

**ERS Research seminar
Post Genome respiratory epidemiology
Abbaye des Vaux de Cernay 25-27 January 2002**

Working group topics program
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Postma DS., Howard TD, Koppelman GH, Xu J, Zheng SL, Meyers DA, Bleecker ER. Gene-gene interaction in asthma

Romieu I. What are the current research questions in respiratory epidemiology regarding nutritional factors ?

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Srivastava P, McNeill G, Shaw D, Stewart D, Bourguignon M, Reid T, Helms P CD14 genotype, atopy and antibodies to *H. Pylori* in young adults.

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PROVIDING INFORMATION USEFUL TO THE UNDERSTANDING OF RHINITIS AND ASTHMA BY EPIDEMIOLOGICAL INVESTIGATION OF THEIR COMORBIDITY.

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Clinical observations suggest that various phenotypes of rhinitis and asthma occur together more often than expected by chance alone. This has been confirmed by epidemiological studies conducted in population-based samples with standardised methods. Twin studies have also provided further evidence. However, available data are still few overall in the respect of indirect evidence of the relationship between the two conditions (see below).

Outnumbering patients suffering from allergic rhinitis and “extrinsic” (allergic) asthma has provided direct evidence of the relationship between rhinitis and asthma. Fewer epidemiological data exist in the case of non-allergic rhinitis and “intrinsic” (non allergic) asthma. Epidemiological studies have also provided arguments supporting indirect evidence of the relationship between rhinitis and asthma such as: 1) the existence of a common aetiology for the two diseases; 2) the association of rhinitis with intermediate phenotypes of asthma (such as specific and non specific bronchial hyper-responsiveness) and *vice versa* of asthma with intermediate phenotypes of rhinitis (such as specific nasal hyper-responsiveness); 3) similarities in the natural history of the two diseases.

Epidemiological investigation of indirect associations between rhinitis and asthma may provide important clues in the understanding of both diseases, among which their respective genetics. In this context, epidemiology ought to contribute appropriate tools to fill information gaps commonly encountered in studies such as lack of standardisation in assessment of diseases, shortage of prospective design, taking into account of confounders.

Examples will be shown and discussed during the seminar.

CD14: A MODEL OF GENE-BY-ENVIRONMENT INTERACTION IN ALLERGIC DISEASE.

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CD14, the receptor for bacterial LPS, appears to be involved in APC-mediated Th1/Th2 cell differentiation. A single nucleotide polymorphism (SNP) in the CD14 promoter (-159 C/T) is associated with soluble CD14 (sCD14) ¹ and total serum IgE levels ¹⁻³. Therefore, -159 C/T or other promoter SNPs in linkage disequilibrium with -159 C/T could affect sCD14 expression thereby influencing a Th2 response such as IgE production. A search for additional promoter SNPs in the CD14 gene was recently extended using denaturing high-performance liquid chromatography and automated sequence analysis of overlapping PCR products ^{4, 5}. Four SNPs were detected; -1619 A/G, -1359 G/T, -1145 A/G and -809 A/C. Total serum IgE and genotypes for -1359 G/T, -1145 A/G and -159 C/T were assessed in an unselected sample of children (mean age: 11 years). Due to extremely tight linkage disequilibrium between the three SNPs, virtually only T/A/C, G/A/C and G/G/T were present among all possible -1359/-1145/-159 haplotypes. Subjects homozygous for the T/A/C haplotype had significantly higher levels of total serum IgE (154.9 ± 4.8 IU/ml, N=27, p=0.01) than homozygotes for both the G/G/T (50.1 ± 6.0 IU/ml, N=78) and the G/A/C haplotype (63.1 ± 4.5 IU/ml, N=28). Although homozygous for the T/A/C haplotype had the lowest concentration of sCD14, pointing to an inverse correlation between total serum IgE and sCD14 levels, such difference was not statistically significant.

-159 C/T alone was the major determinant of variability in sCD14 levels as is supported by recent evidence indicating its role in differential transcriptional activity ⁶. A CD14 promoter reporter construct containing CD14/-159T was 32% more active than the wild-type C allele when transiently transfected into monocytic cells. This increase in activity was paralleled by a decreased affinity of Sp protein (Sp1-3)/DNA interactions at the polymorphic GC box, suggesting that the presence of the T allele resulted in decreased binding of inhibitory Sp family member(s).

Conclusion: variations in a key gene of innate immunity may be important for the pathogenesis of allergy and inflammatory disease through gene-by-gene and/or gene-by-environment interactions.

References

1. Baldini M, Lohman IC, Halonen M, Erickson RP, Holt P, Martinez FD. A polymorphism in the 5'-flanking region of the CD 14 gene is associated with circulating soluble CD14 levels and with total serum IgE. *American Journal of Respiratory Cell and Molecular Biology* 1999; 20:976-983.
2. Gao P, Mao X, Baldini M, et al. Serum total IgE levels and CD14 on chromosome 5q31. *Clinical Genetics* 1999;164-165.
3. Koppelman GH, Reijmerink NE, Colin Stine O, et al. Association of a promoter polymorphism of the CD14 gene and atopy. *American Journal of Respiratory and Critical Care Medicine* 2001; 163:965-969.
4. Baldini M, Kabesch M, Graves PE, Erickson RP, Vercelli D, Martinez FD. Detection of four novel polymorphisms in the CD14 promoter and association of their haplotypes with total serum IgE levels. *American Journal of Respiratory and Critical Care Medicine* 2000; 161:A928.
5. Vercelli D, Baldini M, Martinez FD. The Monocyte/IgE Connection: May Polymorphisms in the CD14 Gene Teach Us about IgE Regulation? *Int Arch Allergy Immunol* 2001; 124:20-24.
6. LeVan TD, Bloom J, Bailey T, et al. A common single nucleotide polymorphism in the CD14 promoter alters the affinity of Sp protein binding and enhances transcriptional activity. *J Immunol* 2001; 167:5838-44.

M. BOEZEN

Abstract will be distributed at the seminar

CURRENT QUESTIONS IN OCCUPATIONAL LUNG DISEASES

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For Post Genome Respiratory Epidemiology
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A Delphi conference in 1993 identified three main areas for epidemiological study. Although the recommendations were for occupational allergic diseases, they can equally be applied to diseases of long latency. The areas for priority research were:

The relationship between exposure and ill health

The natural history of occupational lung diseases after exposure has ceased

The effects of promoting and protective factors which influence dose-response relationships

The conference recommended longitudinal studies as the principal method for filling these gaps in our knowledge. Longitudinal studies have proved difficult to perform. They require long-term commitment from the subjects being studied, the researchers and the funders, and are only really feasible in industries where similar exposures continue for a long time. It is not very helpful to find that anthracite coal causes more COPD than softer coals after the need for anthracite has gone and anthracite mines have closed down. Long-term studies also require the methods of exposure measurement and ill-health outcome measurement to remain stable over long periods of time. In practice valid alternatives to longitudinal studies need developing with surrogate markers for long-term response, so that those taking part in the research can benefit from the risk assessments achieved.

Ill-health outcomes are often difficult to define for epidemiological studies. Asthma and COPD are particular problems. Should every asthmatic have abnormal non-specific reactivity and sputum eosinophilia? How should those with otherwise indistinguishable disease without these features be classified? How can asthma with poor reversibility be distinguished from COPD? Diseases with clear definitions such as bronchial carcinoma and asbestos-related pleural disease are much easier to study. Does the diagnostic label we attach to a worker affect the long-term outcome?

Similar problems relate to exposure assessment. Methods for defining exposure should be easier to standardise; the problems come from estimating individual exposures from limited air measurements, and in particular estimating these from occupational histories. How can we estimate exposures indirectly, from products in the urine or exhaled breath for instance?

There is much enthusiasm for finding personal risk factors for disease over and above the exposures. We already have strong evidence that some sorts of occupational asthma are much more common in atopic as opposed to non-atopic workers. Should we stop atopic workers from taking up occupations where the increased risks are known, or should we make the workplace safe for the majority of workers? Finally having defined the risks, how should we present these to those exposed and to legislators so that the most appropriate decisions can be made?

PETER BURNEY
THE ASTHMA PHENOTYPE

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Asthma is often described but has never been defined. However, a definition is either circular or only meaningful in a particular context. In the context of health care delivery, a definition of asthma might be 'the condition that responds to inhaled bronchodilators'. This would, however, cover a wide range of pathologies. Emphysema, on the other hand, is defined by pathology and, as such, is of little help in analysing health care needs. Whatever way asthma is defined the phenotype is generally assumed to be the result of several causes and many modifying factors.

In the context of genetics this is important. What is being sought is a gene or several genes that have an important contribution to the heterogeneous conditions generally known as asthma. As with any causal agent the more confounding factors there are that are not taken into account, the more difficult it will be to identify any link. Although it would be tempting to think that the need then was to 'adjust for everything', this would be counterproductive if the confounder adjusted for was on the causal path between the gene and the outcome. An alternative strategy is to look for intermediate outcomes that might themselves be linked both to genes and to asthma. An obvious example is the search for links between the genome and 'atopy' and 'airway responsiveness'.

This leads in turn to further definitional problems and problems of obvious potential confounding. Atopy is variously defined and the definitions are not closely related to each other. Indeed, within the asthmatic population high IgE level, positive skin tests to allergens and eosinophilia are approximately independent of each other. Given what is known of their biology this is not altogether surprising. Similarly, different methods of measuring airway responsiveness give different results in the same individuals. While it is clearly important that the same method is used in any one study, and particularly in any family, whether the choice of measurement matters in the search for linkage to genes is uncertain.

While the strategy for searching for genes may be better pursued by looking for intermediate effects close to the genes, it does not follow that the single gene defects will produce a single phenotype. Other effects including those of the environment, changes in other genes and different polymorphisms of the same gene may all provide a very variable phenotype. This has been particularly well illustrated in the case of cystic fibrosis. Returning to the point of departure, a genetic definition of asthma, if any were ever possible, might produce a very indistinct phenotype.

FAMILIAL AGGREGATION OF SMOKING BEHAVIOR IN THE GENERAL POPULATION
Participants to the Seminar: **LAURA CARROZZI, MATTEO BOTTAI**
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What has been done

In order to evaluate familial aggregation of smoking behavior are investigated 2,333 families from two Italian general population samples, living in Po Delta rural area (North of Italy, 2,841 subjects) and in Pisa urban area (Central Italy, 2,838 subjects).

Smoking habit was assessed by an interviewer administered standardized questionnaire (CNR questionnaire).

Overall, prevalence of ever (current or former) smokers was 57%. 21% of the subjects haven't had any smokers in the family; conversely, 26% of the subjects have had all smokers in the rest of the family.

Logistic regression was used to model the probability to have ever been a smoker, including proportion of ever smokers among the family (quartiles), residence (rural/urban), gender, age (quartiles), working status (yes/no) as independent variables: probability increases along with the proportion of ever smokers among the other family members (common OR = 1.35, 95% CI = 1.06-1.71).

In conclusion, the presence of smokers in the family seems to influence smoking behavior in the subjects analyzed.

Issues to solve

Is this type of analysis good enough to capture potential familial aggregation for smoking habits?

Could it be extended to any other possible risk factor or even to subjects' personal characteristics such as symptoms and diseases?

How to separate environmental from genetic components?

PASSIVE SMOKING AND LUNG FUNCTION IN ALPHA-1-ANTITRYPSIN HETEROZYGOTES SCHOOLCHILDREN.

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Our aim was to determine whether Pi heterozygotes exposed to smoking run a greater risk of reduced lung function compared to Pi M homozygotes. We investigate the effect of passive smoking on lung function of schoolchildren take into consideration Pi heterozygosity as a possible effect modifier. A cross-sectional study was performed in primary and secondary schools in Latium region, Central Italy and a total of 997 subjects aged 11-13 years were studied. Data on respiratory health and risk factors were collected by questionnaire; lung function was measured by spirometer; bronchial hyperresponsiveness was evaluated by methacholine test; atopic status was evaluated by skin prick test; a blood sample was collected to determine serum IgE, eosinophils and Pi phenotype. Cotinine and creatinine concentration were determined from spot urine sample. Subjects were categorised according to Pi phenotype as PiM homozygotes and Pi heterozygotes. Exposure assessment was made by questionnaire data and urinary cotinine concentration. Results – A total of 61 subjects (6.1%) were found to be Pi heterozygotes. Lung function did not differ between homozygotes and heterozygotes. There was a reduction in lung function among subjects exposed to parental smoking in the overall sample : FEV₁/FVC ratio (-.838, p < 0.05), FEF₂₅₋₇₅ (-0.106 liters, p < 0.05), and FEF₇₅ (-0.146, p < 0.01). Moreover, interaction terms between ETS and Pi status were significant as regards FEV₁/FVC ratio (p=0.054) and FEF₅₀ (p=0.024). In subjects exposed to ETS the decrement of lung function tended to be greater in Pi heterozygotes (FEV₁/FVC ratio = -2.208, p=0.01, FEF₂₅₋₇₅= -0.251, p=0.06, FEF₅₀=-0.373 p=0.01, and FEF₇₅=-0.258, p=0.035) than in PiM homozygotes. We confirmed detrimental effect of ETS on lung function in schoolchildren. This harmful effect is greater in Pi heterozygotes.

**DOES α_1 -ANTITRYPSIN *MZ* HETEROZYGOSITY INTENSIFY THE EFFECT OF SMOKING ON PULMONARY FUNCTION AND DISEASE?
A STUDY OF 9,187 INDIVIDUALS FROM THE GENERAL POPULATION**

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Tobacco smoking is the most important risk factor for developing chronic obstructive pulmonary disease (COPD), however, only a fraction of tobacco smokers develop clinical symptoms of COPD. We tested whether smokers with the common α_1 -antitrypsin *MZ* genotype had reduced FEV₁% predicted, increased annual decline in FEV₁, or increased risk of airway obstruction or death or hospitalisation due to COPD. For this purpose we did spirometry and genotyped 4,412 current smokers, 2,330 ex-smokers, and 2,282 never smokers from the adult Danish general population, the Copenhagen City Heart Study. Smokers with *MZ* genotype had FEV₁% predicted of 87% compared with 89% in smokers with *MM* genotype (t-test: P=0.13). Smokers with *MZ* genotype had annual decline in FEV₁ of 27 ml compared with 25 ml in smokers with *MM* genotype (P=0.49). Unadjusted odds ratio for airway obstruction was 1.4 (95% CI, 1.1-1.9) in *MZ* smokers vs. *MM* smokers. Unadjusted relative risk for COPD hospitalisation and/or death was 1.6 (1.0-2.4) in *MZ* smokers vs. *MM* smokers. Smoking habits, however, did not interact statistically with α_1 -antitrypsin *MZ* heterozygosity in predicting FEV₁% predicted (P=0.10), annual change in FEV₁ (P=0.23), airway obstruction (P=0.61), and COPD hospitalisation and/or death (P=0.35). In conclusion, α_1 -antitrypsin *MZ* heterozygosity did not intensify the effect of smoking on FEV₁% predicted, annual decline in FEV₁, and risk of airway obstruction or COPD.

MODEL-BASED METHODS TO DETECT GENE-ENVIRONMENT INTERACTIONS IN FAMILY STUDIES.

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INSERM EMI 00-06, Evry, France.

Investigating gene-environment (GxE) interactions in multifactorial diseases becomes of increasing interest once a gene involved in these diseases has been mapped to a chromosomal region and there is linkage disequilibrium (LD) in that region. Two main analytical approaches have been proposed to address this issue: 1) model-free methods that do not assume a genetic model for the disease; 2) model-based methods that specify a genetic model. Our presentation will focus on this latter approach. The regressive models, that can include the effect of the gene under investigation, in linkage disequilibrium with a marker or a set of markers (haplotypes), other sources of familial correlations (due to other genes and/or shared environmental factors), and covariates are particularly suitable to investigate gene-gene and gene-environment interactions. We have implemented these models in the REGESS software and a new software (FINESSE) is under development. Power of this model to detect GxE has been investigated by simulations in nuclear families assuming common genetic variants with small effects underlying the liability to disease (as likely to be encountered in asthma and atopy) and varying the strength of LD and GxE. Power to detect GxE is high (> 80%) when there is complete LD (i.e. confounding between the genetic variant and the marker). The decrease in power observed for incomplete LD or no LD depends on the genetic model and GxE strength. Alternatively, ignoring the presence of GxE in the analysis may affect the detection of the true LD model and, therefore, the identification of the putative functional variant. Thus, use of models taking into account both LD and GxE appears of major importance to disentangle the mechanisms underlying complex diseases. This method will be illustrated by a few examples.

ENVIRONMENTAL AND FAMILIAL DETERMINANTS FOR WHEEZING AND ASTHMA IN CHILDREN IN PALESTINE
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Background Several unknown genetic factors are clearly important in determining the risk for asthma development, but environmental factors are also likely to be primary determinants of disease expression. In the Middle East, asthma prevalence is reported to be lower than in “developed” countries. In Palestine, our prevalence study on children aged 6-12 years showed crude prevalence rates for “wheezing ever”, “wheezing in the previous 12 months”, and “physician-diagnosed asthma” of 17.1%, 8.8% and 9.4% respectively. There were significant differences between cities, villages, and refugee camps, with the latter having highest rates. Reasons for these findings are unknown and need further investigations.

Objective To investigate the familial, early days exposures, and indoor environmental determinants for asthma in Palestinian schoolchildren aged 6-12 years living in the West Bank.

Methods A case-control study of schoolchildren, drawn from the prevalence study, comparing those children reported having wheezing in the previous 12 months (wheezing children, n=237) to controls (non-wheezing children, n=252).

The methods included answers to a detailed parental questionnaire (n=489). In addition, 376 children, those who accepted to participate in complete testing, were tested for pulmonary function (with a 6 minute exercise to assess bronchial hyperresponsiveness); skin prick test (SPT) for 8 allergens; serum for total and specific IgE; and dried blood spots were stored for future genetic analysis. Moreover, home environmental samples were obtained for a subgroup (n=120).

Results In our study, wheezing children’s families showed more asthma, hay fever and eczema (OR 1.57 [95%CI 1.04-2.36], 1.75 [1.22-2.51], respectively) when compared to controls’ families. Among wheezing disorders, the asthmatic children (n=99), i.e. those who had physician-diagnosed asthma in the prevalence questionnaire, showed similar but stronger associations. Previous diagnoses of bronchial allergy, bronchitis, pneumonia, or whooping cough were significantly more likely among wheezing and asthmatic children than controls. After adjustment for several environmental and socio-demographic factors and previous diseases, a family history of asthma continued to be significantly higher among wheezing children than controls.

Children having attended day care centers showed higher risks of physician-diagnosed asthma than controls, but this association was not significant for the wheezing children. Houses of both wheezing and asthmatic children were reported to have more damp spots (OR 1.69 [1.05-2.74], 1.84 [1.01-3.36], respectively) and visible moulds, either recently or in the first year of the child’s life. Neither keeping pets indoor or outdoor, nor using gas or gasoline for cooking and heating showed any significant difference between cases and controls.

Breathing problems related to colds, indoor and outdoor dust, and other provoking factors had often led to indoor changes such as removing pets and stopping smoking. Positive skin prick tests for any of the 8 allergens, such as house dusts mites and cockroaches, were more frequently positive in both wheezing- and asthmatic-children than their controls.

Conclusion Our study confirmed that familial “atopic” diseases are significant predictors of childhood asthma in Palestinian children. However, indoor environment such as domestic moulds also appears to play a role in asthma prevalence, incidence and/or symptoms exacerbation. Our findings are consistent with other studies done in Canada and New Zealand, and we have similar results to studies carried out recently on Estonian and Swedish schoolchildren. Thus, our study material shows promise to explore further the gene-environment interaction in the genesis of asthma.

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NUMBER OF OFFSPRING AND MATERNAL ALLERGY

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The consistent association seen between family size and childhood allergy has led to the “hygiene hypothesis”, namely that a lower exposure to infections in early childhood is associated with an increased risk of asthma and hay-fever. It has been recently observed (Sunyer et al, 2001) that maternal atopy measured with prick test is inversely related to the number of offspring. Since maternal atopy is a strong predictor of childhood hay-fever and asthma, it has been postulated that this observation challenges the role of family size on child atopy. We have tried to replicate the findings by analysing data already collected in a cross-sectional study of non-smoking women in four Italian areas. A total of 1,755 women (35-74 years) filled a questionnaire on reproductive history as well as on lifetime occurrence of symptoms/diseases: wheeze (9.2%), physician-diagnosed asthma (6.2%), symptoms of allergic rhinitis (26.1%), and symptoms allergic conjunctivitis (30.1%). There was no relationship between level of education and wheeze or physician diagnosis of asthma, but occurrence of both allergic rhinitis (p for trend <0.001) and allergic conjunctivitis (p for trend <0.001) raised with increased educational level. We have examined the association between number of pregnancies and number of live births with the four outcomes. After adjustment for area of residence, age, and educational level, the number of live births was inversely related to lifetime allergic rhinitis (p-value for trend=0.037) and allergic conjunctivitis (p-value for trend= 0.018). The odds ratios for those with 4+ children (in comparison with those having 0-1) were: 0.54 (95%CI=0.27-1.06) and 0.44 (95%CI=0.23-0.84), respectively. No association was found between number of children and wheeze or asthma, and between number of pregnancies and all the outcomes investigated. The results suggest that pregnancy itself may have an effect on maternal atopy. Increase in maternal atopy, due to a decrease in women's parity, may be proposed then to explain the growing prevalence of childhood allergy. Alternatively, another causal factor, which is linked with both low SES and high number of children, may be also responsible for the low occurrence of maternal allergic disorders: the same factor which protect children from allergy may decrease the expression of allergy in the mother.

INCIDENCE OF GOLD PHENOTYPES OF COPD IN A GENERAL ADULT POPULATION.

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A stratified random sample of the population aged 18 to 73 years of Bergen and 11 surrounding municipalities in Norway was examined in 1987/88 and re-examined in 1996/97 with the same procedure and equipment for spirometry. COPD was defined as FEV_1/FVC below 0.7 and FEV_1 was adjusted by age, height and sex using reference values developed from asymptomatic, never smokers who had never been exposed to dust.

The participation rates of randomly drawn persons invited to the first and second survey were 83% and 79%, respectively. The prevalence of COPD in 1987/88 was 5,9%. Altogether 915 subjects, who were at risk for developing COPD in 1987/88, were reexamined and their average age (SD) was 41 (18) years while 52% were women. 39%, 24% and 38% of the subjects at risk were never smokers, ex-smokers and smokers, respectively. The overall 9 years incidence (SE) rate of COPD was 9.8 (1.3)%, being 6.5 (1.4)% in women and 13.4 (2.1)% in men. The incidence (SE) in the age groups 18-29, 30-44, 45-59 and 60-74 years were 3.7 (1.5)%, 8.3 (1.9)%, 9.6 (2.5)% and 23.4 (4.7)% respectively and for never smokers, ex smokers and smokers the incidence were 3.9 (1.1)%, 9,8 (2.7)% and 15.8 (2.6)%, respectively. The percentage distribution of incident COPD cases in 1996/97 by GOLDs classification of severity was 5% moderate B, 55% moderate A and 40% mild.

The incidence of GOLD defined phenotypes of COPD increase heavily with age and smoking habits of people in a Norwegian community

WHAT ARE THE CURRENT RESEARCH QUESTIONS IN RESPIRATORY EPIDEMIOLOGY REGARDING AIR POLLUTION?

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Outdoor air pollution is a complex mixture of airborne particles of different composition and size and several gaseous components such as sulfur dioxide, nitrogen dioxide and ozone. In recent years there has been a vast number of epidemiological studies documenting that short-term variations in outdoor air pollution are associated with acute effects on a range of endpoints including acute respiratory symptoms, pulmonary function, respiratory and cardiovascular hospital admissions and mortality. Most studies have identified particulate matter (typically characterized as the mass of particles less than 10 μm , denoted as PM10) as an important constituent. Effects of long-term exposure to air pollution have been documented on chronic respiratory symptoms, lung function and (cardio-pulmonary) mortality. The number of studies assessing long-term air pollution effects (especially on mortality) is very limited, complicating quantitative risk assessment considerably. Important research questions that need to be addressed include:

- ?? Does long-term exposure to outdoor air pollution result in increased cardiovascular and respiratory mortality?
- ?? Does exposure to motorized traffic emissions (including diesel soot) result in an increase in allergy and asthma?
- ?? What mechanisms play a role in explaining the associations between (particulate matter) air pollution and cardiovascular mortality and hospital admissions?
- ?? Which factors explain the heterogeneity in short-term effects observed in different locations (host, air pollution interactions, life-style)?
- ?? Which component(s) of particulate matter play a role in the observed health effects?

SMOKING AND ADULT-ONSET ASTHMA: A POPULATION-BASED INCIDENT CASE-CONTROL STUDY

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Cancelled

LIMITATIONS AND CHANCES OF CANDIDATE GENE STUDIES OF CHILDHOOD ASTHMA

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Asthma and allergy can be viewed as complex diseases in which genetic variations as well as environmental factors play a role. Segregation analyses have indicated that multiple genes are involved in the polygenic pattern of inheritance observed in these diseases. To identify genetic variants that potentially contribute to the development of asthma and atopy strategies have been used which are based on a candidate gene approach. Typically, a single nucleotide polymorphism (SNP) in one candidate gene has been used to genotype a study population to establish an association between the genetic variant and a phenotype of interest. So far, genetic variants in more than 100 genes have been studied this way. Although this is a very powerful method to identify genetic involvement of certain genes in the development of the disease, a number of limitations and pitfalls exist. We would like to discuss study design issues that have to be addressed for candidate gene studies including the selection of the appropriate study design (population based, case-control or trios), the adequate sample size number and methods for genetic sample stratification. Furthermore, problems exist in the interpretation of results stemming from association studies with candidate genes as spurious associations are frequently observed. Confirmation of results in independent populations, ascertainment of linkage disequilibrium with surrounding SNPs and corrections for multiple testing are potential methods to validate the impact of association results. Furthermore, strategies to replace candidate gene studies by candidate system studies involving signalling cascades and the ascertainment of interacting environmental factors are proposed.

HOW MUCH OF COPD RELATES TO ASTHMA PHENOTYPE ?

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COPD phenotype is primarily defined as irreversible airflow obstruction, which means a reduced FEV₁/FVC ratio, reduced FEV₁ in percent of that predicted together with only a small (or no) improvement in FEV₁ after administration of a bronchodilator (no reversibility).

As several studies indicate that asthma may also lead to irreversible airflow limitation, the differentiation between COPD and asthma with irreversible airflow obstruction is difficult. This is especially the case in an epidemiological setting, where information on other pathophysiological characteristics that may help separating asthma from COPD, like for example presence of emphysema on CT scan and/or reduced diffusion capacity, is not available. In addition many population samples do not have information on reversibility to bronchodilators and therefore differentiation between asthma and COPD has to be supported by additional tests and questionnaires. Finally, there is at present no firm consensus on how to label a condition where a previous asthmatic patient (a never smoker or a very light smoker) has developed an irreversible airflow obstruction and markers of asthmatic inflammation like a high level eosinophils are no longer present

Most studies suggest, that "asthma related airflow obstruction" is usually less severe than that caused by smoking alone and that survival of asthmatics with airflow limitation is much better than that of COPD patients with predominantly emphysema. Although the data on the prevalence of irreversible airflow limitation in asthma is sparse, in some studies this figure has been reported to be around 50%. Especially asthmatics with severe and longstanding asthma are at risk of developing permanent airflow obstruction, making this condition more prevalent among elderly asthmatics. Fortunately, it seems likely that early treatment with inhaled steroids may prevent permanent changes in asthmatic airways and thus also prevent development of permanent airflow obstruction, but long-term studies are not available.

How much of COPD may be caused by asthma may therefore vary across different study populations. In epidemiological studies comprising samples of elderly subjects from a general population, a substantial percentage of COPD phenotype may therefore be related to asthma, whereas in a clinical setting, especially among hospitalised patients, only a small percentage of COPD phenotype is likely to be related to asthma. These discrepancies related to the heterogeneity of COPD phenotype may be of importance in order to interpret and compare results from different genetic studies of COPD.

PARENT OF ORIGIN EFFECTS IN ALLERGIC DISEASES

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Maternal effects on the inheritance of atopy are well recognized, although no mechanism to explain the phenomenon has as yet been identified. The first evidence of parent of origin effects arose from phenotypic studies investigating risk factors for the development of childhood atopic disease. Since then numerous genetic linkage and association studies for atopic diseases (asthma and atopic dermatitis) and atopic traits (Immunoglobulin E) have also provided evidence of parent of origin effects on the inheritance of allergic diseases.

These genetic findings are not always consistently found when different populations have been examined for a particular genetic locus, adding a further level of complexity. Interestingly parent of origin effects in other immune diseases has also been reported, notably for Insulin Dependent Diabetes Mellitus (IDDM) and Inflammatory Bowel disease.

Detection of parent of origin effects should therefore be considered when carrying out genetic investigations, including large-scale genetic epidemiological studies. At the moment these effects can only be detected by family based tests of association. Elucidation of the mechanisms by which these epigenetic effects work may allow their detection in individual DNA.

ELASTIC FIBERS SYNTHESIS AND COPD

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BACKGROUND: In spite of a similar pattern of tobacco use only one quarter of the smokers progress to COPD. **AIM:** To determine if individual differences in elastic fiber synthesis, identified using skin morphometry, may be related to the appearance of COPD. **METHOD:** All patients attending a dermatology and/or a pneumology clinic in North Barcelona during a 6 months period were offered to participate. The study included a questionnaire on smoking habits and treatment, lung function testing and skin biopsy. The density of elastic fibers in the skin was determined using static morphometry and expressed as percentage. The correlation between lung function (FEV1% and RV/TVC) and the density of skin elastic fibers was determined, adjusting for covariates. **RESULTS:** 78 subjects (58 SD 8 years; all men) were included in the studied sample, 20 of them were never smokers (FEV1% 85 SD 17; RV/TLC 42 SD 8) and 58 were smokers (35 former, 23 current; median 40 pack-year, interquartile range 26-72; FEV1% 60 SD 30; RV/TLC 50 SD 12). The density of skin elastic fibers was lower in never smokers (12 SD 2%) than in smokers (15 SD 4%) ($p < 0.001$, t test), but in this last subgroup this density was not related to the cumulative dosage of tobacco (40 pack-year: 15 SD 4%; >40 pack-year: 15 SD 3%, ns t test). FEV1% (R^2 0.18, $p < 0.001$) and RV/TLC (R^2 0.18; $p > 0.01$) were related to skin elastic fibers, and these association did not change after adjustment for smoking (R^2 0.15, $p < 0.05$ and R^2 0.20; $p < 0.05$, respectively). The same results were also obtained after the exclusion of patients who have used inhaled or systemic corticosteroids. **CONCLUSION:** The appearance of COPD in smokers is partly related to elastic fiber synthesis and this specific individual pattern of response may be identified through the exam of skin tissue.

Abstract for ERS Research Seminar in Post Genome Respiratory Epidemiology

(Suggested placement: workshop B)

DETERMINANTS OF OCCUPATIONAL ASTHMA : ENVIRONMENTAL AND GENETIC

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Agents inhaled at work can induce asthma either by direct toxic injury to the airways (irritant-induced asthma) or as the outcome of a specific hypersensitivity reaction (hypersensitivity-induced asthma). Hypersensitivity-induced asthma is distinguished by an initial symptom free period of exposure, usually of weeks or months, on occasion years, to the agent in concentrations which subsequently provoke asthma (latency); asthma induced by inhalation of an irritant in toxic concentrations develops within hours (usually less than 24 hours) of the exposure.

Importance of occupational asthma

Several recently published studies have addressed the question of what proportion of asthma which develops or relapses in adult life is attributable to an occupational cause. Blanc and Toren, who evaluated studies published prior to 1999, concluded that the median fraction of new or relapsed asthma in adult life attributable to occupation was 9%¹. A subsequent study of adult asthma in British Columbia estimated the attributable fraction to be 18%². These data imply that between about 1 in 6 and 1 in 10 cases of new or relapsed asthma in adults has an occupational cause is potentially preventable and can be improved in the individual case by avoidance of further exposure.

Causes of occupational asthma and estimated incidence

The Surveillance of Work and Occupational Related Disease (SWORD) is a volunteer reporting scheme in UK to which the majority of chest and occupational physicians report new (incident) cases of occupational lung disease. During the 10 years of the scheme, since 1990, asthma has consistently been the most frequently reported disease category; with the exception of latex, whose incidence has increased markedly during the decade, the frequency of cases attributed the most common agents has changed little, with isocyanates accounting for some 20% of cases and flour, wood dust, laboratory animals and colophony accounting for a further 20%. The estimated incidence of asthma by occupation has also changed little being highest among coach and spray painters with an incidence of 1380/10⁶/yr³.

Determinants :

Exposure

Several studies, reported during the past decade, have shown clear evidence of an exposure-response relationship between estimated or measured exposure to airborne protein or low MW chemical and the prevalence or incidence of IgE sensitisation and associated asthma. Cullinan et al reported a gradient of sensitisation, nasal and asthmatic symptoms in relation to measured airborne exposure to rat urine protein⁴ and to estimated exposure to enzymes protease and amylase in a detergent factory⁵. Barker et al found the risk of developing specific IgE to trimellitic anhydride (TMA) and associated respiratory symptoms increased with increasing exposure to TMA in a linoleum manufacturer⁶.

Atopy

The risk of IgE sensitisation and asthma caused by several inhaled protein causes of occupational asthma is increased in those with specific IgE to common inhalant allergens (atopy). Venables et al found the risk of developing asthma caused by laboratory animal urine proteins was increased some five-fold among atopics⁷; Juniper, in a study of enzyme detergent manufacturers, found that at different levels of exposure to airborne protease, atopics were at increased risk of developing IgE sensitisation⁸. However, atopics have not been found to be at increased risk of developing asthma caused by several low MW chemicals, which include isocyanates, plicatic acid (W Red Cedar) and colophony.

Tobacco smoking

The risk of IgE sensitisation and associated asthma caused by several low MW chemicals is increased in tobacco smokers. Venables et al found that the development of specific IgE to tetrachlorophthalic anhydride (TCPA) in the workforce of an electronics components factory was increased some six-fold in tobacco smokers, most in atopic tobacco smokers⁹. Similarly Venables et al found that the risk of developing an immediate skin prick test

response to ammonium hexachloroplatinate (ACP) was some six-fold greater among tobacco smokers¹⁰. Calverley et al found the prevalence of skin prick test reactions to ACP in employees of a S. African platinum refinery was greater in those exposed to airborne concentrations of ACP greater than 2µg/m³ with an interaction between exposure and tobacco smoking¹¹. The highest risk of sensitisation occurred among tobacco smokers in the high exposure group, no cases in non-smokers in the low exposure group, with intermediate rates in non-smokers in the high exposure group and smokers in the low exposure group.

HLA associations

Several studies have reported evidence of an association of IgE sensitisation or asthma caused by low MW chemicals and HLA Class 2 alleles. Three separate studies have found that HLA DQB1* Asp⁵⁷ is present more frequently in cases of isocyanate-induced asthma than in the exposed referent group^{12,13,14}. The prevalence of HLA DR3 was significantly more frequent in cases of TMA sensitisation than in comparably exposed referents (OR = 16), whereas cases sensitised to phthalic anhydride (PA) were no more likely to be DR3 positive than their referents¹⁵. Similarly an excess of HLA-DR3, and also a deficit of HLA-DR6, was found in cases of sensitisation to platinum salts as compared to matched referents: all cases were DR3 positive or DR6 or DR2 negative; 84% of the referents were DR3 negative or DR6 or DR2 positive. The odds of a case being HLA-DR3 positive or DR6 negative was markedly greater in the 'low' intensity than in the 'high' intensity exposure groups¹⁶.

Conclusion

Occupational asthma probably accounts for some 10% of new or relapsed asthma in adult life. Its major causes and the incidence of disease in high risk occupations have changed little in the past decade. The major, and remediable, determinant of occupational asthma is exposure intensity. Atopy, tobacco smoking and Class 2 alleles also contribute to the risk of sensitisation and asthma caused by some agents. The findings of the South African platinum refinery workers¹⁶ if repeated in other circumstances with other agents, imply that the risk of genetic susceptibility may become increasingly important at low levels of exposure.

References

1. Blanc PD, Toren K. How much adult asthma can be attributed to occupational factors. *Am J Med* 1999 **107** 580-587.
2. Johnson AR, Dimich Ward HD, Manfreda J. *Am J Respir Crit Care Med* 2000 **162** 2058-2062
3. Newman Taylor AJ. Asthma. *Epidemiology of work-related diseases*, 2nd ed. McDonald JC (editor). BMJ Books 2000
4. Cullinan P, Cook A, Gordon S et al. Allergen exposure, atopy and smoking as determinants of allergy to rats in a cohort of laboratory employees. *Eur Respir J* 1999 **13** 1139-1143
5. Cullinan P, Harris JM, Newman Taylor AJ et al. An outbreak of asthma in a modern detergent factory. *Lancet* 2000; **358** 1899-1900.
6. Barker RD, Van Tongeren MJA, Harris JM et al. Risk factors for sensitisation and respiratory symptoms among workers exposed to acid anhydrides: a cohort study. *Occup Environ Med* 1998 **55** 684-696.
7. Venables KM, Tee RD, Hawkins ER et al. Laboratory animal allergy in a pharmaceutical company. *Br J Ind Med* 1988 **45** 667-71.
8. Juniper CP, Howe W, Goodwin BFJ, Kinshott AK. *Bacillus subtilis* enzymes: a 7 year clinical epidemiological and immunological study of an industrial allergen. *J Soc Occup Med* 1977; **27** 3-12.
9. Venables KM, Topping MD, Howe W, Newman Taylor AJ. Interaction of smoking and atopy in producing specific IgE antibody against a hapten protein conjugate. *Br Med J* 1985; **290** 201-204.
10. Venables KM, Dally MB, Nunn AJ et al. Smoking and occupational allergy in a platinum refinery. *BMJ* 1989; **299** 939-42.
11. Calverley AE, Rees D, Dowdeswell RJ, Linnett PJ, Keilkowski J. Platinum salt sensitivity in refinery workers: incidence and effects of smoking and exposure. *Occup Environ Med* 1995; **52** 661-6.
12. Bignon JS, Aron L, JU Y et al. HLA class II alleles in isocyanate induced asthma. *Am J Respir Crit Care Med* 1994; **149** 71-75
13. Balboni A, Baricoidi OR, Fabbri LM et al. Association between toluene diisocyanate induced asthma and DQB1 markers; a possible role for aspartic acid at position 57. *Eur Respir J* 1996; **9** 207-10.
14. Mapp CE, DE Marzo N, Jovine L et al. Association between HLA genes and susceptibility to toluene diisocyanate induced asthma. *Clin Exp Allergy* 2000; **5** 651-656.

15. Young RP, Barker RD, Pile KD, Cookson WOCM, Newman Taylor AJ. The association of HLA DR3 with specific IgE to inhaled acid anhydrides. *Am J Respir Crit Care Med* 1995; 151 219-21.
16. Newman Taylor AJ, Cullinan P, Lympny PA et al. Interaction of HLA phenotype and exposure intensity in sensitisation to complex platinum salts. *Am J Respir Crit Care Med* 1999; 160 435-438.

GENE-GENE INTERACTION IN ASTHMA

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Clinical findings in asthma include bronchial hyperresponsiveness and allergic responses, demonstrated by elevated total serum IgE levels and positive skin tests to common allergens. These closely associated clinical phenotypes have been shown to have a strong genetic component. Binding of IL4 to the IL4 receptor (IL4R) induces the initial response for Th2 polarization. IL4 and IL13 are both produced by Th2 cells and are capable of inducing isotype class-switching of B-cells to produce IgE after allergen exposure. These cytokines also share a common receptor component, IL4R α , which is a potential biological candidate gene for asthma and atopy. We have investigated five IL4R α single-nucleotide polymorphisms in a well-characterized population of Dutch families ascertained through a proband with asthma that was initially studied 25 to 35 years ago. Using the probands and their spouses from this population in a case-control study design, we observed significant associations of atopy and asthma related phenotypes with several IL4R α polymorphisms genotyped within the gene. The most significant association was observed with S478P, which was associated with high IgE levels ($p = 0.0007$). In addition, a significant gene-gene interaction was detected between the S478P variation in IL4R α (significantly associated with high IgE levels) and the -1111 promoter variation in IL13 (significantly associated with hyperresponsiveness). Individuals with the risk genotype for both of these genes were at almost five times higher risk for the development of asthma compared to individuals with both non-risk genotypes ($p = 0.0004$). These data suggest that variations in IL4R α contribute to elevated total serum IgE levels, and interaction between IL4R α and IL13 markedly increases an individual's susceptibility to asthma in this Dutch population. Investigations on gene-gene interaction are promising for better understanding of the pathophysiology and genetics of asthma.

WHAT ARE THE CURRENT RESEARCH QUESTIONS IN RESPIRATORY EPIDEMIOLOGY REGARDING NUTRITIONAL FACTORS (EXCLUDING RESPIRATORY CANCER)?

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In recent studies, dietary factors have been implicated in the cause and prevention of lung diseases. Nutritional factors could significantly modify the host response to environmental toxicants. An adequate diet may be able to inhibit, arrest, or even reverse the chain of events in toxicity, while a deficient diet could increase a person's susceptibility to adverse environmental exposure, such as allergens, environmental tobacco smoke (ETS), infections, and air pollution. Several dietary factors have been implicated in obstructive lung diseases, mainly because of their potential role in inflammatory reaction, and in activities of airway smooth muscle and enzymatic reactions that affect neuromuscular transmission. These factors include the following: a deficiency in anti-oxidant vitamins, a lower consumption of omega-3 fatty acids and higher consumption omega-6 oils, processed foods, and salt, and low magnesium intake.

To date, most of the available evidence on the effect of dietary factors on the risk for obstructive lung diseases comes from cross-sectional studies, with questionable temporal relationship and often poor intake assessment without accounting for intake of other nutrient that may bias the estimates. These studies suggest that antioxidant vitamins, particularly vitamin C, might decrease the prevalence of asthma symptoms, and modulate the decline of pulmonary functions in adults and that omega-3 PUFA might affect blood levels of leukotrienes and other inflammatory markers and modulate as well as the pulmonary function decline among adults. However, these findings are not confirmed in the few longitudinal studies. In addition, larger body mass index (BMI) has been recently linked to asthma. Although results are conflictive, some authors have suggested that obesity and asthma could share common pathogenic factors.

There is a need for further research in experimental and epidemiological settings to better understand the pathophysiological effects of nutritional factors, in particular antioxidant vitamins and omega-3 fatty acids on lung tissues, as well as the role of obesity in the development of asthma. The impact of diet on the incidence and evolution of asthma and chronic obstructive lung diseases should be investigated using a cohort design that accounts for known risk factors. The major issues that should be addressed include:

- ?? Can specific nutrient intake decrease the incidence of childhood asthma and other allergic disease? Is there a window of opportunity (during: pregnancy or early life)?
- ?? Is larger BMI a risk factor for the incidence of asthma? Is there a common mechanism for asthma and obesity? Common genetic susceptibility? Is low birth weight related to asthma in older life (barker hypothesis)?
- ?? Similarly in adult onset of asthma: can specific nutrient affect asthma incidence? Is BMI important? Is there window of opportunity?
- ?? Can the intake of specific nutrient modulate the decline in pulmonary functions in adults? Is there a window o opportunity? Is there specific subgroup that might benefit more?
- ?? Can supplementation with specific nutrient improve the severity of chronic respiratory diseases? Which dose should be use? For how long?
- ?? Is there susceptibility gene to environmental insult (e.g. air pollutant) and what is the potential protective effect of nutrient intake or supplementation?
- ?? If specific nutrient are found to have a protective role, what daily intake should be recommended?

GENETIC FACTORS OF ASPIRIN INDUCED ASTHMA

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Aspirin-induced asthma (AIA), a distinct clinical syndrome, affects about 10-15% of adults with asthma. Acute asthma attacks follow ingestion of non-steroid anti-inflammatory drugs (NSAIDs), known of their cyclooxygenase-1 inhibitory capacity [1]. Cysteinyl leukotrienes (cys-LTs), i.e. leukotriene C₄, D₄ and E₄, are potent bronchoconstrictors, released after NSAIDs challenge from mast cells and eosinophils. Their production at base is increased in aspirin-sensitive asthmatics [2]. In bronchi of AIA patients leukotriene C₄ synthase (LTC₄S), the enzyme controlling cys-LTs biosynthesis, is over-expressed.

A common single nucleotide polymorphism (SNP) was found in the 5' untranslated region of LTC₄S gene, consisting of A to C transversion, 444 nucleotides upstream from the translation start. Initial screening for LTC₄S alleles revealed a genetic association between LTC₄S allele C and the disease only in AIA patients [3]. Further studies limited this finding to a group of patients suffering from moderate to severe asthma. A replication study failed to demonstrate this genetic association in the North American AIA patients, however, LTC₄S allele C homozygotes were more common in this group [4]. Molecular studies suggested a moderate 20% increase of transcriptional activity for LTC₄S variant C. Though the mRNA for LTC₄S was increased in peripheral blood eosinophils of AIA patients, effect of LTC₄S SNP was not found. Activated production of cys-LTs, measured either in vitro or following challenge test with aspirin by urinary metabolite, could distinguish carriers of LTC₄S allele C as having increased capacity for cys-LTs biosynthesis [5, 6, 7].

In a large survey of European Network on Aspirin Induced Asthma, familial occurrence of aspirin hypersensitivity was present in 5.1% [8]. Inheritance of LTC₄S allelic variant C predisposed to more severe variant of the disease, hallmarked by increased cys-LTs biosynthesis. Interestingly, very recent study on response to cys-LTs receptor antagonist, pranlukast in Japanese asthmatics [9], confirmed preliminary observations [6, 7] on pharmacogenetic aspect of LTC₄S SNP. Carriers of the allele C responded better to anti-leukotriene therapy in contrast to the wild allele A homozygotes. A cys-LTs dependent bronchoconstriction is thus controlled by a genetic variance at LTC₄S promoter in general group of asthmatic. AIA represents a subset of asthmatic patients, in whom a predisposing variant of LTC₄S is more frequent, but an unknown environmental effect has triggered the disease.

References:

1. Szczeklik A., Stevenson D.D. Aspirin-induced asthma: advances in pathogenesis and management. *J Allergy Clin Immunol* 1999;104:5-13.
2. Sanak M., Sampson A.P. Biosynthesis of cysteinyl-leukotrienes in aspirin-intolerant asthma. *Clin Exp Allergy* 1999;29:306-313.
3. Sanak M., Simon H.U., Szczeklik A. Leukotriene C₄ synthase promoter polymorphism and risk of aspirin-induced asthma. *Lancet* 1997;350:1599-1600.
4. Van Sambeek R., Stevenson D.D., Baldasaro M., et al. 5'Flanking region polymorphism of the gene encoding leukotriene C₄ synthase does not correlate with the aspirin-intolerant asthma phenotype in the United States. *J Allergy Clin Immunol* 2000;106:72-76.
5. Sanak M., Pierzchalska M., Bazan-Socha S., Szczeklik A. Enhanced expression of the leukotriene C₄ synthase due to overactive transcription of an allelic variant associated with aspirin-intolerant asthma. *Am J Respir Cell Mol Biol* 2000;23:290-296.
6. Sampson A.P., Siddiqui S., Buchanan D., et al. Variant of LTC₄ synthase allele modifies cysteinyl leukotriene synthesis in eosinophils and predict clinical response to zafirlukast. *Thorax* 2000;55(Suppl 2):S28-S31.
7. Szczeklik A., Mastalerz L., Nizankowska E., Sanak M. Montelukast for persistent asthma – Reply. *Lancet* 2001;358:1456-1457.
8. Sanak M., Szczeklik A. Genetics of aspirin induced asthma. *Thorax* 2000;55(Suppl 2):S45-S47.
9. Asano K., Hasegawa N., Nakamura H., et al. Leukotriene C₄ synthase gene polymorphism and clinical response to a Cys-LT₁ antagonist, pranlukast. *Clin Exp Allergy* 2002, in press.

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References:

1. Szczeklik A., Stevenson D.D. Aspirin-induced asthma: advances in pathogenesis and management. *J Allergy Clin Immunol* 1999;104:5-13.
2. Sanak M., Sampson A.P. Biosynthesis of cysteinyl-leucotrienes in aspirin-intolerant asthma. *Clin Exp Allergy* 1999;29:306-313.
3. Sanak M., Simon H.U., Szczeklik A. Leukotriene C₄ synthase promoter polymorphism and risk of aspirin-induced asthma. *Lancet* 1997;350:1599-1600.
4. Van Sambeek R., Stevenson D.D., Baldasaro M., et al. 5'Flanking region polymorphism of the gene encoding leukotriene C₄ synthase does not correlate with the aspirin-intolerant asthma phenotype in the United States. *J Allergy Clin Immunol* 2000;106:72-76.
5. Sanak M., Pierzchalska M., Bazan-Socha S., Szczeklik A. Enhanced expression of the leukotriene C₄ synthase due to overactive transcription of an allelic variant associated with aspirin-intolerant asthma. *Am J Respir Cell Mol Biol* 2000;23:290-296.
6. Sampson A.P., Siddiqui S., Buchanan D., et al. Variant of LTC₄ synthase allele modifies cysteinyl leukotriene synthesis in eosinophils and predict clinical response to zafirlukast. *Thorax* 2000;55(Suppl 2):S28-S31.
7. Szczeklik A., Mastalerz L., Nizankowska E., Sanak M. Montelukast for persistent asthma – Reply. *Lancet* 2001;358:1456-1457.
8. Sanak M., Szczeklik A. Genetics of aspirin induced asthma. *Thorax* 2000;55(Suppl 2):S45-S47.
9. Asano K., Hasegawa N., Nakamura H., et al. Leukotriene C₄ synthase gene polymorphism and clinical response to a Cys-LT₁ antagonist, pranlukast. *Clin Exp Allergy* 2002, in press.

GENE-ENVIRONMENT INTERACTIONS THROUGH CASE CONTROL STUDIES

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Cancelled

CD14 GENOTYPE, ATOPY AND ANTIBODIES TO H. PYLORI IN YOUNG ADULTS.

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The 'hygiene hypothesis' suggests that the increase in asthma and atopy seen in many countries in recent decades is the result of declining exposure to bacterial pathogens in early life. Binding of bacteria to leucocytes involves the protein CD14 found on the cell membrane. The gene coding for CD14 is localised on chromosome 5q, a region which has been linked to asthma and atopy in many studies. The C allele of the -159 C-to-T promoter polymorphism of this gene has been associated with increased severity of atopy and IgE levels in several studies.

In 487 young men and women aged 16-22y we found that carriers of the C allele were more likely to be atopic (one or more positive skin prick tests to common antigens) than TT homozygotes (OR = 1.46; 95% CI 0.97, 2.19; p=0.07). In the same population the risk of atopy was higher in those who did not have antibodies to H Pylori (OR = 1.39; 95% CI 0.99 – 1.93; p=0.05). The aim of this analysis was to assess whether carriers of the C allele were less likely to have antibodies to H Pylori.

The table shows the percentage of subjects with antibodies to H Pylori in the three genotypes:

	Genotype		
	CC (n =117)	CT (n =255)	TT (n =125)
% with antibodies to <i>H. Pylori</i>	30.4%	32.9%	27.4%

There was no difference in the proportion of subjects with antibodies to H. Pylori between the genotype groups ($\chi^2 = 1.21$; p=0.55). This suggests that the increased risk of atopy in carriers of the C allele is not associated with a lower antibody response to H. Pylori as a result of altered binding to CD14 on host cells.

WHICH GENE-ENVIRONMENT INTERACTIONS ARE WORTH DETECTING, AND HOW CAN THEY BE DETECTED?

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Investigation of interactions between genetic and non-genetic (“environmental”) determinants of disease forms an important part of the post-genome research agenda. This begs a number of conceptual and methodological questions:

- a) *What do we mean by “interaction”*. Biologists and statisticians have different perspectives. The most obvious common ground is when the effect of “environmental” factors is concentrated entirely among a subgroup of the population who are genetically susceptible to the exposure. This “risk concentration” model, however, represents an ideal scenario which will rarely be approximated in real life.
- b) *Which interactions are worth detecting?* One criterion would be the “aetiological fraction” (the proportion of the disease which is statistically attributable to the joint effect of genotype and environmental exposure). This requires that either the genotype or the exposure, or both, is common. If susceptibility is widespread, then conventional non-genetic epidemiology will be well placed to detect “environmental” effects. The real benefits of studying gene-environment interactions arise when the genotype is rare (<30%) and the non-genetic exposure is common (>70%: “hazards of everyday life”).
- c) *How do we define genetic susceptibility?* Typically, polymorphisms at many loci may influence biological pathways and thereby the risk associated with an environmental, lifestyle or pharmacological exposure. Examples will be used to show that the statistical power to detect gene-environment interactions depends crucially on aggregating all the genotypes associated with a particular “susceptibility”. This implies that research into gene-environment interactions will need to group genes according to their functional consequences (“phenocopies”), rather than study interactions between polymorphisms at a single locus and non-genetic risk factors for disease.

PET KEEPING IN CHILDHOOD AND ADULTHOOD AND ADULT ASTHMA, HAY FEVER AND ATOPIC DERMATITIS, ACCORDING TO FAMILIAR PREDISPOSITION: RESULTS FROM THE EUROPEAN COMMUNITY RESPIRATORY HEALTH SURVEY.

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The protective effect of siblings on atopy and allergic disease seems to differ according to allergic predisposition. We also found that a protective effect of childhood cat keeping on adult atopy was restricted to individuals with parental allergic disease. In the present study, we address how the associations between pet keeping in childhood (cats, dogs, birds, horses and/ or rabbits) and adult asthma and hay fever differ according to allergic predisposition.

The analyses were based on interview data from 18530 subjects and blood tests from 13932 subjects aged 20-44 years from 36 centres participating in the ECRHS. Subjects with atopy (positive IgE to HDM, cat, grass and/ or mould) and one or more family member with allergic disease were considered to have an allergic predisposition, as opposed to subjects with no atopy and no allergic family member. Associations were adjusted for age, sex, smoking habits, occupation and study centre.

Among subjects with an allergic predisposition, asthma symptoms was more common in those who had kept a cat in childhood (OR_{wheeze} = 1.23, 95%CI = 1.03-1.48), and hay fever was less common in those who had kept a dog (OR = 0.82, 95%CI = 0.69-0.98) or a bird (OR = 0.82, 95%CI = 0.70-0.98) in childhood. Adult pet keeping was not associated with symptoms of asthma or hay fever in this group.

In subjects with no allergic predisposition, asthma symptoms were more common in those who had kept a dog in childhood (OR = 1.26, 95%CI = 1.04-1.52) or who currently kept a dog (OR = 1.40, 95%CI = 1.14-1.72). Hay fever was more common in those who had kept a bird in childhood (OR = 1.29, 95%CI = 1.05-1.59).

“Wheeze with shortness of breath”, “wheeze when not having a cold” and “current asthma medication” showed similar results as “wheeze”.

The effects of childhood exposure to pets on adult asthma and hay fever, varied greatly depending on allergic predisposition. Pets could not be analysed together as each species showed different effects. We speculate the following explanation for our findings: Allergically predisposed individuals have a generally poor Th cell function and may be particularly prone to react to allergic but also to microbial stimulation. Therefore, early exposure to the allergens of cats predisposed for asthma, while the early microbial stimulation of dogs protected against hay fever. In non-atopics with a sufficient Th-cell function, cat allergens or Th1 stimulation from dogs did not make any difference, while the dog microbes increased the risk for asthma through manifest infections.