ERJ Express. Published on June 4, 2010 as doi: 10.1183/09031936.00173009

Title

Association of COPD candidate genes with CT emphysema and airway phenotypes in severe COPD

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Funding: NIH R01 HL075478, P01 HL083069, R01 HL084323, U01 HL089856, K23HL089353, GlaxoSmithKline, and the Parker B. Francis Foundation

Abstract

The principal determining factors influencing the development of the airway disease and emphysema components of COPD have not been clearly defined. Genetic variability in COPD patients might influence the varying degrees of involvement of airway disease and emphysema. Therefore, we investigated genetic association of SNPs in COPD candidate genes for association with emphysema severity and airway wall thickness phenotypes.

Polymorphisms in six candidate genes were analyzed in 379 subjects of the National Emphysema Treatment Trial (NETT) Genetics Ancillary Study with quantitative chest CT data. Genetic association with percent of lung below -950 hounsfield units (LAA950), airway wall thickness (WT), and derived square root wall area of 10 mm internal perimeter airways (SRWA) were investigated.

Three SNPs in *EPHX1*, five SNPs in *SERPINE2*, and one SNP in *GSTP1* were significantly associated with LAA950. Five SNPs in *TGFB1*, two SNPs in *EPHX1*, one SNP in *SERPINE2*, and two SNPs in *ADRB2* were associated with airway wall phenotypes in NETT.

In conclusion, several COPD candidate genes showed evidence for association with airway wall thickness and emphysema severity using CT in a severe COPD population. Further investigation will be required to replicate these genetic associations for emphysema and airway wall phenotypes.

Keywords; Airway, chronic obstructive pulmonary disease, computed tomography, emphysema, genetic association

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by persistent airflow obstruction [1]. Cigarette smoking is a well-known risk factor, but other environmental and genetic factors are likely involved. The relative amount of airway and airspace involvement is heterogeneous in COPD subjects, but the key determinants of these different phenotypes, which largely develop from exposure to cigarette smoke, are not known.

Computed tomography (CT) is a useful way to characterize structural changes including quantitative measurement of emphysema and airway wall phenotypes [2]. A recent paper suggested that genetic components influencing airway wall thickness and emphysema phenotypes were independent [3]. There are several reports of genetic associations with emphysema severity or emphysema distribution [4] [5] [6]. However, combined genetic investigation of both emphysema and airway wall phenotypes in the same population has not been widely reported.

We hypothesized that different genetic variants would influence airway disease and emphysema. We investigated whether single nucleotide polymorphisms (SNPs) in previously reported COPD susceptibility genes were associated with emphysema severity and airway wall phenotypes in a COPD study population. We selected six candidate genes that had been associated with COPD susceptibility in at least two populations.

The National Emphysema Treatment Trial (NETT) is a cohort of emphysema subjects with available chest CT data. We measured airway wall phenotypes using CT scans in a subset of subjects and investigated associations of variants in COPD susceptibility genes

with both emphysema severity and airway wall phenotypes in emphysema subjects from NETT.

Methods

Subjects

The current analysis included 379 non-Hispanic white subjects in the National Emphysema Treatment Trial (NETT) Genetics Ancillary Study with quantitative chest CT data. Subjects enrolled in NETT had severe airflow obstruction (FEV1 ≤ 45% predicted), hyperinflation and bilateral emphysema on high-resolution chest CT [7]. The study was approved by institutional review boards at participating centers. All subjects provided written informed consent.

Chest CT analysis

Emphysema measurements on NETT subjects have been described previously [6]. Computed tomographic images of the chest were acquired at full inspiration with a minimum of 200 mAs and reconstructed using a high spatial frequency algorithm with a 1 to 2 mm collimation at 20 mm intervals. Densitometric measures of emphysema were performed using the Pulmonary Analysis Software Suite (PASS, Iowa City, IA) at a threshold of –950 Hounsfield units. Airway wall thickness and the square root of wall area were assessed using 3D Slicer (www.Slicer.org) and Airway Inspector (www.airwayinspector.org) at Brigham and Women's Hospital. The full width at half-maximum (FWHM) method was used to measure the wall thickness and wall area of each airway. From these measures, the airway wall thickness (WT) and the square root of wall area (SRWA) of a 10mm luminal perimeter airway were calculated [8].

Genotyping

Genotyping in NETT was performed for 122 single nucleotide polymorphisms (SNPs) in six candidate genes (19 in *EPHX1,* 5 in *SFTPB,* 21 in *TGFB1,* 64 in *SERPINE2,* 7 in *GSTP1*, 6 in *ADRB2*) including upstream and downstream chromosomal regions. We used pairwise linkage disequilibrium (LD)-tagging in Tagger [9] with minimum minor allele frequency of 0.10 and r^2-threshold of 0.9 based on genotype data in Phase II of the HapMap Project, in order to select a panel of SNPs to capture the LD information in each gene. Additional SNPs were also genotyped, based on previously reported genetic association analyses of COPD-related phenotypes [10] [11] [12]. SNPs were genotyped using TaqMan (Applied Biosystems, Foster City, CA) or Sequenom (San Diego, CA) assays as previously reported [13] [14].

Statistical analysis

Phenotypes tested for association in NETT included percent of lung under –950 HU (LAA950), wall thickness (WT) and derived square root wall area of 10 mm internal perimeter airways (SRWA). Multivariate analysis was performed using linear regression models with SAS PROC GLM, assuming an additive mode of inheritance, and adjusting for age, gender, weight, pack-years of cigarette smoking, and clinical center. All statistical analyses were performed using SAS (SAS Institute, Cary, NC). Linkage disequilibrium between SNPs was assessed using the Haploview program (include reference).

Results

Demographic characteristics

In NETT, the mean age of 379 subjects who had quantitative CT emphysema and/or airway wall measurements was 67.5 years and mean FEV1 percent of predicted was 24.9% (Table 1). Emphysema data were available from 358 and airway data were available from 338 subjects. Mean number of airways analyzed per subject was 12 (range; 4-23).

Association analysis with CT phenotypes in NETT

As shown in Table 2, three SNPs in *EPHX1* (rs2854450, rs3738042, rs1009668), five SNPs in *SERPINE2* (rs6734100, rs729631, rs975278, rs6436449, rs7608941), and one SNP in *GSTP1* (rs11227844) were significantly associated with LAA950. Five SNPs in *TGFB1* (rs1800469, rs1800470, rs1800471, rs11083616, rs11466321), two SNPs in *EPHX1* (rs2854450, rs3753658), one SNP in *SERPINE2* (rs6436459), and two SNPs in *ADRB2* (rs1042717, rs1042718) were associated with airway wall phenotypes (Table 3). Statistical analysis showed similar results for airway wall thickness and SRWA. Among the significantly associated SNPs in Table 2, rs729631 and rs975278 in *SERPINE2* (pairwise r^2=97%) were in strong LD. Among the significantly associated SNPs in Table 3, rs1800470 and rs11083616 (pairwise r^2=95%), rs1800471 and rs11466321 (pairwise r^2=94%) in *TGFB1*, and rs1042717 and rs1042718 (pairwise r^2=83%) in *ADRB2* were in strong LD. The list of genotyped SNPs and linkage disequilibrium maps are provided in supplementary tables and figures, and genetic association results of all SNPs are shown in a supplementary table.

At the gene level, SNPs in *EPHX1* and *SERPINE2* were associated with both airway wall and emphysema phenotypes; however, only rs2854450 in *EPHX1* was associated with both airway and emphysema phenotypes (Figure 1). The minor allele of rs2854450 was associated with less severe emphysema and thinner airway walls.

Discussion

In this study, SNPs in six genes previously associated with COPD susceptibility were investigated for association with emphysema severity and airway wall thickness. In NETT subjects, three genes included SNPs that were associated with emphysema severity, and four genes included SNPs that were associated with airway wall phenotypes.

There have been several previous reports of genetic associations with emphysema or emphysema distribution. One previous study reported that emphysema severity determined by visual emphysema score was associated with a TNF-α promoter polymorphism in 84 subjects with COPD [4]. Another study revealed that low attenuation area percentage of the lung differed according to *MMP-9* genotype only in the upper lung zones [5]. In NETT subjects, variants in *EPHX1* and *GSTP1* showed significant association with emphysema distribution suggesting that xenobiotic enzymes may contribute to upper lobe dominant emphysema [6].

In the current study, 358 subjects in NETT were investigated for genetic associations with emphysema. SNPs in *EPHX1*, *GSTP1*, and *SERPINE2* were associated with emphysema severity. One of SNPs associated with emphysema in *EPHX1* (rs1009668) was previously reported by our group to be associated with bronchodilator responsiveness in NETT [15].

Airway wall measurements from chest CT scans are being increasingly used and validated as COPD phenotypes [16] [17]. Airway remodeling is associated with airway

narrowing and wall thickening in COPD subjects [18] and this can be evaluated non-invasively using CT [2]. In 338 NETT subjects, we assessed genetic associations with airway wall thickness and airway wall area. SNPs in *EPHX1*, *SERPINE2, TGFB1* and *ADRB2* were associated with airway wall phenotypes. *EPHX1* and *SERPINE2* were associated with both emphysema and airway wall phenotypes; *GSTP1* was associated only with emphysema and *TGFB1* and *ADRB2* were associated only with airway wall phenotypes.

EPHX1 is related to oxidative stress and has been repeatedly associated with COPD susceptibility [19] [10]; however, recently it has also been reported not to be associated with COPD susceptibility in a large number of COPD and control subjects [20]. With CT phenotypes, previously *EPHX1* was associated with upper lobe predominant emphysema distribution in NETT subjects [6]. In the current analysis, SNPs in *EPHX1* were associated with both emphysema severity and airway wall phenotypes in NETT subjects. At a SNP level, only one SNP in *EPHX1* (rs2854450) was associated with both emphysema and airway wall in NETT subjects. This SNP was associated with less emphysema and thinner airway walls, although there was negative overall correlation between emphysema severity and airway wall thickness in NETT subjects [8]. It is not clear whether that promoter SNP has a direct functional effect; it is more likely that it is in linkage disequilibrium with a functional variant or variants in *EPHX1*.

TGFβ1 was previously reported to be associated with COPD by our research group [11]. TGFβ1 has been suggested to be involved in airway remodeling in COPD [21] [22]. In 85

asthma subjects, serum TGFβ1 levels were associated with a promoter polymorphism in *TGFB1,* but there was no association between airway wall thickness and *TGFB1* genetic polymorphisms [23]. However, in 27 subjects with asthma, sputum TGFβ1 levels were associated with airway wall area corrected by body surface area [24]. In the present study, several SNPs in *TGFB1* were associated with airway wall thickness in the NETT cohort. Among SNPs with significance, rs1800469 and rs1800470 were previously reported to be associated with COPD [11] and respiratory symptom severity phenotypes [13] in our group.

One SNP (rs1042713) of *ADRB2* was recently reported to be associated with airway lumen area in Korean COPD subjects [25], however, *ADRB2* SNPs rs1042717 and rs1042718 were associated with airway wall thickness in the current study. The optimal airway phenotypes to reflect airway remodeling or COPD severity have not been determined.

It is interesting that some xenobiotic enzymes (*EPHX1*) and protease-related genes (*SERPINE2*) were associated with both emphysema and airway phenotypes, while possible airway remodeling related genes (*TGFB1* and *ADRB2*) were associated only with airway wall phenotypes. There may be different mechanisms of pathogenesis for emphysema and airway remodeling. Although we recognize that our association results will need to be replicated in larger populations and different ethnic groups, genetic studies may provide insight into these pathogenetic mechanisms. For example, TGFβ1 is known to have a role in immune response, cell growth, and tissue repair [11]. Although, in animal models, TGFB1 was important in the development of emphysema [26], in our study, this gene was associated with airway phenotypes in a severe COPD cohort

suggesting that TGFB1 may be associated with airway remodeling. ADRB2 gene was reported to be associated with COPD susceptibility and beta2 agonist responsiveness [8]. Although this gene may be related to bronchodilation, it may also have a role in airway remodeling.

The statistical significance of the genetic associations to CT phenotypes in this study was not stronger than previous reports from our group using functional impairment [13] and emphysema distribution [6] phenotypes. It is possible that the currently available emphysema and airway phenotypes do not optimally capture these disease processes. We analyzed SNPs in six candidate genes, and it is likely that many other genes also contribute to emphysema and airway disease pathogenesis.

There are several limitations in this study. Subjects were recruited from multiple clinical centers with different CT scanners that may affect the CT parameters. Although we adjusted for clinical center in our association analysis, residual bias could still exist. We analyzed SNPs in six genes, and multiple statistical comparisons are a potential concern. In this initial analysis of genetic determinants of CT-related phenotypes, we did not correct association p values for multiple statistical testing. We acknowledge that many of the SNPs which showed modest significance with p values less than a nominal threshold of 0.05 may be false positives. Though the optimal approach to adjust for multiple testing is not clear, multiple statistical testing may be less of a problem since most of these genes have already been associated with COPD susceptibility in multiple populations. In addition, replication of our results in other populations will be required.

The NETT population is a U.S.-based cohort of patients with advanced COPD and a clinical and subjective imaging diagnosis of emphysema. These subjects were screened

specifically for participation in a clinical trial of lung volume reduction surgery. They are a predominantly emphysematous subgroup of COPD cases. This may have limited the detection power of the genetic data for the airway phenotypes. However, we previously found that lung function was associated with airway wall thickness in NETT subjects [8], and we now report genetic associations with airway wall thickness in this same population. Thus, airway wall phenotypes likely contribute to airflow obstruction even in subjects with severe emphysema.

In conclusion, one SNP in *EPHX1* was associated with both emphysema and airway wall thickness using CT in a severe COPD population. Significant associations with emphysema and airway wall area individually were found for SNPs in several other candidate genes. Different genetic determinants may influence airway remodeling and emphysema development. Further work will be required to confirm the genetic determinants of emphysema and airway disease.

Acknowledgement

The authors would like to sincerely thank the manuscript reviewers for helpful suggestions.

References

1. Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, Fukuchi Y, Jenkins C, Rodriguez-Roisin R, van Weel C, Zielinski J. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am J Respir Crit Care Med* 2007: 176(6): 532-555.
2. de Jong PA, Muller NL, Pare PD, Coxson HO. Computed tomographic imaging of the airways: relationship to structure and function. *Eur Respir J* 2005: 26(1): 140-152.
3. Patel BD, Coxson HO, Pillai SG, Agusti AGN, Calverley PMA, Donner CF, Make BJ, Muller NL, Rennard SI, Vestbo J, Wouters EFM, Hiorns MP, Nakano Y, Camp PG, Nasute Fauerbach PV, Screaton NJ, Campbell EJ, Anderson WH, Pare PD, Levy RD, Lake SL, Silverman EK, Lomas DA, on behalf of the the International CGN. Airway Wall Thickening and Emphysema Show Independent Familial Aggregation in Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med* 2008: 178(5): 500-505.
4. Sakao S, Tatsumi K, Igari H, Watanabe R, Shino Y, Shirasawa H, Kuriyama T. Association of Tumor Necrosis Factor-{alpha} Gene Promoter Polymorphism With Low Attenuation Areas on High-Resolution CT in Patients With COPD. *Chest* 2002: 122(2): 416-420.
5. Ito I, Nagai S, Handa T, Muro S, Hirai T, Tsukino M, Mishima M. Matrix Metalloproteinase-9 Promoter Polymorphism Associated with Upper Lung Dominant Emphysema. *Am J Respir Crit Care Med* 2005: 172(11): 1378-1382.
6. DeMeo DL, Hersh CP, Hoffman EA, Litonjua AA, Lazarus R, Sparrow D, Benditt JO, Criner G, Make B, Martinez FJ, Scanlon PD, Sciurba FC, Utz JP, Reilly JJ, Silverman EK. Genetic determinants of emphysema distribution in the National Emphysema Treatment Trial. *Am J Respir Crit Care Med* 2007: 176(1): 42-48.
7. National Emphysema Treatment Trial Research G. A randomized trial comparing lung-volume-reduction surgery with medical therapy for severe emphysema. *N Engl J Med* 2003: 348(21): 2059-2073.
8. Kim WJ, Silverman EK, Hoffman E, Criner GJ, Mosenifar Z, Sciurba FC, Make BJ, Carey V, San Jose Estepar R, Diaz A, Reilly JJ, Martinez FJ, Washko GR. CT Metrics of Airway Disease and Emphysema in Severe COPD. *Chest* 2009: 136: 396-404.
9. de Bakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D. Efficiency and power in genetic association studies. *Nat Genet* 2005: 37(11): 1217-1223.
10. Hersh CP, DeMeo DL, Lange C, Litonjua AA, Reilly JJ, Kwiatkowski D, Laird N, Sylvia JS, Sparrow D, Speizer FE, Weiss ST, Silverman EK. Attempted replication of reported chronic obstructive pulmonary disease candidate gene associations. *Am J Respir Cell Mol Biol* 2005: 33(1): 71-78.
11. Celedon JC, Lange C, Raby BA, Litonjua AA, Palmer LJ, DeMeo DL, Reilly JJ, Kwiatkowski DJ, Chapman HA, Laird N, Sylvia JS, Hernandez M, Speizer FE, Weiss ST, Silverman EK. The transforming growth factor-β1 (TGFB1) gene is associated with chronic obstructive pulmonary disease (COPD). *Hum Mol Genet* 2004: 13(15): 1649-1656.
12. DeMeo D, Mariani T, Lange C, Srisuma S, Litonjua A, Celedon J, Lake S, Reilly J, Chapman H, Mecham B, Haley K, Sylvia J, Sparrow D, Spira A. The SERPINE2 gene is associated with chronic obstructive pulmonary disease. *Am J Hum Genet* 2006: 78(2): 253-264.

13. Hersh CP, DeMeo DL, Lazarus R, Celedon JC, Raby BA, Benditt JO, Criner G, Make B, Martinez FJ, Scanlon PD, Sciurba FC, Utz JP, Reilly JJ, Silverman EK. Genetic association analysis of functional impairment in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2006: 173(9): 977-984.
14. Hersh C, DeMeo D, Reilly J, Silverman E. Xenobiotic metabolizing enzyme gene polymorphisms predict response to lung volume reduction surgery. *Respir Res* 2007: 8(1): 59.
15. Kim WJ, Hersh CP, DeMeo DL, Reilly JJ, Silverman EK. Genetic association analysis of COPD candidate genes with bronchodilator responsiveness. *Respir Med* 2009: 103(4): 552-557.
16. Hasegawa M, Nasuhara Y, Onodera Y, Makita H, Nagai K, Fuke S, Ito Y, Betsuyaku T, Nishimura M. Airflow Limitation and Airway Dimensions in Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med* 2006: 173(12): 1309-1315.
17. Nakano Y, Muro S, Sakai H, Hirai T, Chin K, Tsukino M, Nishimura K, Itoh H, Pare PD, Hogg JC, Mishima M. Computed Tomographic Measurements of Airway Dimensions and Emphysema in Smokers . Correlation with Lung Function. *Am J Respir Crit Care Med* 2000: 162(3): 1102-1108.
18. James AL, Wenzel S. Clinical relevance of airway remodelling in airway diseases. *Eur Respir J* 2007: 30(1): 134-155.
19. Smith CA, Harrison DJ. Association between polymorphism in gene for microsomal epoxide hydrolase and susceptibility to emphysema. *Lancet* 1997: 350(9078): 630-633.
20. Chappell S, Daly L, Morgan K, Guetta-Baranes T, Roca J, Rabinovich R, Lotya J, Millar AB, Donnelly SC, Keatings V, MacNee W, Stolk J, Hiemstra PS, Miniati M, Monti S, O'Connor CM, Kalsheker N. Genetic variants of microsomal epoxide hydrolase and glutamate-cysteine ligase in COPD. *Eur Respir J* 2008: 32(4): 931-937.
21. Takizawa H, Tanaka M, Takami K, Ohtoshi T, Ito K, Satoh M, Okada Y, Yamasawa F, Nakahara K, Umeda A. Increased Expression of Transforming Growth Factor-{beta}1 in Small Airway Epithelium from Tobacco Smokers and Patients with Chronic Obstructive Pulmonary Disease (COPD). *Am J Respir Crit Care Med* 2001: 163(6): 1476-1483.
22. Churg A, Tai H, Coulthard T, Wang R, Wright JL. Cigarette Smoke Drives Small Airway Remodeling by Induction of Growth Factors in the Airway Wall. *Am J Respir Crit Care Med* 2006: 174(12): 1327-1334.
23. Ueda T, Niimi A, Matsumoto H, Takemura M, Yamaguchi M, Matsuoka H, Jinnai M, Chin K, Minakuchi M, Cheng L, Shirakawa T, Mishima M. TGFB1 promoter polymorphism C-509T and pathophysiology of asthma. *J Allergy Clin Immunol* 2008: 121(3): 659.
24. Yamaguchi M NA, Matsumoto H, Ueda T, Takemura M, Matsuoka H, Jinnai M, Otsuka K, Oguma T, Takeda T, Ito I, Chin K, Mishima M. Sputum levels of transforming growth factor-β1 in asthma:Relation to clinical and computed tomography findings. *J Investig Allergol Clin Immunol* 2008: 18(3): 202-206.
25. Kim WJ, Oh Y-M, Sung J, Lee YK, Seo JB, Kim N, Kim T-H, Huh JW, Lee J-H, Kim E-K, Lee JH, Lee S-M, Lee S, Lim SY, Shin TR, Yoon HI, Kwon S-Y, Lee SD. CT scanning-based phenotypes vary with ADRB2 polymorphisms in chronic obstructive pulmonary disease. *Respir Med* 2009: 103(1): 98-103.

26. Morris DG, uang X, Kaminski N, Wang Y, Shapiro SD, Dolganov G, Glick A, Sheppard D. Loss of integrin alpha(v)beta6-mediated TGF-beta activation causes Mmp 12-dependent emphysema. Nture 2003:22:169-173

Table 1. Demographic characteristics of analyzed subjects in the National Emphysema Treatment Trial (NETT) Genetics Ancillary Study. Data are presented as means (± S.D.), unless otherwise noted.

Characteristics	NETT
Number of Subjects	379
Age (years)	67.5 ± 5.8
Sex (male)	241 (64%)
Cigarette Smoking (pack-years)	66.4 ± 30.5
Pre-bronchodilator FEV1 %	24.9 ± 6.6
Post-bronchodilator FEV1 %	28.1 ± 7.4
LAA -950%	17 ± 11
Wall Thickness (mm)	1.53 ± 0.25
Square Root Wall Area (mm)	4.6 ± 0.5

In NETT, emphysema data were available in 358 subjects and airway wall data (Wall thickness and square root wall area) were available in 338 subjects.

LAA 950%; percent of lung area under –950 HU

Table 2. Genetic association results of quantitative emphysema measurements in subjects in the National Emphysema Treatment Trial Genetics Ancillary Study. Each model was analyzed assuming an additive mode of inheritance adjusting for age, gender, weight, pack-years of smoking, and clinical center. SNPs with p values ≤ 0.05 are shown.

Gene	SNP	Location	MAF	LAA% -950 HU	
				β	p value
EPHX1	rs2854450	promoter	0.21	-1.6	0.04
	rs3738042	promoter	0.32	1.3	0.05
	rs1009668	exon	0.11	3.3	0.002
SERPINE2	rs6734100	intron	0.14	-2.5	0.01
	rs729631	intron	0.19	-2.0	0.02
	rs975278	intron	0.19	-1.6	0.05
	rs6436449	intron	0.17	-2.2	0.02
	rs7608941	intron	0.22	-1.6	0.04
GSTP1	rs11227844	5' flank	0.09	3.2	0.003

MAF: Minor Allele Frequency

Table 3. Genetic association results of airway wall phenotypes in subjects in the National Emphysema Treatment Trial Genetics Ancillary Study. Each model was analyzed assuming an additive mode of inheritance and adjusting for age, gender, weight, pack-years of smoking, and clinical center. SNPs with p values ≤ 0.05 are shown.

Gene	SNP	Location	MAF	WT		SRWA	
				β	p value	β	p value
TGFB1	rs1800469	5' flank	0.30	-0.04	0.05	-0.03	0.36
	rs1800470	exon	0.38	-0.05	0.004	-0.1	0.008
	rs1800471	exon	0.09	-0.06	0.06	-0.2	0.005
	rs11083616	intron	0.38	-0.04	0.05	-0.1	0.04
	rs11466321	intron	0.07	-0.06	0.10	-0.2	0.02
EPHX1	rs2854450	promoter	0.21	-0.05	0.02	-0.1	0.006
	rs3753658	promoter	0.12	0.06	0.02	0.13	0.02
SERPINE2	rs6436459	intron	0.23	0.05	0.03	0.06	0.17
ADRB2	rs1042717	exon	0.20	-0.05	0.01	-0.08	0.06
	rs1042718	exon	0.17	-0.06	0.003	-0.11	0.01

MAF: Minor Allele Frequency
WT: Wall Thickness of a hypothetical 10 mm luminal perimeter airway
SRWA: Square Root of Wall Area of a hypothetical 10 mm luminal perimeter airway

Figure 1. Square Root of Wall Area (SRWA) of a hypothetical 10 mm internal perimeter airway and LAA950 by rs285540 SNP in EPHX1 in NETT subjects. Mean values (+ SEM) for SRWA and LAA950 are shown.