporting of allergic rhinitis and/or hay fever; * Self-reporting of chronic cough and/or phlegm for at least three months for at least two subsequent years.

- Have you ever had asthma?
- *a probable case of allergic rhinitis*, if all the following conditions (1-4) are met:
 1. the subject affirmatively answers to at least one of the following questions:
 - Do you have any nasal allergies, including hay fever?
 - Have you ever had a problem with sneezing, or a runny or a blocked nose when you did not have a cold or the flu?
 2. the subject reports current symptoms or has used drugs for rhinitis in the last 12 months;
 3. the subject has been having symptoms for 2 years or more;
 4. the subject has no sinusitis and no nasal polyps.
- *a probable case of COPD* if the subject is ≥ 35 years old and answers ‘yes’ to at least one the following questions:
 - Has a doctor ever told you that you have chronic bronchitis or chronic obstructive pulmonary disease (COPD) or emphysema?
 - Have you had coughing and phlegm on most days for a minimum of 3 months a year and for at least 2 successive years? and Have you had these symptoms for at least 10 years?
 - Have you had daily wheezing or whistling in your chest in the last 12 months? (Tinkelman et al. [2006])
 - Have you visited a hospital casualty department or emergency room or have you spent a night in hospital because of breathing problems in the last 10 years?
- *a probable control*, if a subject answers negatively to all the previous questions and does not report wheezing nor nocturnal tightness in the chest nor attacks of shortness of breath at night time in the last 12 months.

All the probable cases of asthma and COPD, and a 50% random sample of probable cases of allergic rhinitis and probable controls are referred to clinical centres where they undergo the ‘phenotypization’ protocol.

Based on the clinical ascertainment, a subject will be classified as an asthma, COPD, rhinitis case or control. Table 1.4 shows an example of possible classification of subjects based on the information collected in the clinical stage of GEIRD.

All cases and controls will be matched by study cohort (frequency matching) and centre. For specific studies on smokers affected by COPD, a specific series of pair-matched controls (one control for each case) will be identified. Matching will be based on sex, age (± 5 years), cumulative pack-years (± 5 pack years) and study cohort.

| | |
|-----------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Asthma case</i> if she/he | <p>reports having ever had asthma and at least one of the following:</p> <ul style="list-style-type: none"> - reporting asthma-like symptoms (asthma attack, wheeze, shortness of breath or chest tightness) and/or use of drugs for asthma in the last 12 months; - having a pre-bronchodilator $FEV_1/FVC < \text{Lower Limit of Normal (LLN, Quanjer et al. [1993])}$ or $< 70\%$ and a positive reversibility test (increase in $FEV_1 > 12\%$ and > 200 ml with respect to pre-bronchodilator FEV_1 after 400 mcg of salbutamol); - being positive to the methacholine bronchial provocation test with $PD_{20} < 1$ mg; - has a pre-bronchodilator $FEV_1/FVC < \text{LLN}$ or $< 70\%$, and a post-bronchodilator $FEV_1/FVC \geq \text{LLN}$ and $\geq 70\%$, and a post-bronchodilator $FEV_1 \geq 80\%$ predicted <p>or</p> <p>reports asthma-like symptoms and/or use of drugs for asthma in the last 12 months and at least one of the following conditions:</p> <ul style="list-style-type: none"> - being positive to the methacholine bronchial provocation test with $PD_{20} < 1$ mg; - having a pre-bronchodilator $FEV_1/FVC < \text{LLN}$ or $< 70\%$ and a positive reversibility test; - has a pre-bronchodilator $FEV_1/FVC < \text{LLN}$ or $< 70\%$, and a post-bronchodilator $FEV_1/FVC \geq \text{LLN}$ and $\geq 70\%$, and a post-bronchodilator $FEV_1 \geq 80\%$ predicted. |
| <i>Allergic rhinitis case</i> if she/he | <p>reports having ever had:</p> <ul style="list-style-type: none"> - any nasal allergies, including hay fever or having ever had a problem with sneezing, or a runny or a blocked nose in the absence of a cold or flu, <p>and</p> <ul style="list-style-type: none"> - she/he is positive to at least one allergen (among those considered in the Phenotypization Protocol as illustrated below) at skin prick test. |
| <i>COPD case</i> if she/he | <p>has a post-bronchodilator $FEV_1/FVC < \text{LLN}$ or $< 70\%$;</p> <p>all the subjects without asthma and with post-bronchodilator FEV_1/FVC greater than the maximum between 70% and LLN, and reporting persistent (more than 5 years) cough and phlegm, will be considered as subjects without bronchial obstruction with chronic respiratory symptoms.</p> |
| <i>Control</i> if she/he | <p>has a pre-bronchodilator $FEV_1/FVC \geq 70\%$ and $\geq \text{LLN}$ and she/he answers negatively to all the questions related to respiratory symptoms, asthma and rhinitis, in the clinical interview.</p> |

Table 1.4: Phenotype classification example (based on the information collected in the clinical stage) of the GEIRD Study.

1.5.4 Phenotypization

The phenotypization protocol is a set of tests and questionnaires administered to subjects participating in GEIRD, performed in a clinical setting (Table 1.5 gives the full list of tests and questionnaires that will be administered in the frame of the project).

| | Asthma | Allergic Rhinitis | COPD / Chronic Bronchitis | Controls |
|-------------------------------------------------------------|--------|-------------------|---------------------------|----------|
| <i>Questionnaires</i> | | | | |
| Clinical interview | ✓ | ✓ | ✓ | ✓ |
| Quality of life (SF-36) | ✓ | ✓ | ✓ | ✓ |
| Asthma control test | ✓ | | | |
| St. George's respiratory questionnaire | | | ✓ | |
| MMRC dyspnoea scale§ | | | ✓ | |
| Rhinasthma | ✓ | ✓ | | |
| <i>Clinical laboratory tests</i> | | | | |
| Weight, waist/hip measure, height, blood pressure, oximetry | ✓ | ✓ | ✓ | ✓ |
| Spirometry (slow and forced) | ✓ | ✓ | ✓ | ✓ |
| Methacholine challenge test or Reversibility test | ✓ | ✓ | ✓ | ✓ |
| Allergological test (SPT, RAST) | ✓ | ✓ | ✓ | ✓ |
| Six minute walking test | ✓ | ✓ | ✓ | ✓ |
| Biomarkers of inflammation* | ✓ | ✓ | ✓ | ✓ |
| Biomarkers of oxidative stress† | ✓ | ✓ | ✓ | ✓ |
| Genetic analyses | ✓ | ✓ | ✓ | ✓ |

Table 1.5: (modified from de Marco et al. [2010]). Tests and questionnaires administered at the clinical stage of GEIRD. § Modified Medical Research Council dyspnoea scale. * fractional exhaled nitric oxide (FeNO); pH, hydrogen peroxide, leukotrienes, isoprostanes, tumour necrosis factor-alpha, interleukine-8 in exhaled breath condensate and alveolar air; percentage of eosinophils, neutrophils, macrophages and lymphocytes in the induced sputum. † 8-hydroxy-2'-deoxyguanosine (8-OHdG) and isoprostanes in the urine.

It consists of:

1. **the identification protocol**, which is the minimum set of tests necessary to identify cases and controls and comprises the clinical interview, the lung function tests (slow and forced spirometry), the reversibility test, the methacholine test, a blood sample for genotyping and IgE assay, height, weight and blood pressure measurements, and the skin prick test for common allergens;
2. **the sub-phenotypization protocol**, which is aimed at identifying specific features of cases and controls and comprises diffusing capacity of the lung and 6-minute walking test. Moreover, additional tests mainly related to inflammation and

oxidative stress (Kharitonov et al. [2006]) are included, such as fractional exhaled nitric oxide (FeNO), exhaled breath condensate, exhaled alveolar air measurements and induced sputum cell count, urine collection for 8-hydroxy-2'-deoxyguanosine (8-OHdG) measurement (Pilger et al. [2006]). One general quality of life questionnaire (SF-36) and a set of specific questionnaires for asthma, allergic rhinitis and COPD are also administered: the asthma control test (ACT) (Nathan et al. [2004]), the Rhinasthma (Baiardini et al. [2003]), the St. George's respiratory questionnaire (Meguro et al. [2007]) and the Modified Medical Research Council (MMRC) dyspnoea scale (Mahler et al. [1988]).

The Clinical interview used in GEIRD is a modification of the ECRHS questionnaire (see de Marco et al. [2010], GEIRD Scientific Committee [2007]); the other questionnaires are previously validated instruments and all the measurements use standardized techniques according to the European Respiratory Society (ERS) or the American Thoracic Society (ATS) guidelines. Questionnaire and measurement protocols can be found at <http://www.geird.org>.

Skin Prick Test Protocol

Skin prick test is performed using the following panel of allergens: *Cupressus arizonica*, *Dermatophagoides pteronyssinus*, *Artemisia vulgaris*, *Dermatophagoides farinae*, *Ambrosia artemisiifolia*, *Alternaria tenuis*, *Parietaria judaica*, dog dandruff, *Corylus avellana*, cat (Fel d 1), *Olea europea*, *Betula verrucosa*, *Cladosporium herbarum* and *Phleum pratense*. Positive histamine and negative diluent controls will be used. The results will be read after 20 minutes. Skin prick test will be considered positive if the average wheal diameter is 3 mm greater than the negative control (Malling [1993]).

Blood and Urine Collection

33 ml of venous blood is taken from each subject who consents. 12 ml will be used to extract the serum for IgE titration and 3 ml for complete blood cell count. The serum will be preserved at -80°C and sent to a local laboratory for total and specific IgE measurement (cat (Fel d 1), timothy grass, *D. pteronyssinus*, *Cladosporium herbarum* and a local allergen). 18 ml will be used for genetic analyses (see genetic protocol) and will be sent to the Division of Genetics and Biology (University of Verona). An additional 11 ml blood sample will be collected for the analyses of RNA and serum proteins from a small sub-sample (see Work Package 9 for a specification).

A urine spot sample (50 ml) will be collected from each consenting subject for the purpose of determining the levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) (Pilger et al. [2006], Zanolin [2008]), 8-iso-Prostaglandin $\text{F}_{2\alpha}$ and creatinine. The University of Verona (Unit of Epidemiology and Medical Statistics) will collect the urine samples from all the other participating centres. The samples will be kept at -80°C in a freezer (located in the Unit of Hygiene and Occupational Medicine, Verona). Urine aliquots will be sent in batches to the Molecular Mutagenesis & DNA Repair Unit (Oncology Experimental Laboratory B (Clinic Mutagenesis)) at the Cancer Institute of Genova, where the

measurements will be performed. 8-OHdG levels (ng/ml) and 8-isoprostane levels will be quantified using dedicated ELISA competitive assay kits (Cayman Chem. Co., USA and Assay Designs, USA). The Creatinine content (mg/ml) in the urine samples will be quantified with a different ELISA assay kit (Cayman Chem. Co., Michigan, USA). The resulting creatinine-corrected 8-OHdG and 8-isoprostane concentrations (ng/mg) will be used for data analysis.

1.5.5 Environmental and Dietetic Protocols

House Protocols

All the subjects involved in the phenotypization protocol and all the subjects who agreed to receive a home visit will be administered:

1. a **nutritional questionnaire** aimed at assessing the antioxidant intake with diet. Each subject will be administered the Epic food frequency questionnaire (Kroke et al. [1999]) by trained personnel. Subjects who are taking antioxidant supplements will be excluded. From the dietary questionnaire, the dietary intakes will be computed as energy and nutrient intakes (i.e. vitamin C, vitamin E, beta-carotene, vitamin A, zinc, etc.) and dietary intakes of foods rich in antioxidant substances;
2. **specific occupational modules**, aimed at considering some occupations and occupational exposures (nurses, cleaners, metal workers, soldering irons, housewives, etc), derived from the ECRHS II occupational questionnaire (see ECRHS Committee [2002], ECRHS Committee [2002a]);
3. a **questionnaire about home characteristics**, aimed at characterising sources of indoor air pollutants, and exposure to allergens, derived from the ECRHS II environmental questionnaire (see ECRHS Committee [2002], ECRHS Committee [2002a]);
4. **individual total exposure assessment**. Depending on funds, in some centres cases and controls will be asked to wear personal passive samplers to assess the average concentration of ozone, formaldehyde and PM_{2.5}. The samplers will be withdrawn after 7 days for the assessment of ozone and formaldehyde, and after 24 hours for the assessment of PM_{2.5}. Passive samplers used to assess the ozone, the formaldehyde and the PM_{2.5} concentration, will be sent to a central laboratory. Cases and controls will be matched by date of individual measurement (+/- 2 days).

Outdoor Pollution Protocol

The residential street address of all the cases and controls —collected during the clinical interview— will be geocoded by the GPS technology. The geographical coordinates will be expressed both in the UTM 32 and in Gauss-Boaga systems and will be used to define two proxy variables of the exposure to environmental pollutants:

1. cumulative exposure of the subjects to environmental pollutants. In collaboration with the Regional Agency for Environmental Protection (ARPA), annual mean values of the main pollutants (NO₂, PM₁₀, Total Suspended Particulate (TSP), ozone) will be associated with each geocoded point. These values will be estimated by means of air emission modelling (Industrial Source Complex (ISC) and the Lagrangian particle model (SPRAY)) and will be used as individual indicators of environmental exposure in the subsequent epidemiologic analysis. Meteorological data will be taken into account in the modelling in order to accurately estimate the exposure;
2. exposure to vehicular traffic, based on the distance from heavily trafficked roads. Subjects living within 100 meters of roads with heavy traffic (>10 cars/min) will be considered highly exposed to vehicular pollution (Lindgren et al. [2009]). Also different geographical classifications will be used to rank subjects according to air pollution exposure.

Furthermore, annual summary statistics of air pollutants (NO₂, SO₂, O₃, CO, TSP, and PM₁₀), measured over the years of the study (2006-2010) will be collected in each centre in collaboration with local authorities, for ecological comparisons.

1.5.6 Genetic Protocol

Several studies, aimed at proving a genetic contribution to the pathogenesis of the respiratory disorders, reported various genes in linkage or in association with asthma, COPD or allergic rhinitis (Vercelli [2008], von Mutius [2009]). Moreover, given the similarities of these three inflammatory airway diseases, it is not surprising that some candidate genes were found to be associated with two or all of the three diseases (Vercelli [2008], Meyers et al. [2004]).

Pleiotropy in immunomediated and/or inflammatory diseases is not unusual, and there is increasing evidence that many of these diseases have common aetiologies. Literature data show that common alleles that confer risk to a given disease under a certain combination of interacting genes and environmental conditions, may act in other genetic backgrounds influenced by other environmental factors resulting in different, but possibly related, clinical outcomes (Cookson et al. [2004]).

On chromosome 1, A1 adenosine receptor (A1AR) gene is likely to be important in the pathogenesis of asthma and other respiratory conditions. A1AR signalling may serve to regulate the severity of pulmonary inflammation and remodelling (Malerba et al. [2005]). Moreover, there is evidence that adenosine plays a role in the pathogenesis of allergic rhinitis (Rimmer et al. [2007]).

On chromosome 2, studies have identified the IL-R1N gene (encoding the interleukin 1 receptor antagonist protein) as possibly being responsible for some alterations in the pathogenesis of asthma (Gohlke et al. [2004], Allen et al. [2003]). Increased levels of IL-1 have been measured in sputum of COPD patients, with further increases during exacerbations (Chung [2005]). Moreover, the role of IL-1 have been documented in nasal secretions of patients with allergic rhinitis (Fireman [1996]).

ADRB2 gene is located in the cytokine gene cluster on chromosome 5 (5q3133). ADRB2 encodes the 2-adrenergic receptor and contains several common genetic variations that affect gene expression and receptor function in vitro (Hawkins et al. [2008]). SNPs in ADRB2 were found to be significantly associated with bronchodilator responsiveness in COPD (Kim et al. [2009]), as well as with asthma phenotypes (Vercelli [2008]).

The 6p21 region (containing human major histocompatibility genes) has shown strong linkage to atopic phenotype and asthma in many studies (Cookson [2002], Moffatt [2003], Shiina et al. [2004]). Moreover, the genes in the 6p21 region may also be involved in COPD exacerbations (Recalde et al. [1999]). On chromosome 11, the glutathione-S-transferase (GST) genes and other genes involved in the oxidative stress have been described to be involved in asthma and atopy (Aynacioglu et al. [2004], Tamer et al. [2004]).

Gene polymorphisms and differential expression levels of the GST genes have been associated with asthma, atopy and lung function (Brasch-Andersen et al. [2004]). The nitric oxide synthase (NOS) enzymes are involved in oxidative stress. The gene encoding for the neuronal NOS is located on chromosome 12q32 (Wjst et al. [1999], Cookson et al. [2004]). Another region of the chromosome 12 that seems to have a role in asthma is the vitamin D receptor gene (Raby BA et al. [2003]). Moreover, increasing epidemiologic evidence links vitamin D to pulmonary function and COPD (Wright [2005]).

Gene polymorphisms on both chromosomes 7 and 13 may be involved in the regulation of IgE levels (Hakonarson et al. [2001], Malerba et al. [2005], Vercelli [2008]). A disintegrin and metalloprotease (ADAM)33 gene on chromosome 20 (20p13) has been recognized as a susceptibility gene for asthma (Kere et al. [2004], Malerba et al. [2005], Van Eerdewegh et al. [2002]). Polymorphisms in this gene have been associated with accelerated lung function decline in asthma, in COPD and at a population level (Jongepier et al. [2004]).

Genetic studies of respiratory diseases have been plagued by a remarkable difficulty in constantly replicating results in different populations or even within the same population. This was true even when the statistical power was not an issue (Zhang et al. [2008]). Methodological limitations of the studies, such as genetic heterogeneity and poor definition of phenotypes (Contopoulos-Ioannidis et al. [2007]), may be partly responsible for the failure to replicate the gene-disease associations. Another explanation for inconsistent results is that genetic polymorphisms may modulate the effect of environmental exposures on the onset and occurrence and clinical expression of respiratory diseases (Martinez [2008]). Accordingly, the absence of analysis of gene-environment interactions from many genotyping studies would explain the conflicting data obtained from different studies (Vercelli [2009]).

The GEIRD project will attempt to overcome previous limitations of genetic studies by using large series of unselected phenotypes (from the general population) that will be accurately defined with respect to their

- inflammatory profile and lung function;
- objective biomarkers of oxidative stress and history of environmental exposures

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and lifestyle factors;

- present and past history of respiratory diseases and symptoms;
- treatment and achieved control.

In the GEIRD Project all the measurements are performed and information is collected according to international protocols concerted and standardized in the frame of the European Community Respiratory Health Survey II (see ECRHS Committee [2002a]). Accordingly, the bio-banks and data-bases created by GEIRD will also be shared with other researchers to perform more powerful analyses and to test epidemiological and genetic associations on different populations.

The study will provide a unique integrated set of data that will be suited to analyse functional genomics and gene-environment interactions. In the post-genomic era, identifying genes associated with complex diseases and gene-environment interactions is still one of the great challenges for dissecting human complex diseases. Association analysis is expected to be the most suitable approach to detect a common mild-risk allele which accounts for a substantial population attributable fraction in common complex diseases (Carlson [2004]). Large scale case-control studies, where cases are selected through an accurate characterization of the basic phenotypes, are needed to obtain reliable results on the role of the genetic component and of gene-gene, gene-environment interactions in common diseases (Hoh [2004]). Identifying susceptibility genes and understanding the aetiology of complex diseases will lead to the real potential of diagnosis, treatment, and prevention.

At a local level, the blood sample will be collected in 2x10 ml EDTA tubes and frozen. At a central laboratory, samples will be stored at -20°C until DNA extraction. Genomic DNA will be extracted using a ‘salting out’ standard procedure. DNA samples will be stored at -20°C in a specific freezer. When all the DNA samples from all the Italian centres will be available, candidate gene polymorphisms will be analysed in both cases and controls. The choice of the candidate genes, as well as the polymorphisms, will be based on literature data and will be updated according to preliminary results obtained analysing the Verona samples. The polymorphisms will be analysed using a multiplexed high degree genotyping method, such as the ‘Golden Gate genotyping assay’ (Illumina).

1.5.7 Training and Quality Control

At least one field worker per centre has attended/will attend the clinical lab in Verona for a training session. Adherence to protocol will be assessed through a quality control visit by a member of the co-ordinating centre to each participating centre. Where deviations from the protocol are observed they should be rectified to ensure standardization across the study.

1.5.8 Organisation Structure

Centres and Cohorts Involved in the Study

The Italian centres that will initially contribute to the GEIRD database are Verona, Pavia, Sassari, Torino, Ancona, Palermo and Terni. The screening phase will involve more than 41,000 subjects in the 6 participating centres.

The pre-existing cohorts involved are:

1. The Italian branch of the European Community Respiratory Health Study — ECRHS (ECRHS Italy [1995])— a general population random sample (aged 20-44 at enrollment), who were already studied in 1991 and 2000 (de Marco et al. [2003]);
2. The ISAYA cohort (de Marco et al. [2003, 2002b]) made up of random samples from the general population aged 20-44 years in 1998, when they were first studied.

Seven new random samples from the general population aged 20-64³ years, selected with the same sampling method used in ISAYA and ECRHS, will also be screened in the centres of Ancona, Palermo, Pavia, Sassari, Terni, Turin and Verona.

Using available estimates of prevalence, the expected number of probable cases and controls in the seven Italian centers are 3,682 and 1,700 respectively (see table 1.6).

| | Verona | Torino | Pavia | Sassari | Ancona | Terni | Palermo | Total |
|-----------------------|-------------|------------|-------------|------------|------------|------------|------------|-------------|
| Asthma ¹ | 520 | 138 | 264 | 85 | 169 | 150 | 196 | 1522 |
| COPD ² | 220 | 167 | 320 | 103 | - | 60 | 224 | 1094 |
| Rhinitis ³ | 240 | 146 | 280 | 90 | 160 | 150 | - | 1066 |
| Controls ⁴ | 520 | 160 | 360 | 116 | 170 | 150 | 224 | 1700 |
| Clinical phase | 1500 | 611 | 1224 | 394 | 499 | 510 | 644 | 4768 |

Table 1.6: Expected number, based on the more reliable centre-specific prevalence estimates, of probable phenotypes at the screening questionnaire in the Italian centers initially contributing to the GEIRD project. A participation of 70% has been assumed. Updates are available on the website (GEIRD Scientific Committee [2007]). ¹ self reporting of ever asthma; ² FEV₁/FVC<70% and/or chronic cough and phlegm for at least three months for at least two subsequent years, but no asthma; ³ self reporting of allergic rhinitis and/or hay fever, but neither asthma nor COPD/chronic cough and phlegm; ⁴ neither asthma, nor rhinitis, nor COPD, nor respiratory symptoms nor use of medicine to help breathing in the last year.

³20-84 for the Palermo, Sassari and Verona centres.

Timing

Each Italian centre has started the screening phase before May 2009 and will start the clinical phase before September 2010. The initial data bank is expected to be completed by June 2011.

The study is managed by a provisional scientific Steering Committee (SC) which is responsible for the implementation, the promotion, the funding of the project and the scientific use of the data bank. This SC is also responsible for developing and seeking collaboration with other research teams with the aim to build an international data bank. Before the end of the study (2011), a definite SC will be established, based on all the centres that will participate in the project.

The Public Domain GEIRD Database

This project will make it possible to establish a large database, including information on biomarkers of inflammation and oxidative stress, individual and ecological exposures (outdoor and indoor air pollutants), diet, early-life factors, smoking habits, genetic traits, and medication use in the cases of asthma, allergic rhinitis and COPD and in the controls. Not all centres and not all subjects will perform all the measurements and tests described in the general protocol.

A subject will be included in the GEIRD data-base when she/he will have performed the minimum set of tests and questionnaires to be identified as a case or as a control.

The Italian centres initially contributing to the GEIRD database (Ancona, Palermo, Pavia, Terni, Sassari, Torino, Verona) will carry out the clinical interview, the lung function test, the reversibility test, the methacholine test, the skin prick test, the blood collection for genotyping and IgE assay. Moreover, each centre will have the possibility to extend the study according to its own scientific interests in the frame of the general project.

Other research teams, using case definition and measurement instruments similar to those used in this project, may provide their cases and controls, increasing this database and generating a public domain case-controls database. In this way, the public domain database can become a new and innovative research instrument in the field of respiratory epidemiology. In fact, it will allow each researcher to answer his own research question with increased power. The comparability of the data among the centres is guaranteed by the use of standardised methods. However, a certain degree of variability between centres may persist. In order to overcome this problem, all the analyses will be based on the matching of cases and controls by centre and study cohort (frequency matching).

2 Measurement protocols

According to the project official homepage, we provide for each subsequent section a copy of the official measurement protocols as they appear on-line.

2 Measurement protocols

2.1 Six minute walk test protocol and worksheet



Genes **E**nvironment **I**nteraction on
Respiratory **D**iseases

THE GEIRD PROJECT

SIX-MINUTE WALK TEST PROTOCOL

Researchers using these materials are requested to inform the GEIRD Coordinating Centre and cite the source appropriately. For further information and contacts, please visit the web site at www.geird.org



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This test measures the distance that a patient can quickly walk on a flat, hard surface in a period of 6 minutes (the 6MWD). Walking is an activity performed daily by all but the most severely impaired patients. The self-paced 6MWT assesses the submaximal level of functional capacity.

The 6MWT is a practical simple test that requires a 30-35 m hallway but no exercise equipment or advanced training for technicians.

REQUIRED EQUIPMENT:

1. Countdown timer (or stopwatch)
2. Two small cones to mark the turnaround points
3. A chair that can be easily moved along the walking course
4. Worksheets on a clipboard
5. Sphygmomanometer

As well as for methacholine challenge, testing should be performed in a location where a rapid, appropriate response to an emergency is possible. Source of oxygen, telephone, and automated electronic defibrillator should be available.

ACCEPTANCE CRITERIA: Any subject who fulfils all of the following criteria is accepted:

- 1) is not unable to walk by a condition other than heart or lung disease
- 2) is not excluded by the following exclusion criteria.

EXCLUSION CRITERIA: Any subject who fulfils any one of the following criteria is excluded:

- 1) has had a heart attack in the last three months,
- 2) has any heart disease for which he/she is taking medication,
- 3) is taking medication for epilepsy,
- 4) is pregnant,
- 5) is breast feeding,
- 6) is taking a beta-blocker for any reason (including eye drops).
- 7) has a resting heart rate of more than 120.
- 8) has systolic blood pressure of more than 180 mmHg and/or diastolic blood pressure of more than 100 mm Hg.

General guidelines

The 6MWT is performed before the bronchodilator or methacholine challenge (first day).

- 1) Comfortable clothing and appropriate shoes should be worn.
- 2) A light meal is acceptable before early morning or early afternoon tests.
- 3) Patients should not have exercised vigorously within 2 hours of beginning the test.
- 4) The patient should sit at rest on a chair located near the starting position for at least 10 minutes before the test starts.
- 5) During this time, check for contraindications, measure pulse and blood pressure, and make sure that clothing and shoes are appropriate
- 6) Position the patient at the starting line. You should also stand near the starting line during the test. Do not walk with the patient.
- 7) As soon as the patient starts to walk, start the timer.
- 8) Do not talk to anyone during the walk. Use an even tone of voice when using the standard phrases of encouragement. Watch the patient. Do not get distracted and lose count of the laps.
- 9) Each time the participant returns to the starting line, mark the lap on the worksheet. Let the participant see you do it. Exaggerate using body language, like using a stopwatch at a race.
- 10) After the first minute, tell the patient the following (in even tones):
"You are doing well. You have 5 minutes to go."
(*"Sta andando bene. Mancano 5 minuti."*)
- 11) When the timer shows 4 minutes remaining, tell the patient the following:
"Keep up the good work. You have 4 minutes to go."
(*"Continui così. Mancano 4 minuti."*)
- 12) When the timer shows 3 minutes remaining, tell the patient the following:
"You are doing well. You are halfway done."
(*"Sta andando bene. È a metà del test."*)
- 13) When the timer shows 2 minutes remaining, tell the patient the following:
"Keep up the good work. You have only 2 minutes left."
(*"Continui così. Mancano solo 2 minuti."*)
- 14) When the timer shows only 1 minute remaining, tell the patient:
"You are doing well. You have only 1 minute to go."
(*"Sta andando bene. Manca solo 1 minuto."*)
- 15) When the timer is 15 seconds from completion, say this:

Six Minute Walk Test Protocol – GEIRD project

“In a moment I’m going to tell you to stop. When I do, just stop right where you are and I will come to you.”

(“Fra poco le dirò di fermarsi. Quando lo farò, si fermi dove si trova e io verrò da lei”).

16) When the timer rings (or buzzes), say this: “Stop!” (“Stop!”)

Walk over to the patient. Consider taking the chair if they look exhausted. Mark the spot where they stopped by placing a bean bag or a piece of tape on the floor.

17) Record the number of tick marks on the worksheet. Record the additional distance covered (the number of meters in the final partial lap). Calculate the total distance walked, rounding to the nearest meter, and record it on the worksheet.

18) Congratulate the patient on good effort and offer a drink of water.

Do not use other words of encouragement during the test (or body language to speed up).

If the patient stops walking during the test and needs a rest, say this:

“You can lean against the wall if you would like; then continue walking whenever you feel able.”

(“Può appoggiarsi alla parete se vuole; quando si sente in grado, riprenda a camminare.”).

Do not stop the timer.

If the patient stops before the 6 minutes are up and refuses to continue (or you decide that they should not continue), wheel the chair over for the patient to sit on, discontinue the walk, and note on the worksheet the distance, the time stopped, and the reason for stopping prematurely.

Instructions to subjects

“The object of this test is to walk as far as possible for 6 minutes.
You will walk back and forth in this hallway.”

(“Lo scopo di questo test è camminare il più possibile per 6 minuti. Camminerà avanti e indietro in questo corridoio”)

“Six minutes is a long time to walk, so you will be exerting yourself. You will probably get out of breath or become exhausted.”

(“Sei minuti sono un tempo lungo, dovrà fare uno sforzo. Probabilmente resterà senza fiato o sarà esausto.”)

“You are permitted to slow down, to stop, and to rest as necessary. You may lean against the wall while resting, but resume walking as soon as you are able.”

(“Può rallentare, fermarsi e riposarsi quanto le è necessario. Può appoggiarsi contro la parete mentre si riposa, ma si rimetta a camminare non appena può.”)

“You will be walking back and forth around the cones. You should pivot briskly around the cones and continue back the other way without hesitation.”

(“La farò camminare avanti e indietro intorno ai coni. Dovrebbe girare velocemente intorno ai coni e continuare verso l'altro senso di marcia senza esitazioni.”)

“Now I’m going to show you. Please watch the way I turn without hesitation.”

(“Ora le farò vedere. Osservi come mi giro senza esitazioni.”)

Demonstrate by walking one lap yourself. Walk and pivot around a cone briskly.

“Are you ready to do that? I am going to keep track of the number of laps you complete.”

(“È pronto? Prenderò nota del numero di giri da lei completati.”)

Six Minute Walk Test Worksheet

CENTRE

Personal Number

| | | | | | |
|--|--|--|--|--|--|
| | | | | | |
|--|--|--|--|--|--|

Date of compilation of the form

| | | | | | |
|-----|--|-------|--|------|--|
| | | | | | |
| DAY | | MONTH | | YEAR | |

Reminder: the 6-MWT must be performed before the bronchodilator or methacholine challenge. The patient should sit at rest on a chair located near the starting position for at least 10 minutes before the test starts. During this time check for contraindications (see exclusion criteria), measure pulse (resting heart rate < 120) and blood pressure (systolic b.p. <180 mmHg and diastolic b.p. <100 mm Hg).

Indicate values at rest:

Heart rate

Saturimetry (%)

Systolic blood pressure (mmHg)

Diastolic blood pressure (mmHg)

| | | |
|--|--|--|
| | | |
| | | |
| | | |
| | | |

If the patient has not performed the test, check one (or more) of the following options:

The subject:

- 1) has refused to perform the test
- 2) is unable to walk due to a condition other than heart or lung disease
- 3) has had a heart attack in the last three months
- 4) has any heart disease for which he/she is taking medication
- 5) he/she is taking medication for epilepsy
- 6) is pregnant or breast-feeding
- 7) is taking a beta-blocker for any reason (including eye drops)
- 8) has a resting heart rate of more than 120
- 9) has systolic blood pressure of more than 180 mmHg and/or diastolic blood pressure of more than 100 mmHg
- 10) other (specify)

| |
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Six Minute Walk Test Worksheet

Each time the participant returns to the starting line, mark the lap **here**:

| number of laps (..... meters) completed (check a box any time a lap is completed) | | | | | | | | | |
|------------------------------------------------------------------------------------------|----|----|----|----|----|----|----|----|----|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |

additional distance covered* (in meters):

* Please fill in these fields even if the patient stops before the 6 minutes are up and refuses to continue (or if you decide that they should not continue); in addition, if you stop the test, include the following information:

minutes to go when the test was stopped

reasons for stopping the test

.....

2.2 Anthropometric measurement protocol



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Protocol for anthropometric measurements

Waist circumference, hip circumferences and demispan

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Waist and hip circumferences

Waist (WC) and hip (HC) circumference are measured during the clinical visit with a non-stretchable standard tape placed directly on the skin. The subject is asked to stand relaxed in underclothes only, and to stay balanced on both feet, with the feet touching each other and both arms hanging freely. The measurement is taken at the end of a normal expiration, in centimetres to one decimal place (precision of 0.1 cm), without compressing any underlying soft tissues. Before taking a reading, specific attention is given to placing the tape perpendicular to the long axis of the body and horizontal to the floor. For the latter reason, a second operator is recommended for help, and measurements are taken from a lateral standpoint, with the subject being in profile.

1. Waist circumference

The WC is measured at the horizontal point between the costal margin and iliac crest that yields the minimum measurement (i.e. the narrowest waist, as suggested by the ASM reference Manual¹). The narrowest waist is probably the most frequently recommended site to measure the WC. It is easy to identify the narrowest waist in most subjects: in general, it is situated over the umbilicus. For some subjects, there is no single narrowest point between the lowest rib and the iliac crest because of either a large amount of abdominal fat or extreme thinness.

Additionally, the WC is also measured at the midpoint between the lowest rib and iliac crest (as suggested by the WHO guidelines²).

2. Hip circumference

The HC is measured at the widest diameter over the great trochanters (as suggested by the WHO guidelines²).

Demispan

The demispan³ is measured while the subject is standing against a flat wall, with the arms extended laterally and kept at shoulder height during the measurement, using a non-stretchable standard tape. The starting point is at the centre of the suprasternal notch stretching laterally and ending at the metacarpophalangeal joint between the third and fourth fingers. Demiquet and Mindex are then calculated as follows:

Demiquet (for older men) = body weight (kg) / (demi-span (m))²

Mindex (for older women) = body weight (kg) / demi-span (m)

¹ Lohman TG. Anthropometric standardization reference manual. Champaign, IL: Human Kinetics, 1988:28-80.

² WHO document (1988): Measuring obesity – classification and description of anthropometric data. Copenhagen: WHO Regional Office for Europe, Nutritional Unit. # EUR/ICP/NUT 125.

³ Assantachai P, Yamwong P, Lekhakula S. Alternative anthropometric measurements for the Thai elderly: Mindex and Demiquet. Asia Pac J Clin Nutr. 2006;15(4):521-7.

2.3 Skin prick test protocol



Genes **E**nvironment **I**nteraction on
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SKIN PRICK TEST PROTOCOL

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If the subject has taken medications with antihistamine-like actions (antihistamines, some cold remedies, tricyclic antidepressants) in the 5 days before testing, he/she must be excluded.

Skin prick test has to be performed on the forearm.

The skin must be free of active eczema. The arm is first cleaned with alcohol.

A grid is marked with a pen at 2.5 cm intervals and a drop of the relevant allergen placed on the arm at the end of each line.

The pattern follows the corresponding list of allergens used for easy identification

A sterile lancet with 1 mm point is used to prick the skin through the drop. (With the so-called "prick through drop" method it is unnecessary to scratch or lift the skin and no blood should be drawn).

One new single use lancet is used for each prick, in order to prevent carry-over of allergens.

Reactions should occur within 10-15 minutes after which the results can be assessed.

A positive and negative control must be included in each series of tests. The negative control solution is the diluent used to preserve the allergen extract. The positive control solution is a 1 mg/ml histamine hydrochloride solution.

A reaction of 3 mm greater than the negative control is regarded as positive.

Devices are available to directly measure the transverse and longitudinal diameters of skin prick test wheals or flares in centimetres.

Results may also be recorded using transparent tape at least 25 mm wide over the wheal and flare and marking the size of the wheal and flare using a felt tipped pen.

The drops of allergen solution are always applied in exactly the same order and the results are transferred in the same orientation to the data collection sheet.

ALK kits will be used for the allergens reported in the following sheet.

References

Bousquet J. "In view methods for study of allergy" in *Allergy, Principles and practice*, Ed. Middleton E, Reed CE, Ellis E, Adkinson NF, Yunginger JW. 3rd Edition, Chapter 19, page 427.

The European Community Respiratory Health Survey I, MAIN protocol.

www.geird.org

Skin Prick Test Protocol – GEIRD project

| <i>Elbow</i> RIGHT ARM | <i>Elbow</i> LEFT ARM |
|---------------------------|--------------------------------|
| Positive control | (space) |
| (space) | Cupressus arizonica |
| Graminacee mix | Dermatophagoides pteronyssinus |
| Artemisia vulgaris | Dermatophagoides farinae |
| Ambrosia artemisifolia | Alternaria tenuis |
| Parietaria judaica | Dog dander |
| Corylus avellana | Cat hair |
| Olea europea | Negative control |
| Betula verrucosa | Cladosporium herbarum |

Boxes for recording should be 2.5 cm high

2.4 Lung function protocol



Genes **E**nvironment **I**nteraction on
Respiratory **D**iseases

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LUNG FUNCTION PROTOCOL

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Criteria for baseline forced and slow spirometry

The purpose of baseline forced spirometry is to record an accurate Forced Expiratory Volume in one second (FEV₁) and Forced Vital Capacity (FVC) from every subject who attends the testing centre. The purpose of baseline slow spirometry is to record an accurate Inspiratory Capacity (IC) and Expiratory Vital Capacity (VC) from every subject who attends the testing centre.

ACCEPTANCE CRITERIA:

Subjects who are probable cases or probable controls and are able to attend the testing centre.

EXCLUSION CRITERIA:

If the subject smokes: Lung function testing should be carried out at least one hour after the last cigarette has been smoked.

If the subject has used an inhaler: Lung function testing should be carried out at least one hour after the use of any inhaler.

- *If the subject has used an inhaler that is a long acting beta-2-agonist or a long acting anticholinergic in the last 8 hours:* If the subject is willing to come back at another time when they have not taken their long acting Beta-2-agonist, another appointment should be made. HOWEVER – this may be difficult for them to do, in which case, testing should proceed and medication used should be recorded.
- *If the subject has used an inhaler that is a short acting beta-2-agonist or a short acting anticholinergic inhaler in the last one to four hours:* If the subject is willing to come back another time for lung function testing, another appointment should be made. If the subject is unable or reluctant to return another time, testing should proceed and the medication used should be recorded.
- *If the subject has used an inhaler that is not a beta-2-agonist or an anticholinergic inhaler in the last one to four hours:* Lung function testing is carried out and the data recorded.

If the subject has taken an oral beta-2-agonist or an oral theophylline or an oral antimuscarinic within the last eight hours: If the subject is willing to come back another time for lung function testing, another appointment should be made. If the subject is unable or reluctant to return another time, testing should proceed and the medication used recorded.

If the subject has had a respiratory tract infection in the last three weeks: Another appointment should be made unless the subject is unwilling to come back, in which case testing should continue. The number of days elapsed since the end of the respiratory infection should be recorded.

If, after a total of nine attempts, a subject is unable to produce a technically satisfactory manoeuvre, no FEV₁ or FVC will be recorded.

Predicted FEV₁ values and lower limit of normal for FEV₁/FVC

Normal FEV₁ values will be calculated using the following equations (Quanjer et al, ERJ 1993 [†]):

Males: $4.30 H - 0.029 A - 2.49$

Females: $3.95 H - 0.025 A - 2.60$

where

H = height in metres (males: 1.55-1.95 m; females: 1.45-1.80 m)

A = age in years (range 18-70).

The lower limit of normal (LLN) for the FEV₁/FVC ratio will be calculated using the following equations (Quanjer et al, ERJ 1993 [†]):

Males: $- 0.18 A + 75.41$

Females: $- 0.19 A + 78.40$

where

A = age in years (range 18-70).

These equations are only valid for subjects over the age of 25. Subjects aged 20-24 should have their expected indexes calculated as if their age is 25.

Criteria for bronchodilator challenge

The FEV₁ and FVC will be measured following the administration of 400µg salbutamol by metered dose inhaler (MDI) via a Volumatic spacer.

ACCEPTANCE CRITERIA:

Any subject who fulfils all of the following criteria is accepted:

- 1) has produced technically satisfactory FEV₁ and FVC manoeuvres,
- 2) a) has a FEV₁/FVC ratio less than 0.70 or less than LLN **or** b) has FEV₁ less than 1.5L or less than 70% of the predicted value ^{*},
- 3) has signed a consent form for bronchodilator challenge,
- 4) is not excluded by the following exclusion criteria.

[†] Quanjer PhH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Eur Respir J 1993; 6 suppl. 16: 5-40

^{*} FEV₁ is the maximum assessed during the baseline forced spirometry; FVC is the maximum assessed during the baseline forced spirometry even if it does not come from the same curve of FEV₁.

EXCLUSION CRITERIA:

Any subject who fulfils any one of the following criteria is excluded:

- 1) has had a heart attack in the last three months,
- 2) has any heart disease for which he/she is taking medication,
- 3) has epilepsy for which he/she is taking medication,
- 4) is pregnant,
- 5) is breast feeding,
- 6) is taking a beta-blocker for any reason (including eye drops).

These conditions will be assessed by the Lung Function Questionnaire.

Criteria for methacholine challenge

The aim of methacholine challenge is for subjects to inhale increasing concentrations of methacholine solutions and to monitor any change in FEV₁ by repeated spirometric testing.

ACCEPTANCE CRITERIA:

Any subject who fulfils all three of the following criteria is accepted:

- 1) has been able to perform at least 2 technically satisfactory manoeuvres during baseline spirometry
- 2) has signed a consent form for methacholine challenge,
- 3) is not in the categories for exclusion (see below).

EXCLUSION CRITERIA:

Any subject who fulfils any one of the following criteria is excluded from methacholine challenge:

- 1) has had a heart attack in the last three months,
- 2) has any heart disease for which he/she is taking medication,
- 3) has epilepsy for which he/she is taking medication,
- 4) is pregnant,
- 5) is breast feeding,
- 6) is taking a beta-blocker for any reason (including eye drops).

These criteria will be assessed by the Lung Function Questionnaire.

In addition, any subject who fulfils either of the following is excluded:

- 7) has an FEV₁ less than 70% of the predicted value,
- 8) has an FEV₁ less than 1.5 litres.

FEV₁ is the maximum assessed during the baseline forced spirometry.

Note that any subject who fulfils all the following is accepted for both bronchodilator challenge and methacholine:

- 1) has an FEV_1 greater than 70% of the predicted value **,
- 2) has an FEV_1 greater than 1.5 litres **,
- 3) has an FEV_1/FVC ratio less than 0.7 *.*

Making the appointment(s) for testing

When applicable, bronchodilator challenge (first day) and methacholine challenge (second day), should be performed in two different days.

Ideally, lung function testing should be performed:

- 1) more than four hours after the use of a beta-2-agonist or anticholinergic inhaler,
- 2) more than eight hours after inhaled long acting beta-2-agonist, oral beta-2-agonist or theophylline or oral antimuscarinic.

When the appointment for lung function testing is made the fieldworker should determine if the subject is taking any of the following medications:

- 1) beta-2-agonist inhaler (short or long acting),
- 2) anticholinergic inhaler,
- 3) oral beta-2-agonist,
- 4) oral theophylline,
- 5) oral antimuscarinic.

If the subject is taking any of these medications (or any other inhaler) an appointment time should be agreed that will cause the least disruption to the subject's normal dosing schedule.

One simple way of ensuring compliance with these instructions is to:

- 1) avoid early morning appointments for those using inhalers,
- 2) when fixing a time for an appointment, ask the subject: i) to take their oral medication or long acting beta-2-agonist or long acting anticholinergic inhalers eight hours before testing; ii) to take inhalers other than long acting inhalers four hours before testing .

* FEV_1 is the maximum assessed during the baseline forced spirometry; FVC is the maximum assessed during the baseline forced spirometry even if it does not come from the same curve of FEV_1 .

The fieldworker should ensure that the subject has not had a respiratory tract infection in the three weeks prior to testing and should advise the subject not to smoke for one hour prior to coming to the testing centre. A letter should be sent to the subject explaining this.

Subjects who have not followed guidelines

Those who have had a cigarette in the last hour should have the lung function test delayed until one hour has elapsed. (Most subjects will be in the centre for at least one hour.)

Those who have had an inhaler in the last four hours or oral medication (or long acting beta-2-agonist) in the last eight hours may fall into one or more of the following categories:

- 1) misunderstood the instructions,
- 2) forgot the instructions,
- 3) ignored the instructions,
- 4) may have symptoms too severe to follow the instructions.

Lung function testing may still be carried out unless the subject is excluded for other reasons, and recent medication should be noted in the Lung Function Questionnaire.

General guidelines

All forced and slow manoeuvres will be performed:

- 1) sitting, legs uncrossed
- 2) with noseclip on,
- 3) using a plastic or cardboard mouthpiece without teethgrips,
- 4) tight clothing should be loosened.

Two types of forced expiratory manoeuvre will be used in this protocol:

- 1) During baseline spirometry and bronchodilator challenge FVC will be measured and all subjects must exhale fully.
- 2) During methacholine challenge only the FEV₁ needs to be recorded and the technician may interrupt the exhalation when this has been achieved.

One type of slow manoeuvre will be used in this protocol:

1) During baseline spirometry and bronchodilator challenge IC and VC will be measured. All subjects must breath regularly for several breaths (at least three tidal manoeuvres) and take a slow full inspiration followed by a full expiration in a relaxed (not forced) manner.

A technically unsatisfactory manoeuvre is defined as:

- 1) an unsatisfactory start of expiration characterised by excessive hesitation or false start
- 2) coughing during the first second of the manoeuvre, thereby affecting the measured FEV₁ value, or any cough that interferes with the accurate measurement of FVC, IC or VC
- 3) Valsalva Manoeuvre (glottis closure)
- 4) A leak in the system or around the mouthpiece
- 5) An obstructed mouthpiece, e.g. the tongue in front of the mouthpiece.

Manoeuvres which have these faults are technically unsatisfactory and are rejected as failed attempts.

Evidence of poor compliance is shown by:

- 1) greater than 150 mL variation in FEV₁ and IC (VC) between blows.
- 2) greater than 150 mL or 5% FVC back-extrapolated volume
- 3) peak expiratory flow that is less than 85% of the best record
- 4) expiratory time that is less than six seconds

If these features are noted technicians should encourage the subject to produce a better reading but the blows should not be excluded as failed attempts on these criteria alone.

A manoeuvre may only be rejected as a failed attempt if it is 'technically unsatisfactory'. Manoeuvres with evidence of 'poor compliance' only should not be rejected.

The above protocol is consistent with the current ATS/ERS guidelines (Eur Respir J 2005; 26: 319–338). These state that 'The repeatability criteria are used to determine when more than three acceptable FVC manoeuvres are needed; these criteria are not to be used to exclude results from reports or to exclude subjects from a study. No spirogram should be rejected solely on the basis of its poor repeatability'.

Instructions to subjects

Some of the subjects will never have used any form of lung function testing equipment before and others will be very familiar with it.

Technicians should explain to the subject that the aim of the test is to find out how much air can be blown out of the lungs (slow and forced manoeuvre) and how forcefully it can be blown out (forced manoeuvre).

This can be done by asking the subject to follow these steps:

Forced (FVC and FEV₁) manoeuvre:

- 1) Take in as deep breath as possible when full-
- 2) Place the mouthpiece in his/her mouth.
- 3) Close his/her lips tightly around the mouthpiece.
- 4) Blast or blow through the mouthpiece into the spirometer, blowing air out as hard, fast, smoothly and completely as possible.

Slow (IC and VC) manoeuvre:

- 1) Place the mouthpiece in his/her mouth.
- 2) Close his/her lips tightly around the mouthpiece.
- 3) Breathe regularly for several breaths (at least three tidal manoeuvres)
- 4) Take in as deep breath as possible when full-
- 5) Blow through the mouthpiece into the spirometer, blowing air out in a relaxed (not forced) manner, smoothly and completely as possible.

The subject should continue to push out air actively for as long as possible (FVC manoeuvre and slow VC manoeuvre) or until the technician tells him/her to stop (FEV₁ manoeuvre). During this time the technician must offer positive encouragement to push or squeeze out more air.

Guideline for baseline spirometry

- 1) Ensure that it is appropriate to perform lung function testing.
- 2) Demonstrate the manoeuvre to all subjects at least once (more often if he/she appears uncertain).
- 3) Ask the subject to carry out five FVC manoeuvres and three slow manoeuvres.
- 4) Record the FEV₁ and FVC and Peak Expiratory Flow (in litres per second) from at least two and up to five technically satisfactory manoeuvres.
- 5) If the subject has failed to produce two technically satisfactory FVC manoeuvres after five attempts, the technician should show them again how to conduct the manoeuvre and allow them four more attempts.
- 6) Any subject who is unable to produce two technically satisfactory manoeuvres after nine FVC attempts should not be tested further and no FEV₁ / FVC data should be recorded.
- 7) The number of FVC rejected attempts should be recorded as appropriate on the Lung Function Data Collection Sheet.
- 8) Ask the subject to carry out three slow manoeuvres.
- 9) Record the IC and VC from at least two and up to three technically satisfactory manoeuvres.

- 10) If the subject has failed to produce two technically satisfactory slow manoeuvres after three attempts, the technician should show them again how to conduct the manoeuvre and allow them two more attempts.
- 11) Any subject who is unable to produce two technically satisfactory manoeuvres after five slow attempts should not be tested further and no IC / VC data should be recorded.
- 12) The number of IC / VC rejected attempts should be recorded as appropriate on the Lung Function Data Collection Sheet.

Guideline for bronchodilator challenge

A bronchodilator challenge will be given to those who have an FEV₁/FVC ratio less than 0.7 or less than the LLN, or FEV₁ less than 1.5L or FEV₁ less than 70% of the predicted value.

Any subject who has more than a 10% fall in FEV₁ from baseline during the methacholine challenge test should have their bronchoconstriction reversed at the end of the test and before leaving the test centre, by the same method.

The salbutamol inhaler should be shaken and inserted into the volumatic. One puff should be activated and the subject asked to place their lips around the volumatic and to inhale and exhale five times. The salbutamol inhaler should be activated again and five inhalations/exhalations performed. This should be repeated two more times so that a total of 400ug of salbutamol has been delivered. Subjects who are known asthmatics and familiar with Volumatic usage can self-administer this dose.

The FEV₁ and FVC are measured 10 minutes after the administration of bronchodilator.

During the bronchodilator challenge forced FVC manoeuvres and slow manoeuvres will be used.

Up to nine attempts may be made to obtain two technically satisfactory forced FVC manoeuvres recordings after the inhalation of bronchodilator.

Up to five attempts may be made to obtain two technically satisfactory slow manoeuvres recordings after the inhalation of bronchodilator.

Guideline for methacholine challenge

A methacholine challenge will be given to:

- 1) those who have an FEV₁/FVC ratio more than 0.7 and more than the LLN;
- 2) those who have an FEV₁/FVC ratio less than 0.7 or less than the LLN, but have a FEV₁ more than 1.5 L and more than 70% of predicted. These subjects will perform the reversibility testing on the first day, and will be

invited on a second day for methacholine testing, when, as a precaution, the long protocol for methacholine challenge will only be performed.

During methacholine challenge the subject may need to perform 30 or more expiratory manoeuvres and, to minimise exhaustion, the forced expiration will be abandoned each time after one second when the FEV₁ has been recorded.

- 1) Two minutes after inhalation from the dosimeter up to five attempts should be made to record an FEV₁
- 2) As soon as two technically satisfactory manoeuvres have been achieved these readings are recorded. The next dose can be given as soon as possible after the completion of these measurements.
- 3) Further testing should be abandoned if the subject is unable to produce two technically satisfactory manoeuvres within five attempts.

If a reversal of bronchoconstriction needs to be carried out then the procedure is the same as the bronchodilator challenge.

THE METHACHOLINE SOLUTIONS

Source and supply

Methacholine will be obtained from Lofarma.

Session number and order in session

Each time the nebulisers are filled with fresh methacholine solution a new session of testing is said to have started. Each session should be sequentially numbered. Each challenge within each testing session should also be sequentially numbered and recorded on the Lung Function Data Collection Sheet.

At the beginning of a session all nebulisers contain 3 mL methacholine. Six subjects are tested using a 3-way valve and their order in session is 1-6. After the 6th person has been tested the 12.5 mg/mL solution is discarded, the nebuliser is cleaned and dried, and 3 mL of fresh 12.5 mg/mL solution is added. Six more subjects are tested and they are numbered 7-12. After the 12th person has been tested all solutions are discarded and the nebulisers are cleaned. The next session begins when new solutions are added. A session may be extended over one night only by placing the nebulisers containing solutions upright in the fridge, covered with parafilm.

The standard inhalation

The sequence of inhalation is:

- 1) Slow expiration to functional residual capacity.
- 2) Place lips around mouthpiece to produce airtight seal.
- 3) Slow inspiration to total lung capacity.
- 4) Hold breath for at least three seconds.

- 5) Remove mouthpiece and exhale.

The procedure is repeated after six seconds until sufficient inhalations for the dose have been performed. Inhalations may be performed on consecutive breaths if desired. Spirometric testing is carried out two minutes after the dose. As soon as two FEV₁ manoeuvres have been recorded, the test is continued with the next dose.

The end of the testing session

Solutions remaining in the nebulisers must be discarded and under no circumstances should they be returned to the storage containers. All nebulisers must be cleaned and dried. All mouthpieces must be cleaned, sterilised and thoroughly rinsed to ensure that there is no sterilising solution left on the surface.

The methacholine protocol

Instructions for baseline spirometry

Perform full FVC manoeuvres as described previously for 'Baseline spirometry' (The forced expiratory manoeuvre). Record INITIAL FEV₁ and FVC. Calculate the BEST INITIAL FEV₁ as a percentage of the total predicted.

Measurement of control (post-diluent) FEV₁

The control FEV₁ is the FEV₁ measured following the inhalation of diluent. Four inhalations of diluent are given, as described in 'The standard inhalation'.

Perform FEV₁ manoeuvres as described in 'Methacholine challenge' (The forced expiratory manoeuvre). Record CONTROL (POST-DILUENT) FEV₁. Calculate BEST CONTROL FEV₁ as a percentage of the BEST INITIAL FEV₁.

If the BEST CONTROL FEV₁ is less than 90% of the BEST INITIAL FEV₁ methacholine challenge is not carried out. Bronchoconstriction should be reversed by administering 400 µg salbutamol by MDI via a Volumatic and full FVC manoeuvres should be repeated.

If the BEST CONTROL FEV₁ is within 10% of the best initial FEV₁. Calculate 80% of the BEST CONTROL FEV₁. Calculate 90% of the BEST CONTROL FEV₁. Methacholine challenge may now be conducted following either the short or long protocol.

Choice of long or short protocol

Each subject can be challenged on the short or long protocol. The long protocol will increase by doubling doses and the short by quadrupling doses. Subjects most likely to react to methacholine should be tested on the long protocol. Subjects who are unexpectedly reactive and have been allocated to the short protocol may switch to the long protocol during the challenge to avoid severe bronchoconstriction. The choice of protocol for each subject will be assessed by the Main Questionnaire. The questions to be used to direct subjects to the long protocol may be decided locally, but the following are recommended:

Subjects who answered 'YES' to any one of Questions 1, 2, 3, 5, 11 or 16 in the Main Questionnaire, that is any subject who has:

- 1) had wheezing or whistling in their chest in the last 12 months (Q1)
- 2) woken with tightness of chest in the last 12 months (Q2)
- 3) had an attack of shortness of breath during the day while at rest in the last 12 months (Q3)
- 4) been woken by an attack of shortness of breath in the last 12 months (Q5)
- 5) trouble with their breathing (Q11)
- 6) ever had asthma (Q16)

Methacholine challenge protocol

| CONC (mg/mL) | No of inhalations: | | CUMULATIVE DOSE (mg) | DOSE LEVEL (As per ECRHS I) |
|-----------------|--------------------|-------|-------------------------|--------------------------------|
| | LONG | SHORT | | |
| 0.39 | 2 | | 0.0078 | 3 |
| 0.39 | 2 | 4 | 0.0156 | 4 |
| 1.56 | 1 | | 0.0312 | 5 |
| 1.56 | 2 | 3 | 0.0625 | 6 |
| 6.25 | 1 | | 0.125 | 7 |
| 6.25 | 2 | 3 | 0.25 | 8 |
| 12.5 | 2 | | 0.5 | 9 |
| 12.5 | 4 | 6 | 1.0 | 10 |
| 12.5 | 8 | 8 | 2.0 | 11 |

Changing from Long to Short protocol

If, during the short protocol, the FEV₁ falls 10% or more from the best control FEV₁, the subject should change protocol and receive the next dose level on the long protocol.

For example: A subject following the short protocol shows a fall of 10% after Dose 4 (four inhalations of 0.39 mg/mL). They should inhale Dose 5 (one inhalation of 1.56 mg/mL) next.

Short protocol:

Change to long protocol if FEV₁ falls below 90% of the BEST CONTROL FEV₁. Go to next dose level on long protocol.

STOP challenge if FEV₁ falls below 80% of the BEST CONTROL FEV₁

Long protocol:

STOP challenge if FEV₁ falls below 80% of the BEST CONTROL FEV₁

Completion of test

The methacholine challenge is complete when a cumulative dose 2 mg of methacholine has been reached.

It is stopped sooner if:

- 1) there is greater than 10% fall in FEV₁ from the **BEST BASELINE FEV₁** following inhalation of diluent,
- 2) there is greater than 20% fall in FEV₁ from the **BEST CONTROL FEV₁** following inhalation of any concentration of methacholine solution,
- 3) the subject is not able to perform two technically satisfactory manoeuvres in five attempts following any dose level,
- 4) the subject does not wish to carry on.

Lung Function Protocol – GEIRD project

Subjects may complain of mild chest tightness, coughing or wheezing but if lung function does not demonstrate a 20% fall in FEV₁ this is not an indication to stop the test.

Reversal of bronchoconstriction

Four hundred micrograms of salbutamol will be given via a volumatic (see above for bronchodilator challenge)
Perform full FVC manoeuvres as described in 'Methacholine challenge' 10 minutes after administration.

Record the POST-BRONCHODILATOR FEV₁ and FVC.

Calculate the BEST POST-BRONCHODILATOR FEV₁ as a PERCENTAGE of the BEST INITIAL FEV₁.

If the best post-bronchodilator FEV₁ is more than 90% of the best initial FEV₁ the test is over.

EACH CENTRE SHOULD PREPARE PROTOCOLS TO BE FOLLOWED IN THE EVENT OF A SUBJECT NOT RETURNING TO WITHIN 10% OF THE BASELINE.

Bronchodilator challenge protocol

Four hundred micrograms of salbutamol are administered by MDI as described in 'Bronchodilator challenge'.
Perform full FVC manoeuvres as described in 'Baseline spirometry'. Record the POST-BRONCHODILATOR FEV₁ AND FVC.

Coding for lung function questionnaire

The same general rules apply as for the main questionnaire.

Questions with NO / YES

- | | |
|---|-----|
| 0 | NO |
| 1 | YES |

Questions with ‘TICK ONE BOX ONLY’ instruction:

The number of the box ticked is the code for that answer.

QUESTION 11.1 Inhalers in the last 24 hours

Drug grouping should be consistent with those used for main questionnaire.

- | | |
|----|-----------------------------------------|
| 1 | Beta-2-agonist (short acting) |
| 2 | Beta-2-agonist (long acting) |
| 3 | Non-specific adrenoreceptor agonists |
| 4 | Anticholinergic inhalers (short acting) |
| 5 | Anticholinergic inhalers (long acting) |
| 6 | Inhaled steroids |
| 7 | Sodium cromoglycate |
| 8 | Nedocromil |
| 9 | Compound bronchodilators |
| 98 | Not coded |
| 99 | Not known |

QUESTION 12.1 Oral medications

Drug grouping should be consistent with those used for main questionnaire.

- | | |
|----|---------------------------------------|
| 1 | Beta-2-agonist |
| 2 | Non-specific adrenoreceptor agonist |
| 3 | Oral anticholinergics/antimuscarinics |
| 4 | Oral methylxanthines |
| 5 | Oral steroids |
| 6 | Oral antihistamines |
| 7 | Oral compound bronchodilators |
| 8 | Oral-antileukotrienes |
| 98 | not coded |
| 99 | not known |

Lung Function Protocol – GEIRD project

Appendix: LLN reference values for males and females aged 25-70 years (subjects aged 20-24 should have their LLN calculated as if their age is 25).

| gender | age | LLN (Quanjer) | gender | age | LLN (Quanjer) |
|--------|-----|---------------|--------|-----|---------------|
| M | 25 | 70.9 | F | 25 | 73.7 |
| M | 26 | 70.7 | F | 26 | 73.5 |
| M | 27 | 70.6 | F | 27 | 73.3 |
| M | 28 | 70.4 | F | 28 | 73.1 |
| M | 29 | 70.2 | F | 29 | 72.9 |
| M | 30 | 70.0 | F | 30 | 72.7 |
| M | 31 | 69.8 | F | 31 | 72.5 |
| M | 32 | 69.7 | F | 32 | 72.3 |
| M | 33 | 69.5 | F | 33 | 72.1 |
| M | 34 | 69.3 | F | 34 | 71.9 |
| M | 35 | 69.1 | F | 35 | 71.8 |
| M | 36 | 68.9 | F | 36 | 71.6 |
| M | 37 | 68.8 | F | 37 | 71.4 |
| M | 38 | 68.6 | F | 38 | 71.2 |
| M | 39 | 68.4 | F | 39 | 71.0 |
| M | 40 | 68.2 | F | 40 | 70.8 |
| M | 41 | 68.0 | F | 41 | 70.6 |
| M | 42 | 67.9 | F | 42 | 70.4 |
| M | 43 | 67.7 | F | 43 | 70.2 |
| M | 44 | 67.5 | F | 44 | 70.0 |
| M | 45 | 67.3 | F | 45 | 69.9 |
| M | 46 | 67.1 | F | 46 | 69.7 |
| M | 47 | 67.0 | F | 47 | 69.5 |
| M | 48 | 66.8 | F | 48 | 69.3 |
| M | 49 | 66.6 | F | 49 | 69.1 |
| M | 50 | 66.4 | F | 50 | 68.9 |
| M | 51 | 66.2 | F | 51 | 68.7 |
| M | 52 | 66.1 | F | 52 | 68.5 |
| M | 53 | 65.9 | F | 53 | 68.3 |
| M | 54 | 65.7 | F | 54 | 68.1 |
| M | 55 | 65.5 | F | 55 | 68.0 |
| M | 56 | 65.3 | F | 56 | 67.8 |
| M | 57 | 65.2 | F | 57 | 67.6 |
| M | 58 | 65.0 | F | 58 | 67.4 |
| M | 59 | 64.8 | F | 59 | 67.2 |
| M | 60 | 64.6 | F | 60 | 67.0 |
| M | 61 | 64.4 | F | 61 | 66.8 |
| M | 62 | 64.3 | F | 62 | 66.6 |
| M | 63 | 64.1 | F | 63 | 66.4 |
| M | 64 | 63.9 | F | 64 | 66.2 |
| M | 65 | 63.7 | F | 65 | 66.1 |
| M | 66 | 63.5 | F | 66 | 65.9 |
| M | 67 | 63.4 | F | 67 | 65.7 |
| M | 68 | 63.2 | F | 68 | 65.5 |
| M | 69 | 63.0 | F | 69 | 65.3 |
| M | 70 | 62.8 | F | 70 | 65.1 |

2.5 Alveolar air: equipment, sampling and storing

ALVEOLAR AIR: EQUIPMENT, SAMPLING AND STORING

Equipment : storage

Before using the 20 ml-glass vials and caps (Teflon caps with aluminium ring), they have to be stored at least three hours at 80 °C until collection time.

If you use the electronic crimper (electronic crimper Agilent), which works with battery, remember it has to be recharged every once in a while, generally every two months.

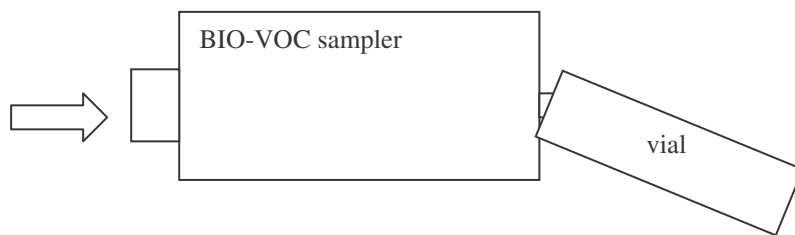
The Bio-Voc Sampler is a device with uni-directional valves and we use it to prevent the air flow inversion during the sample collection, ensuring the sample only composed by alveolar air. The Bio-Voc sampler could be periodically disinfected by germicidal solutions (i.e. sodium hypochlorite: Amuchina with recommended concentrations) after disassembling its components by unscrewing the rings.

SAMPLING OF THE ALVEOLAR AIR

The fieldworker should execute 2-3 refilling – emptying operations of air in the Bio-Voc sampler, then unscrew the green plunger and put the disposable mouthpiece.

It's necessary to explain to the patient that he/she has to hold the Bio-Voc Sampler and to execute a deep inspiration and then to breath out continuously and gently into the mouthpiece, until the lungs are empty: the fieldworker need to capture the end tidal air which consists in alveolar air. In case of error or doubt, You can repeat the sampling in the same vial.

During the expiration of the patient the fieldworker has to lean the glass vial against the opposite part of the Bio-Voc sampler and make to get in the vial the expired air with an angle of 120-150°. (see below).



Straight after the end of expiration the vial must be plugged up with Teflon cap, which will well-tightened by the crimper . To verify if vial has been sealed try to unscrew the cap: it could be fixed; otherwise repeat the sealing operation. (Sometimes the electronic crimper needs to be set up for tightening pushing the button + - on the handle of the crimper).

The samples collected have to be stored in freezer until analysis, at -18/20°C.

2.6 Exhaled nitric oxide protocol



Genes **E**nvironment **I**nteraction on
Respiratory **D**iseases

THE GEIRD PROJECT

EXHALED NITRIC OXIDE (FE_{NO}) PROTOCOL

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EXHALED NITRIC OXIDE (FENO)

BACKGROUND:

Exhaled nitric oxide (FENO) is reliable non-invasive marker of airway inflammation^[1] and it has been demonstrated to be elevated in several airway diseases such as asthma^[2], eosinophilic bronchitis^[3], allergic rhinitis^[4] and chronic rhinosinusitis with and without nasal polyps^[5]. In the last 15 years FENO has emerged as a potentially useful clinical tool being successfully used to guide asthma treatment^[6] and demonstrating to have an excellent negative predictive value for the diagnosis of asthma both in clinical settings^[4] and in epidemiological studies^[7]. Several epidemiological studies have confirmed that FENO levels are raised in atopic subjects, whether or not they have significant lower respiratory tract symptoms^[8], and during acute airway infections, while it is decreased by the influence of tobacco smoke^[9] and inhaled^[10] and oral corticosteroid^[11].

OPERATIVE PROTOCOL:

All selected subjects will undergo to FENO measurement accordingly to international guidelines^[12] using a chemiluminescence analyser or a hand held device^[13]

In details:

- a. **Inhalation phase:** the patient should be seated comfortably, with the mouthpiece at the proper height and position. A nose clip should not be used, because this may allow nasal NO to accumulate and promote leakage of this NO via the posterior nasopharynx. However, if a subject cannot avoid nasal inspiration or nasal exhalation, a nose clip may be used. The patient inserts a mouthpiece and inhales over 2 to 3 seconds through the mouth to total lung capacity (TLC), or near TLC if TLC is difficult, and then exhales immediately, because breath holding may affect FENO. TLC is recommended because this is the most constant point in the respiratory cycle and patients accustomed to spirometry are familiar with inhaling to this volume.
- b. **Exhalation phase:** Three factors are critical in ensuring reproducible and standardized measurements of lower respiratory tract exhaled NO: (1) exclusion of nasal NO, (2) standardization of exhalation flow rate and (3) exclusion of environmental NO.
 - i. **Exclusion of nasal NO:** closure of the velopharyngeal aperture during exhalation is one way to minimize the nasal NO leakage. This can be achieved by exhaling against an expiratory resistance with subjects asked to maintain a positive mouthpiece pressure. It is common practice to display pressure or expiratory flow rate to the subject, who is requested to maintain these within a certain range. The resultant mouthpiece pressure should be at least 5 cm H₂O to ensure velum closure and exclude contamination of the expirate with nasal NO. However, a pressure above 20 cm H₂O should be avoided because this may be uncomfortable for patients to maintain.
 - ii. **Recommended respiratory flow rate:** A flow rate of 0.05 L/second is a reasonable compromise between measurement sensitivity and patient comfort. There are now reports that this flow rate is acceptable to children and adults, and reproducible. A constant expiratory flow rate can be achieved in different ways. One commonly used method to achieve a constant expiratory flow rate is by displaying a target mouthpiece pressure or flow rate

to the subject (e.g., using a gauge or computer display), while the subject exhales via a fixed expiratory resistance. In general, an exhalation is deemed adequate if the mean exhalation flow rate is 0.05 L/second ($\pm 10\%$) during the time of the NO plateau generation, and instantaneous flow rate is not less than 0.045 L/second or greater than 0.055 L/second at any time during the exhalation.

- iii. **Exclusion of environmental NO:** to obtain it, repeat the points *i.* and *ii.* of this protocol for three complete respiratory cycles asking the patient to breathe uniquely in the breathing-circuit of the machine (which has a NO-filter that provides NO-free air).

Interpretation of FE_{NO} results:

Constant flow rate exhalations, however achieved, result in a single-breath NO profile (exhaled NO vs. time plot) that consists of a washout phase followed by an NO plateau.

The duration of exhalation must be sufficient (at least 4 seconds for children < 12 years and > 6 seconds for children > 12 years and adults). This corresponds to an exhaled volume of at least 0.3 L in adults at an exhalation flow rate of 0.05 L/second to allow the airway compartment to be washed out and a reasonable plateau achieved. In general, patients can exhale comfortably up to 10 seconds, and this may be necessary for the achievement of a stable NO plateau. The plateau concentration in NO should be evaluated over a 3-second (0.15 L) window of the exhalation profile.

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2.7 Methods for blood sampling and genetic analyses

THE GEIRD PROJECT

METHODS FOR BLOOD SAMPLING AND CONSERVATION FOR MOLECULAR GENETIC ANALYSES

1) ALL THE SUBJECTS: SAMPLES FOR DNA ANALYSIS

Collect 20ml of peripheral whole blood in 1 or more tubes containing an anticoagulant solution (e.g. 3 EDTA-K2 Vacutainer tubes yellow cap, 6 ml each).

After blood collection, gently invert the tubes 8-10 times to avoid clots that would make the DNA extraction difficult, and store the tubes immediately at -20°C.

It is possible to keep the samples at -80°C.

Do NOT use polystyrene tubes-holders for storage.

Absolutely avoid the use of HEPARIN, as it inhibits the enzymes used for DNA analysis.

2) 50 RANDOM SUBJECTS: 13 WITH MILD ASTHMA , 13 WITH SEVERE ASTHMA RESISTANT TO THE TREATMENT, 13 WITH COPD, 11 CONTROLS.

2.1) BLOOD SAMPLES FOR RNA ANALYSIS:

Collect 5 ml of blood in 2 tubes of 2.5ml volume containing a specific additive to preserve RNA from degradation.

Gently invert the tubes 8-10 times. Storage conditions depend on the kind of additive * and are fundamental to guarantee the integrity of the sample.

RNA is very delicate and it is necessary to avoid samples' thawing, even partially, during storage and shipment to the laboratory.

*SAMPLES TUBES

PAXgene Blood RNA tube (cod. 762165; PreAnalytiX- QIAGEN/BD Company). Follow instructions about PAXgene-Blood RNA tubes in *How to collect blood with PAXgene tube*.

Store the samples at room temperature for at least 2 hrs (max 24 hrs) to inactivate RNAase before freezing at -20°C.

It is possible to store the tubes at -80°C, but only after 24hrs at -20°C. Freezing the tubes directly at -80°C may damage the tubes and lose the sample.

Don't use polystyrene tubes-holders to storage the tubes.

2.2 BLOOD SAMPLES FOR PROTEIN ANALYSIS

Collect 6ml of blood in EDTA tubes (e.g. Vacutanier Purple or lavender cap tubes, do not use Heparin) to obtain at least 2ml of plasma.

Gently invert the tube several times and leave it at +4°C for a maximum of 20 minutes. Spin at 2000 x g +4°C for 10 minutes.

Aliquot plasma in 2 ml sterile tubes (0.2 ml plasma/each tube) and immediately freeze at -80°C.

Keep also the tube containing the residual cellular fraction. Freeze it at -20°C (it also possible to store it at -80°C, as blood samples for DNA analysis).

Don't use polystyrene tubes-holders to storage the tube containing the cellular fraction.

FOR ALL THE SAMPLES:

Use preferentially 20-21G needles to avoid blood cell damage.

In order to perform all the sampling at the same time it is possible to use a Vacutainer system with the same plastic holder for each subject and connecting the tubes in the following order:

- a) Tubes without anticoagulant (e.g. in Verona : haemochrome and serology tubes)
- b) 1 or more Vacutainer yellow cap (for DNA. In Verona 3 tubes, 6 ml each)
- c) 1 or more Vacutainer purple or lavender cap (for Proteins. In Verona 1 tube, 6 ml)
- d) 2 PAXgene RNA tubes (for RNA)

The PAXgene tube will never be the first to be used in order to avoid to lose the blood corresponding to the air volume in the butterfly needle connecting tube.

When possible, use polypropylene tubes that better resists freezing.

SHIPMENT OF SAMPLES TO THE LABORATORIES

Blood samples must arrive frozen to the laboratory; therefore, thawing should be avoid during the trip.

All the samples should be frozen at -20°C and shipped on dry ice.

Please advise laboratory before shipment so that we can monitor the arrival of samples.

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3 Questionnaires

According to the project official homepage, we provide for each subsequent section a copy of the official questionnaires as they appear on-line.

3 Questionnaires

3.1 Screening questionnaire of the cross-sectional survey



Università degli Studi
di Verona

ITALIAN SURVEY ON RESPIRATORY DISEASES



Azienda Ospedaliera
"Istituti Ospitalieri di Verona"

The confidentiality of the data you have provided is protected according to the existing laws (Law by Decree n° 196, 30 June 2003).

We remind you that the data you have provided will be handled by health personnel who must keep professional secrecy and will be stored with maximum security. The data you have provided will be anonymously transferred into a computer.

However we inform you that the questionnaire contains personal information that could allow identification of the interviewed, even in the absence of name and surname.

For those reasons at any time you have the right to access your data and/or ask for their modification or cancellation, writing to the following address:

If you don't mind being contacted by one of the study team for the purpose of the investigation we would be grateful if you could give us your telephone number and/or e-mail

Telephone number *home* _____

work _____

mobile phone _____

E-mail: _____

Please be sure you have answered all the questions and check
you have written your birth date properly.
Please mail back this questionnaire using pre-paid envelope attached

Thank you for your kind cooperation.



Università degli Studi
di Verona

ITALIAN SURVEY ON RESPIRATORY DISEASES



Azienda Ospedaliera
"Istituti Ospitalieri di Verona"

*To answer the questions please choose the appropriate box
If you are unsure of the answer please check 'no'*

What is today's date:

| | | | |
|-----|-------|------|--|
| | | | |
| day | month | year | |

What is your date of birth?

| | | | |
|-----|-------|------|--|
| | | | |
| day | month | year | |

Sex: ☐ male ☐ female

Are you currently:

- | | |
|-----------------------------------------|-----------------------------------------------------------------|
| <input type="checkbox"/> 1 housewife | <input type="checkbox"/> 6 self-employed person |
| <input type="checkbox"/> 2 manager | <input type="checkbox"/> 7 workman |
| <input type="checkbox"/> 3 unemployed | <input type="checkbox"/> 8 retired → previous profession: _____ |
| <input type="checkbox"/> 4 clerk | <input type="checkbox"/> 9 student |
| <input type="checkbox"/> 5 entrepreneur | <input type="checkbox"/> 10 other: _____ |

What is your
educational level?

- | | | | |
|-------------------------------------------------------|---------------------------------------------------|------------------------------------------------|----------------------------------------------|
| <input type="checkbox"/> 1 Elementary school graduate | <input type="checkbox"/> 2 Middle school graduate | <input type="checkbox"/> 3 High school diploma | <input type="checkbox"/> 4 University degree |
|-------------------------------------------------------|---------------------------------------------------|------------------------------------------------|----------------------------------------------|

How many years have you lived in your current home? years

(please write 0 if you have lived in your current home for less than one year)

What term best describes
the place you live?:

- | | | |
|---------------------------------------|---------------------------------------------|----------------------------------------------------|
| <input type="checkbox"/> 1 inner city | <input type="checkbox"/> 2 suburb of a city | <input type="checkbox"/> 3 village in a rural area |
|---------------------------------------|---------------------------------------------|----------------------------------------------------|

Are there any factories near your home?

☐ No ☐ Yes

How often do cars
pass your house?

- | |
|---------------------------------------|
| <input type="checkbox"/> 1 constantly |
| <input type="checkbox"/> 2 frequently |
| <input type="checkbox"/> 3 seldom |
| <input type="checkbox"/> 4 never |

How often do heavy vehicles
(e.g. trucks/ buses) pass your house?

- | |
|---------------------------------------|
| <input type="checkbox"/> 1 constantly |
| <input type="checkbox"/> 2 frequently |
| <input type="checkbox"/> 3 seldom |
| <input type="checkbox"/> 4 never |

1. Have you had wheezing or whistling in your chest at any time in the last 12 months? ☐ No ☐ Yes
(wheezing means a whistling sound, however high or low pitched and however faint)
If 'NO' go to question 2, if 'Yes':

1.1 How many times did you have wheezing or whistling in your chest in the last 12 months?
 sometimes at least once a week every day

1.2 Have you been at all breathless when the wheezing noise was present? ☐ No ☐ Yes

1.3 Have you had this wheezing or whistling when you did not have a cold? ☐ No ☐ Yes

2. Have you woken up with a feeling of tightness in your chest at any time in the last 12 months? ☐ No ☐ Yes

3. Have you been woken by an attack of shortness of breath at any time in the last 12 months? ☐ No ☐ Yes

4. Have you had an attack of asthma in the last 12 months? ☐ No ☐ Yes

5. Are you currently taking any medicines for asthma?
(including inhalers, aerosols or tablets) ☐ No ☐ Yes

6. Have you ever had asthma? ☐ No ☐ Yes

If 'NO' go to question 7, if 'Yes':

6.1 Was this confirmed by a doctor? ☐ No ☐ Yes

6.2 How old were you when you had your first attack of asthma? years

6.3 How old were you when you had your most recent attack of asthma? years

6.4 How many attacks of asthma have you had in the last 12 months? number of attacks of asthma

6.5 Have you used any medicines for asthma in the last 12 months (including inhalers, aerosols or tablets)? ☐ No ☐ Yes

7. Do you have any nasal allergies including hay fever? ☐ No ☐ Yes

8. Have you ever had a problem with sneezing, or a runny or a blocked nose when you did not have a cold or the flu? ☐ No ☐ Yes

If 'NO' to question 7 and 8 go to question 9, if 'Yes' to at least one:

8.1 How old were you when you first had this nose problem? years

8.2 Do you still have this nose problem? ☐ Yes ☐ No → 8.2.1 How old were you when this nose problem disappeared? years

8.3 Do you mainly suffer from: runny nose blocked nose

8.4 Have you used any medicines for rhinitis in the last 12 months (including inhalers, aerosols or tablets)? ☐ No ☐ Yes

8.5 Do you suffer from sinusitis? ☐ No ☐ Yes

8.6 Do you suffer from nasal polyps? ☐ No ☐ Yes

9. Have you ever had eczema or any kind of skin allergy, confirmed by a doctor? ☐ No ☐ Yes

If 'NO' go to question 10, if 'Yes':

9.1 How old were you when you first had these problems? years

9.2 Do you still have these problems? ☐ Yes ☐ No → 9.2.1 How old were you when these problem disappeared? years

10. Have you had coughing and phlegm on most days for a minimum of 3 months a year and for at least 2 successive years? ☐ No ☐ Yes

If 'NO' go to question 11, if 'Yes':

10.1 How many years have you been suffering from these problems? years

11. Have you ever been told by a doctor that you have or had chronic bronchitis, chronic obstructive pulmonary disease (COPD) or emphysema? ☐ No ☐ Yes

12. Do you walk slower than contemporaries on level ground because of breathlessness, or do you have to stop for breath when walking at own pace? ☐ No ☐ Yes

13. Have you ever smoked for as long as a year?
(yes' means at least one cigarette per day or one cigar a week for one year) ☐ No ☐ Yes

If 'NO' go to question 14, if 'Yes':

13.1 How old were you when you started smoking? years

13.2 Do you now smoke, as of one month ago? ☐ Yes ☐ No → 13.2.1 How old were you when you stopped smoking? years

13.3 On average, how much do you smoke or did you smoke? number of cigarettes per day

14. Have you visited an emergency room at least once or have you spent at least one night as an inpatient in hospital because of breathing problems in the last 10 years? ☐ No ☐ Yes

15. Have you visited an emergency room at least once or have you spent at least one night as an inpatient in hospital in the last 3 months? (for any reason, excluding accidents and work injuries) ☐ No ☐ Yes

If 'NO' go to question 16, if 'Yes':

15.1 Was this due to breathing problems? ☐ No ☐ Yes

16. If you are currently working, how many days of work have you lost because of health problems in the last 3 months?(excluding accident and work injuries) (write 0 if you did not lose days of work)

If you lost at least one day of work:

16.1 How many days of work have you lost because of breathing problems in the last 3 months? (write 0 if you did not lose days of work)

17. Whatever your working situation, how many days have you had to give up activities other than work (e.g. looking after children, the house, studying) because of health problems in the last 3 months? (excluding accidents and work injuries) (write 0 if you did not lose any day)

If You lost at least one day:

17.1 How many days have you had to give up activities other than work (e.g looking after children, the house, studying) because of breathing problems in the last 3 months? (write 0 if you did not lose any day)

3.2 GEIRD clinical questionnaire (all cohorts)

THE GEIRD PROJECT

Genes Environment Interaction on Respiratory Diseases

Clinical Questionnaire

Researchers using these materials are requested to inform the GEIRD Coordinating Centre and cite the source appropriately. For further information and contacts, please visit the GEIRD web site at the following URL:
www.geird.org



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 YEAR

0.6 FOR THE INTERVIEWER: Please indicate the sex of interviewee.

0.7 When were you born?

0.8 What is the address of the house in which you live?

City: _____

Street / Square / Avenue / Etc.: _____

House Number:

| | | | |
|--|--|--|--|
| | | | |
|--|--|--|--|

 Zip Code:

| | | | | |
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 Province/District:

| | |
|--|--|
| | |
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THIS INFORMATION IS NEEDED IN ORDER TO HAVE A PRECISE GEOGRAPHICAL REFERENCE THAT IDENTIFIES THE AREA OF RESIDENCE.

1. Have you had wheezing or whistling in your chest at any time in the last **12 months?** ('Wheeze' can be described as 'A whistling sound, whether high or low pitched and however faint')
- IF 'NO' GO TO QUESTION 2, IF 'YES':**

NO YES

- | | | | |
|------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------|-------------------------------------------------------------|--------------------------------------|
| 1.1 How many times have you had wheezing or whistling in the last 12 months ? | Sometimes <input type="checkbox"/> | One time a week <input type="checkbox"/> | Everyday <input type="checkbox"/> |
| 1.2 Have you been at all breathless when the wheezing noise was present? | | NO YES <input type="checkbox"/> <input type="checkbox"/> | |
| 1.3 Have you had this wheezing or whistling also when you did not have a cold? | | NO YES <input type="checkbox"/> <input type="checkbox"/> | |
| 2. Have you woken up with a feeling of tightness in your chest at any time in the last 12 months ? | | NO YES <input type="checkbox"/> <input type="checkbox"/> | |
| 3. Have you had an attack of shortness of breath that came on during the day when you were at rest at any time in the last 12 months ? | | NO YES <input type="checkbox"/> <input type="checkbox"/> | |
| 4. Have you had an attack of shortness of breath that came on following strenuous activity at any time in the last 12 months ? | | NO YES <input type="checkbox"/> <input type="checkbox"/> | |
| 5. Have you been woken by an attack of shortness of breath at any time in the last 12 months ? | | NO YES <input type="checkbox"/> <input type="checkbox"/> | |

IF 'NO' GO TO QUESTION 6, IF 'YES':

5.1 Have you been woken by an attack of shortness of breath in the last **3 months**?

NO YES
☐ ☐

IF 'NO' GO TO QUESTION 6, IF 'YES':

5.1.1 **On average** have you been woken by an attack of shortness of breath **at least once a week** in the last **3 months**?

NO YES
☐ ☐

IF 'NO' GO TO QUESTION 6, IF 'YES':

5.1.1.1 How many times a week **on average** have you been woken by shortness of breath in the last **3 months**?

TIMES

6. Have you been woken by an attack of coughing at any time in the last **12 months**?

NO YES
☐ ☐

7. Do you **usually** cough first thing in the morning in the winter?

NO YES
☐ ☐

[IF 'YES' OR DOUBTFUL, USE QUESTION 8.1 TO CONFIRM]

8. Do you **usually** cough during the day, or at night, in the winter?

NO YES
☐ ☐

IF 'NO' GO TO QUESTION 9, IF 'YES':

8.1 Do you cough like this on most days for as much as three months each year?

NO YES
☐ ☐

IF 'NO' GO TO QUESTION 9, IF 'YES':

8.1.1 How many years have you been affected by this problem?

YEARS

9. Do you **usually** bring up any phlegm from your chest first thing in the morning in the winter?

NO YES
☐ ☐

[IF 'YES' OR DOUBTFUL, USE QUESTION 10.1 TO CONFIRM]

10. Do you **usually** bring up any phlegm from your chest during the day, or at night, in the winter?

NO YES
☐ ☐

IF 'NO' GO TO QUESTION 11, IF 'YES':

10.1 Do you bring up phlegm like this on most days for as much as three months each year?

NO YES
☐ ☐

IF 'NO' GO TO QUESTION 11, IF 'YES':

10.1.1 How many years have you been affected by this problem?

YEARS

11. Do you ever have trouble with your breathing?

NO YES
☐ ☐

IF 'NO' GO TO QUESTION 12, IF 'YES':

11.1 Do you have this trouble:

- A) continuously so that your breathing is never quite right?
B) repeatedly, but it always gets completely better?
C) only rarely?

TICK ONE BOX ONLY

1 ☐
2 ☐
3 ☐

12. Are you disabled from walking by a condition **other than** heart or lung disease?

NO YES
☐ ☐

IF 'YES': STATE CONDITION (12.0) _____

**IF THE INTERVIEWEE HAS NEGATIVELY ANSWERED TO ALL QUESTIONS 1-11,
GO TO QUESTION 14,
OTHERWISE (AT LEAST ONE POSITIVE ANSWER TO QUESTIONS 1-11)
GO TO QUESTION 13**

IF 'NO':

12.1 Are you troubled by shortness of breath when hurrying on level ground or walking up a slight hill?

NO YES
☐ ☐

**IF 'NO' AND THE INTERVIEWEE HAS NEGATIVELY ANSWERED TO ALL QUESTIONS 1-11,
GO TO QUESTION 14,**

**IF 'NO' AND THE INTERVIEWEE HAS POSITIVELY ANSWERED AT LEAST
TO ONE QUESTION FROM 1 TO 11, GO TO QUESTION 13**

IF 'YES':

12.1.1 Do you get short of breath walking with other people of
your own age on level ground?

NO YES
☐ ☐

IF 'NO' GO TO QUESTION 13, IF 'YES':

12.1.1.1 Do you have to stop for short of breath when walking at
your own pace on level ground?

NO YES
☐ ☐

13. In the last **12 months**, have you had any episodes/times when
your symptoms (cough, phlegm, shortness of breath) were
a lot worse than usual?

NO YES
☐ ☐

IF 'NO' GO TO QUESTION 14 IF 'YES':

In the last **12 months**:

13.1 How many times have these episodes occurred?

TIMES

13.2 How many times have these episodes forced you to consult your doctor?

13.3 How many times was your therapy changed after these episodes?

13.4 How many times have you visited a hospital casualty department or emergency room
or have you spent a night in hospital after these episodes?

14. FOR WOMEN ONLY – FOR MEN GO TO QUESTION 15

Have you ever noticed that you had respiratory symptoms (such as wheeze, tightness in your
chest or shortness of breath) at a particular time of your monthly cycle?

TICK ONE BOX ONLY

- A) Yes, in the week before my period
- B) Yes, during my period
- C) Yes, in the week after my period
- D) Yes, another time of the month
- E) Does not apply to me (i.e., amenorrhoeal)
- F) No

1 ☐
2 ☐
3 ☐
4 ☐
5 ☐
6 ☐

15.1-3 Has a doctor ever said that you have or have had:

15.1 Chronic bronchitis?

15.2 COPD (Chronic Obstructive Pulmonary Disease)?

15.3 Emphysema?

NO YES
☐ ☐
☐ ☐
☐ ☐

16. Have you ever had asthma?

NO YES
☐ ☐

IF 'NO' GO TO QUESTION 17, IF 'YES'

**PLEASE GIVE AND MAKE THE PEOPLE WHO DECLARED THAT THEY HAVE HAD
ASTHMA DURING THEIR LIFETIME FILL IN THE ASHTMA CONTROL TEST (QUESTION 16)**

16.1 Was this confirmed by a doctor?

NO YES
☐ ☐

16.2 How old were you when you had your first attack of asthma?

YEARS

16.3 How old were you when you had your most recent attack of asthma?

YEARS

16.4.1-6 Which months of the year do you usually have attacks of asthma?

- 16.4.1 January / February
- 16.4.2 March / April
- 16.4.3 May / June
- 16.4.4 July / August
- 16.4.5 September / October
- 16.4.6 November / December

| NO | YES |
|--------------------------|--------------------------|
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |

16.5 Have you had one or more attacks of asthma in the last **12 months**?

IF 'NO' GO TO QUESTION 16.6, IF 'YES'

16.5.1 Did they occur in every month of the year?

IF 'NO' GO TO QUESTION 16.5.3, IF 'YES':

16.5.2 Were your asthma attacks more severe or frequent certain months of the year?

IF 'NO' GO TO QUESTION 16.5.4, IF 'YES':

16.5.3.1-12 In which months?

- 16.5.3.1 January
- 16.5.3.2 February
- 16.5.3.3 March
- 16.5.3.4 April
- 16.5.3.5 May
- 16.5.3.6 June
- 16.5.3.7 July
- 16.5.3.8 August
- 16.5.3.9 September
- 16.5.3.10 October
- 16.5.3.11 November
- 16.5.3.12 December

| NO | YES |
|--------------------------|--------------------------|
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |

| NO | YES |
|--------------------------|--------------------------|
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
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| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |

16.5.4 How many attacks of asthma have you had in the last **12 months**?

| | |
|---------|----------------------|
| ATTACKS | <input type="text"/> |
|---------|----------------------|

16.5.5 How many attacks of asthma have you had in the last **3 months**?

| | |
|---------|----------------------|
| ATTACKS | <input type="text"/> |
|---------|----------------------|

16.6 How many times have you woken up because of your asthma in the last **3 months**?

- A) every night or almost every night
- B) more than once a week, but not most nights
- C) at least twice a month, but not more than once a week
- D) less than twice a month
- E) not at all

TICK ONE BOX ONLY

| | |
|---|--------------------------|
| 1 | <input type="checkbox"/> |
| 2 | <input type="checkbox"/> |
| 3 | <input type="checkbox"/> |
| 4 | <input type="checkbox"/> |
| 5 | <input type="checkbox"/> |

16.7 How often have you had trouble with your breathing because of your asthma in the last **3 months**?

- A) Continuously
- B) about once a day
- C) at least once a week, but less than once a day
- D) Less than once a week
- E) Not at all

TICK ONE BOX ONLY

| | |
|---|--------------------------|
| 1 | <input type="checkbox"/> |
| 2 | <input type="checkbox"/> |
| 3 | <input type="checkbox"/> |
| 4 | <input type="checkbox"/> |
| 5 | <input type="checkbox"/> |

16.8 Are you currently taking any medicines (including inhalers, aerosols or tablets) for asthma?

| NO | YES |
|--------------------------|--------------------------|
| <input type="checkbox"/> | <input type="checkbox"/> |

16.9 Do you have a peak flow meter of your own?

NO YES
☐ ☐

IF 'NO' GO TO QUESTION 16.10, IF 'YES':

16.9.1 How often have you used it over the last **3 months**?

- A) Never
B) some of the days
C) most of the days

TICK ONE BOX ONLY

1 ☐
2 ☐
3 ☐

16.10 Do you have written instructions from your doctor on
how to manage your asthma if it gets worse or if you have an attack?

NO YES
☐ ☐

16.11 **FOR WOMEN ONLY - MEN GO TO QUESTION 17**

Have you ever noticed that your asthma got worse with your monthly cycle?

- A) Yes, in the week before my period
B) Yes, during my period
C) Yes, in the week after my period
D) Yes, another time of the month
E) Does not apply to me (i.e., amenorrhoeal)
F) No

TICK ONE BOX ONLY

1 ☐
2 ☐
3 ☐
4 ☐
5 ☐
6 ☐

16.12 Have you been pregnant (at least 25 weeks) since your asthma started?

NO YES
☐ ☐

IF 'NO' GO TO QUESTION 17, IF 'YES':

16.12.1. What happened to your asthma during your pregnancies?

- A) got better
B) got worse
C) stayed the same
D) not the same for all pregnancies
E) Don't know

TICK ONE BOX ONLY

1 ☐
2 ☐
3 ☐
4 ☐
5 ☐

17. Do you have any nasal allergies including hay fever?

NO YES
☐ ☐

IF 'NO' GO TO QUESTION 18, IF 'YES':

17.1 How old were you when you first had hay fever or nasal allergy?

YEARS

NOW GO TO QUESTION 19

18. ***During your lifetime*** have you ever had any nasal allergies including hay fever?

NO YES
☐ ☐

IF 'NO' GO TO QUESTION 19, IF 'YES':

18.1 How old were you when you first had hay fever or nasal allergy?

YEARS

18.2 How old were you when you had hay fever or nasal allergy for the last time?

YEARS

19. Have you ever had a problem with sneezing, or a runny or a blocked nose when you
did not have a cold or the flu?

NO YES
☐ ☐

**IF THE INTERVIEWEE REPLIED 'NO' TO QUESTION 17 AND 'NO' TO QUESTION 19,
GO TO QUESTION 21 (NASAL POLYPOSIS)**

**IF THE INTERVIEWEE REPLIED 'YES' TO QUESTION 17 AND 'NO' TO QUESTION 19,
GO TO QUESTION 20**

IF 'YES' (TO QUESTION 19):

19.1 Have you had a problem with sneezing, or a runny or a blocked nose when you
did not have a cold or the flu in the last **12 months**?

NO YES
☐ ☐

**IF 'NO' TO QUESTION 17 AND 'NO' TO QUESTION 19.1,
GO TO QUESTION 21 (NASAL POLYPOSIS)**

OTHERWISE:

20.1-9 Which of these symptoms occurred in the last **12 months**?

- 20.1 Blocked nose (both nostrils)
- 20.2 Now one blocked nostril, now the other one
- 20.3 Dripping nose (watery mucus)
- 20.4 Mucus dripping / phlegm from the nose
- 20.5 Mucus dripping / phlegm from the nose into the throat
- 20.6 Sneezes
- 20.7 Nose itch
- 20.8 Smell reduction or complete smell loss
- 20.9 Facial ache or forehead ache

| NO | YES |
|--------------------------|--------------------------|
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |

20.10.1-2 Has/have this/these nose problem/s been accompanied by:

- 20.10.1 Itchy or watery eyes?
- 20.10.2 Itchy throat or palate?

| NO | YES |
|--------------------------|--------------------------|
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |

20.11 In which months of the year did this/these nose problem/s occur?

- 20.11.1 January
- 20.11.2 February
- 20.11.3 March
- 20.11.4 April
- 20.11.5 May
- 20.11.6 June
- 20.11.7 July
- 20.11.8 August
- 20.11.9 September
- 20.11.10 October
- 20.11.11 November
- 20.11.12 December

| NO | YES |
|--------------------------|--------------------------|
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |

20.12 Did your problems occur for more than 4 days a week and for more than 4 consecutive weeks in the last **12 months**?

| NO | YES |
|--------------------------|--------------------------|
| <input type="checkbox"/> | <input type="checkbox"/> |

20.13 How much did hay fever or nasal problems limit your abilities in each of these fields in the last **12 months**?

| | Not at all | Not so much | Moderately | Very much |
|-----------------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| 20.13.1 Sport and recreation activities | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 20.13.2 Work or school attendance | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 20.13.3 Friendships | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 20.13.4 Sleeping at night | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 20.13.5 Other daily activities | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

21. Have you ever suffered from nasal polyps?

| NO | YES |
|--------------------------|--------------------------|
| <input type="checkbox"/> | <input type="checkbox"/> |

IF 'NO' GO TO QUESTION 22, IF 'YES':

NO ONCE MORE THAN
ONE TIME

21.1 Have you been operated to remove nasal polyps?

| | |
|--------------------------|--------------------------|
| <input type="checkbox"/> | <input type="checkbox"/> |
|--------------------------|--------------------------|

☐

22. Have you ever had sinusitis?

| NO | YES |
|--------------------------|--------------------------|
| <input type="checkbox"/> | <input type="checkbox"/> |

IF 'NO'
FOR THE SUBJECTS OF:
THE SARA COHORT.

THE ECRHS COHORT THAT DID NOT PARTICIPATE IN ECRHS II.
THE ISAYA COHORT THAT DID NOT ANSWER TO THE TELEPHONE INTERVIEW ON ANTI-ASTHMATIC DRUGS
GO TO QUESTION 23.G,

IF 'NO'
FOR THE SUBJECTS OF:
THE ECRHS II COHORT.
THE ISAYA COHORT (CURRENT DIAGNOSED ASTHMA) THAT ANSWERED TO THE TELEPHONE INTERVIEW
ON ANTI-ASTHMATIC DRUGS
GO TO QUESTION 23.E,

IF 'YES':

| | | |
|------------------------------------------------------------|--------------------------|--------------------------|
| | NO | YES |
| 22.1 Was this confirmed by a doctor? | <input type="checkbox"/> | <input type="checkbox"/> |
| | NO | SI |
| 22.2 Have you had sinusitis in the last 12 months ? | <input type="checkbox"/> | <input type="checkbox"/> |

ONLY FOR THE SUBJECTS OF:
THE SARA COHORT.
THE ECRHS COHORT THAT DID NOT PARTICIPATE IN ECRHS II.
THE ISAYA COHORT THAT DID NOT ANSWER TO THE TELEPHONE
INTERVIEW ON ANTI-ASTHMATIC DRUGS:

| | | |
|-----------------------------------------------------------------------|--------------------------|--------------------------|
| 23.G Have you ever used any medication to treat your nasal disorders? | NO | YES |
| | <input type="checkbox"/> | <input type="checkbox"/> |

IF 'NO' GO TO QUESTION 24, IF 'YES':

23.1-2.G Have you used any of the following nasal medicines (e.g. nasal sprays, inhaled powders or drops) for the treatment of your nasal disorders?
[SHOW LIST OF STEROID/VASOCONSTRICTOR NASAL MEDICINES]

| | | | |
|---------------------------------------------|---------------------------------------------------|----------------------------------------------------------|---------------------------------------------------------|
| | | How many years have you been taking them? | Have you used them in the last 12 months ? |
| | NO YES | YEARS | NO YES |
| 23.1.G Steroids | <input type="checkbox"/> <input type="checkbox"/> | IF YES <input type="checkbox"/> <input type="checkbox"/> | <input type="checkbox"/> <input type="checkbox"/> |
| 23.2.G Decongestionnant Vasoconstrictors | <input type="checkbox"/> <input type="checkbox"/> | IF YES <input type="checkbox"/> <input type="checkbox"/> | <input type="checkbox"/> <input type="checkbox"/> |

| | | |
|-----------------------------------------------------------------------------------------------------------------|--------------------------|--------------------------|
| 23.3.G Have you used any of the following pills, capsules or tablets for the treatment of your nasal disorders? | NO | YES |
| | <input type="checkbox"/> | <input type="checkbox"/> |

[SHOW LIST OF ANTIHISTAMINES]

IF 'NO' GO TO QUESTION 24, IF 'YES':

| | |
|-------------------------------------------------------------------------------------------|---------------------------------------------------|
| 23.3.1.G How many years have you been taking these sort Of pills, capsules or tablets? | YEARS |
| | <input type="checkbox"/> <input type="checkbox"/> |

| | | |
|-------------------------------------------------------------------------------------------------------|--------------------------|--------------------------|
| 23.3.2.G Have you used any of the following pills, capsules or tablets in the last 12 months ? | NO | YES |
| | <input type="checkbox"/> | <input type="checkbox"/> |

ONLY FOR THE SUBJECTS OF:

THE ECRHS II COHORT,

THE ISAYA COHORT (CURRENT DIAGNOSED ASTHMA) THAT ANSWERED TO THE TELEPHONE INTERVIEW
ON ANTI-ASTHMATIC DRUGS:

23.E Since the last survey have you used any medication to treat your nasal disorders? NO YES
☐ ☐

IF 'NO' GO TO QUESTION 24, IF 'YES':

23.1-2.E Have you used any of the following nasal medicines (e.g. nasal sprays, inhaled powders or drops) for the treatment of your nasal disorders?

[SHOW LIST OF STEROID/VASOCONSTRICTOR NASAL MEDICINES]

| | NO | YES | How many years have you been taking them? | YEARS | Have you used them in the last 12 months? |
|---------------------------------------------|--------------------------|--------------------------|-------------------------------------------------|--------------------------|-------------------------------------------------------------|
| | <input type="checkbox"/> | <input type="checkbox"/> | | <input type="checkbox"/> | NO YES <input type="checkbox"/> <input type="checkbox"/> |
| 23.1.E Steroids | <input type="checkbox"/> | <input type="checkbox"/> | IF YES | <input type="checkbox"/> | <input type="checkbox"/> |
| 23.2.E Decongestionnant vasoconstrictors | <input type="checkbox"/> | <input type="checkbox"/> | IF YES | <input type="checkbox"/> | <input type="checkbox"/> |

23.3.E Have you used any of the following pills, capsules or tablets for the treatment of your nasal disorders? NO YES
☐ ☐

[SHOW LIST OF ANTIHISTAMINES]

IF 'NO' GO TO QUESTION 24, IF 'YES':

23.3.1.E How many years have you been taking these sort of pills, capsules or tablets? YEARS
☐

23.3.2.E Have you used any of the following pills, capsules or tablets in the last **12 months?** NO YES
☐ ☐

24. Have you **ever** had eczema or any kind of skin allergy? NO YES
☐ ☐

IF 'NO' GO TO QUESTION 25, IF 'YES':

24.1 Was this confirmed by a doctor? NO YES
☐ ☐

24.2 How old were you when you had your first disorders? YEARS
☐

24.3 Do you still suffer from this? NO SI
☐ ☐

IF 'YES' GO TO QUESTION 25, IF 'NO':

24.3.1 How old were you when they disappeared? YEARS
☐

25. Have you **ever** had an itchy rash that was coming and going **for at least 6 months?** NO YES
☐ ☐
IF 'NO' GO TO QUESTION 26, IF 'YES':

25.1 Have you had this itchy rash in the last **12 months?** NO YES
☐ ☐
IF 'NO' GO TO QUESTION 26, IF 'YES':

| | | |
|---------------------------------------------------------------------------------------|--------------------------|--------------------------|
| 25.1.1-7 Has this itchy rash at any time affected any of the following places: | NO | YES |
| 25.1.1 The folds of the elbows | <input type="checkbox"/> | <input type="checkbox"/> |
| 25.1.2 Behind the knees | <input type="checkbox"/> | <input type="checkbox"/> |
| 25.1.3 In front of the ankles | <input type="checkbox"/> | <input type="checkbox"/> |
| 25.1.4 Under the buttocks | <input type="checkbox"/> | <input type="checkbox"/> |
| 25.1.5 Around the neck | <input type="checkbox"/> | <input type="checkbox"/> |
| 25.1.6 Around the ears | <input type="checkbox"/> | <input type="checkbox"/> |
| 25.1.7 Around the eyes | <input type="checkbox"/> | <input type="checkbox"/> |

| | | |
|----------------------------------------------------------------------------------|--------------------------|--------------------------|
| 26. Have you ever had any difficulty with your breathing after taking medicines? | NO | SI |
| | <input type="checkbox"/> | <input type="checkbox"/> |

IF 'NO' GO TO QUESTION 27, IF 'YES':

| | |
|-------------------------------------|--------------------------|
| 26.1-2 Which medicines?(26.1) _____ | CODE |
| (26.2) _____ | <input type="checkbox"/> |
| | <input type="checkbox"/> |

| | | |
|------------------------------------------------------------------------------------------------------------------------------|--------------------------|--------------------------|
| 27. Have you ever had nasal disorders or swelling or skin soreness after taking aspirin or other antiinflammatory medicines? | NO | YES |
| | <input type="checkbox"/> | <input type="checkbox"/> |

| | | |
|---------------------------------------------------------------------|--------------------------|--------------------------|
| 28. Has a doctor ever told you that you have or have had: | NO | YES |
| 28.1 Gastritis or stomach ulcer (confirmed by a gastroscopy)? | <input type="checkbox"/> | <input type="checkbox"/> |
| 28.2 Gastroesophageal reflux disease, hiatal hernia or esophagitis? | <input type="checkbox"/> | <input type="checkbox"/> |
| 28.3 Osteoporosis? | <input type="checkbox"/> | <input type="checkbox"/> |
| 28.4 Gout? | <input type="checkbox"/> | <input type="checkbox"/> |
| 28.5 Arthritis or osteoarthritis? | <input type="checkbox"/> | <input type="checkbox"/> |
| 28.6 Pulmonary embolism? | <input type="checkbox"/> | <input type="checkbox"/> |

| | | |
|-----------------------------------------------------------------------------------|--------------------------|--------------------------|
| 29. During the last years have you been told more than once that you have: | NO | YES |
| 29.1 High triglycerides (dyslipidemia)? | <input type="checkbox"/> | <input type="checkbox"/> |
| 29.2 High cholesterol (hypercholesterolemia)? | <input type="checkbox"/> | <input type="checkbox"/> |

| | | |
|----------------------------------------------------------------------------------------------------|--------------------------|--------------------------|
| 30. Have you had any fracture not caused by road/work/sport accidents in the last 5 years ? | NO | YES |
| | <input type="checkbox"/> | <input type="checkbox"/> |

| | | |
|------------------------------------------------------------|--------------------------|--------------------------|
| 31. Have you ever been told that you snore when you sleep? | NO | YES |
| | <input type="checkbox"/> | <input type="checkbox"/> |

IF 'NO' GO TO QUESTION 32, IF 'YES':

| | | | | | |
|----------------------------------------------------------------------------------------------------------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| | Never | Seldom | Sometimes | Frequently | Every Time |
| 31.1 In the last 12 months have you been told that you stop breathing or have irregular breathing while you are sleeping? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 31.2 Have you woken up all of a sudden with a choking sensation or not being able to breathe in the last 12 months ? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 31.3 Have you ever that you snore loudly or that your snoring disturbs other people in the last 12 months ? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

| | | |
|-----------------------------------------------------------------|--------------------------|--------------------------|
| 32. Do you get a pain or discomfort in your legs when you walk? | NO | YES |
| | <input type="checkbox"/> | <input type="checkbox"/> |

IF 'NO' GO TO QUESTION 33, IF 'YES':

| | | |
|------------------------------------------------------------------------|--------------------------|--------------------------|
| 32.1 Does this pain ever begin when you are standing still or sitting? | NO | YES |
| | <input type="checkbox"/> | <input type="checkbox"/> |
| | NO | SI |

32.2 Do you get it if you walk uphill or hurry?

| | |
|--------------------------|--------------------------|
| <input type="checkbox"/> | <input type="checkbox"/> |
|--------------------------|--------------------------|

32.3 Do you get it if you walk at an ordinary pace on the level?

| | |
|--------------------------|--------------------------|
| NO | SI |
| <input type="checkbox"/> | <input type="checkbox"/> |

32.4 What happens to it if you stand still?

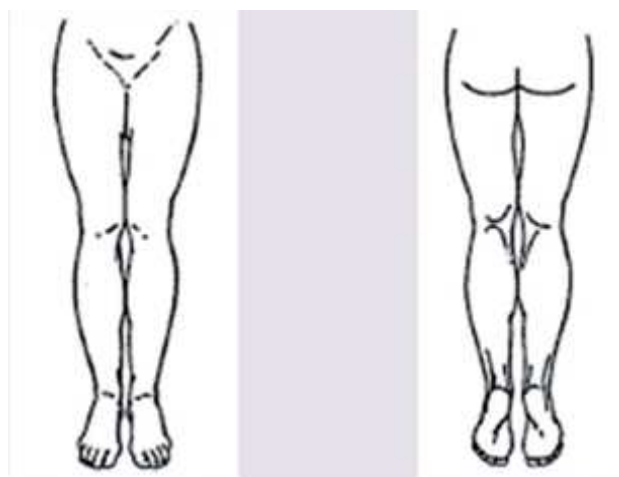
- A) Usually disappears in 10 minutes or less
B) Usually continues for more than 10 minutes

TICK ONE BOX ONLY

| | |
|---|--------------------------|
| 1 | <input type="checkbox"/> |
| 2 | <input type="checkbox"/> |

32.5.1-3 Where do you get this pain or discomfort?

Using the following picture, indicate where you get/feel the pain (more than one part if necessary).



FOR THE INTERVIEWER: FILL IN THE PARTS OF THE BODY AFFECTED BY PAIN

32.5.1 Calves

32.5.2 Thighs or buttocks

32.5.3 Hamstrings, joints, feet, legs or other parts of the body

| | |
|--------------------------|--------------------------|
| NO | YES |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |

33. Has a doctor ever told you that you have or have had:

33.1 A heart attack (coronary thrombosis)

33.2 Angina

33.3 Arrhythmia (e.g. atrial fibrillation)

33.4 Other heart problems (specify): _____

| | |
|--------------------------|--------------------------|
| NO | YES |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |

34. Have you ever had a heart or aorta operation?

IF 'NO' GO TO QUESTION 35, IF 'YES':

34.1-4 Have you ever undergone the following operations:

34.1 Aortocoronary bypass or coronary angioplasty

34.2 Pacemaker implant

34.3 Heart valves surgeries

34.4 Aortic aneurysm surgery

| | |
|--------------------------|--------------------------|
| NO | YES |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |

35. Have you ever had an ictus? NO YES
☐ ☐
(sometimes denominated as cerebral hemorrhage, cerebral thrombosis, subarachnoid hemorrhage, cerebrovascular accident, brain ischemia, transient ischemic attack)

36. Has your doctor ever told you that you have high blood pressure? NO YES
☐ ☐
IF 'NO' GO TO QUESTION 37, IF 'YES':
36.1 Are you taking any medicines for high blood pressure? NO YES
☐ ☐

IF 'NO' GO TO QUESTION 37, IF 'YES':

36.1.1-3 Which ones?

[RECORD HERE BELOW THE MEDICINES THAT THE INTERVIEWEE TAKES]

| | NO | YES |
|-------------------------------|--------------------------|--------------------------|
| 36.1.1 ACE inhibitors | <input type="checkbox"/> | <input type="checkbox"/> |
| 36.1.2 Beta blockers | <input type="checkbox"/> | <input type="checkbox"/> |
| 36.1.3 Other (specify): _____ | <input type="checkbox"/> | <input type="checkbox"/> |

37. Has a doctor ever told you that you have diabetes? NO YES
☐ ☐
IF 'NO' GO TO QUESTION 38, IF 'YES':
37.1 How old were you when you started to suffer from diabetes? YEARS

| | NO | YES |
|-------------------------------------------------------|--------------------------|--------------------------|
| 37.2 Are you going on a diet recommended by a doctor? | <input type="checkbox"/> | <input type="checkbox"/> |
| 37.3 Are you taking oral medicines for your diabetes? | <input type="checkbox"/> | <input type="checkbox"/> |
| 37.4 Are you taking insulin? | <input type="checkbox"/> | <input type="checkbox"/> |

38. Has a doctor ever told you that you have or have had a tumour, or cancer or a neoplasia? NO YES
☐ ☐
IF 'NO' GO TO QUESTION 39, IF 'YES':

38.1.1-15 In which part of the body? NO YES
[FOR THE INTERVIEWER: DO NOT READ THE LIST]

| | | |
|---------------------------------------------------------------|--------------------------|--------------------------|
| 38.1.1 Mouth and oropharynx; | <input type="checkbox"/> | <input type="checkbox"/> |
| 38.1.2 Larynx; | <input type="checkbox"/> | <input type="checkbox"/> |
| 38.1.3 Oesophagus; | <input type="checkbox"/> | <input type="checkbox"/> |
| 38.1.4 Stomach; | <input type="checkbox"/> | <input type="checkbox"/> |
| 38.1.5 Colon and rectus; | <input type="checkbox"/> | <input type="checkbox"/> |
| 38.1.6 Liver; | <input type="checkbox"/> | <input type="checkbox"/> |
| 38.1.7 Pancreas; | <input type="checkbox"/> | <input type="checkbox"/> |
| 38.1.8 Trachea; | <input type="checkbox"/> | <input type="checkbox"/> |
| 38.1.9 Bronchi, lungs; | <input type="checkbox"/> | <input type="checkbox"/> |
| 38.1.10 Breasts; | <input type="checkbox"/> | <input type="checkbox"/> |
| 38.1.11 Uterus; | <input type="checkbox"/> | <input type="checkbox"/> |
| 38.1.12 Prostate; | <input type="checkbox"/> | <input type="checkbox"/> |
| 38.1.13 Bladder; | <input type="checkbox"/> | <input type="checkbox"/> |
| 38.1.14 Blood or lymphatic organs (e.g. leukaemia, lymphoma); | <input type="checkbox"/> | <input type="checkbox"/> |
| 38.1.15 Skin; | <input type="checkbox"/> | <input type="checkbox"/> |
| 38.1.16 Bones; | <input type="checkbox"/> | <input type="checkbox"/> |
| 38.1.17 Other part of the body (specify): _____ | <input type="checkbox"/> | <input type="checkbox"/> |

39. In the **last month**: NO YES
☐ ☐
39.1 Did you get down, have you been depressed or without hope?
39.2 Have you often felt low interest or pleasure in doing thigs? ☐ ☐

YEAR

40. Which year was your mother born?

41. Where was your mother born? (Tick **one box only**)

| | | | |
|-----------------------|--------------------------|---------------------|--------------------------|
| Abruzzo | <input type="checkbox"/> | Molise | <input type="checkbox"/> |
| Basilicata | <input type="checkbox"/> | Piemonte | <input type="checkbox"/> |
| Calabria | <input type="checkbox"/> | Puglia | <input type="checkbox"/> |
| Campania | <input type="checkbox"/> | Sardegna | <input type="checkbox"/> |
| Emilia-Romagna | <input type="checkbox"/> | Sicilia | <input type="checkbox"/> |
| Friuli-Venezia Giulia | <input type="checkbox"/> | Toscana | <input type="checkbox"/> |
| Lazio | <input type="checkbox"/> | Trentino-Alto Adige | <input type="checkbox"/> |
| Liguria | <input type="checkbox"/> | Umbria | <input type="checkbox"/> |
| Lombardia | <input type="checkbox"/> | Valle d'Aosta | <input type="checkbox"/> |
| Marche | <input type="checkbox"/> | Veneto | <input type="checkbox"/> |

IF SHE WAS NOT BORN IN ITALY:

42. Specify the foreign country of birth: _____

COUNTRY CODE

| | | |
|----------------------|----------------------|----------------------|
| <input type="text"/> | <input type="text"/> | <input type="text"/> |
|----------------------|----------------------|----------------------|

YEARS

| | |
|----------------------|----------------------|
| <input type="text"/> | <input type="text"/> |
|----------------------|----------------------|

If illiterate enter 0

43. At what age did your mother stop studying (approximatively)?

44. When you were a child, did your mother smoke regularly?

NO

☐

YES, outside
home only

☐

YES, inside
home as well

☐

45. Where was your father born? (Tick **one box only**)

| | | | |
|-----------------------|--------------------------|---------------------|--------------------------|
| Abruzzo | <input type="checkbox"/> | Molise | <input type="checkbox"/> |
| Basilicata | <input type="checkbox"/> | Piemonte | <input type="checkbox"/> |
| Calabria | <input type="checkbox"/> | Puglia | <input type="checkbox"/> |
| Campania | <input type="checkbox"/> | Sardegna | <input type="checkbox"/> |
| Emilia-Romagna | <input type="checkbox"/> | Sicilia | <input type="checkbox"/> |
| Friuli-Venezia Giulia | <input type="checkbox"/> | Toscana | <input type="checkbox"/> |
| Lazio | <input type="checkbox"/> | Trentino-Alto Adige | <input type="checkbox"/> |
| Liguria | <input type="checkbox"/> | Umbria | <input type="checkbox"/> |
| Lombardia | <input type="checkbox"/> | Valle d'Aosta | <input type="checkbox"/> |
| Marche | <input type="checkbox"/> | Veneto | <input type="checkbox"/> |

IF HE WAS NOT BORN IN ITALY:

46. Specify the foreign country of birth: _____

COUNTRY CODE

| | | |
|----------------------|----------------------|----------------------|
| <input type="text"/> | <input type="text"/> | <input type="text"/> |
|----------------------|----------------------|----------------------|

YEARS

| | |
|----------------------|----------------------|
| <input type="text"/> | <input type="text"/> |
|----------------------|----------------------|

If illiterate enter 0

47. At what age did your father stop studying (approximatively)?

48. When you were a child, did your father smoke regularly?

NO

☐

YES, outside
home only

☐

YES, inside
home as well

☐

49. Where were you born? (Tick **one box only**)

| | |
|-----------------------|--------------------------|
| Abruzzo | <input type="checkbox"/> |
| Basilicata | <input type="checkbox"/> |
| Calabria | <input type="checkbox"/> |
| Campania | <input type="checkbox"/> |
| Emilia-Romagna | <input type="checkbox"/> |
| Friuli-Venezia Giulia | <input type="checkbox"/> |
| Lazio | <input type="checkbox"/> |
| Liguria | <input type="checkbox"/> |
| Lombardia | <input type="checkbox"/> |
| Marche | <input type="checkbox"/> |

| | |
|---------------------|--------------------------|
| Molise | <input type="checkbox"/> |
| Piemonte | <input type="checkbox"/> |
| Puglia | <input type="checkbox"/> |
| Sardegna | <input type="checkbox"/> |
| Sicilia | <input type="checkbox"/> |
| Toscana | <input type="checkbox"/> |
| Trentino-Alto Adige | <input type="checkbox"/> |
| Umbria | <input type="checkbox"/> |
| Valle d'Aosta | <input type="checkbox"/> |
| Veneto | <input type="checkbox"/> |

IF S/HE WAS NOT BORN IN ITALY:

50. Specify the foreign country of birth: _____

COUNTRY CODE

| | | |
|----------------------|----------------------|----------------------|
| <input type="text"/> | <input type="text"/> | <input type="text"/> |
|----------------------|----------------------|----------------------|

51. Which is your citizenship?

[If s/he has another citizenship other than the italian one, tick the 'Italian' box only]

- A) Italian
- B) Foreign
- C) Displaced (no citizenship)

TICK ONE BOX ONLY

| | |
|---|--------------------------|
| 1 | <input type="checkbox"/> |
| 2 | <input type="checkbox"/> |
| 3 | <input type="checkbox"/> |

IF 'ITALIAN'

51.1 **SPECIFY:**

- A) From birth
- B) Acquired (e.g. through marriage, naturalization, etc.)

TICK ONE BOX ONLY

| | |
|---|--------------------------|
| 1 | <input type="checkbox"/> |
| 2 | <input type="checkbox"/> |

IF 'ACQUIRED'

51.1.1 Specify the foreign country
of previous citizenship: _____

COUNTRY CODE

| | | |
|----------------------|----------------------|----------------------|
| <input type="text"/> | <input type="text"/> | <input type="text"/> |
|----------------------|----------------------|----------------------|

IF 'FOREIGN':

51.2 **SPECIFY:**

Foreign country of citizenship: _____

COUNTRY CODE

| | | |
|----------------------|----------------------|----------------------|
| <input type="text"/> | <input type="text"/> | <input type="text"/> |
|----------------------|----------------------|----------------------|

GRAMS

| | | | |
|----------------------|----------------------|----------------------|----------------------|
| <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |
|----------------------|----------------------|----------------------|----------------------|

52. What was your weight at birth?

IF S/HE DOES NOT REMEMBER THE WEIGHT AT BIRTH:

52.1 Were you born underweight (weight at birth under 2500 g)?

| | | |
|--------------------------|--------------------------|--------------------------|
| NO | YES | DO NOT REMEMBER |
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

53. With respect to the estimated birthdate, were you born:

- A) Regularly (**no more than 3 weeks** before than, or **no more than 2 weeks** later than the estimated birthdate)
- B) More than 3 weeks before
- C) More than 2 weeks later
- D) I do not know

TICK ONE BOX ONLY

| | |
|---|--------------------------|
| 1 | <input type="checkbox"/> |
| 2 | <input type="checkbox"/> |
| 3 | <input type="checkbox"/> |
| 4 | <input type="checkbox"/> |

54. Were you born by:
 A) Natural birth
 B) Caesarian section
 C) Forceps

TICK ONE BOX ONLY

| | |
|---|--|
| 1 | |
| 2 | |
| 3 | |

55. In the months before your birth, during pregnancy
 have your mother had loss or great discomfort episodes
 (mourning, personal or spouse job, separation, etc.)?

| | |
|----|-----|
| NO | YES |
| | |

56. Did you have a serious respiratory infection before the age of five years?

| | | |
|----|-----|----|
| NO | YES | DK |
| | | |

57. Were you hospitalized before the age of two years for lung disease?

| | |
|----|-----|
| NO | YES |
| | |

58. At what age did you first attend a school, play school, day care or nursery?

| | |
|-------|--|
| YEARS | |
| | |

59. How many *other* children regularly slept in your bedroom before
 you were *five years old*?

| | |
|----------|--|
| CHILDREN | |
| | |

60. For every member of your family I will ask you some anagraphical data and possible respiratory pathologies.

| Family member | Sex | Year of Birth | <u>Only for Brothers/sisters.</u> Is/was s/he your omozygotic twin (almost similar to you)? | <u>Only for Brothers/sisters.</u> Is/was s/he your eterozygotic twin (not similar to you)? | Has s/he ever had asthma? | Has s/he ever had nasal allergies or hay fever? | Has s/he ever had skin allergies or eczema? |
|---------------|-----|---------------|------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|---------------------------|-------------------------------------------------|---------------------------------------------|
|---------------|-----|---------------|------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|---------------------------|-------------------------------------------------|---------------------------------------------|

| | | | NO YES | NO YES | NO YES | NO YES | NO YES |
|----------------------|--|--|--------|--------|--------|--------|--------|
| 60.1 Father | | | | | | | |
| 60.2 Mother | | | | | | | |
| 60.3 Brother/Sister | | | | | | | |
| 60.4 Brother/Sister | | | | | | | |
| 60.5 Brother/Sister | | | | | | | |
| 60.6 Brother/Sister | | | | | | | |
| 60.7 Brother/Sister | | | | | | | |
| 60.8 Brother/Sister | | | | | | | |
| 60.9 Brother/Sister | | | | | | | |
| 60.10 Brother/Sister | | | | | | | |
| 60.11 Brother/Sister | | | | | | | |
| 60.12 Brother/Sister | | | | | | | |

61. Which among these expressions best describe you present condition?

- A) I am married and my partner and I live together
 B) I share my house with someone who is not my partner
 C) I live alone

TICK ONE BOX ONLY

| | |
|---|--|
| 1 | |
| 2 | |
| 3 | |

62. Some stressful events that could have caused you great uneasiness and pain are reported below.

If they occurred, please record the age when they occurred:

62.1 Involuntary job dismissal

62.2 Separation or divorce

62.3 Death of partner or lover

62.4 Family mourning

| NO | YES | YEARS | |
|----------------------|----------------------|----------------------|----------------------|
| <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |
| <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |
| <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |
| <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |

63. At what age did you complete full time education?

YEARS

| | |
|----------------------|----------------------|
| <input type="text"/> | <input type="text"/> |
|----------------------|----------------------|

If full-time student enter 88

64. Have you been employed in any job for three continuous months or longer (these jobs may be outside the house or at home, full-time or part-time, paid or not paid, including self-employment, for example in a family business.

Please include part time jobs only if you had been doing them for more then eight hours per week)?

| NO | YES |
|----------------------|----------------------|
| <input type="text"/> | <input type="text"/> |

65. Are you currently:

A) employed (including military service)

B) self employed

C) unemployed, looking for work

D) not working because of poor health

E) full-time house-person

F) full-time student

G) retired

H) other. Specify: _____

TICK ONE BOX ONLY

| | |
|---|----------------------|
| 1 | <input type="text"/> |
| 2 | <input type="text"/> |
| 3 | <input type="text"/> |
| 4 | <input type="text"/> |
| 5 | <input type="text"/> |
| 6 | <input type="text"/> |
| 7 | <input type="text"/> |
| 8 | <input type="text"/> |

IF S/HE IS 'EMPLOYED':

65.1 Are you currently:

A) manager

B) employee

C) workman

D) other. Specify: _____

TICK ONE BOX ONLY

| | |
|---|----------------------|
| 1 | <input type="text"/> |
| 2 | <input type="text"/> |
| 3 | <input type="text"/> |
| 4 | <input type="text"/> |

IF S/HE IS 'SELF-EMPLOYED':

65.2 Are you currently:

A) entrepreneur

B) freelance

C) other. Specify: _____

TICK ONE BOX ONLY

| | |
|---|----------------------|
| 1 | <input type="text"/> |
| 2 | <input type="text"/> |
| 3 | <input type="text"/> |

IF 'YES' TO QUESTION 64, OR IF S/HE ANSWERED 'EMPLOYED' OR 'SELF-EMPLOYED' OR 'FULL-TIME HOUSE-PERSON' TO QUESTION 65, GO TO OCCUPATIONAL MATRIX (QUESTION 66).

OTHERWISE GO TO QUESTION 68

66. If you had more than one job in the same company, or if you were doing more than one job at the same time, we would like to talk about them separately. Please start with your current or last job.

| JOB | 66.1 What is (was) the title of your current (last) job? | 66.2 What did the firm, company or organisation do or what services did it provide? | 66.3 In what month and year did you start working in this job? | | 66.4 In what month and year did you stop working in this job? | | 66.5 Do (did) you work full-time or part-time? | | |
|--------|----------------------------------------------------------|-------------------------------------------------------------------------------------|----------------------------------------------------------------|------|---------------------------------------------------------------|------|------------------------------------------------|-----------|--|
| | | | MONTH | YEAR | MONTH | YEAR | Full-time | Part-time | |
| JOB 1 | | | | | | | | | |
| JOB 2 | | | | | | | | | |
| JOB 3 | | | | | | | | | |
| JOB 4 | | | | | | | | | |
| JOB 5 | | | | | | | | | |
| JOB 6 | | | | | | | | | |
| JOB 7 | | | | | | | | | |
| JOB 8 | | | | | | | | | |
| JOB 9 | | | | | | | | | |
| JOB 10 | | | | | | | | | |

67. Have you ever been **regularly** exposed to vapours, gas dust or fumes at work? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 68, IF 'YES' PLEASE ANSWER TO QUESTIONS 67.1-5:

*With reference to each job indicated in the previous table, please tick the boxes that match with an affirmative answer.
If the answer is negative, please do not tick the box.*

67.1 Which of these jobs exposed you to vapours, gas dust or fumes regularly?

67.2 Were air vents functioning in the area where you were working?

67.3 Have you been using safety measures for the airways?

67.4 Have you undergone spirometric trials?

67.5 Have any of these jobs ever caused you breathing problems (chest tightness, wheezing, breathing problems, coughing)?

IF 'NO' FOR ALL JOBS GO TO QUESTION 68,

IF 'YES' FOR AT LEAST ONE JOB:

67.5.1 Did these breathing problems start from the first days of work?

67.5.2 Did these breathing problems diminish or stop during the week-end or during holidays and then start again when you went back to work?

67.5.3 Have you ever had to leave any of these jobs because they caused you respiratory problems?

IF 'NO' FOR ALL JOBS GO TO QUESTION 68,

IF 'YES' FOR AT LEAST ONE JOB:

67.5.3.1 Did the respiratory problems stop or diminish with the new job?

| JOB 1 | JOB 2 | JOB 3 | JOB 4 | JOB 5 | JOB 6 | JOB 7 | JOB 8 | JOB 9 | JOB 10 |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------|
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68. Have you ever been involved in an accident at home, work or elsewhere that exposed you to **high levels** of vapours, gas, dust or fumes?

NO ☐ YES ☐

IF 'NO' GO TO QUESTION 69, IF 'YES':

68.1 When did it happen to you?

MONTH

| | |
|--|--|
| | |
|--|--|

YEAR

| | | | |
|--|--|--|--|
| | | | |
|--|--|--|--|

TICK ONE BOX ONLY

68.2 Where did it happen to you?

- A) In my house
- B) In the workplace
- C) Indoors (another place)
- D) Outdoors

1

| |
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2

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4

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HOURS AND/OR MINUTES

| | |
|--|--|
| | |
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| | |
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| | |
|--|--|

68.3 How long were you exposed?

68.4 Were you exposed to:

68.4.1 Vapours?

68.4.2 Gas?

NO YES

| | |
|--|--|
| | |
| | |

68.4.3 Dust?
68.4.4 Fumes?

| | |
|--|--|
| | |
| | |

TICK ONE BOX ONLY

68.5 Could you briefly describe what happened during the accident that you had?

- A) A fire or an explosion
B) A leakage of liquid or gas
C) A mixture of cleaning products
D) Other (specify): _____

| | |
|---|--|
| 1 | |
| 2 | |
| 3 | |
| 4 | |

68.6 Did you have respiratory problems within the 24 hours after the accident?

IF 'NO' GO TO QUESTION 69, IF 'YES':

68.6.1 Have you been to the Emergency Room or have you been hospitalized at least one night for these respiratory problems?

| | |
|----|-----|
| NO | YES |
| | |
| NO | YES |
| | |

69. Do (did) you drink alcohol?

IF 'NO' GO TO QUESTION 70, IF 'YES':

| | | |
|----|-----|-------------|
| NO | YES | IN THE PAST |
| | | |

| | | |
|------------------|------------------|------|
| DURING LUNCHTIME | NOT AT LUNCHTIME | BOTH |
| | | |

69.1 When do (did) you drink alcohol?

69.2 How much alcohol do you drink when you have each of the following drinks?

[For each type of drink please indicate respectively how many days per week you drink, how many units you drink, when you started to drink regularly and (if applicable) when you gave up drinking regularly]

| Type of drink | Days/week | Units/week | Age when you started drinking regularly | If ex drinker, age when you stopped drinking regularly |
|---------------------------------------------------------|----------------------|----------------------|-----------------------------------------|--------------------------------------------------------|
| 69.2.1 Wine (125 ml) | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |
| 69.2.2 Beer (330ml) | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |
| 69.2.3 After dinner drinks (30 ml) | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |
| 69.2.4 Grappa (30 ml) | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |
| 69.2.5 Whisky, cognac and brandy (30 ml) | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |
| 69.2.6 Other (at least one per week), specify: _____ | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |

70. How often do you usually exercise so much that you get out of breath or sweat?

- A) every day
- B) 4-6 times a week
- C) 2-3 times a week
- D) once a week
- E) once a month
- F) less than once a month
- G) never

TICK ONE BOX ONLY

| | |
|---|--|
| 1 | |
| 2 | |
| 3 | |
| 4 | |
| 5 | |
| 6 | |
| 7 | |

71. How many hours a week do you usually exercise so much that you get out of breath or sweat?

- A) none
- B) about ½ hour
- C) about 1 hour
- D) about 2-3 hours
- E) about 4-6 hours
- F) 7 hours or more

TICK ONE BOX ONLY

| | |
|---|--|
| 1 | |
| 2 | |
| 3 | |
| 4 | |
| 5 | |
| 6 | |

72. Do you avoid taking vigorous exercise because of breathing problems?

| | |
|--------------------------|--------------------------|
| NO | YES |
| <input type="checkbox"/> | <input type="checkbox"/> |

73. When was your present home built?

YEAR

| | | | |
|--|--|--|--|
| | | | |
|--|--|--|--|

ONLY FOR THE SUBJECTS OF THE ECRHS AND ISAYA COHORTS:

73.FU Do you live in the same home *as when you were last surveyed?*

| | |
|--------------------------|--------------------------|
| NO | YES |
| <input type="checkbox"/> | <input type="checkbox"/> |

74. How many years have you lived in your present home?

| |
|----------------------|
| YEARS |
| <input type="text"/> |

75. Which best describes the building in which you live?

- A) a mobile home or trailer
- B) a one family house detached from any other house
- C) a one family house attached to one or more houses
- D) a building for two families
- E) a building for three or four families
- F) a building for five or more families
- G) a boat, tent or van
- H) other (specify): _____

TICK ONE BOX ONLY

| | |
|---|--|
| 1 | |
| 2 | |
| 3 | |
| 4 | |
| 5 | |
| 6 | |
| 7 | |
| 8 | |

76. What term best describes the area where your house is situated?

- A) country or small village surrounded by open areas or fields
- B) city suburb, with parks and gardens
- C) city suburb, without parks and gardens
- D) inner city, with parks and gardens
- E) inner city, without parks and gardens
- F) other (specify): _____

TICK ONE BOX ONLY

| | |
|---|--|
| 1 | |
| 2 | |
| 3 | |
| 4 | |
| 5 | |
| 6 | |

77. Are there any industrial plants near your house?

| | |
|--------------------------|--------------------------|
| NO | YES |
| <input type="checkbox"/> | <input type="checkbox"/> |

78. How often do cars pass your house?

- A) constantly
- B) frequently
- C) seldom
- D) never

TICK ONE BOX ONLY

| | |
|---|--|
| 1 | |
| 2 | |
| 3 | |
| 4 | |

79. How often do heavy vehicles (e.g. trucks/buses) pass your house?

- A) constantly
- B) frequently
- C) seldom
- D) never

TICK ONE BOX ONLY

| | |
|---|--|
| 1 | |
| 2 | |
| 3 | |
| 4 | |

80. How old is your mattress?

- A) less than one year
- B) 1-5 years
- C) more than 5 years

TICK ONE BOX ONLY

| | |
|---|--|
| 1 | |
| 2 | |
| 3 | |

81. What is your mattress made of?

- A) Springs
- B) Foam rubber (polyurethane)
- C) Latex
- D) Polyester (cored fiber)
- E) Wool
- F) Do not know
- G) Other material, please describe it: _____

TICK ONE BOX ONLY

| | |
|---|--|
| 1 | |
| 2 | |
| 3 | |
| 4 | |
| 5 | |
| 6 | |
| 7 | |

82. How old is the pillow that you use when you sleep?

- A) less than one year
- B) 1-5 years
- C) more than 5 years

TICK ONE BOX ONLY

| | |
|---|--|
| 1 | |
| 2 | |
| 3 | |

83. What is the pillow that you use when you sleep made of?

- A) Goose feather
- B) Foam rubber (polyurethane)
- C) Latex
- D) Polyester (cored fiber)
- E) Wool
- F) Do not know
- G) Other material, please describe it: _____
- H) Do not use pillows to sleep

TICK ONE BOX ONLY

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| |

84. Within the last **12 months** have you had wet or damp spots on surfaces inside your home other than in the basement (for example on walls, wall paper, ceilings or carpets)?

| | |
|--------------------------|--------------------------|
| NO | YES |
| <input type="checkbox"/> | <input type="checkbox"/> |

85. Has there been mould or mildew on any surfaces (other than food) inside the home in the last **12 months**?

| | |
|--------------------------|--------------------------|
| NO | YES |
| <input type="checkbox"/> | <input type="checkbox"/> |

86. Do you keep a cat/cats?

IF 'NO' GO TO QUESTION 87, IF 'YES':

86.1 How many years have you been keeping the cat(s)?

| | |
|--------------------------|--------------------------|
| NO | YES |
| <input type="checkbox"/> | <input type="checkbox"/> |
| YEARS | |
| <input type="text"/> | <input type="text"/> |

| | | |
|--------------------------------------------------------------------------------------------------------------|--------------------------|--------------------------|
| 86.2 Is your cat (are your cats) allowed inside the house? | NO | YES |
| 86.3 Is your cat (are your cats) allowed in your bedroom? | <input type="checkbox"/> | <input type="checkbox"/> |
| 87. Do you keep a dog/dogs? | NO | YES |
| IF 'NO' GO TO QUESTION 88, IF 'YES': | <input type="checkbox"/> | <input type="checkbox"/> |
| 87.1 How many years have you been keeping the dog(s)? | YEARS | |
| | <input type="text"/> | <input type="text"/> |
| 87.2 Is your dog (are your dogs) allowed inside the house? | NO | YES |
| 87.3 Is your dog (are your dogs) allowed in your bedroom? | <input type="checkbox"/> | <input type="checkbox"/> |
| 88. Was there a cat in your home when you were a child? | NO | YES |
| | <input type="checkbox"/> | <input type="checkbox"/> |
| 89. Was there a dog in your home when you were a child? | NO | YES |
| | <input type="checkbox"/> | <input type="checkbox"/> |
| 90. Do you regularly use antidust or antiacari sprays? | NO | YES |
| 91. Do you regularly use an anallergic mattress cover for your mattress? | <input type="checkbox"/> | <input type="checkbox"/> |
| 92. What term best describes the place you lived most of the time when you were under the age of five years? | TICK ONE BOX ONLY | |
| A) farm | 1 | <input type="checkbox"/> |
| B) village in a rural area | 2 | <input type="checkbox"/> |
| C) small town | 3 | <input type="checkbox"/> |
| D) suburb of a city | 4 | <input type="checkbox"/> |
| E) inner city | 5 | <input type="checkbox"/> |
| 93. When you are near trees, grass or flowers, or when there is a lot of pollen about, do you ever: | NO | YES |
| 93.1 start to cough? | <input type="checkbox"/> | <input type="checkbox"/> |
| 93.2 start to wheeze? | <input type="checkbox"/> | <input type="checkbox"/> |
| 93.3 get a feeling of tightness in your chest? | <input type="checkbox"/> | <input type="checkbox"/> |
| 93.4 start to feel short of breath? | <input type="checkbox"/> | <input type="checkbox"/> |
| 93.5 get a runny or stuffy nose or start to sneeze? | <input type="checkbox"/> | <input type="checkbox"/> |
| 93.6 get itchy or watery eyes? | <input type="checkbox"/> | <input type="checkbox"/> |
| IF THE INTERVIEWEE ANSWERED 'YES' TO ANY OF THE ABOVE QUESTIONS (FROM 93.1 TO 93.6): | | |
| 93.7.1-4 Which time of the year does this happen? | NO | YES |
| 93.7.1 winter | <input type="checkbox"/> | <input type="checkbox"/> |
| 93.7.2 spring | <input type="checkbox"/> | <input type="checkbox"/> |
| 93.7.3 summer | <input type="checkbox"/> | <input type="checkbox"/> |
| 93.7.4 autumn | <input type="checkbox"/> | <input type="checkbox"/> |
| 94. Do your respiratory symptoms get worse during thunderstorms? | NO | YES |
| | <input type="checkbox"/> | <input type="checkbox"/> |
| 95. When you are near animals, such as cats or dogs, do you ever: | NO | YES |
| 95.1 start to cough? | <input type="checkbox"/> | <input type="checkbox"/> |
| 95.2 start to wheeze? | <input type="checkbox"/> | <input type="checkbox"/> |
| 95.3 get a feeling of tightness in your chest? | <input type="checkbox"/> | <input type="checkbox"/> |
| 95.4 start to feel short of breath? | <input type="checkbox"/> | <input type="checkbox"/> |
| 95.5 get a runny or stuffy nose or start to sneeze? | <input type="checkbox"/> | <input type="checkbox"/> |
| 95.6 get itchy or watery eyes? | <input type="checkbox"/> | <input type="checkbox"/> |

96. When you are in a dusty part of the house, or near pillows or duvets do you ever:

96.1 start to cough?

96.2 start to wheeze?

96.3 get a feeling of tightness in your chest?

96.4 start to feel short of breath?

96.5 get a runny or stuffy nose or start to sneeze?

96.6 get itchy or watery eyes?

NO YES

| | |
|--|--|
| | |
| | |
| | |
| | |
| | |
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NO YES

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|--|--|
| | |
|--|--|

97. Have you ever smoked for as long as a year?

('YES' means at least 20 packs of cigarettes or 12 oz (360 grams) of tobacco in a lifetime, or at least one cigarette per day or one cigar a week for one year)

IF 'NO' GO TO QUESTION 98, IF 'YES':

YEARS

| | |
|--|--|
| | |
|--|--|

97.1 How old were you when you started smoking?

NO YES

| | |
|--|--|
| | |
|--|--|

97.2 Do you **now** smoke, as of **one month ago**?

IF 'NO' GO TO QUESTION 97.3, IF 'YES':

97.2.1-4 How much do you now smoke on average?

97.2.1 Number of cigarettes per day

97.2.2 Number of cigarillos per day

97.2.3 Number of cigars a week

NUMBER

| | |
|--|--|
| | |
| | |
| | |

GRAMS

| | | |
|--|--|--|
| | | |
|--|--|--|

97.2.4 Pipe tobacco, in grams/week

NO YES

| | |
|--|--|
| | |
|--|--|

97.3 Have you stopped or cut down smoking?

IF 'NO' GO TO QUESTION 97.4, IF 'YES':

NO YES

| | |
|--|--|
| | |
|--|--|

97.3.1 Have you stopped or cut down smoking due to breathing problems?

YEARS

| | |
|--|--|
| | |
|--|--|

97.3.2 How old were you when you stopped or cut down smoking?

97.3.3.1-4 On average of the entire time you smoked, before you stopped or cut down, how much did you smoke?

97.3.3.1 Number of cigarettes per day

97.3.3.2 Number of cigarillos per day

97.3.3.3 Number of cigars a week

NUMBER

| | |
|--|--|
| | |
| | |
| | |

GRAMS

| | | |
|--|--|--|
| | | |
|--|--|--|

97.3.3.4 Pipe tobacco, in grams/week

NO YES

| | |
|--|--|
| | |
|--|--|

97.4 Do (did) you inhale the smoke?

98. Have you been **regularly** exposed to tobacco smoke in the last **12 months**?

('Regularly' means on most days or nights)

NO YES

| | |
|--|--|
| | |
|--|--|

IF 'NO'

ONLY FOR THE SUBJECTS OF:

THE SARA COHORT,

THE ECRHS COHORT THAT DID NOT PARTICIPATE IN ECRHS II,

THE ISAYA COHORT THAT DID NOT ANSWER TO THE TELEPHONE

INTERVIEW ON ANTI-ASTHMATIC DRUGS

GO TO QUESTION 99.G

IF 'NO'

ONLY FOR THE SUBJECTS OF:

THE ECRHS II COHORT,

**THE ISAYA COHORT (CURRENT DIAGNOSED ASTHMA) THAT ANSWERED
TO THE TELEPHONE INTERVIEW ON ANTI-ASTHMATIC DRUGS:**

GO TO QUESTION 99.E

IF 'YES':

98.1 Not counting yourself, how many people in your household smoke regularly?

NUMBER

| | |
|--|--|
| | |
|--|--|

NO YES

98.2 Do people smoke regularly in the room where you work?

| | |
|--|--|
| | |
|--|--|

HOURS

98.3 How many hours per day are you exposed to *other people's* tobacco smoke?

| | |
|--|--|
| | |
|--|--|

98.4 Please provide more information. How many hours per day, are you exposed to other people's tobacco smoke in the following locations?

HOURS

98.4.1 at home

| | |
|--|--|
| | |
|--|--|

98.4.2 at workplace

| | |
|--|--|
| | |
|--|--|

98.4.3 in bars, restaurants, cinemas or similar social settings

| | |
|--|--|
| | |
|--|--|

98.4.4 elsewhere

| | |
|--|--|
| | |
|--|--|

ONLY FOR THE SUBJECTS OF:

THE SARA COHORT,

THE ECRHS COHORT THAT DID NOT PARTICIPATE IN ECRHS II,

**THE ISAYA COHORT THAT DID NOT ANSWER TO THE TELEPHONE
INTERVIEW ON ANTI-ASTHMATIC DRUGS**

99.G Have you ever used inhaled steroids?

NO YES

| | |
|--|--|
| | |
|--|--|

[SHOW THE LIST OF INHALED STEROIDS]

IF 'NO' GO TO QUESTION 100, IF 'YES':

99.1.G How old were you when you first started to use inhaled steroids?

YEARS

| | |
|--|--|
| | |
|--|--|

99.2.G Have you used inhaled steroids *every year since you started to use them*?

NO YES

| | |
|--|--|
| | |
|--|--|

IF 'NO' GO TO QUESTION 99.3.G, IF 'YES':

99.2.1.G On average how many months each year have you taken them?

MONTHS

| | |
|--|--|
| | |
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GO TO QUESTION 100

99.3.G How many years have you been taking inhaled steroids?

YEARS

| | |
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| | |
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99.4.G On average how many months of each of these years have you taken them?

MONTHS

| | |
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ONLY FOR THE SUBJECTS OF:
THE ECRHS II COHORT,
THE ISAYA COHORT (CURRENT DIAGNOSED ASTHMA) THAT
ANSWERED TO THE TELEPHONE INTERVIEW
ON ANTI-ASTHMATIC DRUGS:

99.E *Since the last survey* have you used inhaled steroids?

NO YES
☐ ☐

[SHOW THE LIST OF INHALED STEROIDS]

IF 'NO' GO TO QUESTION 100, IF 'YES':

99.1.E How old were you when you first started to use inhaled steroids?

YEARS

99.2.E Have you used inhaled steroids *every year since the last survey*?

NO YES
☐ ☐

IF 'NO' GO TO QUESTION 99.3.E, IF 'YES':

99.2.1.E On average how many months each year have you taken them?

MONTHS

GO TO QUESTION 100

99.3.G How many of the years *since the last survey* have you taken inhaled steroids?

YEARS

99.4.G On average how many months of each of these years have you taken them?

MONTHS

100. In the last **12 months** have you used *spray or aerosol* to help your breathing?

NO YES
☐ ☐

IF 'NO' GO TO QUESTION 101, IF 'YES':

| | | |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------|----|-----|
| 100.1 Do you remember the name/s of the <i>spray/s or aerosol/s</i> that you have been using in the last 12 months ? | NO | YES |
| IF 'NO' READ THE LIST OF SPRAY/S AND AEROSOL/S. GIVE A DETAILED RECORD OF THE COMMERCIAL NAME/S OF THE MEDICINE/S SUGGESTED BY THE INTERVIEWEE | | |

| | | | | | | | |
|-----------------------------------------------------------------------------------------------|--------------------------------------------------------|-----------------------------------------------------------|--------------------------------------------------------------|-------------------|-----------------------------------|-------------------------|-------------------------|
| Commercial name of the medicine (e.g. Ventolin, aerosol dos. 200 inal. 20 mg (MDI)) | If s/he uses MDI Do you also use the spacer? | In the last 3 months have you taken this medicine? | In the last 3 months how have you used this medicine? | | | | |
| | | | When Needed | In short courses | | Continuously | |
| | | | Number of puffs per month | Number of courses | Average number of days per course | Number of puffs per day | Number of puffs per day |
| 100.1.1 | NO YES | NO YES | IF YES | | | | |
| 100.1.2 | | | IF YES | | | | |
| 100.1.3 | | | IF YES | | | | |
| 100.1.4 | | | IF YES | | | | |
| 100.1.5 | | | IF YES | | | | |

101. Have you used any *pills, capsules, tablets or medicines*, other than sprays or aerosols, to help your breathing at any time in the last **12 months**? NO YES
☐ ☐
IF 'NO' GO TO QUESTION 102, IF 'YES':

| | | |
|------------------------------------------------------------------------------------------------------------------------------------------------------|----|-----|
| 101.1 Do you remember the name of the oral medicine/s that you have taken in the last 12 months ? | NO | YES |
| IF 'NO' READ THE ORAL MEDICINE/S LIST. GIVE A DETAILED RECORD OF THE COMMERCIAL NAME/S OF THE MEDICINE/S SUGGESTED BY THE INTERVIEWEE | | |

| | |
|-----------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|
| Commercial name of the medicine (e.g. Tefamin pills 200 mg) | Is it a steroid? |
| | NO YES |
| 101.1.1 | <input type="checkbox"/> <input type="checkbox"/> IF 'YES' GO TO QUESTION 101.2, IF 'NO' GO TO QUESTION 101.3 |
| 101.1.2 | <input type="checkbox"/> <input type="checkbox"/> IF 'YES' GO TO QUESTION 101.2, IF 'NO' GO TO QUESTION 101.3 |
| 101.1.3 | <input type="checkbox"/> <input type="checkbox"/> IF 'YES' GO TO QUESTION 101.2, IF 'NO' GO TO QUESTION 101.3 |
| 101.1.4 | <input type="checkbox"/> <input type="checkbox"/> IF 'YES' GO TO QUESTION 101.2, IF 'NO' GO TO QUESTION 101.3 |
| 101.1.5 | <input type="checkbox"/> <input type="checkbox"/> IF 'YES' GO TO QUESTION 101.2, IF 'NO' GO TO QUESTION 101.3 |

101.2 In the last **12 months** how frequently have you used this/these medicine/s?

| Commercial name of the medicine/s | When needed | In short courses | | Continuously | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|-----------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------|-----------------------------------|-------------------------|-------------------------|--|--|--|--|--|--|--------------------------------------------------------------------------------------------------------------------------------------------------------|--|--|--|--|--|--|--|--|--|--|--------------------------------------------------------------------------------------------------------------------------------------------------------|--|--|--|--|--|--|--|--|--|--|--------------------------------------------------------------------------------------------------------------------------------------------------------|--|--|--|--|--|--|--|--|--|--|--------------------------------------------------------------------------------------------------------------------------------------------------------|--|--|--|--|--|--|--|--|--|--|
| | Number of pills per month | Number of courses | Average number of days per course | Number of pills per day | Number of pills per day | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| 101.2.3 | <table><tr><td></td><td></td></tr><tr><td></td><td></td></tr><tr><td></td><td></td></tr><tr><td></td><td></td></tr><tr><td></td><td></td></tr></table> | | | | | | | | | | | <table><tr><td></td><td></td></tr><tr><td></td><td></td></tr><tr><td></td><td></td></tr><tr><td></td><td></td></tr><tr><td></td><td></td></tr></table> | | | | | | | | | | | <table><tr><td></td><td></td></tr><tr><td></td><td></td></tr><tr><td></td><td></td></tr><tr><td></td><td></td></tr><tr><td></td><td></td></tr></table> | | | | | | | | | | | <table><tr><td></td><td></td></tr><tr><td></td><td></td></tr><tr><td></td><td></td></tr><tr><td></td><td></td></tr><tr><td></td><td></td></tr></table> | | | | | | | | | | | <table><tr><td></td><td></td></tr><tr><td></td><td></td></tr><tr><td></td><td></td></tr><tr><td></td><td></td></tr><tr><td></td><td></td></tr></table> | | | | | | | | | | |
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101.3

| Commercial name of the medicine (e.g. Ventolin, aerosol dos. 200 inal. 20 mg (MDI)) | In the last 3 months have you taken this medicine? | In the last 3 months how frequently have you used this medicine? | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| | | Number of items per month | Number of courses | Average number of days per | Number of items per day | Number of items per day | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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'Items' stands for pills, capsules, tablets, spoons, drops or other types of oral medicine taking.

ONLY FOR THE SUBJECTS OF:
THE SARA COHORT,
THE ECRHS COHORT THAT DID NOT PARTICIPATE IN ECRHS II,
THE ISAYA COHORT THAT DID NOT ANSWER TO THE TELEPHONE
INTERVIEW ON ANTI-ASTHMATIC DRUGS

102.G Have you ever been vaccinated for allergy?

NO

YES

DK

IF 'NO' OR 'DO NOT KNOW' GO TO QUESTION 103, IF 'YES':

YEARS

102.1.G How many years have you had vaccinations?

| | |
|--|--|
| | |
|--|--|

ONLY FOR THE SUBJECTS OF:
THE ECRHS II COHORT.
THE ISAYA COHORT (CURRENT DIAGNOSED ASTHMA) THAT
ANSWERED TO THE TELEPHONE INTERVIEW
ON ANTI-ASTHMATIC DRUGS:

102.E Have you been vaccinated for allergy *since the last survey*?

| | | |
|--------------------------|--------------------------|--------------------------|
| NO | YES | DK |
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

IF 'NO' OR 'DO NOT KNOW' GO TO QUESTION 103, IF 'YES':

102.1.E For how many years have you been vaccinated?

| |
|----------------------|
| YEARS |
| <input type="text"/> |

102.2 Have you been vaccinated for allergy in the last **12 months**?

| | |
|--------------------------|--------------------------|
| NO | YES |
| <input type="checkbox"/> | <input type="checkbox"/> |

**IF 'NO' GO TO QUESTION 103, IF 'YES' RECORD THE REASON
WHY THE INTERVIEWEE HAS BEEN VACCINATED:**

102.2.1 for asthma

| | |
|---|--------------------------|
| 1 | <input type="checkbox"/> |
|---|--------------------------|

102.2.2 for rhinitis

| | |
|---|--------------------------|
| 2 | <input type="checkbox"/> |
|---|--------------------------|

102.2.3 for rhinitis and asthma

| | |
|---|--------------------------|
| 3 | <input type="checkbox"/> |
|---|--------------------------|

102.2.4 other (specify): _____

| | |
|---|--------------------------|
| 4 | <input type="checkbox"/> |
|---|--------------------------|

103. Have you been vaccinated for flu in the last **12 months**?

| | |
|--------------------------|--------------------------|
| NO | YES |
| <input type="checkbox"/> | <input type="checkbox"/> |

104. Are you *usually (every year)* vaccinated for flu?

| | | |
|--------------------------|--------------------------|--------------------------|
| NO | YES | DK |
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

105. Have you been vaccinated for pneumococcus in the last **5 years**?

| | | |
|--------------------------|--------------------------|--------------------------|
| NO | YES | DK |
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

106. Have you had any antibody anti-IgE (Omalizumab-Xolair) injections
to help your breathing in the last **12 months**?

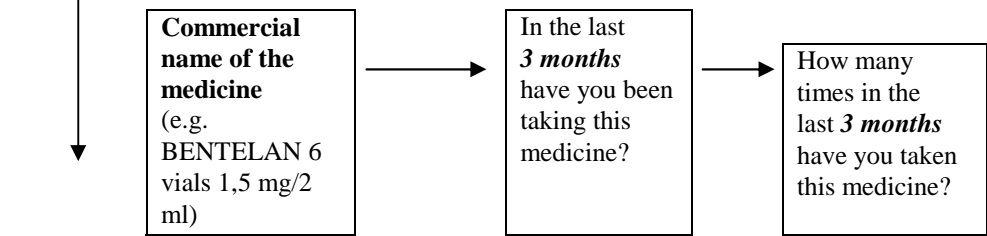
| | |
|--------------------------|--------------------------|
| NO | YES |
| <input type="checkbox"/> | <input type="checkbox"/> |

107. Have you had any other *injections* to help your breathing at any time
in the last **12 months**?

| | |
|--------------------------|--------------------------|
| NO | YES |
| <input type="checkbox"/> | <input type="checkbox"/> |

IF 'NO' GO TO QUESTION 108, IF 'YES':

| | | |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|-----|
| 107.1 Do you remember the name of the injections (other than the allergy vaccinations) that you have had in the last 12 months ? IF 'NO' READ THE INJECTIONS LIST. GIVE A DETAILED RECORD OF THE COMMERCIAL NAME/S OF THE MEDICINE/S SUGGESTED BY THE INTERVIEWEE | NO | YES |
| | | |



| | | | | | |
|---------|--|----|-----|--------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 107.1.1 | | NO | YES | IF YES | <div style="border: 1px solid black; width: 20px; height: 20px; display: inline-block;"></div> <div style="border: 1px solid black; width: 20px; height: 20px; display: inline-block;"></div> |
| 107.1.2 | | NO | YES | IF YES | <div style="border: 1px solid black; width: 20px; height: 20px; display: inline-block;"></div> <div style="border: 1px solid black; width: 20px; height: 20px; display: inline-block;"></div> |
| 107.1.3 | | NO | YES | IF YES | <div style="border: 1px solid black; width: 20px; height: 20px; display: inline-block;"></div> <div style="border: 1px solid black; width: 20px; height: 20px; display: inline-block;"></div> |
| 107.1.4 | | NO | YES | IF YES | <div style="border: 1px solid black; width: 20px; height: 20px; display: inline-block;"></div> <div style="border: 1px solid black; width: 20px; height: 20px; display: inline-block;"></div> |
| 107.1.5 | | NO | YES | IF YES | <div style="border: 1px solid black; width: 20px; height: 20px; display: inline-block;"></div> <div style="border: 1px solid black; width: 20px; height: 20px; display: inline-block;"></div> |

108. Have you ever used any *other remedies* to help your breathing in the last **12 months**?
IF 'NO' GO TO QUESTION 109, IF 'YES':

NO YES

| |
|------------------------------------------------------------------------------|
| 108.1 In the last 12 months , which other remedy have you used? |
|------------------------------------------------------------------------------|

| |
|--------------------------------------------------------------------|
| In the last 3 months have you used this remedy? |
|--------------------------------------------------------------------|

| |
|------------------------------------------------------------------------|
| In the last 3 months how many sessions have you attended? |
|------------------------------------------------------------------------|

| | | | | | | | | |
|----------------------|----|-----|--------|----|-----|--------|----------------------|----------------------|
| 108.1.1 HYPNOTHERAPY | NO | YES | IF YES | NO | YES | IF YES | <input type="text"/> | <input type="text"/> |
|----------------------|----|-----|--------|----|-----|--------|----------------------|----------------------|

| | | | | | | | | |
|---------------------|----|-----|--------|----|-----|--------|----------------------|----------------------|
| 108.1.2 ACUPUNCTURE | NO | YES | IF YES | NO | YES | IF YES | <input type="text"/> | <input type="text"/> |
|---------------------|----|-----|--------|----|-----|--------|----------------------|----------------------|

| | | | | | | | | |
|---------------------|----|-----|--------|----|-----|--------|----------------------|----------------------|
| 108.1.3 REFLEXOLOGY | NO | YES | IF YES | NO | YES | IF YES | <input type="text"/> | <input type="text"/> |
|---------------------|----|-----|--------|----|-----|--------|----------------------|----------------------|

| |
|--------------------------------------------------------------------------------|
| In the last 3 months how many times have you used this remedy? |
|--------------------------------------------------------------------------------|

| | | | | | | | | | |
|---------------------------------|----|-----|--------|----|-----|--------|---|----------------------|----------------------|
| 108.1.4 HOMEOPATHY, SPECIFY: | NO | YES | IF YES | NO | YES | IF YES | → | <input type="text"/> | <input type="text"/> |
| (108.1.4.1) | | | | | | | | <input type="text"/> | <input type="text"/> |
| (108.1.4.2) | | | | | | | | <input type="text"/> | <input type="text"/> |
| (108.1.4.3) | | | | | | | | <input type="text"/> | <input type="text"/> |

| | | | | | | | | | |
|------------------------------------|----|-----|--------|----|-----|--------|---|----------------------|----------------------|
| 108.1.5 HERB REMEDIES, SPECIFY: | NO | YES | IF YES | NO | YES | IF YES | → | <input type="text"/> | <input type="text"/> |
| (108.1.5.1) | | | | | | | | <input type="text"/> | <input type="text"/> |
| (108.1.5.2) | | | | | | | | <input type="text"/> | <input type="text"/> |
| (108.1.5.3) | | | | | | | | <input type="text"/> | <input type="text"/> |

| | | | | | | | | | |
|----------------------------------|----|-----|--------|----|-----|--------|---|----------------------|----------------------|
| 108.1.6 RESPIRATORY EXERCISES | NO | YES | IF YES | NO | YES | IF YES | → | <input type="text"/> | <input type="text"/> |
|----------------------------------|----|-----|--------|----|-----|--------|---|----------------------|----------------------|

| |
|---------------------------------------------------------------------------------------------|
| In the last 3 months how many times have you been to the gym/swimming pool? |
|---------------------------------------------------------------------------------------------|

| | | | | | | | | | |
|------------------|----|-----|--------|----|-----|--------|---|----------------------|----------------------|
| 108.1.7 SWIMMING | NO | YES | IF YES | NO | YES | IF YES | → | <input type="text"/> | <input type="text"/> |
|------------------|----|-----|--------|----|-----|--------|---|----------------------|----------------------|

| | | | | | | | | | |
|-------------------------|----|-----|--------|----|-----|--------|---|----------------------|----------------------|
| 108.1.8 OTHER EXERCISES | NO | YES | IF YES | NO | YES | IF YES | → | <input type="text"/> | <input type="text"/> |
|-------------------------|----|-----|--------|----|-----|--------|---|----------------------|----------------------|

| | | | | | |
|-------------------------|----|-----|--------|----|-----|
| 108.1.9 DIET CONTROL | NO | YES | IF YES | NO | YES |
|-------------------------|----|-----|--------|----|-----|

| | | | | | |
|--------------------------------------------------|----|-----|--------|----|-----|
| 108.1.10 THERMAL INHALINGS TREATMENT CYCLE | NO | YES | IF YES | NO | YES |
|--------------------------------------------------|----|-----|--------|----|-----|

| | | | | | |
|-----------------------------|----|-----|--------|----|-----|
| 108.1.11 OTHER, SPECIFY: | NO | YES | IF YES | NO | YES |
|-----------------------------|----|-----|--------|----|-----|

109. In the last **3 months**, have you regularly (every day or every week) taken medicines, including eye drops, containing beta blockers?

| | |
|----------------------|----------------------|
| NO | YES |
| <input type="text"/> | <input type="text"/> |

110. Has your doctor ever prescribed medicines for your breathing?

| | |
|----------------------|----------------------|
| NO | YES |
| <input type="text"/> | <input type="text"/> |

IF 'NO' GO TO QUESTION 111, IF 'YES':

110.1 Has your doctor ever explained to you how to use the different types of spray nozzles for the prescribed inhalers?

| | |
|----------------------|----------------------|
| NO | YES |
| <input type="text"/> | <input type="text"/> |

110.2 If you are prescribed medicines for your breathing,
do you **normally** take:

- A) all of the medicine?
- B) most of the medicine?
- C) some of the medicine?
- D) none of the medicine?

TICK ONE BOX ONLY

| | |
|---|--------------------------|
| 1 | <input type="checkbox"/> |
| 2 | <input type="checkbox"/> |
| 3 | <input type="checkbox"/> |
| 4 | <input type="checkbox"/> |

110.3 **When your breathing get worse**, and you are prescribed
medicines for your breathing, do you normally take:

- A) all of the medicine?
- B) most of the medicine?
- C) some of the medicine?
- D) none of the medicine?

TICK ONE BOX ONLY

| | |
|---|--------------------------|
| 1 | <input type="checkbox"/> |
| 2 | <input type="checkbox"/> |
| 3 | <input type="checkbox"/> |
| 4 | <input type="checkbox"/> |

110.4 Do you think it is bad for you to take medicines all the time
To help your breathing?

| | |
|--------------------------|--------------------------|
| NO | YES |
| <input type="checkbox"/> | <input type="checkbox"/> |

110.5 Do you think you should take as much medicine as you need to get
rid of all your breathing problems?

| | |
|--------------------------|--------------------------|
| NO | YES |
| <input type="checkbox"/> | <input type="checkbox"/> |

ONLY FOR THE ISAYA AND THE ECRHS COHORTS' SUBJECTS:

111.FU **Since the last survey**, have you visited a hospital casualty department
or emergency room (for whichever reason, apart from accidents or injuries)?

IF 'NO' GO TO QUESTION 112.FU, IF 'YES':

111.1.FU Was this due at least once to **breathing problems**?

| | |
|--------------------------|--------------------------|
| NO | YES |
| <input type="checkbox"/> | <input type="checkbox"/> |
| NO | YES |
| <input type="checkbox"/> | <input type="checkbox"/> |

111. In the last **12 months** have you visited a hospital casualty department
or emergency room (for whichever reason, apart from accidents or injuries)?

IF 'NO' GO TO QUESTION 112.FU, IF 'YES':

111.1 How many times in the last **12 months**?

| | |
|--------------------------|--------------------------|
| NO | YES |
| <input type="checkbox"/> | <input type="checkbox"/> |
| TIMES | |
| <input type="text"/> | <input type="text"/> |

111.2 Among these ones, how many times because of **breathing problems**?

[Write '0' if s/he had not visited the emergency room for respiratory problems]

| | |
|----------------------|----------------------|
| TIMES | |
| <input type="text"/> | <input type="text"/> |

112.FU **Since the last survey**, have you spent a night in hospital
(for whichever reason, apart from accidents or injuries)?

IF 'NO' GO TO QUESTION 113, IF 'YES':

112.1.FU Was this due at least once to **breathing problems**?

| | |
|--------------------------|--------------------------|
| NO | YES |
| <input type="checkbox"/> | <input type="checkbox"/> |
| NO | YES |
| <input type="checkbox"/> | <input type="checkbox"/> |

ONLY FOR THE SARA COHORT SUBJECTS:

111. In the last **12 months** have you visited a hospital casualty department
or emergency room (for whichever reason, apart from accidents or injuries)?

IF 'NO' GO TO QUESTION 112, IF 'YES':

111.1 How many times in the last **12 months**?

| | |
|--------------------------|--------------------------|
| NO | YES |
| <input type="checkbox"/> | <input type="checkbox"/> |
| TIMES | |
| <input type="text"/> | <input type="text"/> |

111.2 Among these ones, how many times because of **breathing problems**?

[Write '0' if s/he had not visited the emergency room for respiratory problems]

| | |
|----------------------|----------------------|
| TIMES | |
| <input type="text"/> | <input type="text"/> |

112. In the last **12 months**, have you spent a night in hospital
(for whichever reason, apart from accidents or injuries)?

IF 'NO' GO TO QUESTION 113, IF 'YES':

112.1 How many times in the last **12 months**?

112.2 Was this due at least once to **breathing problems**?

IF 'NO' GO TO QUESTION 113, IF 'YES':

112.2.1-5 In the last **12 months** how many times have you been hospitalized
in each of the following types of ward for **breathing problems**?

112.2.1 general

112.2.2 chest medicine

112.2.3 rehabilitation

112.2.4 intensive care unit

112.2.5 other

NO YES
☐ ☐

TIMES

☐ ☐

NO YES
☐ ☐

TIMES

| | |
|--|--|
| | |
| | |
| | |
| | |
| | |

113. In the last **12 months** have you been seen by your general practitioner
(for whichever reason, apart from accidents or injuries)?

IF 'NO' GO TO QUESTION 114, IF 'YES':

113.1 How many times in the last **12 months**?

113.2 Among these ones, how many times because of **breathing problems**?

[Write '0' if s/he had not been seen by her/his general practitioner for respiratory problems]

NO YES
☐ ☐

TIMES

☐ ☐

TIMES

☐ ☐

114. In the last **12 months** have you seen a specialist
(for whichever reason, apart from accidents or injuries)?

IF 'NO' GO TO QUESTION 115, IF 'YES':

114.1 How many times in the last **12 months**?

114.2 Among these ones, how many times have you seen a specialist
(chest physician, allergy specialist, internal medicine specialist,
ENT doctor) because of your **breathing problems**?

[Write '0' if s/he had not been seen by a specialist for respiratory problems]

NO YES
☐ ☐

TIMES

☐ ☐

TIMES

☐ ☐

115. Are you given regular appointments to be seen by a doctor
because of **breathing problems**?

NO YES
☐ ☐

116. In the last **12 months** how many times have you visited the following
because of **breathing problems**?

116.1 nurse

116.2 physiotherapist

116.3 practitioner of 'alternative' medicine

TIMES

| | |
|--|--|
| | |
| | |
| | |

117. In the last **12 months** have you had any clinical or laboratory test
because of health problems (apart from accidents or injuries)?

IF 'NO' GO TO QUESTION 118, IF 'YES':

117.1 Was this happened at least once for **breathing problems**?

IF 'NO' GO TO QUESTION 118, IF 'YES':

117.1.1-5 In the last **12 months** how many times have you had the following test
for **breathing problems**?

117.1.1 breathing test in a laboratory specially for lung function measures

117.1.2 skin test for allergy

117.1.3 blood test for allergy

117.1.4 x-rays

117.1.5 thorax CT

NO YES
☐ ☐

NO YES
☐ ☐

TIMES

| | |
|--|--|
| | |
| | |
| | |
| | |
| | |

NO YES

118. Have you worked in the last **12 months**?

| | |
|--|--|
| | |
|--|--|

IF 'NO'

ONLY FOR THE ISAYA AND THE ECRHS COHORTS' SUBJECTS
GO TO QUESTION 119.FU

IF 'NO'

ONLY FOR THE SARA COHORT'S SUBJECTS
GO TO QUESTION 119

IF 'YES':

118.1 In the last **12 months** have you lost days of work because of health problems
(apart from accidents or injuries)?

| | |
|----|-----|
| NO | YES |
| | |

IF 'NO'

ONLY FOR THE ISAYA AND THE ECRHS COHORTS' SUBJECTS
GO TO QUESTION 119.FU

IF 'NO'

ONLY FOR THE SARA COHORT'S SUBJECTS
GO TO QUESTION 119

IF 'YES':

118.1.1 How many days in the last **12 months**?

| | | |
|------|--|--|
| DAYS | | |
| | | |

118.1.2 Among these ones, how many because of **breathing problems**?

[Write '0' if s/he had not lost days of work for respiratory problems]

| | | |
|------|--|--|
| DAYS | | |
| | | |

ONLY FOR THE ISAYA AND THE ECRHS COHORTS' SUBJECTS:

119.FU **Since the last survey** were you forced to **give up working**

Because of health problems (apart from accidents or injuries)?

IF 'NO' GO TO QUESTION 120, IF 'YES':

119.1.FU When?

| | |
|-------|------|
| NO | YES |
| | |
| MONTH | YEAR |
| | |

119.2.FU Did it happen because of **respiratory problems**?

| | |
|----|-----|
| NO | YES |
| | |

ONLY FOR THE SARA COHORT'S SUBJECTS:

119. Were you forced to **give up working** in the last **12 months**
because of health problems (apart from accidents or injuries)?

IF 'NO' GO TO QUESTION 120, IF 'YES':

119.1 When?

| | |
|-------|------|
| NO | YES |
| | |
| MONTH | YEAR |
| | |

119.2 Did it happen because of **respiratory problems**?

| | |
|----|-----|
| NO | YES |
| | |

120. Whichever is your professional condition, in the last **12 months**,
have there been any days when you have had to **give up activities other than work**
(e.g. looking after children, the house, study)

because of health problems (apart from accidents or injuries)?

IF 'NO' YOU HAVE FINISHED THE QUESTIONNAIRE, IF 'YES':

120.1 How many days on average each month?

| | |
|------|-----|
| NO | YES |
| | |
| DAYS | |
| | |
| DAYS | |

120.2 Among these ones, how many because of *respiratory problems*?

| | |
|--|--|
| | |
|--|--|

END

FIELDWORK NUMBER

| | |
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| | |
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