ERJ Express. Published on February 5, 2009 as doi: 10.1183/09031936.00120408

Leptin Receptor Polymorphisms and Lung Function Decline in Chronic Obstructive Pulmonary Disease

Nadia N. Hansel, MD MPH[1], Li Gao, MD, PhD[1], Nicholas M. Rafaels, MS[1], Rasika A. Mathias, ScD[2], Enid R. Neptune, MD[1], Clarke Tankersley, PhD[3], Audrey V. Grant, PhD[1], John Connett, MD[4], Terri H. Beaty, PhD[3], Robert A. Wise[1], MD, Kathleen C. Barnes, PhD[1,3]

[1]Department of Medicine, School of Medicine, Johns Hopkins University, Baltimore, MD; [2]Inherited Disease Research Branch, National Human Genome Research Institute (NHGRI), National Institutes of Health; [3]Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD; [4]Division of Biostatistics, School of Public Health, University of Minnesota, St. Paul, Minnesota

Running Head: Leptin Receptor and COPD

Grant funding: This research was supported by NHLBI (HL076322, HL066583, HL010342), NIA (AG21057). This research was supported in part by the Intramural Research Program of the National Human Genome Research Institute, National Institutes of Health. Genotyping services were provided by the Johns Hopkins University under U.S. Federal Government contract number N01-HV-48195 from the NHLBI. KCB was supported in part by the Mary Beryl Patch Turnbull Scholar Program.

Word count: 2,704 (<3,000)

Corresponding Author: Kathleen C. Barnes, PhD, The Johns Hopkins Asthma & Allergy Center, 5501 Hopkins Bayview Circle, Room 3A.62, Baltimore, MD 21224, Telephone: 410-550-2071 / Fax: 410-550-2130, Email: kbarnes@jhmi.edu

ABSTRACT

Background: Only a fraction of all smokers develop COPD, suggesting a large role for genetic susceptibility. The leptin receptor (LEPR) is present in human lung tissue and may play a role in COPD pathogenesis. This study examined the association between genetic variants in the *LEPR* gene and lung function decline in COPD.

Methods: 429 European Americans were randomly selected from the NHLBI Lung Health Study. Thirty-six single nucleotide polymorphisms (SNPs) in *LEPR* were genotyped using the Illumina™ platform. Mean annual decline in FEV_1 % predicted over the five-year period was calculated using linear regression. Linear regression models were also used to adjust for potential confounders. In addition, *in vivo* expression of the receptor gene was assessed with immunohistochemistry on lungs from smoke-exposed inbred mice.

Results: We identified significant associations ($P<0.05$) between lung function decline and 21 SNPs. Haplotype analyses confirmed several of these associations seen with individual markers. Immunohistochemistry results in inbred mice strains support a potential role of LEPR in COPD pathogenesis.

Conclusions: We identified genetic variants in the *LEPR* gene significantly associated with lung function decline in a population of smokers with COPD. Our results support a role for *LEPR* as a novel candidate gene for COPD. **Word Count: 199 (<200)**

KEY WORDS

Chronic obstructive pulmonary disease (COPD), leptin receptor, lung function decline, polymorphisms

INTRODUCTION

COPD is the fourth leading cause of death in the United States and the fifth leading cause of death worldwide, and its prevalence is expected to increase in upcoming decades.(1;2) The overwhelming majority of COPD is caused by environmental exposures. In the United States, this exposure is primarily cigarette smoke; however only 15% of all smokers develop COPD. This suggests a large role of genetic susceptibility.

In addition to its role in obesity (appetite suppression), leptin has been shown to have multiple other functions, including increasing sympathetic nerve activity, maintaining reproductive function, immunity, angiogenesis, preserving normal respiratory function in the presence of obesity and cell proliferation of tracheal epithelial cells and lung growth.(3) After adjustment for obesity, the leptin pathway has been associated with inflammatory markers as well as multiple inflammatory conditions, including cardiovascular disease.(4;5) Its potential role in the systemic inflammatory response in patients with COPD is evident from the correlation of leptin with other inflammatory markers.(6-8) Leptin has been shown to directly stimulate phagocytic activity of macrophages and enhance endotoxin induced production of TNFα, IL-6, and IL-12, cytokines typically involved in COPD pathogenesis.(9-12) Conversely, deficiency in leptin or its receptor may predispose to both immunodeficiency and infection.(10) Serum leptin levels are elevated in exacerbations of COPD and have also been associated with bacterial pulmonary infections, which are associated with COPD exacerbations and decline in lung function.(7;13-15) Leptin exerts multiple effects through its leptin receptor (LEPR), located on human chromosome 1p31. The leptin receptor is produced in several alternatively spliced forms that share extracellular and transmembrane domains

but have varying cytoplasmic residues(16). Leptin receptor has a wide tissue distribution, including lung tissue.(17)

We tested for association between genetic variants in the *LEPR* gene and lung function decline in a subset of the multicenter NHLBI-supported Lung Health Study (LHS) cohort. In addition, the potential role of LEPR in COPD pathogenesis was further evaluated in smoke-exposed AKR/J mice which display marked airspace enlargement by histologic and morphometric criteria.(18) To determine whether the leptin receptor is differentially localized to resident lung cells under conditions which simulate human COPD, we performed immunohistochemistry on murine lung sections obtained from AKR/J mice exposed to four months of cigarette smoke versus room air controls.

Identifying pathways and novel molecular targets that modify the clinical course of disease is fundamental to developing preventive strategies and novel therapies.

METHODS

Subjects

Subjects participating in the current study included 429 European Americans (EA) randomly selected from a group of 4,287 participants of the multi-center NHLBI-supported Lung Health Study (LHS) for whom DNA was available. Lung function was measured annually over five years and conducted according to ATS guidelines using identical spirometers, software, procedures and reading center personnel.(19;20) The quality of the spirometry testing conducted by the technicians was monitored centrally throughout the testing and comparison of baseline spirometry measures showed good reproducibility with very small mean short-term intra-individual variations in FEV_1.(21)

Lung function data from Annual Visit 1 to Annual Visit 5 was used for the current analyses and has been shown to have a good linear fit in previous LHS analyses.(19;22) Subjects with < 3 annual lung function measurements were excluded from analysis (n=15).

SNP Selection and Genotyping

Single nucleotide polymorphisms (SNPs) representing the *LEPR* gene were selected from Goldenpath (http://genome.ucsc.edu/) and/or NCBI (http://www.ncbi.nlm.nih.gov/). Criteria included 1) SNPs approximating inter-SNP distance as close to 5 kb as were available at the time of the dbSNP Build 124; 2) representation of SNPs in the promoter, coding and 3'UTR regions; and 3) SNPs with acceptable design scores according to the Illumina Assay Design Tool for genotyping on the Illumina™ GoldenGate platform. Priority for selecting SNPs included: 1) regulatory and coding SNPs; 2) highly polymorphic SNPs, preferably ≥10% minor allele frequency; 3) validated SNPs; and 4) SNPs at intron/exon boundaries. A total of 36 *LEPR* SNPs spanning 228,294 bp on human chromosome 1p31 with an average inter-SNP distance of 6.53 kb (ranging from 1.9–14 kb) are summarized in **Table 1**.

Statistical Analysis

Mean annual decline in lung function (post-bronchodilator %predFEV_1) was calculated as a linear regression slope over the five-year study period. Linear regression models were used to adjust for potential confounders, including baseline characteristics: smoking history (pack-years), age, sex, %predFEV_1 and airway reactivity (AR). AR was calculated as a quantitative measure, using the two-point slope.(22). In addition to adjusting for baseline characteristics 1) change in body mass index (BMI), calculated as a

linear regression slope, and 2) smoking status at Year 5, defined as 'continuous smoker', 'intermittent smoker', and 'sustained quitter' were also analyzed.

Forward and backward selection were used to develop a parsimonious model. Residuals from the regression were included in a genetic additive model and the most common homozygote genotype for each SNP served as the reference category. Interaction term for smoking and genetic effect were tested using PLINK while testing for quantitative interaction. Each SNP locus was evaluated for Hardy-Weinberg equilibrium. All analyses were performed with StataSE, version 8.0 (Stata Corp, College Station, TX) and PLINK(23).

Individual haplotypes were analyzed using PLINK. Pairwise linkage disequilibrium (LD) based on the D' statistic was measured using Haploview.(24) LD blocks were defined using their default algorithm.(25) Sliding windows of 2 to 4 adjacent SNPs were used to test for association. Haplotype estimates were computed using PHASE assuming no recombination.(26)

Murine Model

Ten week old AKR/J mice were exposed to cigarette smoke 5 hours/day, 5 days/week, for 6 months. The exposure was conducted by burning 2R4F reference cigarettes (University of Kentucky, Tobacco Research Institute), using a TE-10 smoking machine (Teague Enterprises). Each cigarette was puffed for 2 seconds, once every minute for a total of 8 puffs, at a flow rate of 1.05 l/min, to provide a standard puff of 35 cm^3. The smoke machine was adjusted to produce a mixture of sidestream smoke (89%) and mainstream smoke (11%). The smoke chamber was monitored daily for total

suspended particles and carbon monoxide, with concentrations of 90 mg/m^3 and 350 ppm, respectively. Air-exposed control mice were housed in a filtered air environment.

Immunohistochemistry: Five micron paraformaldehyde-fixed, paraffin-embedded tissue sections from smoke-exposed and room air control AKR/J mice were deparaffinized and rehydrated in an ethanol series. Sections were blocked for non-specific binding with 3% normal serum from chicken and incubated with the primary antibodies for 1 hour at room temperature. Following incubation with the primary antibody overnight at 4°C, slides were washed with PBST, incubated for 30 minutes at RT with an appropriate biotinylated secondary antibody and developed by using DAB (3,3'-diaminobenzidine) substrate and chromagen from Dako. Antigen retrieval was performed using citrate buffer for 30 minutes. Antibodies were used at the following concentrations: leptin receptor, ObR (goat polyclonal, I-17, 1:100, Santa Cruz), donkey anti-goat secondary antibody (sc2020, 1:400, Santa Cruz). .

RESULTS

Clinical characteristics of subjects are presented in **Table 2.** There were no statistical differences in baseline characteristics between those included in the final analyses (n=414) compared to those excluded because more than two data points in lung function were missing (n=15, data not shown). Distributions of baseline characteristics and lung function measurements were also similar to those in the full LHS cohort from which this subset was randomly selected (n=5,887, data not shown). Age, baseline lung function, airway reactivity (AR), smoking status at Year 5, and change in BMI were independently associated with lung function decline, and were included in the final regression analyses.

There was no significant interaction between smoking status at year 5 and LEPR polymorphisms.

Single-marker analyses

All 36 *LEPR* SNPs were in Hardy-Weinberg Equilibrium (HWE). All results of the two-point tests for association between *LEPR* markers and %predFEV$_1$ decline are presented in **Table 3**. We found evidence for significant associations between 21 SNPs in the *LEPR* gene and %predFEV$_1$ decline, spanning the length of the gene from intron 2 to the 5' end. The minor alleles of most significant SNPs were associated with attenuation in lung function decline. Of particular interest, each G allele at the functional marker rs1137100, in exon 4, which creates a lysine to arginine amino acid change, was associated with a 0.33%/ yr attenuation in annual loss of %predFEV$_1$.

Examination of the linkage disequilibrium (LD) structure across these SNPs revealed high LD (D' ranging from 0.80 to 1.0, with R^2 ranging from 0.65 to 0.96) for 31 of 35 pairs of contiguous SNPs. **(Figure 1).** In fact, 33 of the SNPs fell into one of 6 LD blocks, and most of the 21 SNPs with association signal are noted to have similar effect sizes for the minor allele and fall into one of three LD blocks (blocks 2, 3 and 4, annotated in Table 3).

Haplotype analyses

Considering the clustering of most genotyped SNPs within six LD blocks with high levels of LD also observed between SNPs across different blocks, a systematic sliding window approach was implemented, considering windows of 2-4 SNPs/window beginning with the first (5') marker, and working across the gene, one marker at a time. Haplotype tests revealed multiple association signals in three specific regions that

overlapped with the single-SNP results described above. Twenty-five haplotypes showed stronger association than single SNP results. **(Figure 2).** One of the most compelling regions was at the 5' end of *LEPR* (region 1) and the haplotype GCCT (rs7531867, rs1805096, rs1892535, rs6691346), which revealed a strong association with lung function decline (P=0.003) and includes the coding-synonymous SNP, rs1805096. Furthermore, one 3-SNP and two 4-SNP window haplotypes in region 2 spanning rs10443259 to rs10889562 (introns 2 through 5) were most strongly associated with lung function decline (P=0.002), and includes the functional marker rs1137100, described above.

Validation of *LEPR* as a candidate gene for COPD phenotypes in inbred mice

AKR/J mice, a well-characterized inbred strain, exposed to 4 months of cigarette smoke (CS) not only exhibit airspace enlargement but also develop airway thickening and inflammation that is highly reminiscent of COPD.(18) We examined whether CS exposure altered leptin receptor expression in the lungs of this strain. Using immunohistochemistry, we observed that leptin receptor expression was evident in macrophages and the airway and airspace compartments in room air-exposed AKR/J mice. However, upon smoke exposure, there was a reduction of staining in the airspace and airway wall **(Figure 3)**. These findings are consistent with cigarette-smoke induced down-regulation of leptin receptor expression in the epithelial compartments of cigarette smoke exposed mice.

DISCUSSION

We identified 21 SNPs in the *LEPR* gene that were significantly associated with lung function decline in a European American population with COPD and our haplotype

analysis supported results from single SNP analysis. The association signals observed across these SNPs likely represent two or three signals in the gene. Furthermore, the potential role of the leptin receptor in lung architecture and COPD phenotypes is supported by an AKR/J murine model showing decreased *Lepr* expression in airway wall and epithelium after smoke exposure. Our results identify *LEPR* as a novel candidate gene for COPD.

To our knowledge, no previous studies have investigated the role of genetic polymorphisms in the *LEPR* gene and COPD or its associated phenotypes. The phenotype that we studied was rate of decline of lung function rather than COPD *per se*. The rationale for this was that rate of decline in lung function is a more precise phenotype than a single cross-sectional measurement of lung function needed to define COPD, and may serve as a more genetically homogeneous phenotype. We identified 21 SNPs in the *LEPR* gene that were significantly associated with lung function decline in a European American population with COPD. One of the most compelling regions was at the 5' end of LEPR (region 1), which includes part of the intracellular domain of the receptor(16) and may result in different signaling potency. Most variants identified in *LEPR* were associated with an attenuation of lung function decline, and notably, the G variant at the non-synonymous SNP rs1137100, which codes for an amino acid substitution in the extracellular domain of the leptin receptor(27), was associated with a 0.33%/ yr attenuation in loss of %pred FEV_1. Airflow obstruction that occurs in COPD is caused by a mixture of small airway disease, parenchymal destruction and increased airways responsiveness that develops over decades.(28) Thus, over time, these genetic variants may have substantial impact on disease progression. Over a 40 year period, a person

homozygous for the G allele at rs1137100 will have a %pred FEV_1 which is 26.4% higher compared to a homozygote for the major allele. Given a MAF of 0.26, as seen in our cohort, we might extrapolate 7% of the population to be homozygote for the minor allele (GG), 38% to be heterozygote (AG) and 55% to be homozygote for the major allele (AA). Even after adjusting for smoking status, this genetic variant could explain the difference between mild and moderate or moderate and very severe COPD under current GOLD criteria.(29)

The dearth of well-characterized populations of COPD subjects with longitudinal lung function data comparable to the LHS adds to the uniqueness of our population, but limits the opportunity to replicate these results. However, finding multiple significant SNPs in a single gene reduces the likelihood of spurious results due to multiple testing, though it may also be due in part to the high degree of LD in this gene. Furthermore, when using a False Discovery Rate (FDR) of 0.2, six SNPs would remain statistically significantly associated with lung function decline ($P<0.006$) in this study. Previous studies have shown genetic variants in *LEPR* to be associated with markers of inflammation, including C-reactive protein (CRP) and fibrinogen levels, lending support to the hypothesis that the leptin pathway has a physiological influence on inflammatory traits. Specifically, the minor allele (T) for the rs1805096 locus was associated with lower levels of fibrinogen, CRP and IL-6 levels(30) in a previous study of healthy European Americans. Both, lower CRP and IL-6 levels have been associated with attenuated lung function decline in the LHS cohort.(31) Interestingly, the minor allele (T) in the coding SNP rs1805096 was also associated with lower rates of lung function decline in our study (0.295 %/yr, p=0.007). Therefore, we hypothesize one potential

mechanism of the disease modifying-effects of the *LEPR* gene may be mediated through inflammatory mechanisms. The notion that leptin may function as an immunomodulatory cytokine has become increasingly accepted. The association between leptin and muscle wasting and cachexia in COPD is well established;(32) and the presence of leptin in induced sputum of patients with moderate COPD and its association with other inflammatory markers(33) suggests leptin may be involved in the local inflammatory response in COPD.

Inbred mice or guinea pigs subjected to chronic cigarette smoke (CS) exposure have been shown to be invaluable models of CS-induced parenchymal lung disease.(34;35) Furthermore, the use of animal models of complex human diseases to parse candidate genes identified in broad genetic or genomic surveys is a standard approach to initial pathway validation, especially if additional populations are not available for replication and therapeutic targets are an ostensible goal.(36;37) We noted a reduction in staining in the airspace wall and airway epithelial compartment, but retained expression in macrophages in AKR/J smoke-exposed mice. This is consistent with findings of Bruno et al. who found decreased expression of leptin and its receptor in smokers and subjects with mild-to-severe COPD as compared to healthy nonsmoking subjects.(13) Accordingly, reduced leptin receptor expression (acquired or genetic) may be a critical predisposing factor to cigarette smoke induced lung disease.

In summary, the current study is the first to report an association between *LEPR* polymorphisms and COPD. Our results support the role of the leptin pathway, and particularly the leptin receptor (*LEPR*) in COPD and lung function decline. Specifically, individuals with the minor allele at the selected polymorphisms were less susceptible to

loss of lung function and COPD progression. The single-SNP and haplotype tests point to three clusters of signal highlighting two potential loci considering the linkage disequilibrium between these signals: the functional SNP rs137100 and at the synonymous coding SNP rs1805096. While these two SNPs are not in LD with each other (D'=0.45, R^2=0.125), haplotypes of significance that include each SNP overlap neighboring LD blocks making it somewhat difficult to tease apart these two signals. It is necessary to replicate our findings in other populations; however our results identify *LEPR* as a novel target in COPD and lung function loss.

Acknowledgements

We would like to thank Peter Chi and Monica Campbell; Alan L Scott, PhD, Anne E. Jedlicka and Margaret V. Mintz of the Malaria Research Institute, Gene Array Core Facility, Johns Hopkins University; Alan F. Scott, PhD, Kimberly Doheny, PhD, Roxann Ashworth and Corinne Boehm of the Center for Inherited Disease Research Institute; and Helen Voelker and Kathy Farnell of the LHS Data Coordinating Center, University of Minnesota. The principal investigators and senior staff of the clinical and coordinating centers, the NHLBI, and members of the Safety and Data Monitoring Board of the Lung Health Study are as follows:

Case Western Reserve University, Cleveland, OH: M.D. Altose, M.D. (Principal Investigator), C.D. Deitz, Ph.D. (Project Coordinator); *Henry Ford Hospital, Detroit, MI*: M.S. Eichenhorn, M.D. (Principal Investigator), K.J. Braden, A.A.S. (Project Coordinator), R.L. Jentons, M.A.L.L.P. (Project Coordinator); *Johns Hopkins University School of Medicine, Baltimore, MD*: R.A. Wise, M.D. (Principal Investigator), C.S. Rand, Ph.D. (Co-Principal Investigator), K.A. Schiller (Project Coordinator); *Mayo Clinic, Rochester, MN*: P.D. Scanlon, M.D. (Principal Investigator), G.M. Caron (Project Coordinator), K.S. Mieras, L.C. Walters; *Oregon Health Sciences University, Portland*: A.S. Buist, M.D. (Principal Investigator), L.R. Johnson, Ph.D. (LHS Pulmonary Function Coordinator), V.J. Bortz (Project Coordinator); *University of Alabama at Birmingham*: W.C. Bailey, M.D. (Principal Investigator), L.B. Gerald, Ph.D., M.S.P.H. (Project Coordinator); *University of California, Los Angeles*: D.P. Tashkin, M.D. (Principal Investigator), I.P. Zuniga (Project Coordinator); *University of Manitoba, Winnipeg*: N.R. Anthonisen, M.D. (Principal Investigator, Steering Committee Chair), J. Manfreda, M.D. (Co-Principal Investigator), R.P. Murray, Ph.D. (Co-Principal Investigator), S.C. Rempel-Rossum (Project Coordinator); *University of Minnesota Coordinating Center, Minneapolis*: J.E. Connett, Ph.D. (Principal Investigator), P.L. Enright, M.D., P.G. Lindgren, M.S., P. O'Hara, Ph.D., (LHS Intervention Coordinator), M.A. Skeans, M.S., H.T. Voelker; *University of Pittsburgh, Pittsburgh, PA*: R.M. Rogers, M.D. (Principal Investigator), M.E. Pusateri (Project Coordinator); *University of Utah, Salt Lake City*: R.E. Kanner, M.D. (Principal Investigator), G.M. Villegas (Project Coordinator); *Safety and Data Monitoring Board*: M. Becklake, M.D., B. Burrows, M.D. (deceased), P. Cleary, Ph.D., P. Kimbel, M.D. (Chairperson; deceased), L. Nett, R.N., R.R.T. (former member), J.K. Ockene, Ph.D., R.M. Senior, M.D. (Chairperson), G.L. Snider, M.D., W. Spitzer, M.D. (former member), O.D. Williams, Ph.D.; *Morbidity and Mortality Review Board*: T.E. Cuddy, M.D., R.S. Fontana, M.D., R.E. Hyatt, M.D., C.T. Lambrew, M.D., B.A. Mason, M.D., D.M. Mintzer, M.D., R.B. Wray, M.D.; *National Heart, Lung, and Blood Institute staff, Bethesda, MD*: S.S. Hurd, Ph.D. (Former Director, Division of Lung Diseases), J.P. Kiley, Ph.D. (Former Project Officer and Director, Division of Lung Diseases), G. Weinmann, M.D. (Former Project Officer and Director, Airway Biology and Disease Program, DLD), M.C. Wu, Ph.D. (Division of Epidemiology and Clinical Applications).

Table 1: Location, minor allele frequency, and type of selected *LEPR* SNPs

SNP ID	SNP	Position	Region	Minor Allele	MAF
1	RS7531867	65819567	Downstream	A	0.39
2	RS1805096	65814278	Coding exon (1019 P/P)	T	0.39
3	RS1892535	65809202	Intron	T	0.18
4	RS6588153	65804038	Intron	A	0.38
5	RS1938484	65793303	Intron	A	0.18
6	RS8179183	65787973	Coding exon (656 K/N)	C	0.18
7	RS3790419	65779130	Coding exon (343 S/S)	G	0.22
8	RS3828034	65774346	Intron (boundary)	C	0.19
9	RS12564626	65768563	Intron	A	0.45
10	RS10443259	65763371	Intron	A	0.28
11	RS6691346	65758418	Intron	A	0.28
12	RS4655680	65753490	Intron	T	0.29
13	RS1137100	65748462	Coding exon (109 K/R)	G	0.26
14	RS10889562	65742466	Intron	A	0.22
15	RS6702028	65731912	Intron	C	0.28
16	RS1782763	65719921	Intron	C	0.33
17	RS1171265	65715273	Intron	A	0.36
18	RS1171271	65710811	Intron	C	0.27
19	RS1782754	65705369	Intron	G	0.27
20	RS1171279	65700514	Intron	T	0.27
21	RS1171274	65692859	Intron	C	0.27
22	RS10889558	65688987	Intron	A	0.27
23	RS6694528	65675037	Intron	T	0.13
24	RS1327121	65669358	Intron	C	0.34
25	RS17412682	65664314	Intron	C	0.46
26	RS2025804	65658142	Intron	C	0.34
27	RS17127652	65647164	Intron	G	0.02
28	RS4655811	65635178	Intron	C	0.34
29	RS6657868	65625728	Intron	A	0.39
30	RS9436746	65620494	Intron	A	0.4
31	RS970468	65618511	Downstream	G	0.39
32	RS17097182	65615466	Downstream	T	0.05
33	RS1045895	65610002	3' UTR	A	0.38
34	RS9436299	65604909	Intron	C	0.33
35	RS1327118	65597590	Promoter	C	0.46

SNP – single nucleotide polymorphism
MAF – minor allele frequency

Table 2: Patient Characteristics

	European Americans (n=414)
Baseline Characteristics	
Mean age (SD)	48.6 (7.0)
Male, %	65.0
BMI	25.4 (3.6)
Smoking (pack-years)	40.5 (19.2)
Pre-BD %FEV_1	76.3 (6.4)
Post-BD %FEV_1	79.2 (6.2)
Longitudinal Characteristics at 5 years	
Smoking history, %	
Continuous	50
Intermittent, now smoking	12
Intermittent, now quit	19
Sustained quitter	19
Post-BD ΔFEV_1, %/yr	-0.74 (1.63)

BD – bronchodilator
BMI – body mass index
SD – standard deviation

Table 3: Association of *LEPR* polymorphisms with lung function decline in a subset of the LHS European American (n=414) cohort

LD Block	SNP ID	SNP	Beta	95% CI	p-val
1	1	RS7531867	0.298	-0.111 - 0.298	**0.006**
	2	RS1805096	0.295	-0.082, 0.339	**0.007**
2	3	RS1892535	0.314	-0.008, 0.435	**0.018**
	4	RS6588153	0.292	-0.381, 0.039	**0.007**
	5	RS1938484	0.351	-0.459, 0.469	**0.007**
	6	RS8179183	0.109	-0.014, 0.410	0.429
	7	RS3790419	-0.09	**0.013, 0.432**	0.473
	8	RS3828034	0.026	-0.008, 0.419	0.848
	9	RS12564626	0.25	-0.004, 0.435	**0.015**
	10	RS10443259	0.334	-1.011, 0.509	**0.004**
	11	RS6691346	0.334	**0.039, 0.477**	**0.004**
	12	RS4655680	0.312	**-0.436, -0.027**	**0.007**
3	13	RS1137100	0.33	**0.019, 0.456**	**0.005**
	14	RS10889562	-0.044	-0.350, 0.277	0.73
	15	RS6702028	0.32	**0.085, 0.538**	**0.006**
	16	RS1782763	0.318	**0.111, 0.565**	**0.003**
	17	RS1171265	0.321	-0.329, 0.138	**0.004**
	18	RS1171271	0.32	**0.091, 0.543**	**0.006**
	19	RS1782754	0.317	**0.093,0.547**	**0.006**
	20	RS1171279	-0.095	**0.105, 0.537**	0.425
	21	RS1171274	0.338	**0.106, 0.529**	**0.004**
	22	RS10889558	0.311	**0.093, 0.547**	**0.007**
4	23	RS6694528	-0.037	-0.296, 0.207	0.818
	24	RS1327121	0.238	**0.103, 0.557**	**0.033**
	25	RS17412682	-0.232	**0.087, 0.537**	**0.027**
	26	RS2025804	0.258	**0.106, 0.562**	**0.022**
	27	RS17127652	-0.251	**0.106, 0.562**	0.518
5	28	RS4655811	0.216	**0.049, 0.452**	0.055
	29	RS6657868	0.206	-0.240, 0.292	0.06
	30	RS9436746	0.223	-0.334, 0.155	**0.039**
	31	RS970468	0.198	-0.161, 0.378	0.068
6	32	RS17097182	0.005	**0.096, 0.607**	0.983
	33	RS1045895	-0.171	**0.082, 0.502**	0.111
	34	RS9436299	0.214	**0.056, 0.572**	0.059
	35	RS1327118	0.129	**0.084, 0.507**	0.231
	36	RS10493377	0.093	**0.088, 0.508**	0.373

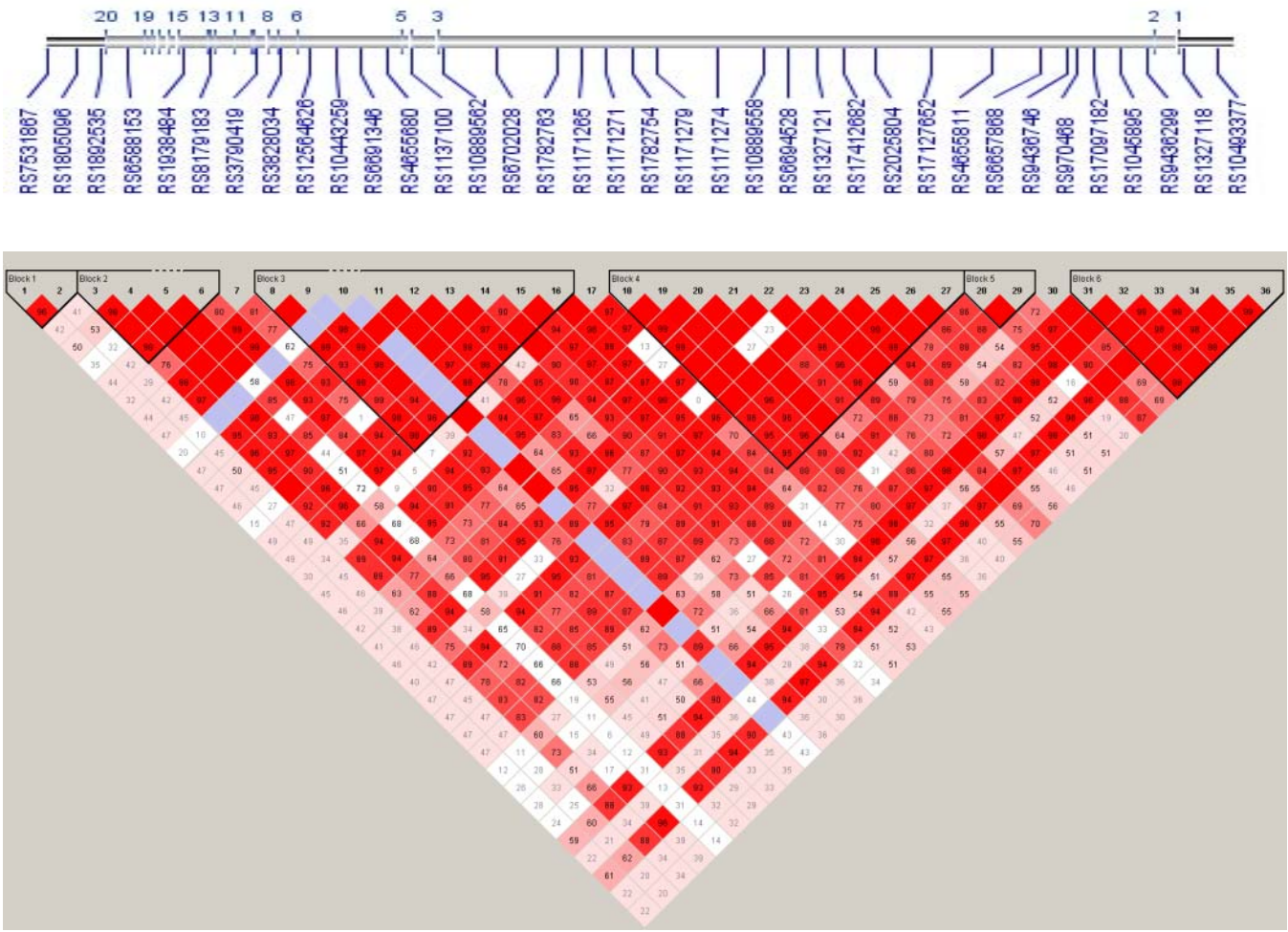

Figure 1. Pairwise LD in subjects is represented as red squares for strong LD, blue squares for non-significant LD, and white squares for little or no LD. LD blocks are identified as noted.

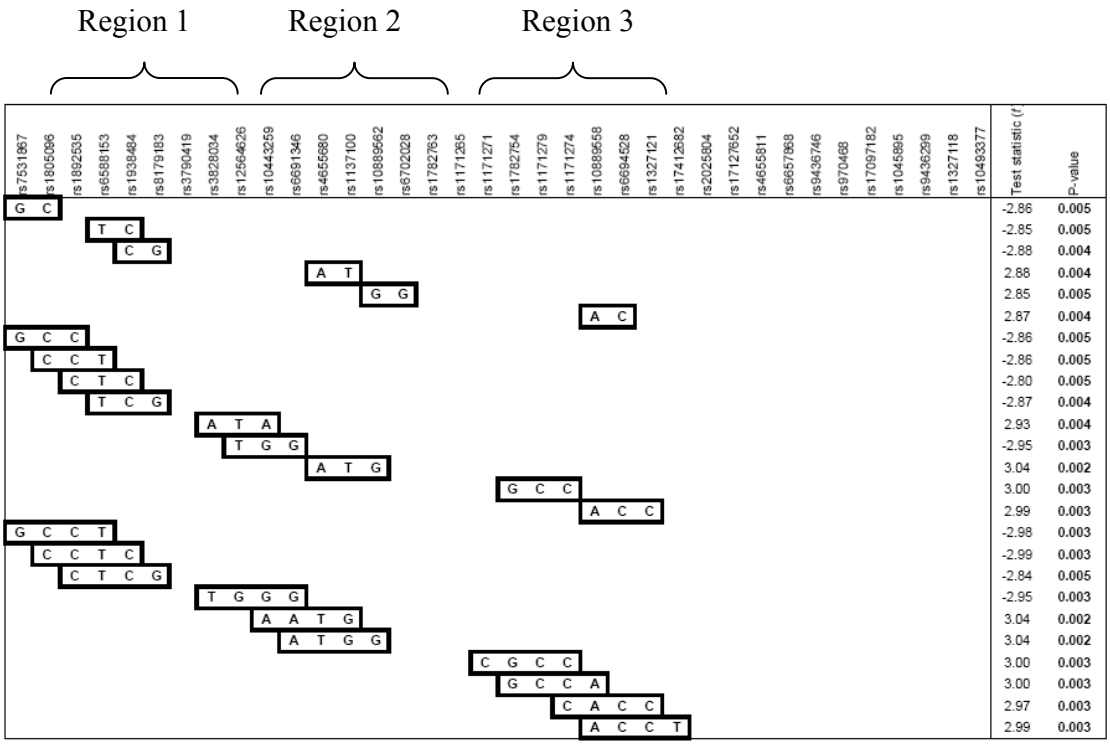

Figure 2: Haplotypes (n=25) that show greater association than single SNP results.

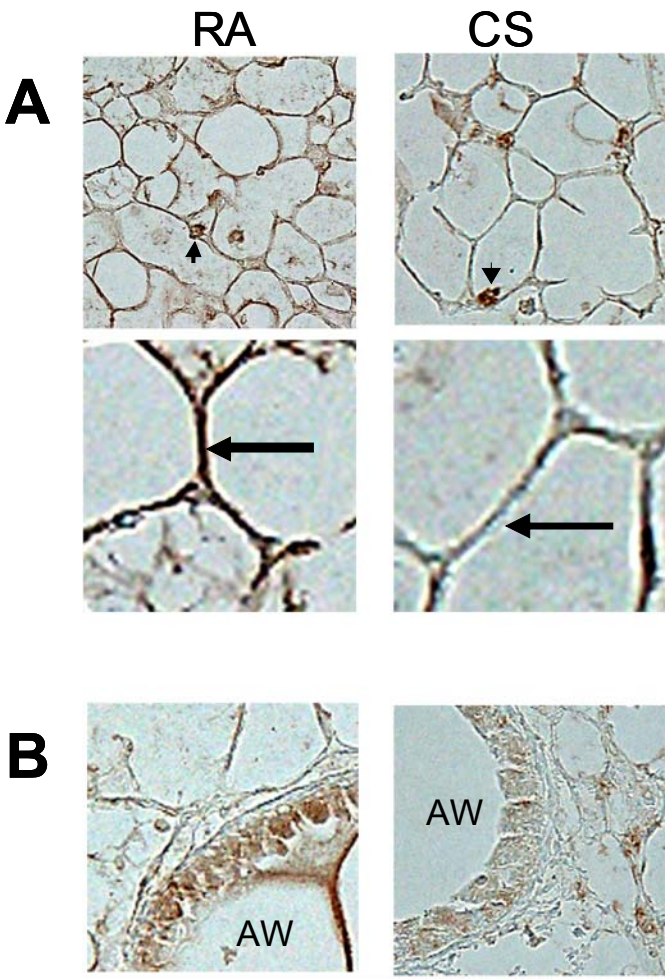

Figure 3: Reduced leptin receptor expression in lung parenchyma of AKR/J mice exposed to 4 months of cigarette smoke. Representative images of a minimum of three mice per condition. (A) Decreased immunohistochemical staining of leptin receptor (brown) in alveolar walls of smoke-exposed mice using polyclonal antibody against ObR and avidin-biotin-peroxidase complex method. Short arrows denote preserved leptin receptor staining of alveolar macrophages after cigarette smoke exposure. Long arrows show reduced airspace wall staining in smoke-exposed mice. (B) Reduced leptin receptor expression is also evident in airway epithelial cells of smoke-exposed mice. RA-room air exposed, CS-cigarette smoke exposed, AW-airway lumen.

Reference List

(1) Mannino DM, Homa DM, Akinbami LJ, Ford ES, Redd SC. Chronic obstructive pulmonary disease surveillance--United States, 1971-2000. Respir Care 2002 Oct;47(10):1184-99.

(2) Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. Lancet 1997 May 24;349(9064):1498-504.

(3) O'Donnell CP, Tankersley CG, Polotsky VP, Schwartz AR, Smith PL. Leptin, obesity, and respiratory function. Respir Physiol 2000 Feb;119(2-3):163-70.

(4) izawa-Abe M, Ogawa Y, Masuzaki H, Ebihara K, Satoh N, Iwai H, et al. Pathophysiological role of leptin in obesity-related hypertension. J Clin Invest 2000 May;105(9):1243-52.

(5) Barouch LA, Berkowitz DE, Harrison RW, O'Donnell CP, Hare JM. Disruption of leptin signaling contributes to cardiac hypertrophy independently of body weight in mice. Circulation 2003 Aug 12;108(6):754-9.

(6) Calikoglu M, Sahin G, Unlu A, Ozturk C, Tamer L, Ercan B, et al. Leptin and TNF-alpha levels in patients with chronic obstructive pulmonary disease and their relationship to nutritional parameters. Respiration 2004 Jan;71(1):45-50.

(7) Creutzberg EC, Wouters EF, Vanderhoven-Augustin IM, Dentener MA, Schols AM. Disturbances in leptin metabolism are related to energy imbalance during acute exacerbations of chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2000 Oct;162(4 Pt 1):1239-45.

(8) Schols AM, Creutzberg EC, Buurman WA, Campfield LA, Saris WH, Wouters EF. Plasma leptin is related to proinflammatory status and dietary intake in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1999 Oct;160(4):1220-6.

(9) Loffreda S, Yang SQ, Lin HZ, Karp CL, Brengman ML, Wang DJ, et al. Leptin regulates proinflammatory immune responses. FASEB J 1998 Jan;12(1):57-65.

(10) Madiehe AM, Mitchell TD, Harris RB. Hyperleptinemia and reduced TNF-alpha secretion cause resistance of db/db mice to endotoxin. Am J Physiol Regul Integr Comp Physiol 2003 Mar;284(3):R763-R770.

(11) Gan WQ, Man SF, Senthilselvan A, Sin DD. Association between chronic obstructive pulmonary disease and systemic inflammation: a systematic review and a meta-analysis. Thorax 2004 Jul;59(7):574-80.

(12) Willemse BW, ten Hacken NH, Rutgers B, Lesman-Leegte IG, Postma DS, Timens W. Effect of 1-year smoking cessation on airway inflammation in COPD and asymptomatic smokers. Eur Respir J 2005 Nov;26(5):835-45.

(13) Bruno A, Chanez P, Chiappara G, Siena L, Giammanco S, Gjomarkaj M, et al. Does leptin play a cytokine-like role within the airways of COPD patients? Eur Respir J 2005 Sep;26(3):398-405.

(14) Kanner RE, Anthonisen NR, Connett JE. Lower respiratory illnesses promote FEV(1) decline in current smokers but not ex-smokers with mild chronic obstructive pulmonary disease: results from the lung health study. Am J Respir Crit Care Med 2001 Aug 1;164(3):358-64.

(15) Wedzicha JA. Airway infection accelerates decline of lung function in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2001 Nov 15;164(10 Pt 1):1757-8.

(16) Lee GH, Proenca R, Montez JM, Carroll KM, Darvishzadeh JG, Lee JI, et al. Abnormal splicing of the leptin receptor in diabetic mice. Nature 1996 Feb 15;379(6566):632-5.

(17) Tsuchiya T, Shimizu H, Horie T, Mori M. Expression of leptin receptor in lung: leptin as a growth factor. Eur J Pharmacol 1999 Jan 22;365(2-3):273-9.

(18) Guerassimov A, Hoshino Y, Takubo Y, Turcotte A, Yamamoto M, Ghezzo H, et al. The development of emphysema in cigarette smoke-exposed mice is strain dependent. Am J Respir Crit Care Med 2004 Nov 1;170(9):974-80.

(19) Anthonisen NR, Connett JE, Kiley JP, Altose MD, Bailey WC, Buist AS, et al. Effects of smoking intervention and the use of an inhaled anticholinergic bronchodilator on the rate of decline of FEV1. The Lung Health Study. JAMA 1994 Nov 16;272(19):1497-505.

(20) Kanner RE, Connett JE, Williams DE, Buist AS. Effects of randomized assignment to a smoking cessation intervention and changes in smoking habits on respiratory symptoms in smokers with early chronic obstructive pulmonary disease: the Lung Health Study. Am J Med 1999 Apr;106(4):410-6.

(21) Enright PL, Connett JE, Kanner RE, Johnson LR, Lee WW. Spirometry in the Lung Health Study: II. Determinants of short-term intraindividual variability. Am J Respir Crit Care Med 1995 Feb;151(2 Pt 1):406-11.

(22) Tashkin DP, Altose MD, Connett JE, Kanner RE, Lee WW, Wise RA. Methacholine reactivity predicts changes in lung function over time in smokers with early chronic obstructive pulmonary disease. The Lung Health Study Research Group. Am J Respir Crit Care Med 1996 Jun;153(6 Pt 1):1802-11.

(23) Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007 Sep;81(3):559-75.

(24) Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005 Jan 15;21(2):263-5.

(25) Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, et al. The structure of haplotype blocks in the human genome. Science 296[5576], 2225-2229. 6-21-2002.
Ref Type: Journal (Full)

(26) Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001 Apr;68(4):978-89.

(27) Thompson DB, Ravussin E, Bennett PH, Bogardus C. Structure and sequence variation at the human leptin receptor gene in lean and obese Pima Indians. Hum Mol Genet 1997 May;6(5):675-9.

(28) Mannino DM, Buist AS. Global burden of COPD: risk factors, prevalence, and future trends. Lancet 2007 Sep 1;370(9589):765-73.

(29) Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med 2007 Sep 15;176(6):532-55.

(30) Zhang YY, Gottardo L, Mlynarski W, Frazier W, Nolan D, Duffy J, et al. Genetic variability at the leptin receptor (LEPR) locus is a determinant of plasma fibrinogen and C-reactive protein levels. Atherosclerosis 2007 Mar;191(1):121-7.

(31) Man SF, Connett JE, Anthonisen NR, Wise RA, Tashkin DP, Sin DD. C-reactive protein and mortality in mild to moderate chronic obstructive pulmonary disease. Thorax 2006 Oct;61(10):849-53.

(32) Takabatake N, Nakamura H, Minamihaba O, Inage M, Inoue S, Kagaya S, et al. A novel pathophysiologic phenomenon in cachexic patients with chronic obstructive pulmonary disease: the relationship between the circadian rhythm of circulating leptin and the very low-frequency component of heart rate variability. Am J Respir Crit Care Med 2001 May;163(6):1314-9.

(33) Broekhuizen R, Vernooy JH, Schols AM, Dentener MA, Wouters EF. Leptin as local inflammatory marker in COPD. Respir Med 2005 Jan;99(1):70-4.

(34) Churg A, Cosio M, Wright JL. Mechanisms of cigarette smoke-induced COPD: insights from animal models. Am J Physiol Lung Cell Mol Physiol 2008 Apr;294(4):L612-L631.

(35) Brusselle GG, Bracke KR, Maes T, D'hulst AI, Moerloose KB, Joos GF, et al. Murine models of COPD. Pulm Pharmacol Ther 2006;19(3):155-65.

(36) Shapiro SD, Demeo DL, Silverman EK. Smoke and mirrors: Mouse models as a reflection of human chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2004 Nov 1;170(9):929-31.

(37) Debouck C, Goodfellow PN. DNA microarrays in drug discovery and development. Nat Genet 1999 Jan;21(1 Suppl):48-50.