

# Children's Hospital of Philadelphia

## Annual Progress Report: 2007 Formula Grant

### Reporting Period

July 1, 2009 – June 30, 2010

### Formula Grant Overview

The Children's Hospital of Philadelphia received \$3,499,480 in formula funds for the grant award period January 1, 2008 through December 31, 2011. Accomplishments for the reporting period are described below.

### **Research Project 1: Project Title and Purpose**

*MRI Studies of System Level Brain Dysfunction in Autism Spectrum Disorders* - Neuroimaging research is beginning to characterize several important ways in which the brains of persons with an Autism Spectrum Disorder (ASD) develop and function differently. Magnetic resonance imaging (MRI) has been particularly important in this regard. It is also now widely recognized that ASD is a heterogeneous disorder, with individual differences in severity of symptoms, other aspects of behavior and cognitive functioning. This project will use MRI to study the brain anatomy and function, and will correlate neuroanatomy and function with differences in symptomatology and behavior, in order to enhance our understanding of the brain-bases of the ASDs. This research is guided by the belief that understanding the fundamental mechanisms that cause ASDs will allow for the development of better interventions and treatments.

### Anticipated Duration of Project

1/1/2008 - 12/31/2011

### Project Overview

The autism spectrum disorders (ASDs) involve disruption of the child's ability to interact with others, communicate, play and learn. These are neurobiological disorders that appear to have multiple causes and that involve a good deal of phenotypic heterogeneity. The broad objective of this project is to use magnetic resonance imaging (MRI) to study the ways in which the brains of children with an ASD differ in their neuroanatomical structure and their neural function from typically developing children (TDC). Because the ASDs are heterogenous, large samples are required to discover coherent sets of brain-behavior relationships and to derive coherent subgroups. This project will entail establishing a systematic MRI program of research at Children's Hospital of Philadelphia (CHOP), including the clinical infrastructure necessary to evaluate large numbers of children with an ASD. Using structural MRI (sMRI), functional MRI (fMRI) and diffusion tensor imaging (DTI), we will test for significant brain based differences between ASD and TDC.

The specific aims of this project entail collecting sMRI, DTI and fMRI data from 100 children with an ASD and 100 TDC, matched on age, ethnicity, IQ and gender. While each of these three imaging approaches independently has revealed clues about the underlying neural systems abnormalities in the ASDs, no study to date has combined all 3 sources of imaging data in the same sample. Results from our prior studies using these methods in isolation have converged to suggest that abnormal white matter growth in the temporal lobes may be coupled with abnormalities of long range fiber connections between posterior visual perceptual areas and anterior limbic areas; these anatomical deficits may be responsible for the under-activation of key nodes in the temporal lobes during social perceptual and social cognitive mental activity in research volunteers with an ASD. By combining these three sources of imaging data in a large ASD sample, we expect to find more definitive evidence for the role of temporal lobe neural connectivity problems in causing the primary social deficits in the ASDs. Moreover, because we will collect a large sample, we will be able to characterize differences between individuals with an ASD in terms of different patterns of brain-behavior relationships.

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### **Expected Research Outcomes and Benefits**

This study aims to be the first large-scale effort of its kind to understand and characterize the neuroanatomical bases of the autism spectrum disorders (ASDs). Prior attempts to characterize brain based causal mechanisms have yielded some important clues, but none has attempted the more comprehensive approach to be adopted here. The current project will employ a large sample of children with an ASD and will explicitly attempt to address the heterogeneity in symptoms and levels of functioning. We now believe that there are several and perhaps even many different causal pathways to what we call “autism” or the “ASDs”. Past research has relied on small study samples that likely mixed different types of ASD cases, thereby obscuring our ability to clearly find correlations between brain and behavior. Our strategy will be to create a suitably large clinical infrastructure to enable enrollment of a large sample of children affected with an ASD. Each child will have a broad based expert clinical assessment of their difficulties, as well as of areas of cognitive strengths and behavioral competencies. One immediate health benefit of the research process is that we will give expert clinical evaluations and feedback free of charge to a large community of research volunteers and their families. Scientifically, health benefits include a much deeper understanding of the particular neural systems that are affected by the ASDs. Our research is guided by a fundamental belief that it is difficult and perhaps

impossible to develop effective treatments without first laying out in detail the nature of the problem in terms of causal mechanisms. We expect that not all persons with an ASD will show the exact same neural system abnormalities, and that differences in brain system abnormalities will be mirrored by differences in behavioral and symptomatic features. Better characterization of these brain based mechanisms will facilitate the development of novel and more effective treatments and aid in the process of gene discovery.

### **Summary of Research Completed**

As the Center for Autism Research (CAR) is new and having been created only a little more than 2 years ago, progress on this grant has paralleled progress in growing the infrastructure of the Center. This grant has been instrumental in establishing a major new research center in the Commonwealth of Pennsylvania that already is an international leader in autism research. In the last year, CAR expanded its infrastructure by hiring ~ 20 new employees, bringing its total faculty and staff as of this reporting date to 65. Of note for the progress of this grant is our enhanced recruitment and clinical infrastructure. The rate limiting step for scientific advancement in this field is (a) access to research participants and (b) establishment of clinical research infra-structure manned by expert autism clinicians, who are reliable with other national centers on gold standard diagnostic instruments. During this reporting period the researchers made great strides in increasing our recruitment capacity. The researchers have made more than 100 presentations about our research programs to parent and professional groups in the greater Delaware Valley and now have a database of more than 4,000 parents and professionals that have expressed interest in helping refer patients for research participation or in having their child evaluated as part of this CAR research study. Financial support for these efforts is shared across the researchers' current grants, including this grant.

During this reporting period, the researchers also made considerable progress in recruiting typically developing controls for this study. These study participants need to be matched on important demographic and cognitive variables, and thus recruiting participants in parallel with the ASD sample is tricky. Based on demographic and cognitive variables of other ASD samples at CAR, the researchers have projected what the typically developing control sample will need to be, and based on those projections purchased marketing lists for direct mailing to households in the Delaware Valley with children in the age range, and with the desired characteristics for matching. In June of 2010, the researchers mailed out 35,000 recruitment letters to putative control families and in two weeks since had more than 200 families express interest, 100 of which the researchers have already screened with a 30-45 minute phone interview. The researcher's final sample of 100 TDCs for this study will come from these efforts.

The researchers have also increased their clinical infrastructure greatly in the last year. In the fall of 2009 the researchers hired 2 new PhD autism experts (Drs. Rosemarie Manfredi and Leandra Berry) into their postdoctoral training program, to work part of their time on this grant for its duration. Drs. Manfredi and Beery are focusing their efforts on diagnostic and phenotypic assessment of the study participants, and will be looking at the correlations between signs, symptoms and behavioral manifestations of autism, to better understand the heterogeneity of the disorder. The researchers also engaged in a very thorough national search for additional clinical experts in autism, and successfully recruited 5 new PhDs who are starting at various times during

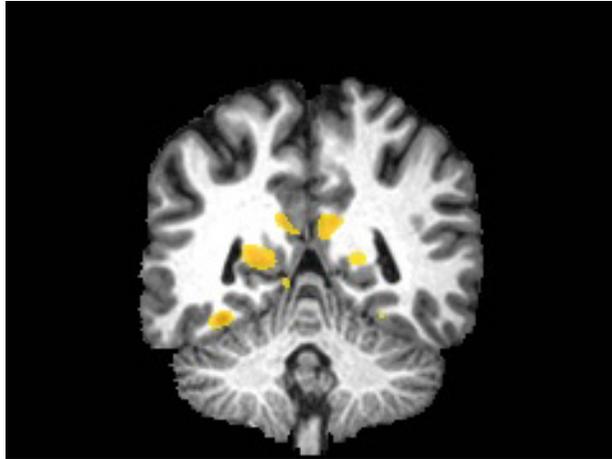
the summer of 2010; these are experienced autism clinicians who will work on phenotypic characterization for this project. Of the 5, two will have faculty appointments and three will be clinical postdocs with CAR.

During the reporting period, CAR's research clinic evaluated 62 children (45 male, 17 female) for this study. These are full day assessments replete with detailed gold standard diagnostic and neurocognitive evaluation (per the research plan). Ethnic breakdown is as follows: 43 Caucasian, non-Hispanic (69.5%), 11 African American, non-Hispanic (17.7%), 2 Asian, non-Hispanic (3.2%), 1 Biracial (1.6%), 5 Hispanic (8%). Of the 62 children evaluated, 15 were typically developing controls (TDCs) and 47 had a community based diagnosis of an autism spectrum disorder (ASD). However, using gold standards for diagnostic assessment, the researchers could confirm an ASD diagnosis in only 35 of the 47 children evaluated. All participants received comprehensive written feedback describing the evaluation findings, as well as individually tailored treatment recommendations.

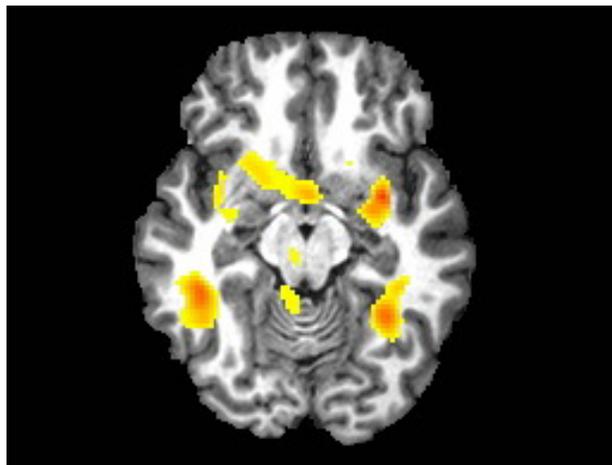
During the reporting period the researchers have made substantial progress with acquisition of the structural magnetic resonance imaging data (sMRI), the functional MRI (fMRI) data, as well collection of the diffusion tensor imaging (DTI) data. Forty-seven participants were scanned on the protocol that collects all three types of MRI data (34 males, 13 females). Ethnic breakdown is as follows: 3 Hispanic (6.4%), 11 African-American (23.4%), 1 Biracial (2.1%) and 32 Caucasian, non-Hispanic (68.1%). Of those scanned, 30 were participants with ASD and 17 were TDCs. Four of the participants failed the fMRI and DTI portion of the exam.

MRI data analysis is ongoing. Nearly all participants have had their data evaluated at the individual level through processing pipelines. It is a bit premature to focus much on group results. Nonetheless, several summary statements are possible. The fMRI data are not yet yielding the predicted group differences. Current efforts are looking at sample heterogeneity to see if there are moderating variables. The sMRI and DTI results are currently more promising (24 ASD and 15 TDC are included so far). The researchers are finding the predicted significant tissue enlargement in the right fusiform gyrus (see *Figure 1*).

Analyses of the DTI data includes voxel level group comparisons using fractional anisotropy (FA) and DTI trace (a scalar measure of total diffusivity in a voxel). *Figure 2* shows that the white matter in the region of fusiform gyrus has greater DTI trace in ASD vs. TDC (consistent with predictions), suggesting less coherent fiber inputs to this cortical processing node of interest. Additional preliminary results (not shown in the figures) include: increased brain tissue in ASD vs. TDC in the precuneus, right amygdala, hippocampus (bilateral) middle temporal gyrus (bilateral), and decreased white matter in the genu and splenium of the corpus callosum, and in white matter regions adjacent to the amygdala. In addition, the researchers find widespread increased Trace DTI in the white matter inputs and crossing pathways throughout the basal ganglia, including ventral reward regions, and decreased FA in white matter adjacent to the amygdala (bilateral). All of these results should be treated as very preliminary as the full sample is not yet collected and in some cases the results do not survive correction for multiple comparisons. Nevertheless these results are consistent with hypothesis and thus very promising.



*Figure 1.* Significant sMRI voxel level group differences (ASD > TDC, False Discovery Rate < .05) on a coronal slice at the level of the fusiform gyrus (right and left reversed by radiological convention). Corrected for group differences in age and full scale IQ.



*Figure 2.* Voxel level DTI trace group differences (ASD > TDC,  $p < .05$ , uncorrected) on an axial slice at the level of the fusiform gyrus (right and left reversed by radiological convention). Not corrected for group differences in age and full scale IQ.

## **Research Project 2: Project Title and Purpose**

*Ethnic Diversity and Gene-Environment Interactions in Childhood Asthma* - The purpose of this research is to use a genome-wide association (GWA) approach to uncover the genetic factors that predispose to asthma, the most common chronic disease in children, and to thoroughly examine gene-environment interaction in asthma patients of different ethnic background. The utilization of the GWA approach has excellent potential of identifying the genetic determinants of asthma predisposition and to subsequently dissect out the role of second hand smoke and potentially other specific environmental exposures in the pathogenesis of asthma in subjects from different ethnic background, including Caucasian, African-American and Hispanic Caucasian descent. The study will utilize a well-characterized pediatric asthma cohort from the Children's Hospital of Philadelphia (CHOP) to drive the discovery phase.

## **Anticipated Duration of Project**

1/1/2008 - 12/31/2011

## **Project Overview**

Our primary objectives are directed at unveiling the genes that underlie the pathobiology of asthma and to shed light on how specific environmental factors interact with these genetic factors to trigger the expression of the asthma phenotypes. In our first specific aim we propose to analyze a pediatric asthma cohort from Philadelphia using a GWA approach. We propose to genotype approximately 1000 children with asthma and 1000 non-asthmatic controls and analyze the dataset for single SNP (single nucleotide polymorphism) and SNP- environmental interaction effects on the phenotypic characteristics that will be gathered on the study cohort. In specific aim 2 we propose to select SNPs that show the highest association in specific aim 1 and genotype these selected SNPs in another cohort of 1200 asthma patients and 1200 controls from Philadelphia and analyze the data jointly with the original cohort in specific aim 1 to find single SNPs and SNP-environmental factor interactions that are genome-wide significant after correction for multiple tests. We estimate that approximately 1500 SNPs will have p value of  $10^{-5}$  or lower from where a subset of SNPs (~ 10-15) will be true positives. In specific aim 3 we propose to validate and refine disease-associated SNP alleles and SNP-environmental factor interactions in independent sample sets consisting of 500 Hispanic Caucasian and 500 African-American (AA) subjects. In specific aim 4 we will examine the interplay between genes that harbor the at-risk variants that replicate in specific aims 2 and 3 and specific environmental factors with respect to the expression of the asthma phenotypes and we will re-sequence the most significant genes to identify the functional causative mutations that predispose to asthma. The institutional resources available to pursue this project are outstanding, most notably with the establishment at CHOP of one of the world's largest genotyping centers (Center for Applied Genomics or CAG) for GWA studies and analyses. CAG has already genotyped over 80,000 individuals and produced over 50 billion genotypes that are being leveraged for this project as control subjects. By incorporating these outstanding resources and expertise available at CHOP we believe that this proposal will effectively address the above specific aims and, thereby, provide critical new insights into gene-environment interactions that underlie asthma in children.

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## **Expected Research Outcomes and Benefits**

Asthma is a major health problem in children and an immense economic burden on the health care systems in the US and in the rest of the world. Given the important need for effective translational research in asthma, we believe that recent advances in genotyping technology, coupled to the fundamental information provided by the Human Genome and International HapMap Projects, makes the timing right for this ambitious project. Unique to the project are its cohort study populations, a very large and carefully annotated collection of samples already in hand and a successful strategy for continued collection of samples, and its application of the latest developments in genotyping technology. In view of these strengths of the project, we anticipate that our proposed studies to associate genome-wide individual SNPs and their interactions with environment exposures with varying phenotypes will be successful in identifying genetic variants and gene-environment interactions that underlie the development of asthma in childhood in subjects of different ethnic background. Indeed, we have already observed that exposures to smoking by asthmatic children modifies the predisposition risk of certain genotypes at the ORMDL3 locus and that these effects are differentially expressed in asthmatic children of European vs African ancestry. As such, we have great optimism that other such variants can be identified in pediatric asthma. Thus, we strongly suspect that our findings will ultimately significantly contribute to the health of children with asthma since, by providing an improved understanding of the gene-environment interactions that initiate these interrelated disorders, the findings will serve to identify molecular pathways critical to regulating their phenotypes and, thereby, facilitate the development of more effective new therapeutic and environmental control interventions and preventative measures to alleviate this most common chronic disease in children.

## **Summary of Research Completed**

In accordance with specific aim 1 and in order to identify novel asthma predisposition loci the researchers carried out a genome wide association (GWA) study in the large, well phenotyped cohort of pediatric asthma cases of Caucasian and African American ancestry collected at the Children's Hospital of Philadelphia. The sample included 793 North American children of European ancestry with persistent asthma who required daily inhaled glucocorticoid therapy and 1988 matched controls (the discovery set). The researchers also tested for genome wide association in an independent cohort of 917 persons of European ancestry who have had asthma and 1546 matched controls (the replication set). Finally, the researchers tested for an association between 20 single-nucleotide polymorphisms (SNPs) at chromosome 1q31 and asthma in 1667 North American children of African ancestry who have had asthma and 2045 ancestrally matched controls. In the meta-analysis of all samples from persons of European ancestry, the researchers observed an association, with genome-wide significance, between asthma and SNPs at the previously reported locus on 17q21 and an additional eight SNPs at a novel locus on 1q31. The SNP most strongly associated with asthma was rs2786098 ( $P=8.55 \times 10^{-9}$ ). The researchers observed replication of the association of asthma with SNP rs2786098 in the independent series of persons of European ancestry (combined  $P=9.3 \times 10^{-11}$ ). The alternative allele of each of the eight SNPs on chromosome 1q31 was strongly associated with asthma in the children of African ancestry ( $P=1.6 \times 10^{-13}$ ) for the comparison across all samples. The 1q31 locus contains DENND1B, a gene that is expressed by natural killer cells and dendritic cells and that encodes a

protein that is predicted to interact with the tumor necrosis factor alpha receptor. The researchers have identified a locus containing DENND1B on chromosome 1q31.3 that is associated with susceptibility to asthma (see publication 2).

Also in accordance with specific aim 1, to identify genes that underlie atopic disposition and asthma, the researchers carried out a genome-wide association study of eosinophilic esophagitis (EoE) which is a rare condition characterized by difficulty of feeding, failure to thrive, vomiting, epigastric or chest pain, dysphagia and food impaction. The majority of EoE patients are atopic individuals with a high rate of food allergen sensitization, based on skin prick and patch testing; in addition, patients have a higher rate of food anaphylaxis compared with the general population. Unlike classic anaphylaxis that typically involves a limited set of foods, EoE patients are often sensitized to a myriad of foods, often including food groups not typically associated with eliciting anaphylaxis. Experimental modeling in mice has demonstrated a key role for adaptive immunity, Th2 cell cytokines (especially IL-5 and IL-13) and a strong connection between allergic sensitization and inflammation in the respiratory tract and skin. Approximately 20 to 40% of EoE patients also present with asthma.

The EoE discovery cohort consisted of 181 clinically confirmed EoE patients of European ancestry recruited at CCHMC. The subjects in the discovery cohort had an eosinophils/hpf (400X) count of  $\geq 24$ . The mean age of these cases was  $11.3 \pm 10.4$  (SD) years. The replication cohort consisted of 170 cases recruited at CHOP. Cases were defined as those having an eosinophils/hpf (400X) count of  $\geq 24$  and on PPI therapy for at least 8 weeks. The mean age of the replication cohort cases was  $7.8 \pm 4.9$  SD years. Most of the EoE subjects in both the CCHMC and the CHOP cohorts were male making up 70% in the discovery cohort (CCHMC) and 75% in the replication cohort (CHOP). Moreover, 73% of the discovery cohort and 72% of the replication cohort had asthma, allergic rhinitis or atopic dermatitis.

The control samples used in both the discovery and replication phase were recruited within the CHOP Health Care Network by CHOP clinicians and nursing staff. The patients were all of self-reported Caucasian ancestry and screened negative for a diagnosis of asthma, dermatitis or other atopic conditions, as well as for other chronic medical conditions based on questionnaire responses. The mean age of the control group ( $n=2,096$ ) used for the discovery phase was  $8.54 \pm 5.65$  SD years and consisted of an equal percentage of males and females (50.4% and 50.6%, respectively). There was no overlap between patients or controls in the discovery and replication sets.

Single marker analyses for genome-wide data were carried out using the Cochran-Armitage trend test as implemented in PLINK. In the discovery cohort, two SNPs remained significantly associated with EoE following multiple testing correction. One of the two SNPs, rs3806932 (uncorrected  $P$ -value =  $8.81 \times 10^{-8}$ , MAF of 31.2% in cases and 45.8% in controls, OR=0.53, [95% CI 0.42 – 0.67]), maps to an LD block on 5q22.1 that contains eleven other SNPs that also are associated with EoE ( $P$ -value range =  $4.2 \times 10^{-3}$  –  $4.7 \times 10^{-7}$ ; OR range = 0.55 – 1.55). The associated LD block spans the TSLP/WDR36 gene region.

The researchers next sought to replicate the findings in the CHOP EoE cohort. While none of the SNPs surpassed genome wide significance, of the 12 associated SNPs at the 5q22.1 locus in the

discovery cohort, 4 were also associated with EoE in the replication cohort ( $P$ -value range = 0.05 –  $4.8 \times 10^{-3}$ ; OR range = 0.70 – 1.3). Combining the  $P$ -values across the two cohorts using Fisher's method, three SNPs surpassed genome wide significance ( $P$ -value range =  $2.37 \times 10^{-8}$  -  $3.19 \times 10^{-9}$ ). The most significant SNP in the discovery cohort rs3806932, was also significantly associated in the replication cohort ( $P$ -value =  $8 \times 10^{-3}$ ; MAFs 37.7% in cases and 45.2% in controls; OR 0.73; combined  $P$ -value for rs3806932 across CCHMC and CHOP cohorts =  $3.19 \times 10^{-9}$ ).

The researchers have identified and replicated a genome-wide significant locus at 5q22.1 in EoE patients of European ancestry; two genes map to this locus, *TSLP* and *WDR36*. The researchers are currently sequencing the two genes to identify causal mutations that may shed further light on the etiology of the atopic phenotype (see publication 3).

The researchers have also contributed our genotyped asthma and control cohorts, including African American and Caucasian individuals, to two multi-center studies, where the researchers have collaborated with several other teams.

The first was a genome-wide association (GWA) study of asthma with 359 cases from the Childhood Asthma Management Program (CAMP) and 846 genetically matched controls from the Illumina ICONdb public resource. The strongest region of association seen was on chromosome 5q12 in PDE4D. The phosphodiesterase 4D, cAMP-specific (phosphodiesterase E3 duncce homolog, *Drosophila*) gene (PDE4D) is a regulator of airway smooth-muscle contractility, and PDE4 inhibitors have been developed as medications for asthma. Allelic  $p$  values for top SNPs in this region were  $4.3 \times 10^{-7}$  for rs1588265 and  $9.7 \times 10^{-7}$  for rs1544791. Replications were investigated in ten independent populations with different ethnicities, study designs, and definitions of asthma. In seven white and Hispanic replication populations, two PDE4D SNPs had significant results with  $p$  values less than 0.05, and five had results in the same direction as the original population but had  $p$  values greater than 0.05. Combined  $p$  values for 18,891 white and Hispanic individuals (4,342 cases) in our replication populations were  $4.1 \times 10^{-4}$  for rs1588265 and  $9.2 \times 10^{-4}$  for rs1544791. In three black replication populations, which had different linkage disequilibrium patterns than the other populations, original findings were not replicated. Further study of PDE4D variants might lead to improved understanding of the role of PDE4D in asthma pathophysiology and the efficacy of PDE4 inhibitor medications see the following paper *Himes BE, Hunninghake GM, Baurley JW et al (2009). Genome-wide association analysis identifies PDE4D as an asthma-susceptibility gene. Am J Hum Genet May;84(5):581-593.*

The second was a study of asthma in African American populations. A genome-wide association study was performed in 2 independent populations of African ancestry (935 African American asthmatic cases and control subjects from the Baltimore-Washington, DC, area and 929 African Caribbean asthmatic subjects and their family members from Barbados) to identify single-nucleotide polymorphisms (SNPs) associated with asthma. A meta-analysis combining these 2 African-ancestry populations yielded 3 SNPs with a combined  $P$  value of less than  $10^{-5}$  in genes of potential biologic relevance to asthma and allergic disease: rs10515807, mapping to the alpha-1B-adrenergic receptor (*ADRA1B*) gene on chromosome 5q33 ( $3.57 \times 10^{-6}$ ); rs6052761, mapping to the prion-related protein (*PRNP*) gene on chromosome 20pter-p12 ( $2.27 \times 10^{-6}$ ); and

rs1435879, mapping to the dipeptidyl peptidase 10 (DPP10) gene on chromosome 2q12.3-q14.2. The generalizability of these findings were tested in family and case-control panels of United Kingdom and German origin, respectively, but none of the associations observed in the African groups were replicated in these European studies. Evidence for association was also examined in 4 additional case-control studies of African Americans; however, none of the SNPs implicated in the discovery population were replicated. This study illustrates the complexity of identifying true associations for a complex and heterogeneous disease, such as asthma, in admixed populations, especially populations of African descent (see publication 5).

The past year has seen some major advances in the genetics of asthma, the majority of which the researchers have driven or contributed too. The researchers have recently published a review article (see publication 5) summarizing these advances.

### Publications

1. Flory, J.H., Sleiman, P.M., Christie, J.D., Annaiah, K., Bradfield, J., Kim, C.E., Glessner, J., Imielinski, M., Li, H., Frackelton, E.C., Cuiping, H., Otieno, G., Thomas, K., Smith, R., Glaberson, W., Garris, M., Chiavacci, R., Allen, J., Spergel, J., Grundmeier, R., Grunstein, M., Magnusson, M., Grant, S.F., Bonnelykke, K., Bisgaard, H., and Hakonarson, H. (2009) 17q12-21 variants interact with smoke exposure as a risk factor for pediatric asthma but are equally associated with early vs late onset asthma in North-Americans of European Ancestry. *J Allergy Clin Immunol* Sep;124(3):605-607
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5. Sleiman PM, Hakonarson H. (2010) Recent advances in the genetics and genomics of asthma and related traits. *Curr Opin Pediatr* Jun;22(3):307-312