Loci Identified by Genome-wide Association Studies Influence Different Disease-related Phenotypes in COPD

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Online Data Supplement

Online Supplementary Methods

ECLIPSE (Clinicaltrials.gov identifier NCT00292552) is a longitudinal prospective study being conducted at 46 clinical centers in 12 countries. The aims, operational aspects and population description of the ECLIPSE cohort have been reported elsewhere[1]. Briefly, ECLIPSE is a multi-center 3 year longitudinal prospective study to identify novel endpoints in COPD. Individuals aged 40–75 years were recruited to the study. One thousand seven hundred and nineteen Caucasian subjects had COPD as defined by a smoking history of ≥ 10 pack-years, a post-bronchodilator ratio between forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) < 0.7 and GOLD stage II (FEV_1 50-80% predicted), III (FEV_1 30-50% predicted), or IV (FEV_1 <30% predicted) COPD. All consented subjects underwent standardised spirometry following 180 mcg (2 puffs) of salbutamol and all underwent a low dose computed tomography (CT) scan of the chest (120 kVp, 40 mAs, 1 or 1.25 mm slice thickness at full inspiration) to exclude non-COPD related disease and to evaluate the severity and distribution of emphysema and airway disease. The CT scans were evaluated at the central imaging unit at the University of British Columbia in Vancouver. The extent of emphysema was assessed in two ways. Firstly, it was scored independently by two radiologists who were blind to the individual's lung function. Emphysema was reported as trivial, mild, moderate, severe and very severe if it affected <5%, 5-25%, 25-50%, 50-75% and >75% of the lungs, respectively. A consensus reading was obtained when there was a difference of more than 1 emphysema category between the 2 observers. Otherwise, the average of the 2 readings was used in the analysis. Subjects with >5% emphysema were considered as emphysema subjects and those with ≤ 5% emphysema were considered as

controls in the emphysema binary phenotype analysis. Secondly, the extent of emphysema was assessed using the percentage of lung voxels with x-ray attenuation values less than -950 Hounsfield units (HU). The airway wall measurements were undertaken as described previously[2]. Airways were assessed by plotting lumenal perimeter (Pi) against the square root of wall area for all airways with a Pi >6 mm and estimating the square root of the wall area at a Pi of 10 mm[3]. Significant reversibility to salbutamol was defined as greater than or equal to 12% improvement from the baseline FEV_1. The Body-Mass Index, Airflow Obstruction and Exercise Capacity (BODE) Index was calculated using the method previously described[4]. The body mass index (BMI) was calculated using the formula weight (in Kg)/ height^2 (in m). Whole body impedance (expressed in Ω) was measured using the bio-electrical impedance method (BIA, Bodystat®) in each subject by lying on the bed and with separated legs. Fat free Body Mass (FFM) was calculated using the method published elsewhere [5].

COPD exacerbation frequency in ECLIPSE was assessed in two ways. Retrospective exacerbations were defined by a questionnaire administered at the time of recruitment and represent COPD-related exacerbations in the previous 12 months (defined as subject-reported episodes that required antibiotics, systemic corticosteroids or hospitalization). The subjects were also assessed for exacerbations prospectively for 2 years based on monthly phone calls (exacerbations over the first 2 years of the study that required antibiotics, systemic corticosteroids or hospitalization).

Individuals with COPD were recruited as probands to the ICGN study[6], and siblings and available parents were ascertained through the probands. Inclusion criteria for probands were post-bronchodilator FEV_1< 60% predicted and FEV_1/VC < 90%

predicted at a relatively early age (45 to 65 years), a $\geq$ 5 pack-year smoking history, and at least one eligible sibling with a $\geq$ 5 pack-year smoking history. COPD in siblings was defined by a post-bronchodilator FEV_1< 80% predicted and FEV_1/VC< 90% predicted. In total, 1891 Caucasian individuals from 606 pedigrees were included in the ICGN family-based association analysis. High resolution CT measurements were available on 561 probands [48.5%] and 663 siblings [34.2%] as described elsewhere (1-mm sections at 20-mm intervals, 120 kilovolts [peak] [kVp], 200 mAs)[7]. All the HRCT scans from the ECLIPSE and ICGN studies were processed at University of British Columbia, Vancouver, Canada. The ICGN population was originally used to identify the HHIP and CHRNA3/5 SNPs using the COPD phenotype in follow-up to genome-wide association analysis[8], and a previously reported association analyses of FEV_1 in the ICGN is included for comparison in this current manuscript. The ECLIPSE population was not a part of the original genome-wide association study. The ICGN HRCT analysis was conducted using a software developed at University of British Columbia and the ECLIPSE data was analyzed using the VIDA software(VIDA Diagnostics, Iowa City, IA 52242)

Genotyping

The Illumina 550Kv3 SNP array was used for SNP genotyping in the ECLIPSE subjects. Details on the procedures used for genotyping and quality control evaluation are described elsewhere[9]. Subject samples that had a call rate for the SNPs in the genome-wide panel <98% were deleted. Data from 2 SNPs in the CHRNA3/5 locus (rs8034191 and rs1051730) and 2 SNPs in the HHIP locus (rs1828591 and rs13118928) were used in the current analysis. The ICGN subjects were genotyped using Sequenom´s iPLEX SNP

genotyping protocol developed for measurement with the MassARRAY mass spectrometer[10].

Statistical Analyses

In the ECLIPSE population, logistic regression models for the binary phenotypes and linear regression models for the quantitative phenotypes were used for association analysis; covariates included age, sex, pack-years of smoking, current smoking status, and EIGENSTRAT vectors for control of population stratification. The analyses were undertaken using SAS® software 9.1 with an additive genetic model. To control for the possibility of spurious associations resulting from population stratification in the ECLIPSE cohort, we used a modified EIGENSTRAT method[11]using selected SNPs from the QC passed data from the Illumina 550K genotyping as described elsewhere[12] This showed that there were 9 significant principal component axes, all of which were included in the model. Multivariate regression models were evaluated to identify the contribution of the HHIP and CHRNA3/5 SNPs to phenotypes including spirometric measures, fat-free body mass, BMI, bronchodilator reversibility and quantitative measures of emphysema. The associations of the HHIP and the CHRNA SNPs with retrospective and prospective exacerbations in ECLIPSE were assessed with negative binomial regression models. Robust standard errors for the model coefficients were determined by generalized estimating equations (GEE). Each regression model included age, gender, and pack-years smoked as covariates. An offset variable based on the log of the number of days on study was included in the model for prospective exacerbations.

Family-based association analysis was conducted in the ICGN families using PBAT version 3.6[13]. In the analysis of quantitative phenotypes, adjustments for age, gender, pack-years of smoking and current smoking status were performed. Associations with FEV_1 and FEV_1 /FVC were tested using PBAT with age, gender, pack-years of smoking, current smoking status and height as covariates. Since the family-based association analysis is immune to population stratification, we did not adjust for stratification in the ICGN analyses.

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This article has online data supplement tables which are available from this issue's table of content online at www.atsjournals.org.

Online Supplementary Figure 1

a. Linkage disequilibrium plot of the *CHRNA3/5* locus in the ECLIPSE cohort

b. Linkage disequilibrium plot of the *HHIP* locus in the ECLIPSE cohort

c. Linkage disequilibrium plot of the *FAM13A* locus in the ECLIPSE cohort

The SNPs analyzed in the manuscript for the COPD related phenotypes are labeled in green (CHRNA rs8034191; HHIP rs13118928; FAM13A rs7671167)

Imputation was conducted using HAPMAP ver 2 data 200kb up and down-stream of the SNP of interest at each locus (CHRNA rs8034191; HHIP rs13118928; FAM13A rs7671167)

Figure 1a

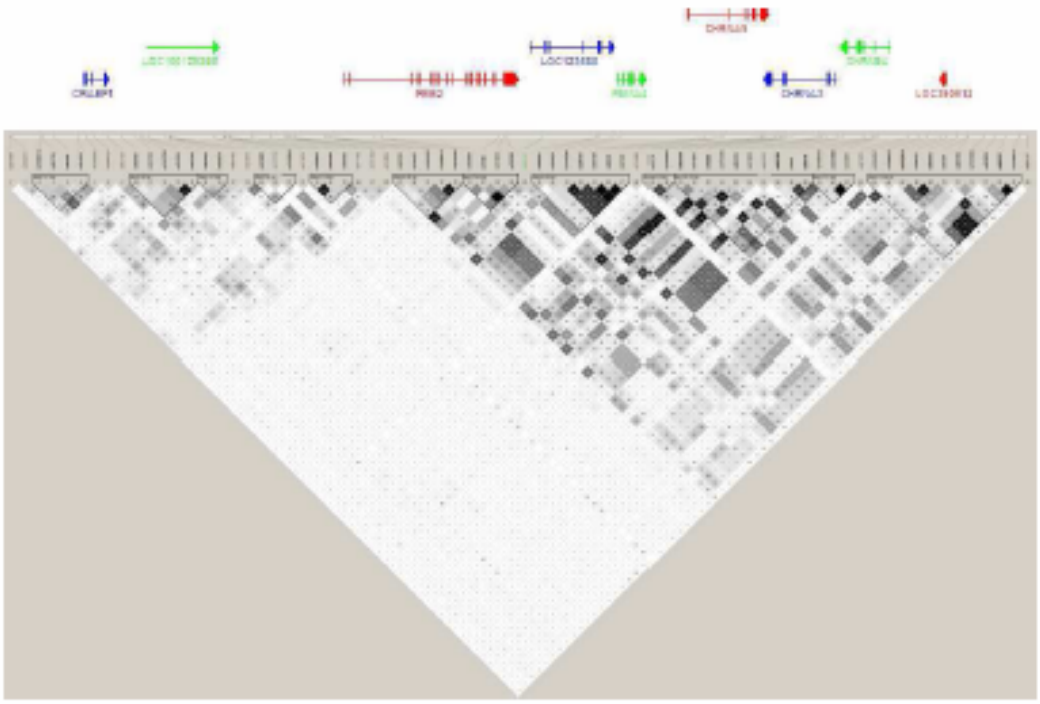

Figure 1b

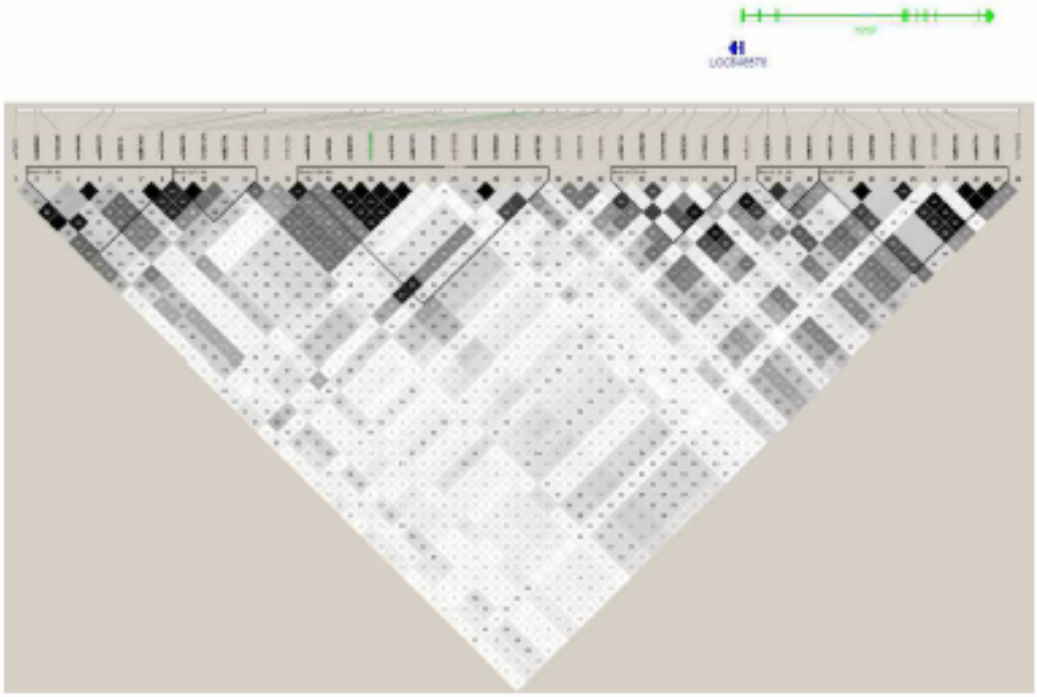

Figure 1c