2814 in silico mining to prioritize functional sequence variants
Eric Schauberg1, Marianne Hurben2, S. Hasan Arshad3, Marcha Walls-Karp4, Karen Fridric5, Susan Ewart6. 1Genetics Program, Michigan State University, East Lansing, MI, United States of America; 2Biomedical Statistics and Informatics, Mayo Clinic, Rochester, MN, United States of America; 3The David Hide Asthma and Allergy Research Centre, St Mary’s Hospital, Newport, Isle of Wight, United Kingdom; 4Immunohematology, Cincinnati Children’s Hospital Medical Center, Cincinnati, OH, United States of America; 5Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI, United States of America; 6Large Animal Clinical Sciences, Michigan State University, East Lansing, MI, United States of America

A genome-wide association study found a 120kb linkage disequilibrium (LD) block on chromosome 1 associated with asthma. The LD block contained 650 variants, including 533 SNPs and 117 indels. The goal of this study was to prioritize variants most likely underlying gene function in asthma. Variants within the LD block were screened via the annotated and/or inferred function of the sequence in which they were located. Regulatory regions were predicted from known and predicted promoter sites and conserved transcri tion factor binding sites. Control and control elements were predicted via consensus sequence motif identification of cis-acting splicing modulating sequence. Conserved regions were determined by overlapping variant locations with 28-way conservation data and used to indicate functionality in any of the categories. Coding variants were examined for impact on protein via prediction engines. For each source a score was assigned based on the strength of the data. Scores were summed within categories and thresholds set to identify variants with multiple supportive evidence. The in silico screen prioritized 27 variants in the asthma-associated LD block. Meeting threshold values were: 23 regulatory/conserved variants, 16 splice control/conserved variants, and 1 coding mutation. Thirteen variants were in -1 category. Recsequencing will validate variants in our population and cell culture assays will be used to verify variant function. In silico screen is a powerful tool for narrowing the search for functional variants within regions of genetic association. Multiple sources of experimentally derived data combined within silico predictions can identify synergistic support for individual variants.

2815 Age-specific effects of NPSR1 and DPP10 in asthma and atopy: an international population-based cohort study (ECRHS)
Francesca Castro-Giner1,2,3, Manolis Kogevinas1,2,4, Juan Ramón González1,2,3, Deborah Jarvis4, Rafael De Cid1,5, Juan Ramon Gonzalez1,2,3, Christfer Janson1, Josep Maria Antó1,2,3, Matthias Wjst1, Xavier Estivill4,5, Rafael De Cid1,2, CISERESP, CIBER Epidemiología y Salud Pública, Barcelona, Spain; 2Genes and Disease Program, Center for Genomic Regulation, Barcelona, Spain; 3CREAL, Centre for Research in Environmental Epidemiology, Barcelona, Spain; 4IMIM-Hospital del Mar, Municipal Institute of Medical Research, Barcelona, Spain; 5Medical School, University of Crete, Heraklion, Greece; 6Respiratory Epidemiology and Public Health Group, National Heart and Lung Institute, Imperial College, London, United Kingdom; 7Institute of Epidemiology, Helmholtz; Zentrum München, Munich, Germany; 8German Research Center for Environmental Health, Helmholtz; Centre GSF, Munich, Germany; 9Department of Health and Experimental Sciences, University Pompeu Fabra, Barcelona, Spain

Background: Several genes identified by positional cloning have been associated with asthma and atopy although few findings have been replicated. Age at onset of asthma has been associated with different phenotypic characteristics and a gene (ORMDL3) identified through genome-wide scan was shown to be associated with early onset asthma. We tested associations and identified age-specific effects of five genes previously identified by association studies and positional cloning approaches (ADAM33, PHF11, NPSR1, DPP10 and SPINK5) with asthma, bronchial hyperresponsiveness (BHR) and atopy.

Methods: We studied 54 single nucleotide polymorphisms (SNPs) in 5,065 participants from 13 countries who took part in the European Community Respiratory Health Survey (1990-2000). Asthma and age at onset of asthma were assessed by questionnaire data, BHR by methacholine challenge test and atopy by specific IgE to five common allergens.

Results: Significant associations with asthma and atopy were observed for a large region of 47 kb in the NPSR1 gene even after Bonferroni correction. A polymorphism in ADAM33 was associated with an excess risk for BHR. After stratification by age at onset, the pattern of association was stronger for NPSR1 in those reporting first attack of asthma before age of 15, with statistically significant interaction with age of onset for three SNPs. Risk associated with two SNPs in DPP10 gene was greater for adult onset of asthma.

Conclusion: This study replicates previous evidences of the effect of NPSR1 on asthma and atopy and ADAM33 on BHR, and suggests an age-dependent effect of NPSR1 on asthma.
2816 ILIRL1 polymorphisms are associated with the level of soluble ILIRL1 and asthma development in children
O.E.M. Savenije1, M. Kerkhof1, B. Brunekree2, J.C. De Jongste1, A.H. Wijga1, D.S. Postma1, G.H. Koppelman1, Dept. of Epidemiology, UMC Groningen, Groningen, Institute for Risk Assessment Sciences, University of Utrecht, Utrecht, Dept. of Pneumatics, Erasmus MC -Sophia, Rotterdam, Center for Prevention and Health Services Research, National Institute of Public Health and the Environment, Biltoven, Dept. of Palmonology, UMC Groningen, Groningen, Beatrix Children’s Hospital, UMC Groningen, Groningen, Netherlands

Objective: The gene ILIRL1 (alias STZ) is a new candidate gene for asthma development (Keijerink et al, J Allergy Clin Immunol, 2008). ILIRL1 has recently been identified through a genome wide association study for the number of blood eosinophils and asthma development (Gudbjartsson et al, Nature Genetics, 2009). ILIRL1-a (alias soluble STZ) is a soluble isoform of ILIRL1 and is measurable in serum. The aim of this study was to assess associations of polymorphisms of ILIRL1 with asthma, and with ILIRL1-a in a prospective birth cohort.

Methods: The effects of 14 SNPs of ILIRL1 on ‘doctor diagnosed asthma in the last 12 months’ (DDA) were tested longitudinally with generalized estimating equations in the prospective birth cohort PIIMA (n=957; 58% boys; 5.0% DDA at age 8). DDA was assessed by a yearly questionnaire from birth up to age 8. At age 4, serum levels of ILIRL1-a were measured in a subset of 433 subjects.

Results: Two SNPs were associated with DDA. In a dominant model, fewer individuals with the minor allele (allele A) for rs1041973 had DDA (Odds Ratio (95% confidence interval) =0.58 (0.37-0.91)), than wild-type individuals. More individuals with genotype TT for rs6719130 had DDA (OR (95% CI) = 3.25 (1.24-8.53)), compared to individuals with other genotypes. 13 SNPs were associated with serum levels of ILIRL1-a (p = 0.025 to <0.0001).

Conclusions: This study demonstrates that 10 of the 14 SNPs are associated with asthma in children. Additionally, this study shows for the first time that ILIRL1 SNPs are associated with the level of soluble ILIRL1.

2817 The relationship between TLR4-299 variant polymorphisms and atopic wheeze in women
Donna Rennie1, Chandoa Karunamayake1, Yue Chen2, Pannun Padma1, David Schwartz3, Ambalapain Senthilvelan3, Don Cockcroft4, Jim Dosman5, Canadian Centre for Health and Safety in Agriculture, University of Saskatchewan, Saskatoon, SK, Canada; 2Department of Epidemiology and Community Medicine, University of Ottawa, Ottawa, ON, Canada; 3National; Jewish Medical Centre, National Jewish Health, Denver, CO, United States of America; 4Division of Public Health Sciences, University of Alberta, Edmonton, AB, Canada

Human response to endotoxin exposure includes respiratory symptoms such as wheeze. Response to endotoxin has been shown to be varied by TLR4-299 genotype. Recently atopy has been reported as modifying the relationship between endotoxin and -299 genotypes. We examined the associations between atopic wheeze or asthma and TLR4 299 variant polymorphisms in a general population of adults.

Method: A cross-sectional survey was conducted in 2003 in Humboldt, Saskatchewan, Canada. Of the 2990 participants, there were 1887 adults who answered survey questionnaire, completed skin prick testing (SPT) to 4 common allergens, and provided blood samples for genotyping of TLR4-299. Asthma was defined by a report of physician diagnosed asthma. Atopic wheeze was defined as a SPT ≥4mm and a history of wheeze with or without a cold, wheeze with shortness of breath or wheeze most days or nights.

Results: The distribution of TLR4-299 was in Hardy Weinberg equilibrium. There were 86.3% with the homoygous wild type, 13.4% who were heterozygous variant and 0.3% who were homoygous variant. The prevalence of asthma, atopy and atopic wheeze was 8.2%, 19.3% and 8.5%, respectively. Interaction was found between TLR4 status and gender for atopic wheeze (Odds Ratio: 2.84, 95% confidence interval: 1.16, 3.60). Compared to women with the TLR4 wild type polymorphism, women with the variant polymorphism reported more atopic wheeze (14.3% versus 7.3%, p < 0.007). No such association was seen for men.

Conclusion: Gender should be taken into consideration in studies investigating the association between TLR4 and asthma and allergy phenotypes.

Funding Source: Canadian Institutes of Health Research MOP-57907.

2818 Sex-specific effect of IL19 polymorphisms on lung function and pollen sensitization in French EGEA families
Hugues Aschard1,2,3, Emmanuel Bouzigon1,2,3, Eve Corda1,2, Ayse Ulgen1,2,3, Marie-Hélène Dieter1,2, Frederic Gormand1, Mark Lathrop3,4, France, Dr Franck Kuilman1,2,3,5,6, Florence Demenais1,2,3,5,6,946, Inserm, Paris, France; 2IGM, Fondation Jean Dausset-CEPH, Paris, France; 3Université d’Evry, Université d’Evry, Evry, France; 5UM3, Inserm, Villejuif, France; 6Université Paris Sud-11, Université Paris Sud 11, Villejuif, France; 7Centre Hospitalier Lyon-Sud, Centre Hospitalier Lyon-Sud, Pierre-Bénite, France; 8UM2, Inserm, Lyon, France; 9Centre National de Génopostage, Commissariat à l’Energie Atomique, Institut de Génomique, Evry, France; 10UM3, Inserm, Villejuif, France.

Sex differences in asthma-associated phenotypes are well known but the genetic factors which may account for these differences have received little attention. This study aimed to characterize sex-specific and pleiotropic genetic factors underlying four quantitative phenotypes involved in the main asthma pathophysiological pathways: IgE levels, a measure of polysensitization (SPTQ), eosinophil counts (EOS) and a measure of lung function (FEV1 divided by height squared [HF]). Sex-stratified univariate and bivariate linkage analyses were conducted in 295 families from the EGEA study. We found genome-wide significant evidence for a male specific locus on 5q31 (P = 7.10-9) influencing both FEV1/HF and SPTQ. To identify the genetic variants associated with these traits, we conducted a family-based association test (FBAT) in males only, analyzing jointly FEV1/HF and SPTQ, and using 24 SNPs belonging to five candidate genes (IFRI, IL13, IL4, IL9, CD14) located in the 5q31 region. Significant association signals were found with two SNPs within IL9 gene, rs2069885 and rs2069882 (P = 0.001 and P = 0.0008 respectively) that remained significant after Bonferroni correction (P = 0.02 and P = 0.002, respectively). This study underlies the importance of taking into account complex mechanisms such as heterogeneity according to sex and pleiotropy to unravel genes involved in asthma phenotypes. Funded by: French Ministry of Higher Education and Research, AFSET-APR-SE-2004, FRM, ANR 06-CBES, GABRIEL.