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A polymorphism in the thyroid hormone receptor gene is associated with bronchodilator response in asthmatics

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Abstract

A pro-asthmatic culture milieu and β_2-agonist (isoproterenol) were previously shown to regulate the expression of select transcription factors (TFs) within human airway epithelial and smooth muscle cells. This study tests 1116 single nucleotide polymorphisms (SNPs) across 98 of these TF genes for association with bronchodilator response (BDR) in asthma patients. Genotyping was conducted using the Illumina HumanHap550v3 Beadchip in 403 non-Hispanic White asthmatic children and their parents. SNPs were evaluated for association with BDR using family and population-based analyses. Forty-two SNPs providing p values < 0.1 in both analyses were then genotyped in three adult asthma trials. One SNP 5' of the thyroid hormone receptor beta gene was associated with BDR in the childhood population and two adult populations (p value = 0.0012). This investigation identified a novel locus for inter-individual variability in BDR and represents a translation of a cellular drug-response study to potential personalization of clinical asthma management.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

Bronchodilator response; transcription factor; association; thyroid hormone receptor β; asthma; pharmacogenetics

Introduction

Asthma is a chronic disorder characterized by inflammation, hyper-responsiveness of the bronchial muscles and narrowing of the airways that affects approximately 300 million individuals worldwide.[1] The increasing prevalence of asthma in recent decades has resulted in high rates of morbidity, mortality and annual health care costs estimated to be tens of billions within the United States alone.[2–4] Despite the availability of several classes of asthma therapies, large inter-individual variability in drug response has been described, which may be attributed in part to genetic factors.[5, 6] Pharmacogenetic studies of β_2-agonists, the most common asthma therapy, have identified several genes associated with bronchodilator response (BDR).[5] The loci described to date, however, explain only a fraction of the variability in drug response, suggesting that other factors modulate BDR.

We previously described the differential expression of transcription factors (TFs) in two types of human airway (epithelial and smooth muscle) cell lines that are regulated by a pro-asthmatic culture milieu and β_2-agonist.[6] Specifically, the expression of 307 TFs was quantified following incubation with pro-inflammatory cytokines (Interleukins 4 and 13, transforming growth factor-β), the mediator Leukotriene D4 (LTD4) and the β_2-agonist isoproterenol. Under these pro-asthmatic conditions, isoproterenol evoked changes (≥50% difference) in TF gene expression. Given that the role of these two airway cell types in asthma pathophysiology (i.e. inflammation, remodeling and bronchoconstriction), we hypothesized that genes regulated by *in vitro* exposure to isoproterenol and a pro-asthmatic culture milieu would be good candidates for modulating drug response to β_2-agonists in asthmatics. The aim of this study is to test the association of single nucleotide polymorphisms in these TF genes with BDR in asthma trial populations treated with a short-acting β_2-agonist.

Subjects and Methods

Study Populations

The Childhood Asthma Management Program (CAMP) was a clinical trial of 1,041 asthmatic children over an average period of 4.3 years.[7, 8] A total of 403 non-Hispanic White probands and their parents were successfully genotyped on the Illumina HumanHap550v3 BeadChip (Illumina Inc., SanDiego, CA). Each of the three replication trials consisted primarily of white adults with mild to severe asthma but no other significant comorbid medical conditions: Sepracor asthma trial (n=435);[9] Leukotriene Modifier or Corticosteroid or Corticosteroid Salmeterol (LOCCS) trial (n=159);[12] Effectiveness of Low Dose (LODO) Theophylline as Add-on Treatment in Asthma trial (n=155).[9] Sepracor participants were selected to have BDR≥15%. LOCCS patients were treated with a low dose inhaled corticosteroid during a 4 to 6-week run-in period prior to randomization, which

improved lung function in the range of 85 to 92 % predicted.[10] In all 4 trial populations, bronchodilator response (BDR) was measured as the percentage difference in forced expiratory volume in one second (FEV_1) after administration of two inhalations of albuterol (180 ug total) via a metered dose inhaler ($BDR=100*(postFEV_1 - preFEV_1)/preFEV_1$). All participants or their guardians provided written informed consent and all protocols were approved by the Institutional Review Board.

Gene Selection and Genotyping

We selected 98 candidate genes which code for isoforms of 59 TFs that were previously shown to be differentially expressed in lung cells (≥50% up- or down-regulation) in response to isoproterenol and pro-asthmatic conditions.[6] A total of 1116 single nucleotide polymorphisms (SNPs) across these candidate genes and 20 kb on either side were successfully genotyped in CAMP using the Illumina HumanHap550v3 BeadChip (Illumina Inc., SanDiego, CA). Data cleaning and quality control of this genotyping data has been previously reported.[14] Follow-up genotyping in the three replication populations used a Sequenom MassARRAY MALDI-TOF mass spectrometer (Sequenom, San Diego, CA). Each SNP had a greater than 95% completion rate and a Hardy-Weinberg equilibrium p-value of > 0.01.

Statistical Analyses

The primary outcome measure of the association analyses was acute bronchodilator response to the inhaled ß$_2$-agonist albuterol, dichotomized by the median value in each population (as shown in Table 1) due to variability in BDR distribution across the four trial populations. CAMP was used for discovery analysis using the available genome-wide SNP data and the top SNPs were then genotyped in the three adult asthma trial populations for replication analysis of BDR. Given that the sample size of the CAMP trial limits the power to detect a genetic association, we performed both a population-based association test as well as a family-based association test and selected the top loci identified by both analyses to carry forward for replication in additional asthma populations. Specifically, 42 SNPs which provided p values < 0.1 in both the family-based analysis (PBAT) of the trios[11] and population-based analysis of the probands using PLINK v1.5 (http://pngu.mgh.harvard.edu/purcell/plink/)[12] were carried forward for genotyping and replication analysis. Our rationale for using both tests was to confirm that the loci identified in the population-based test were not the result of population stratification.

The Haplo.stats package in R was used to estimate the haplotype structure, which applies the expectation-maximization algorithm.[13] Haplotype estimates with posterior probabilities of ≥96% were used to calculate its prevalence in each population. The haplotype effect was specified as an additive model and adjusted for nongenetic covariates including sex, height, pre- bonchodilator FEV_1, and age. Haplotype associations were considered significant only if a global haplogroup test and a subsequent specific haplogroup test each provided p-values < 0.05.

Replication analysis in the three adult asthma trial populations consisted of population-based tests using PLINK. In all analyses, the additive model was used and adjusted for non-

genetic covariates including sex, age, height and prebronchodilator FEV_1. Combined p values were calculated from the one-sided p values of the replication populations using Fisher's method.[14] The allelic and summary odds ratios (ORs) of the mutant allele was estimated using the DerSimonian-Laird random effects meta-analysis approach as implemented in the rmeta package in R.[15] The variation in drug response phenotype attributed by the rs892940 genotype by estimated using a logistic regression model (lrm) in the Design package within R. Linkage Disequilibrium (LD) among SNPs was determined by correlation coefficient values (r2) as calculated using PLINK.

Results

Baseline characteristics of the four asthma trial populations are detailed in Table 1. CAMP consisted of children ranging from ages 5–13 whereas the three replication populations were composed primarily of adult asthma cases. Other distinctions among the four clinical trials include the gender composition with LOCCS and LODO recruiting fewer males than CAMP and Sepracor, as well as differences in the mean and distribution of BDR across the four trials. For example, Sepracor participants were selected to have BDR ≥15%, reflected in a higher mean BDR of 40.3% (SD = 21.6). In addition, LOCCS participants were previously treated with an inhaled corticosteroid, which may have improved their lung function and explain in part their lower mean and more normally distributed BDR (i.e. skewness = 0.038 and kurtosis = 0.444). Given the phenotypic variability across our asthma trial populations, we did not apply conventional clinical thresholds of BDR for classifying patients as "responders" (12% or greater BDR).[16, 17] Instead, the median BDR value of each trial was used to distinguish responders within that trial.

A total of 1116 SNPs across the 98 candidate genes were tested for association with BDR in the CAMP trial using both family-based and population-based methods. SNPs providing p-values < 0.1 using both analytical methods were considered to be the most robustly associated polymorphisms. Table 2 lists 42 such markers that were not correlated (linkage disequilibrium) with each other, indicated by correlation coefficients ($r^2 < 0.8$). These were subsequently genotyped in the three adult asthma trials (listed in Table 2). SNP association analyses in these follow-up populations identified 5 SNPs which provided p values < 0.1 in one or more of the replication populations (Table 3). SNP rs892940 in the thyroid hormone receptor B (*THRB*) locus is associated with BDR in CAMP, and replicated in LODO and Sepracor with a Fisher's combined p value of 0.0012, which meets Bonferroni's significance threshold (≤0.0012). This is a common SNP (minor allele frequency of 41.7% in CAMP) is located 2.5 kb 5' of the *THRB* gene. Figure 1 shows that individuals with the minor allele are 33% more likely to respond to BDR compared to those with the major allele, with a summary odds ratio (OR) of 1.33 (95 % CI 1.11–1.58) (Table 4). However, the percentage of phenotypic variation attributed to the rs892940 is small with estimates of 0.75%, 0.30%, 1.04%, and 0.245% in CAMP, LOCCS, LODO and Sepracor, respectively. Using SNP genotype data from the hapmap CEU population (www.hapmap.org), this SNP was determined to be in linkage disequilibrium (LD, $r^2 > 0.8$) with another SNP (rs4858119), located 2.8 kb 5' of *THRB*. In addition, genotype data from the 1000 Genomes Project confirms the LD between these 2 SNPs and identifies 14 other SNPs in the LD block,

located within 50kb of the *THRB* gene. However, none of these SNPs are coding. It remains to be determined if any of these SNPs regulate the expression of *THRB*.

As multiple markers providing modest associations were identified across genes (Table 2), haplotype analysis was conducted to determine if the haplotypic effects were stronger than single marker associations. Although significant haplotypic effects (p values < 0.05) were found for several candidate genes (*RUNX1*, *TCF12*, *PARP1*, and *AP3*) in CAMP, none of these haplotype associations replicated in the additional asthma populations.

Two SNPs in the vitamin D receptor (*VDR*), with the lowest p values in CAMP, were nominally associated with BDR in LODO but did not meet significance criteria when the p values were combined using Fisher's method. Similarly, rs3858444 in the Wilms tumor 1 (*WT1*) isoform B was moderately associated with BDR in CAMP and replicated in LODO only, yielding a high combined p value. Finally, rs2249650 in the runt-related TF 1 (*RUNX1*) gene replicated in Sepracor but was non-significant when the p values were combined across the replication trials.

Discussion

In this manuscript, we identified a non-coding SNP (rs892940) located 5' of the *THRB* gene that is associated with response to β_2-agonists in the childhood asthma trial (FBAT p value = 0.001, population-based p value = 0.09) and replicated this association in two adult asthma populations (combined p value of 0.0012 in three replication populations and 0.0007 in all populations). Previous work by our group demonstrated that the expression of this gene is altered by exposure to a β_2-agonist in human airway epithelial and smooth muscle cells, co-treated with pro-inflammatory cytokines and LTD4, which are known to be elevated in asthmatic patients.[6] Taken together, this thyroid hormone receptor gene is a novel candidate for regulation of variable response to a common asthma therapy. Further studies are necessary to determine if the associated SNP or any variant in LD with it may regulate the expression or activity of the *THRB* gene in response to bronchodilators. Genetic variants associated with BDR may facilitate genetic tests for predicting individual asthma therapy outcomes.

The *THRB* gene is located on chromosome 3p24.2, encoding for the β subunit of the thyroid hormone receptor, which is one of 2 genes (α and β) that code for several isoforms.[18] The thyroid hormone receptor is located in the nucleus and upon binding to the thyroid hormone, regulates (both repress and activate) transcription through binding to T3 response elements either as a homodimer or heterodimer with retinoid X receptor beta (*RXRB*). The thyroid hormone, mediated through activation of its receptor, has been implicated in the growth and development of the lung as well as other organs in pre- and post-natal stages.[19, 20] In a study of rats treated with this hormone, one group showed increased relaxation of the renal artery smooth muscle along with elevated cyclic AMP, nitric oxide synthase (NOS) and NO, which is a potent vasodilator.[21] Thus, genetic variants in *THRB* may affect the expression of this receptor and have wide-spread downstream effects on transcription regulation that may contribute to inflammation, constriction of the bronchial smooth muscle and obstruction of the airways. However, given the multiple protein isoforms, an earlier knockout mouse study

demonstrated biological redundancy of the receptor activity.[2222] In addition, the biological effect of a potentially regulatory mutation, which may alter the level of the wild-type protein in specific cells depending on the available transcription machinery, likely differs from a non-synonymous variant that alters the protein function in all cells expressing the gene. Thus, variable expression of the thyroid hormone receptor beta isoform may be cell specific and may not have the detrimental effects of a coding variant or another gene without functional redundancy. The mechanism by which *THRB* modulates BDR is unknown and further investigations are necessary to determine its role in β_2-agonist response.

A limitation of our study was the sample sizes of the asthma trials, especially for LOCCS (n = 159) and LODO (n=155), which may have reduced the power to detect genetic associations. To compensate for the reduced power, we selected only those SNPs associated with BDR in both family-based and population-based analyses in CAMP to carry forward for replication. In addition, there were ascertainment biases of the replication populations, which may have contributed to heterogeneity across the cohorts. Specifically, participants in the LOCCS trial were previously treated with glucocorticoids and consequently, had well-controlled asthma compared to the other trials. Glucocorticoid treatment has been shown to alter arginine metabolism by inhibiting the induction of NOS by cytokines, thereby reducing NO production, resulting in improved lung function.[23] This may explain, in part, for the lower mean BDR and more normalized BDR distribution observed in the LOCCS trial compared to the other populations. Also, approximately 60% of LODO participants were taking a controller medication such as a long-acting β_2-agonist that could modify BDR. Finally, the Sepracor trial recruited only high responders to albuterol (BDR ≥15%). As a result of the heterogeneity in BDR distributions across these studies, we dichotomized the phenotype using the median value of each study to distinguish responders from non-responders, which differ from the conventional thresholds for classifying responders from non-responders.[16, 17] The reproducibility of our association results across the three replication trials, given the population heterogeneity, makes our study more robust. Moreover, whereas the initial association analyses were conducted in a childhood asthma population, the replication trials were composed primarily of adults, but each included some childhood cases.

The fact that multiple SNPs across a number of genes were only modestly associated with BDR and no stronger haplotype effect within these genes were found suggests that the genetic associations identified in this manuscript are likely due to linkage disequilibrium (LD) with the causative variant(s). Further studies are necessary to determine the functional role, if any, of the associated SNP in *THRB* on the expression of this gene or if it is in LD with other potentially functional variants.

The identification of TFs which modulate BDR provides a better understanding of the inter-individual variability in response to β_2-agonists, the most common class of asthma medications, as well as novel therapeutic targets for better symptom control. For example, antagonists, inhibitors or small interfering RNAs may be used to alter the expression of a specific TF gene. However, to date, few general TFs have been associated with asthma and asthma pharmacogenetics (that is, vitamin D receptor) since over-expression or suppression of such proteins are expected to result in wide-spread adverse effects. Therapeutic

interventions to regulate the expression of TFs (e.g. antisense oligonucleotides, TF decoys) would have to be cell-specific such as via aerosol or intra-tracheal administration, which specifically targeting TF expression in human lung cells such as airway epithelial and ASMCs only, without affecting gene expression in other cell types or organs. Further studies are necessary to improve the administration of such therapies in humans to minimize adverse effects and optimize therapeutic benefits.

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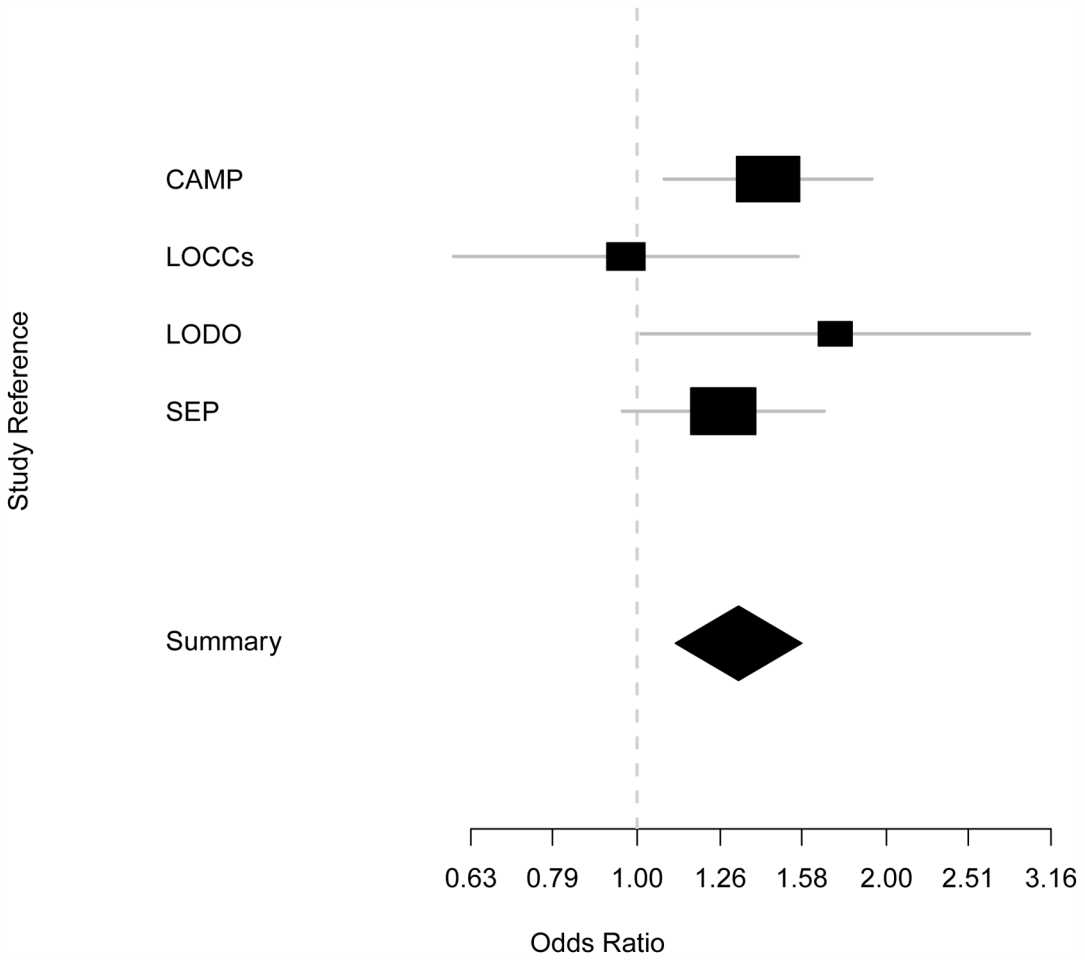

Figure 1.

Odds Ratios (OR) indicate greater likelihood of a high β_2-agonist bronchodilator response for the mutant allele of rs892940 in CAMP, LODO and Sepracor (SEP) trials. Boxes represent the point estimate for each study, the width of which is proportional to the standard error. The summary OR is represented as a diamond, the width of which is proportional to the standard error. Horizontal lines represent 95% confidence intervals.

Table 1

Baseline characteristics of four asthma trial populations.

	CAMP (n=403)	LOCCS (n=159)	LODO (n=155)	Sepracor (n=435)
Age, mean yr (SD)	8.7 (2.1)	34.9 (15.2)	43.0 (14.7)	32.4 (13.6)
Range	5.2 to 13.2	7 to 71	15 to 76	12 to 80
Male, n (%)	266 (63.0)	54 (34.0)	39 (25.2)	214 (49.2)
Pre-bronchodilator FEV_1 % predicted*, mean (SD)	93.4 (14.0)	84.3 (12.3)	78.8 (17.7)	61.5 (6.8)
BDR				
Mean % (SD)	10.8 (10.4)	6.3 (6.1)	9.7 (11.1)	40.3 (20.6)
Median	8	5.9	7.5	35.6
Skewness	1.5	0.04	1.3	1.5
Kurtosis	3.9	0.4	5.9	3.1

Abbreviations: SD = standard deviation; FEV_1 = forced expiratory volume in 1 second.

*Pre-bronchodilator FEV_1 % predicted = (pre-bronchodilator FEV_1/predicted FEV_1) × 100%.

Table 2

Differentially expressed TF genes associated with BDR in CAMP.

				CAMP	
TF*	**Iso Effect on Asthma***	**Gene**	**SNP rs#**	**POP p-value**	**FBAT p-value**
AP3	D	AP3B1	rs13163558	0.017	0.027
		AP3B1	rs355412	0.077	0.042
		AP3B1	rs9790855	0.006	0.010
		AP3S1	rs156666	0.012	0.092
		AP3S1	rs3797568	0.010	0.064
		AP3S1	rs6882704	0.008	0.005
CCAAT-BF	I	NFIX	rs931067	0.070	0.019
Evi-1	I	EVI1	rs3851378	0.055	0.057
HMG-1	I	HMGB1	rs4353357	0.081	0.044
KKLF-15	I	KLF15	rs9838915	0.074	0.017
NRF-1	I	NRF1	rs10276606	0.094	0.031
PARP	I	PARP1	rs12567614	0.008	0.069
		PARP1	rs6426551	0.076	0.086
		PARP1	rs874583	0.003	0.011
PAX8	D	PAX8	rs12620738	0.040	0.088
		PAX8	rs2863244	0.093	0.046
PEBP2alpha	I	RUNX1	rs2249650	0.017	0.031
		RUNX1	rs2253319	0.025	0.046
		RUNX1	rs2834645	0.001	0.072
PTF1-beta	I	TCF12	rs4494480	0.063	0.072
		TCF12	rs8037469	0.063	0.072
RAR	D	RARB	rs12635379	0.025	0.065
		RARB	rs1286654	0.027	0.005
		RARB	rs1299407	0.099	0.042
		RARB	rs1406575	0.088	0.017
		RARB	rs1529672	0.049	0.017
		RARB	rs2056777	0.022	0.041

				CAMP	
TF*	**Iso Effect on Asthma***	**Gene**	**SNP rs#**	**POP p-value**	**FBAT p-value**
		RARB	rs922939	0.071	0.076
		RARG	rs1554753	0.080	0.057
SREBP-1c	D	SREBF1	rs9902941	0.079	0.082
T3R	D	THRA	rs868150	0.010	0.017
		THRB	rs892940	0.086	0.041
VDR	D	VDR	rs1540339	0.024	0.008
		VDR	rs1544410	0.063	0.013
		VDR	rs2107301	0.056	0.092
		VDR	rs2189480	0.032	0.027
		VDR	rs2239179	0.034	0.005
		VDR	rs2239182	0.017	0.007
		VDR	rs2239186	0.027	0.015
		VDR	rs3819545	0.009	0.004
		VDR	rs757343	0.019	0.049
WT1	I	WT1	rs3858444	0.064	0.093

* Data from Panebra et al. 2007 [6]: D=decrease, I=increase, Iso=Isoproterenol.

42 SNPs providing p values < 0.1 in both the population-based (POP, population-based association test; derived using PLINK) and family-based analyses (FBAT p) in CAMP were selected for follow-up genotyping in three adult asthma trials.

Table 3

SNPs associated with BDR in the four asthma trials.

		CAMP		LOCCS	LODO	Sepracor	Combined REP	Combined ALL
SNP	**Gene**	**POP**	**FBAT**					
rs892940	THRB	0.086	0.041	0.431	0.004	0.011	0.0012	0.0007
rs3819545	VDR	0.009	0.004	0.938	0.043	0.857	0.347	0.040
rs2189480	VDR	0.032	0.026	0.949	0.048	0.937	0.390	0.106
rs3858444	WT1	0.064	0.093	0.811	0.048	0.459	0.235	0.094
rs2249650	RUNX1	0.017	0.031	0.798	0.426	0.028	0.157	0.026

P-values shown are two-sided for CAMP and one-sided for LOCCS, LODO and Sepracor, based on the direction of association in CAMP. The Fisher's method was used to calculate the combined p-values for LOCCS, LODO and Sepracor (REP) as well as for all four populations (ALL).

Table 4

Odds Ratios, OR (95% CI), of the minor allele relative to major allele of rs892940 for each asthma trial population, summary OR and test for heterogeneity.

	OR	lower 95% CI	upper 95% CI
CAMP	1.44	1.08	1.92
LOCCs	0.97	0.61	1.55
LODO	1.68	0.97	2.9
SEP	1.32	1	1.74
Summary	1.34	1.12	1.59
Heterogeneity (p-value)	0.4395		