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Association Between the MUC5B Promoter Polymorphism rs35705950 and Idiopathic Pulmonary Fibrosis

A Meta-analysis and Trial Sequential Analysis in Caucasian and Asian Populations

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Abstract: Idiopathic pulmonary fibrosis (IPF) is a progressive disease with a poor prognosis. A number of studies reported the association between MUC5B promoter polymorphism rs35705950 and IPF, but substantial inconsistent findings were observed and the strength of association remains unclear.

The aim of the study was to investigate the association between rs35705950 and IPF in different ethnic populations.

PubMed, EMBASE, Web of Science, and CENTRAL were searched from their inception to April 15, 2015. Allelic and phenotypic comparisons were conducted separately, as were comparisons in Caucasian and Asian populations. A meta-analysis with trial sequential analysis was conducted.

Nine studies presented in 7 full-text articles were included, encompassing 2733 IPF patients and 5044 controls. Six studies were carried out in the Caucasian population, and 3 in the Asian population. Minor *T* allele was associated with an increased risk of IPF compared with G allele (odds ratio [OR] 4.85, 95% confidence interval [CI] 3.79–6.21, $P = 5.88 \times 10^{-36}$), as were TG and TT genotypes compared with GG genotype (TG vs GG: OR 6.20, 95% CI 5.14–7.48, $P = 1.70 \times 10^{-81}$; TT vs GG: OR 11.29, 95% CI 5.69–22.40, $P = 4.22 \times 10^{-12}$), in an allele dose-dependent manner. These observations were confirmed in trial sequential analysis in both populations. The strength of association was more remarkable in the Caucasian population than in the Asian population, and no homozygous *TT* genotype was detected in the Asian population in our study.

Our study revealed strong association between the *MUC5B* promoter rs35705950 polymorphism and the risk of IPF. The strength of association between rs35705950 minor *T* allele and IPF susceptibility was particularly evident in the Caucasian population, and milder but still significant in the Asian population.

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Abbreviations: CI = confidence interval, DLCO = diffusion capacity of lung for carbon monoxide, FVC = forced vital capacity, HWE = Hardy–Weinberg equilibrium, IIP = idiopathic interstitial pneumonia, IPF = idiopathic pulmonary fibrosis, OR = odds ratios, TSA = trial sequential analysis.

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) remains the most common and severest form of idiopathic interstitial pneumonia (IIP) with a complex and yet poorly understood pathophysiology.[1] Although occurring primarily in older adults, IPF is a progressive disease with an extremely poor prognosis: the mortality rate of IPF is similar to that of end-stage lung cancer,[2] partially because that IPF minimally responses to pharmacological interventions.[1,3]

Genetic studies have added new knowledge of IPF target genes and might provide target for novel treatment.[4] Recently, a variant in the promoter region of a mucin gene (*MUC5B*) showed strong linkage with IIP in 82 families, and was associated with increased risks of both familial and sporadic IPF[5]: heterozygous carriers of the minor *T* allele of this polymorphism (rs35705950) increased IPF risk by 9-fold and homozygous carriers by 22-fold.[5] Thereafter, a host of similar designed case-control studies were carried out, with the majority performed in the Caucasian population[4–9] and several in the Asian population.[8–10] The rs35705950 polymorphism was more common in Caucasian population, and studies in this population generated largely consistent finding, but the extent of association varied. While in the Asian population, the variation was rare and substantial inconsistent findings were observed across studies.

It is notable that the sample sizes of IPF patients were small to modest in most of the studies (particularly studies in the Asian population), which limited the power of detection in each individual study. Meta-analysis could increase the statistical power and decrease random errors, and thus was recommended by the Human Genome Epidemiology Network to confirm the

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strength of a genetic association.[11–12] Therefore, in this study, we performed a meta-analysis to evaluate the association between *MUC5B* rs35705950 and risk of IPF. Provided the vast difference of genetic profiles in different ethnic populations, we also performed stratified analyses to determine whether a significant association exists in the Asian population and to determine the strength of association in the Caucasian population. Meanwhile, trial sequential analysis (TSA) was conducted to confirm the robustness of findings from meta-analysis.

MATERIALS AND METHODS

Literature Search

We sought to identify published studies evaluating the association between *MUC5B* promoter polymorphism rs35705950 and IPF. We searched PubMed, EMBASE, Web of Science, and the Cochrane Central Register of Controlled Trials (CENTRAL) from their inception to April 15, 2015, using the following search terms and key words: MUC5B; rs35705950; idiopathic pulmonary fibrosis; IPF; and polymorphism (Search strategy in Supplemental content, http://links.lww.com/MD/A490). We also manually checked references of the identified reports and relevant reviews. No language and ethnicity restrictions were imposed. Ethical approval and informed consent were not necessary because our analyses were based on data from previously published studies.

Study Selection

Two investigators (Q-QZ and X-LZ) independently assessed the eligibility of studies. For inclusion, studies had to meet the following criteria: included IPF patients and healthy controls; genotyped *MUC5B* promoter polymorphism rs35705950 in both groups; provided the genotypic or allelic distribution of rs35705950, that is, reported the number of patients with each genotype (allele) or directly provided odds ratios (ORs) with corresponding confidence intervals (CIs); and were published as either full-text articles or abstracts. We excluded studies without a control group; studies performed in patients with mixed interstitial lung abnormalities in which data on IPF patients could not be independently obtained; and studies conducted on the same cohort, but with smaller sample size.

Outcomes

The prespecified primary endpoint was to investigate whether MUC5B rs35705950 could increase the risk of IPF in the overall population. The secondary endpoint was to determine whether there was a difference on the strengths of association between MUC5B rs35705950 and IPF among different ethnic populations.

Data Collection and Quality Assessment

Two reviewers (Q-QZ and X-LZ) independently extracted the following information: first author, year of publication, the performing country of the study, the ethnicity of IPF patients and controls, number of IPF patients and controls, clinical characteristics of IPF patients (including age, sex, smoking status, forced vital capacity [FVC], and diffusion capacity of lung for carbon monoxide [D_LCO]), genotyping methods, and whether Hardy–Weinberg equilibrium (HWE) was achieved in the control group. For each study, we recorded the number of patients harboring each genotype and the number of each allele, or ORs with their corresponding 95% CIs when raw genotypic or allelic distribution could not be obtained. The quality of included studies was evaluated by 2 reviewers (Q-QZ and H-YM) with the Newcastle–Ottawa Scale criteria.

Statistical Analysis

To assess the association between *MUC5B* rs35705950 and the risk of IPF, we analyzed the genotypic (additive model: *GT* vs *GG*, and *TT* vs *GG*) and allelic (allele *T* vs allele *G*) frequencies between IPF patients and controls separately. We chose the additive model because previous studies revealed that the observed genotypic frequencies of rs35705950 were consistent with an additive genotypic effect on the risk of IPF.[5] ORs and their corresponding 95% CIs were pooled across studies using the DerSimonian–Laird random-effects models, which took account into possible heterogeneity.[13] Studies in which 1 genotype was not detected in any of the groups (case and control) were excluded in the analysis of that comparison. For studies in which 1 genotype was not detected in only one of the groups, the estimate effect and its 95% CIs were calculated after adding 0.5 to each cell of the 2×2 table for that study.[14,15] The I^2 statistic and chi-square–based Q test were used to assess the heterogeneity across studies.[16,17] In case I^2 was higher than 50% or P value of Q statistics test was below 0.10, significant heterogeneity was indicated. Publication bias was assessed by visual inspection of the funnel plot and by performing Egger test.[18] Sensitivity analyses were carried out to evaluate the consistency of the results by omitting one study at a time.[18] All meta-analyses were conducted with the STATA version 11.0 (STATA Corporation, College Station, TX) software.

Repetitive significance test of sparse and accumulated data may result in type I errors.[19] TSA could reduce type I error because it combines estimation of required information size (RIS) with adjusted threshold for statistical significance.[20] TSA was performed by anticipating a 20% relative risk reduction, an overall 5% risk of a type I error, and a statistical test power of 80%. Accordingly, the required diversity-adjusted information size was estimated.[21]

The meta-analysis was in accordance with the Meta-analysis of Observational Studies in Epidemiology (MOOSE) checklist.[22]

RESULTS

Study Selection and Characteristics

The flow diagram of the meta-analysis was shown in Figure 1. Our systematic literature search generated 138 studies. After excluding 59 duplicate publications and 50 review articles or editorials, 29 records remained. In these 29 records, 16 clearly did not meet the inclusion criteria based on titles and abstracts, leaving 13 studies for full-text review.[4–10,23–28]Six studies were further excluded, in which 2 studies evaluated the effect of *MUC5B* rs35705950 on symptom severity or survival without control groups,[23,24] 2 studies were conducted in patients with mixed interstitial lung abnormalities in which data on IPF patients could not be independently obtained,[25,26] and 2 studies[27,28] were conducted on the same but smaller cohorts as with other study with larger population.[4] (Fig. 1) Thus, 9 studies presented in 7 full-text articles were included, encompassing 2733 IPF patients and 5044 controls.[4–10] Six studies were carried out in the Caucasian population,[4–9] and 3 were in the

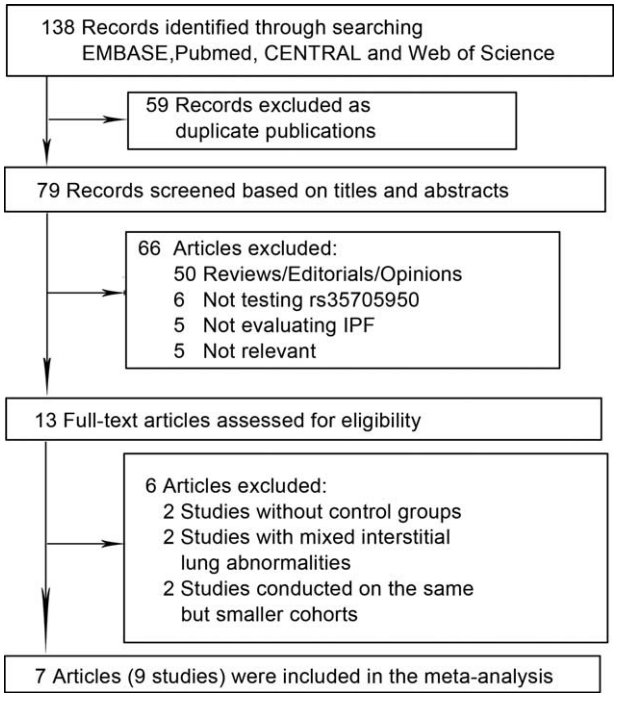

FIGURE 1. Flow diagram of the study selection in the meta-analysis.

Asian population[8–10]: 2 articles reported associations in both the Caucasian and the Asian populations.[8,9]

Baseline characteristics of individual study were shown in Table 1. All studies were published between 2011 and 2015. In IPF patients, the mean age ranged from 61.8 to 69.8 years and the percentage of women from 18% to 72%, and 49% to 73% of IPF patients were current smokers or ever-smokers. A total of 5919 participants of Caucasian ethnicity (2285 IPF patients and 3634 controls) and 1858 individuals of Asian origin (448 IPF patients and 1410 controls) were included in our analyses. All studies had a moderate-to-good quality according to the Newcastle–Ottawa Scale, as detailed in the Supplemental content Table S1 (http://links.lww.com/MD/A490).

MUC5B Minor T Allele Increased Risk of IPF Compared With G Allele

Nine studies involving 2733 IPF patients and 5044 controls were included in the overall analysis. The *MUC5B* rs35705950 minor *T* allele was associated with an increased risk of IPF compared with *G* allele (OR 4.85, 95% CI 3.79–6.21, $P = 5.88 \times 10^{-36}$; Fig. 2). TSA showed that the pooled sample size of alleles exceeded the estimated RIS, and the cumulative Z-curve crossed both the conventional boundary and the trial sequential monitoring boundary (Fig. 3), confirming the results of the meta-analysis. There was significant heterogeneity across these studies ($I^2 = 61.4\%$, 95% CI 47%–74%, $P = 0.008$). Sensitivity analysis revealed that no study could affect the direction of the result. We therefore performed subgroup analyses based on the ethnic origin of participants because ethnicity could substantially affect the genetic profiles. A total of 2285 IPF patients and 3634 controls from 6 studies were of Caucasian origin, and 448 IPF patients and 1410 controls from 3 studies were of Asian origin. Compared with rs35705950 *G*

TABLE 1. Characteristics of Studies Included in the Meta-analysis

First Author	Year	Country	Ethnicity	IPF Number	Control Number	IPF Age (y)	Case Female (%)	IPF Smoking (%)	IPF FVC (%)	IPF D_LCO (%)	Genotyping Method	HWE
Seibold	2011	America	Caucasian	492	322	67.2 (8.1)	72	70	NA	NA	Sequenom iPLEX assays	Yes
Borie	2013	France	Caucasian	142	1383	69.8 (8.9)	18	68	76 (20)	47 (19)	TaqMan genotyping	Yes
Noth	2013	USA	Caucasian	1387	1367	67 (61–73)	27	68	64.9 (18.1)	46.5 (17.8)	Taqman, iPLEX Gold Platform	Yes
Stock	2013	UK	Caucasian	110	416	64.6 (45–85)	28	NA	69.3 (41–99)	40 (18–77)	TaqMan genotyping	Yes
Horimasu	2014	Japan	Asian	44	310	67.5 (13.5)	20	73	73.9 (25.3)	46.2 (23.6)	TaqMan genotyping	Yes
Horimasu	2014	Japan	Caucasian	71	35	67.6 (10.1)	28	51	66.4 (20.0)	45.6 (18.5)	TaqMan genotyping	Yes
Wang	2014	China	Asian	165	1013	61.8 (12.7)	39	NA	NA	NA	RFLP, TaqMan and sequencing	Yes
Peljto	2015	USA	Caucasian	83	111	66.0 (7.7)	29	49	53.9 (15.2)	51.0 (15.3)	TaqMan genotyping	NA
Peljto	2015	USA	Asian	239	87	65.1 (7.7)	25	67	69.0 (19.9)	55.5 (21.5)	TaqMan genotyping	NA

Data are mean (SD), or mean (IQR), % or n, unless otherwise stated. DLCO = diffusion capacity of lung for carbon monoxide, FVC = forced vital capacity, HWE = Hardy–Weinberg equilibrium, IQR = interquartile range, NA = not available, RFLP = restriction fragment length polymorphism, SD = standard deviation.

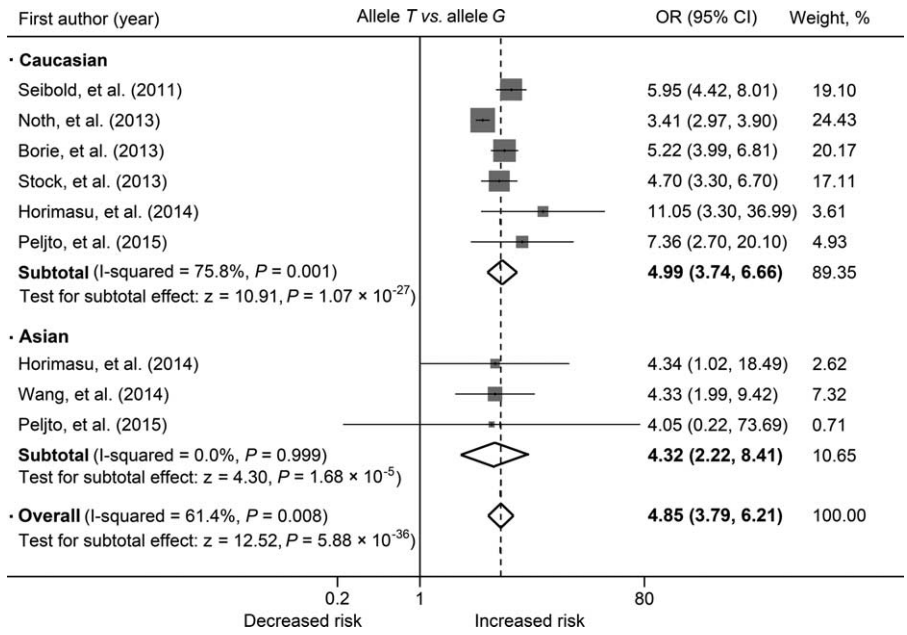

FIGURE 2. MUC5B rs35705950 minor *T* allele increased risk of IPF compared with *G* allele. Forest plot of odds ratios (ORs) and 95% confidence intervals (95% CIs) from each study, subgroup, and overall analysis were shown. Subgroup analyses were stratified by ethnicity. IPF = idiopathic pulmonary fibrosis.

allele, *T* allele significantly increased IPF susceptibility in both the Caucasian population (OR 4.99, 95% CI 3.74–6.66, $P = 1.07 \times 10^{-27}$; Figure 2) and the Asian population (OR 4.32, 95% CI 2.22–8.41, $P = 1.68 \times 10^{-5}$; Fig. 2). These significant associations were confirmed by TSA in both populations (Figure S1 and S2 in supplemental content, http://links.lww.com/MD/A490). Although the accrued sample size did not reach RIS in the Caucasian population, the association was prior established (Figure S1, http://links. lww.com/MD/A490). Significant level of heterogeneity was detected in analysis of the Caucasian population ($I^2 = 75.8\%$, 95% CI 46%–89%, $P = 0.001$), but not in the Asian population ($I^2 = 0$, $P = 0.999$). The funnel plot seemed asymmetric (Figure S3, http://links.lww.com/MD/A490), but Egger test did not reveal significant publication bias ($P = 0.104$).

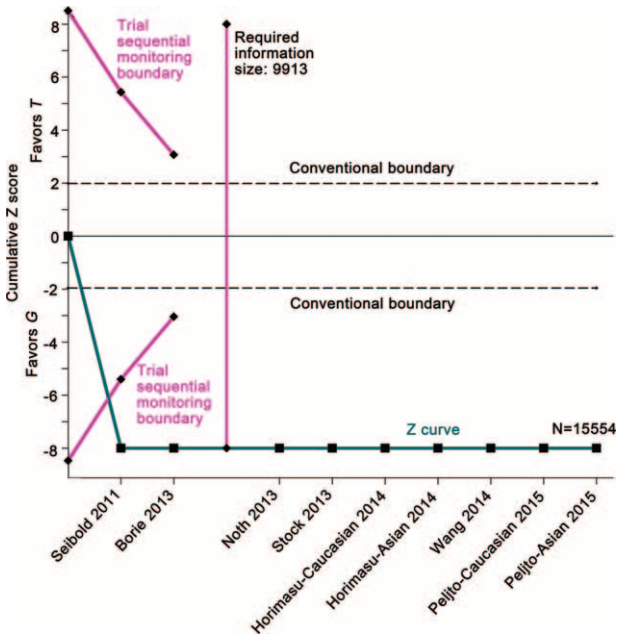

FIGURE 3. Trial sequential analysis of rs35705950 polymorphism and IPF risk using the allelic model (*T* allele vs *G* allele). TSA confirmed results from meta-analysis in Figure 2. IPF = idiopathic pulmonary fibrosis, TSA = trial sequential analysis.

MUC5B rs35705950 GT Genotype Increased Risk of IPF Compared With GG Genotype

Eight studies involving 2650 IPF patients and 4933 controls contributed to the overall analysis. The *MUC5B* rs35705950 *GT* genotype significantly increased the risk of IPF as compared with *GG* genotype (OR 6.20, 95% CI 5.14–7.48, $P = 1.70 \times 10^{-81}$; Fig. 4). In the TSA, the calculated RIS was 12,848. Although the pooled sample size did not exceed the RIS, the cumulative crossed the traditional boundary, the trial sequential monitoring boundary, and prior established the association (Fig. 5), suggesting further studies are not required as this significant association is unlikely to be changed. There was no significant heterogeneity across these studies ($I^2 = 19.9\%$, 95% CI 0%–62%, $P = 0.272$). No publication bias was detected by visual inspection of funnel plot (Figure S4, http://links.lww.com/MD/A490) or Egger test ($P = 0.498$). Sensitivity analysis showed that no study could significantly affect the result. Subgroup analyses stratified by ethnicity demonstrated that *GT* genotype was associated with a significantly higher IPF susceptibility in the Caucasian population (OR 6.59, 95% CI 5.15–8.43, $P = 9.68 \times 10^{-51}$; Fig. 4), and less remarkable but still significantly higher risk of IPF in the Asian population (OR 4.43, 95% CI 2.26–8.70, $P = 1.49 \times 10^{-5}$; Figure 4). TSA confirmed these positive results in both populations (Figure S5 and S6 in supplemental content, http://

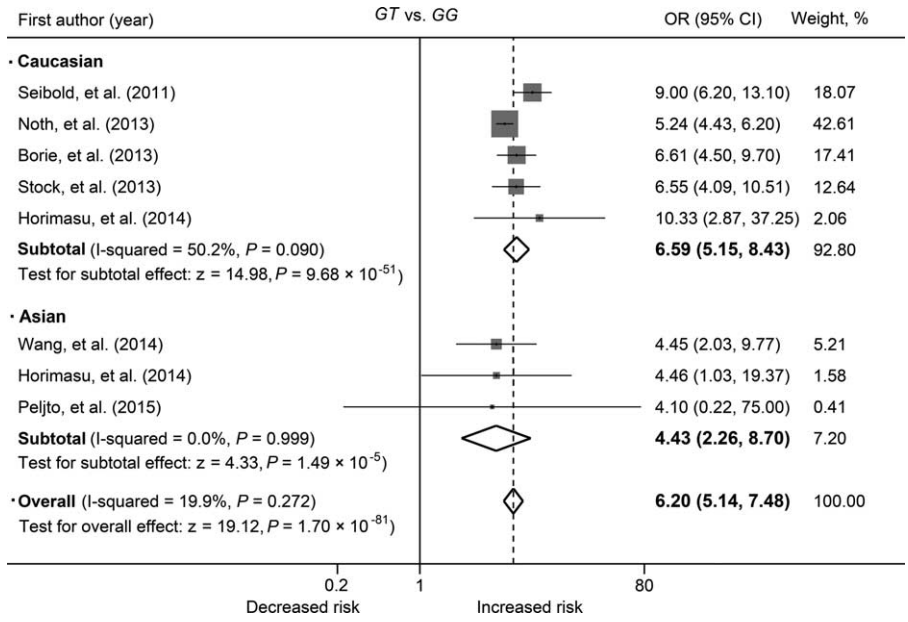

FIGURE 4. MUC5B rs35705950 *GT* genotype increased risk of IPF compared with *GG* genotype. Forest plot of odds ratios (ORs) and 95% confidence intervals (95% CIs) from each study, subgroup and overall analysis were shown. Subgroup analyses were stratified by ethnicity. IPF = idiopathic pulmonary fibrosis.

links.lww.com/MD/A490). A moderate level of heterogeneity was detected in the Caucasian population ($I^2 = 50.2\%$, 95% CI 25%–72%, $P = 0.090$) and no heterogeneity in the Asian population ($I^2 = 0$, $P = 0.999$).

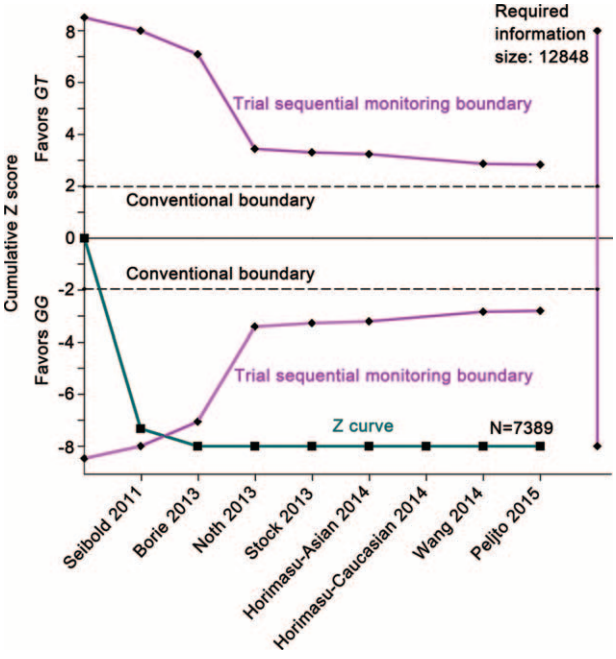

FIGURE 5. Trial sequential analysis of rs35705950 polymorphism and IPF risk using the genotypic model (*GT* genotype vs *GG* genotype). TSA confirmed results from meta-analysis in Figure 4. IPF = idiopathic pulmonary fibrosis, TSA = trial sequential analysis.

Muc5b Rs35705950 TT Genotype Increased Risk of IPF in Caucasian Population Compared With GG Genotype

All 3 studies conducted in the Asian population did not report any individual carrying a homozygous *TT* genotype in both IPF patients and controls, and thus were not included in the analyses of association between *TT* genotype and IPF risk. Five studies involving 2202 IPF patients and 3523 controls in the Caucasian population were included in the analysis. The *MUC5B* rs35705950 *TT* genotype was associated with a significantly higher risk of IPF in the Caucasian population compared with GG genotype (OR 11.29, 95% CI 5.69–22.40, $P = 4.22 \times 10^{-12}$; Fig. 6), with a risk even higher than *TG* genotype. Moreover, TSA provided reliable evidence that this association was true positive (Fig. 7). A significant heterogeneity was detected ($I^2 = 60.2\%$, 95% CI 39%–77%, $P = 0.040$). Sensitively analysis revealed no significant difference.

DISCUSSION

We made a comprehensive meta-analysis with TSA of 9 studies, including 2733 IPF patients and 5044 controls, and found strong association between *MUC5B* rs35705950 polymorphism and IPF susceptibility. The strength of association between rs35705950 minor *T* allele and IPF susceptibility was particularly evident in the Caucasian population, and milder but still significant in the Asian population.

To investigate the difference of strength association among different ethnic populations is important and one of the major endpoints in our study. We revealed that the frequency of minor *T* allele was substantially lower in the Asian population (40/3716 [1.08%]) than in the Caucasian population (2397/11838 [20.2%]), with regard to both IPF patients (19/896 [2.1%] vs 1583/4570 [34.5%]) and healthy controls (21/2820 [0.74%] vs 814/7268 [11.2%]). All 3 studies conducted in the Asian

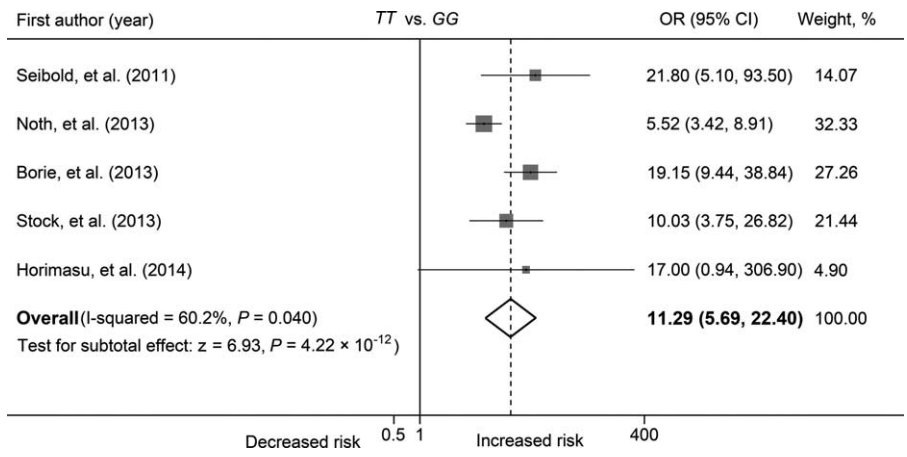

FIGURE 6. MUC5B rs35705950 *TT* genotype increased risk of IPF compared with *GG* genotype. Forest plot of odds ratios (ORs) and 95% confidence intervals (95% CIs) from each study and overall analysis were shown. IPF = idiopathic pulmonary fibrosis.

population did not detect any participants carrying the homozygous *TT* genotype, including IPF patients. These observations were in accordance with those from the single-nucleotide polymorphism database (dbSNP) and 1000 Genomes project for the Asian population.[29,30] Although the minor *T* allele was infrequent in the Asian population, our meta-analysis demonstrated that this variant still significantly increased the risk of IPF. Nevertheless, provided the rarity of rs35705950 polymorphism in the Asian population, the genetic contribution of this variant to IPF might not be as weighted as in the Caucasian population, indicating the presence of other unidentified genetic and/or environmental factors in the Asian population.[9]

Pathologically in IPF lungs, a body of mucin-laden cells were detected, which are normally absent from distal lungs.[31,32]

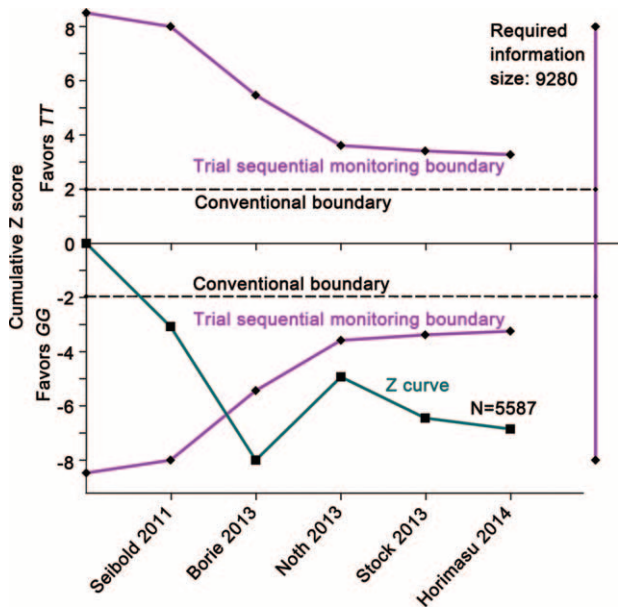

FIGURE 7. Trial sequential analysis of rs35705950 polymorphism and IPF risk using the genotypic model (*TT* genotype vs *GG* genotype). TSA confirmed results from meta-analysis in Figure 6. IPF = idiopathic pulmonary fibrosis, TSA = trial sequential analysis.

Immunohistochemistry analysis identified that MUC5B, but not MUC5AC or MUC2, was highly expressed in IPF lungs.[31] Indeed, IPF patients had a 14 times higher level of MUC5B protein in lung tissue as compared with healthy controls, and MUC5B expression in healthy controls carrying rs35705950 minor *T* allele (*TT* or *TG* genotype) was 37 times higher than the wild-type controls (*GG* genotype).[5] The dysregulated MUC5B expression could contribute to the pathogenesis of IPF, but the molecular mechanism remains poorly understood. A recent study in mice demonstrated that MUC5B was critical for mucociliary clearance (MCC), controlling infections, and maintaining immune homeostasis,[33] indicating a complex function of MUC5B. Several potential mechanisms could be involved. First, excessive MUC5B secretion might lead to bronchiolar plugging and impaired clearance of inhaled materials, resulting in chronic inflammatory and toxicity.[5,34,35]Second, MUCB hypersecretion might activate the unfolded protein response (UPR) through upregulating inositol-requiring enzyme 1β.[36] UPR was an important process in IPF caused by surfactant protein C gene (*SPC*) mutations,[37,38] another important genetic etiology of IPF, and the activation led to endoplasmic reticulum (ER) stress and ultimately apoptosis.[38] Additionally, MUC5B might impede alveolar repair,[5] stimulate a fibroproliferative response,[39] or increase bacterial burden[40] to promote IPF occurrence.

Although the minor *T* allele increased the risk of developing IPF, it was also a predictor of favorable prognosis.[24] In a retrospective study of survival in 586 IPF patients, Peljto et al[24] demonstrated that IPF patients with *TT* and *TG* genotypes were associated with improved survival compared with patients with *GG* genotype independent of other risk factors, which was similar to rs5743890 in *TOLLIP* gene discovered in a genome-wide association study, with the rs5743890_*G* allele being the protective allele to IPF and also increasing mortality of IPF patients.[4] Consistently in several studies, patients with the minor *T* allele had a longer time to decline in FVC,[6] and better lung function.[28] It is conceivable and valuable to separate IPF patients with a comprehensive method of stratification combining numerous risk factors including *MUC5B* rs35705950 polymorphism and other genetic factors, because it might enable prediction of prognosis at early stage or subclinical stage of IPF.[4] Indeed, in the retrospective study mentioned above,[4] similar results were obtained in IPF patients at mild and moderate stages, and in a study in the general population

(including 2633 participants in the Framingham Heart Study), *MUC5B* rs35705950 polymorphism was associated with interstitial lung abnormity—an subclinical stage of pulmonary fibrosis.[26] Future work is needed to define these findings.

Several limitations should be acknowledged in our study. First, the current study was based on study-level data, whereas patient-level data were not available; therefore, we did not perform adjustment for various covariates except ethnicity, which might bring in bias into our study. However, it is notable that several studies selected age and sex-matched IPF patients and controls. Second, significant heterogeneity was detected in numerous comparisons. We attempted to address these issues by conducting subgroup analyses stratified by ethnicity. Although no heterogeneity was detected in the Asian population, significant heterogeneity still existed in the Caucasian population. We were unable to address other sources of heterogeneity because of limited number of studies, but sensitivity analyses did not reveal any study that could change the direction of these meta-analysis results. The diagnosis variability (Supplemental content Table S1, http://links.lww.com/MD/A490), particularly the rate variability of surgical lung biopsy among these studies, might account for part of this heterogeneity, but this effect could not be determined in our study and is unlikely to be large. Third, although Egger test did not reveal significant publication bias, funnel plot seemed asymmetric in the comparison of *T* allele and *G* allele; therefore, we could not fully rule out the possibility of publication bias. Fourth, our study was performed only in the Caucasian population and the Asian population, the association between *MUC5B* rs35705950 and IPF susceptibility could not be simply translated to other uninvestigated ethnicity such as the African population because genetic profiles could be substantially affected by ethnicities, which was also confirmed in our study.

CONCLUSIONS

Our meta-analysis showed a strong association between *MUC5B* promoter rs35705950 polymorphism and the risk of IPF. The strength of association between rs35705950 minor *T* allele and IPF susceptibility was particularly evident in the Caucasian population, and milder but still significant in the Asian population.

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