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Review

REVIEW

CHRNA5 Risk Variant Predicts Delayed Smoking Cessation and Earlier Lung Cancer Diagnosis—A Meta-Analysis

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Abstract

Background: Recent meta-analyses show strong evidence of associations among genetic variants in *CHRNA5* on chromosome 15q25, smoking quantity, and lung cancer. This meta-analysis tests whether the *CHRNA5* variant rs16969968 predicts age of smoking cessation and age of lung cancer diagnosis.

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Methods: Meta-analyses examined associations between rs16969968, age of quitting smoking, and age of lung cancer diagnosis in 24 studies of European ancestry (n = 29 072). In each dataset, we used Cox regression models to evaluate the association between rs16969968 and the two primary phenotypes (age of smoking cessation among ever smokers and age of lung cancer diagnosis among lung cancer case patients) and the secondary phenotype of smoking duration. Heterogeneity across studies was assessed with the Cochran Q test. All statistical tests were two-sided.

Results: The rs16969968 allele (A) was associated with a lower likelihood of smoking cessation (hazard ratio [HR] = 0.95, 95% confidence interval [CI] = 0.91 to 0.98, $P = .0042$), and the AA genotype was associated with a four-year delay in median age of quitting compared with the GG genotype. Among smokers with lung cancer diagnoses, the rs16969968 genotype (AA) was associated with a four-year earlier median age of diagnosis compared with the low-risk genotype (GG) (HR = 1.08, 95% CI = 1.04 to 1.12, $P = 1.1*10^{-5}$).

Conclusion: These data support the clinical significance of the *CHRNA5* variant rs16969968. It predicts delayed smoking cessation and an earlier age of lung cancer diagnosis in this meta-analysis. Given the existing evidence that this *CHRNA5* variant predicts favorable response to cessation pharmacotherapy, these findings underscore the potential clinical and public health importance of rs16969968 in *CHRNA5* in relation to smoking cessation success and lung cancer risk.

Tobacco smoking is one of the modifiable risk factors that most greatly contribute to cancer and many other severe medical illnesses. Clear evidence demonstrates that genetic variation in the α5 nicotinic cholinergic receptor subunit gene (*CHRNA5*) is associated with heaviness of smoking and nicotine dependence (1,2). A series of meta-analyses based on tens of thousands of subjects of European ancestry confirmed the association with smoking quantity, defined by number of cigarettes smoked per day (3–6), with the most robust associations reported for rs16969968 and rs1051730, two highly correlated genetic variants (5). The nonsynonymous variant rs16969968 results in an amino acid change in the α5 nicotinic cholinergic receptor subunit, which alters nicotinic receptor conductance in vitro (7,8). These *CHRNA5* variants also are consistently associated with smoking-related disorders including lung cancer and chronic obstructive pulmonary disease (COPD) (9–11).

However, an important gap in our understanding of variation in *CHRNA5* is its contribution to smoking cessation. For example, these *CHRNA5* variants are less consistently associated with smoking cessation outcomes. Some studies show an association between the *CHRNA5* region and successful smoking cessation (12–18), finding that the same risk variants that contribute to smoking quantity and nicotine dependence also predict smoking cessation. Other studies, however, fail to confirm this association (19–21). A genome-wide association study (GWAS) of three treatment cohorts did not identify any nicotinic receptor genes as predictors of prospectively measured smoking cessation (21). Another large genome-wide association meta-analysis that strongly supported the association between 15q25.1 and smoking quantity ($P < 10^{-32}$) reported a below GWAS-level association between *CHRNA5* and smoking cessation ($P < 10^{-4}$), defined as the contrast between current vs former smoking (5).

This gap in knowledge of the relation between *CHRNA5*, smoking cessation, and smoking-related disorders inspired this current research. We meta-analyzed results from 24 studies of European ancestry in collaboration with the International Lung Cancer Consortium (ILCCO) and Genetic Associations and Mechanisms in Oncology (GAME-ON) Consortia (Transdisciplinary Research in Cancer of the Lung [TRICL] and Discovery, Biology, and Risk of Inherited Variants in Breast Cancer [DRIVE]). We explored two linked aims: 1) Does variation in *CHRNA5* affect the age that smokers quit smoking successfully? 2) Does variation in *CHRNA5* affect the onset of lung cancer?

Methods

Samples

In order to examine *CHRNA5*, age of smoking cessation, and age of lung cancer diagnoses, we invited studies in the ILCCO, the GAME-ON Consortia TRICL and DRIVE, and the Genetic Epidemiology of COPD (COPDgene) Study. In addition, we included the Collaborative Genetic Study of Nicotine Dependence (COGEND) and three studies from the dbGap database: Atherosclerosis risk in Communities (ARIC), Multi-Ethnic Study of Atherosclerosis (MESA), and Study of Addiction: Genetics and Environment (SAGE). Results from 24 datasets (n = 29 072 unrelated smokers of European ancestry) contributed to the meta-analyses. Informed consent was obtained from participants, and all studies received approval from the appropriate institutional review board. Standardized quality control measures were applied to all studies. Of these datasets, seven were studies ascertained by conditions other than smoking-related diseases, and 17 were studies of smoking-related diseases: lung cancer (15 studies), COPD (one study), and coronary heart disease (one study). These different study designs allowed us to conduct stratified meta-analysis in patients ascertained for smoking-related diseases and patients ascertained for other reasons. To be included in analyses, each patient was required to have reported smoking cigarettes in his/her lifetime. Additional details for each dataset are provided in Supplementary Table 1 and Supplementary Methods (available online). Supplementary Table 2 shows the sample size, demographics, cigarettes per day, percentage of smokers who have quit smoking, and minor allele frequency for rs16969968 for each dataset.

Phenotypes

The primary phenotypes were: 1) time (in years) from birth to age of smoking cessation, defined as self-reported age of stopping smoking among ever smokers, and 2) time (in years) from birth to age of lung cancer diagnosis among lung cancer cases. A secondary phenotype defined as the duration from age of smoking onset (not available in all studies) to age of smoking cessation was examined in a subset of studies. Smoking quantity when subjects smoked regularly was assessed with cigarettes smoked per day (CPD), defined as a four-level ordered trait (CPD≤10; 11≤CPD≤20; 21≤CPD≤30; CPD≥31, coded as 0, 1, 2, 3, respectively).

Variants for Analyses

Because of its biological significance, we targeted the *CHRNA5* variant rs16969968 for association testing. The variant rs16969968 was available in all datasets except ARIC, MESA, and the German study. To examine ARIC and MESA, we imputed the rs16969968 genotype based on 1000 Genomes (http://www.1000genomes.org/) for analyses. In addition, we used proxy variants (rs951266, $r^2 = 0.98$, in ARIC, and rs9788721, $r^2 = 0.83$, in MESA, r^2 estimated based on 1000 Genomes for the EUR samples including CEU, GBR, TSI, IBS, FIN) (22,23) for analyses and reached similar results. In the German study, imputed genotype was not available and we used a proxy variant (rs1051730, $r^2 = 0.99$ estimated based on 1000 Genomes for the EUR samples) for analyses. In addition, we conducted the meta-analyses with and without the German Studies and reached similar results.

Statistical Analyses and Meta-Analyses

In each dataset, we used Cox regression models to evaluate the association between rs16969968 and the two primary phenotypes (age of smoking cessation among ever smokers, and age of lung cancer diagnosis among lung cancer case patients), and the secondary phenotype of smoking duration. The assumption of proportionality was examined by several different approaches (plotting the log(-log(survival) against log(time) for different groups, ASSESS statistics, and testing the interactions between the covariate and time). There was a trend of decreasing genetic effects in the very old age group when the cancer incidence decreases. When truncating after age 82.5 (1% of the sample), the proportionality assumption was verified while all results were similar with/without the truncation. Age as a continuous variable and sex were included as covariates. Additional covariates included cigarettes smoked per day (a continuous variable). Genotypes were coded additively as the number of minor alleles (A), where the reference allele was defined as the major allele (G) in the European ancestry population (24). Consistency of allelic coding was confirmed by comparing allele labels and frequencies across datasets (Supplementary Table 2, available online).

Standardized scripts were used for analyses of all participating datasets at each individual research center. For the ILCCO studies, analyses were performed at Washington University as individual level data was provided for data pooling. Results were returned to Washington University for quality checks and meta-analyses. Individual SNP analyses were performed using SAS (SAS Institute, Cary, NC).

We used PLINK to perform variance-based meta-analyses (25). The R package, rmeta, was used to confirm results and generate meta-analysis plots (26). The heterogeneity across studies was assessed with the Cochran Q test and reported for each analysis. There was evidence of heterogeneity across datasets for analyses of age of smoking cessation, but not for the analyses of age at lung cancer diagnosis. This heterogeneity may be because of the varying study designs and ascertainment strategies. We report results from random effects models for all meta-analyses. All statistical tests were two-sided,

Results

The meta-analysis included studies not ascertained for smoking related-disorders, case patients with smoking-related disorders (lung cancer, COPD, coronary heart disease), and matched control patients without these disorders. This design allowed us to conduct meta-analyses of the genetic associations of rs16969968, stratified by ascertainment of individuals with and without smoking-related disorders that may alter smoking cessation rates. This comparison is of interest because illness could affect smoking behaviors. First, we confirmed a consistent association between *CHRNA5* rs16969968 and heaviness of smoking in subjects ascertained with smoking-related disease (meta-analysis $\beta = 0.10$, 95% confidence interval [CI] = 0.07 to 0.13, $P = 3.5^{*}10^{-10}$) (Supplementary Figure 1, available online), as well as in subjects ascertained without such disease (meta-analysis $\beta = 0.10$, 95% CI = 0.06 to 0.14, $P = 1.1^{*}10^{-6}$).

The *CHRNA5* rs16969968 Risk Allele and Delayed Smoking Cessation in Smokers Without Smoking-Related Disorders

Among smokers in the studies not ascertained for smoking-related illnesses, the rs16969968 risk allele (A) was associated with a lower likelihood of smoking cessation, adjusted for age and sex. The meta-analysis across seven datasets resulted in a hazard ratio (HR) of 0.95 (95% CI = 0.91 to 0.98, $P = .0042$, $P_{heterogeneity} = .32$). In addition, we examined this association in subjects who were control patients in studies ascertained for smoking-related illnesses. We saw a similar association in control participants in the studies of lung cancer with significant heterogeneity across these studies (HR = 0.94, 95% CI = 0.87 to 1.02, P = .15, $P_{heterogeneity} = .027$). Non-statistically significant associations were found for control patients in the COPD and coronary heart disease studies. Figure 1 provides a forest plot summary for rs16969968 and age of quitting. The summary median age of smoking cessation was 56 years for individuals with the high-risk genotype (AA), which was a four-year delay compared with individuals with the low-risk genotype (GG), who had a summary median quit age of 52 years. At age 50 years, 42% of individuals with the AA genotype, compared with 48% of individuals with the GG genotype, successfully quit smoking. In addition, rs16969968 was associated with delayed cessation (n = 13 258, HR = 0.90, 95% CI = 0.88 to 0.93, $P = 1.93^{*}10^{-5}$) in a pooled analysis using available individual-level data adjusted for age, sex, and study. These analyses were repeated with smoking duration as the outcome, and similar results were seen.

To further understand the genetic association between rs16969968 and smoking cessation, we meta-analyzed results from models adjusting for smoking quantity (the four-level cigarettes smoked per day variable). In smokers not ascertained for smoking-related disorders, smoking a higher number of cigarettes per day was associated with a later quitting age (meta-analysis random effect HR = 0.87, 95% CI = 0.76 to 0.99, $P = .037$) with significant heterogeneity ($I^2 = 38.3$, $P_{heterogeneity} = .033$). Rs16969968 showed a weakened, non-statistically significant association with age of quitting after adjusting for smoking quantity (HR = 0.98, 95% CI = 0.96 to 1.01, $P = .25$).

The *CHRNA5* rs16969968 Risk Allele Predicts Earlier Age of Lung Cancer Diagnosis

The rs16969968 risk allele (A) was associated with earlier age of lung cancer diagnosis, adjusted for sex. The overall meta-analysis across all 15 datasets of lung cancer case patients gave a hazard ratio of 1.08 (95% CI = 1.04 to 1.12, $P = 1.1^{*}10^{-5}$, $P_{heterogeneity} = .53$) (Figure 2). The summary median age at lung cancer diagnosis was 61 years for individuals with the high-risk genotype (AA), which was a four-year earlier age at onset compared with individuals with the low-risk genotype (GG), who had a summary median

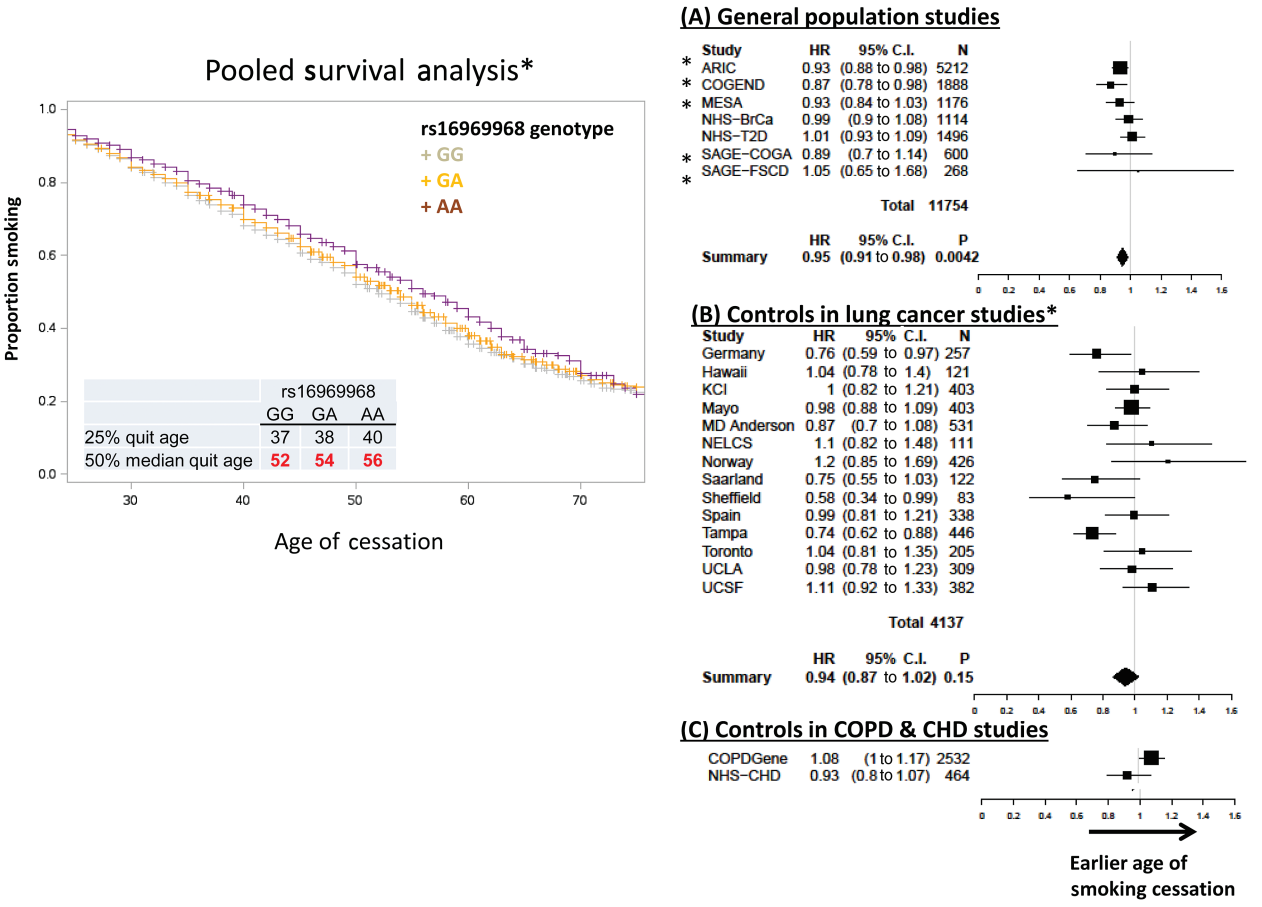

Figure 1. Effect of *CHRNA5* rs16969968 on delayed smoking cessation among subjects without smoking-related disorders. Smoking-related disorders were lung cancer, chronic obstructive pulmonary disorder, and coronary heart disease. All models adjusted for age, sex, and rs16969968. Random effect models are shown, $P_{heterogeneity}$ = .32 for group A, .027 for group B, and .0058 when all groups combined. * Individual-level data for these marked studies were available and used for the pooled analysis (n = 13 285). All statistical tests were two-sided. CHD = coronary heart disease; COPD = chronic obstructive pulmonary disorder.

age of diagnosis of 65 years. In addition, in a pooled analysis using available individual level data adjusted for sex and study, rs16969968 was associated with earlier diagnoses of lung cancer (n = 7074, HR = 1.11, 95% CI = 1.07 to 1.15, $P = 2.49*10^{-9}$).

The genetic effect of *CHRNA5* on age at onset of lung cancer remained after adjusting for smoking quantity (four levels of cigarettes smoked per day). Smoking a higher number of cigarettes per day was associated with an earlier age of lung cancer diagnosis (meta-analysis HR = 1.07, 95% CI = 1.02 to 1.13, P = .0072), and rs16969968 (A) remained a statistically significant predictor of an earlier age of lung cancer diagnosis (HR = 1.08, 95% CI = 1.04 to 1.11, $P = 3.1*10^{-5}$).

Predictors for Smoking Cessation Change Among Patients With Smoking-Related Disorders

In contrast to what was found in patients not ascertained for smoking-related disease, neither rs16969968 nor heaviness of smoking predicted smoking cessation in patients ascertained with lung cancer, COPD, or coronary heart disease (rs16969968 association with age of quitting: meta-analysis HR = 0.98, 95% CI = 0.91 to 1.05, P = .50) (Supplementary Figure 2, available online) (cigarettes per day association with age of quitting: meta-analysis HR = 0.995, 95% CI = 0.90 to 1.11, P = .93) (Supplementary Figure 3, available online). In our secondary analyses using meta-regression, we found a statistically significant interaction in that smoking heaviness was associated with delayed quitting only in patients not ascertained for smoking-related disease, but no association was seen in patients with smoking-related disease (F = 148.23, df = 1, $P < 1.0*10^{-5}$). The difference in association of *CHRNA5* and delayed quitting between patients with and without smoking-related disease was non-statistically significant (F = 1.70, df = 1, P = .21).

Discussion

This research shows a complex relation amongst smoking quantity, smoking cessation, genetic variation, and lung cancer risk. We found that genetic variation in *CHRNA5*, which strongly predicts risk for nicotine dependence (3–6), lung cancer (9,11), and COPD (10), also predicts delayed smoking cessation. The high-risk rs16969968 A allele is common in individuals of European ancestry (42.5%, dbSNP CEU). Having the high-risk genotype (AA, 18% of European ancestry) was associated with a four-year delay in median age of quitting smoking, compared with the low-risk genotype (GG, 33% of European ancestry) amongst individuals without diagnosed smoking-related disease. This delay in quitting results in a longer exposure to carcinogens in cigarettes, which predicts an increased risk for cancer, pulmonary, and cardiovascular disorders (27,28).

Importantly, the *CHRNA5* variant rs16969968 also predicts an earlier diagnosis of lung cancer, manifested as a median age of

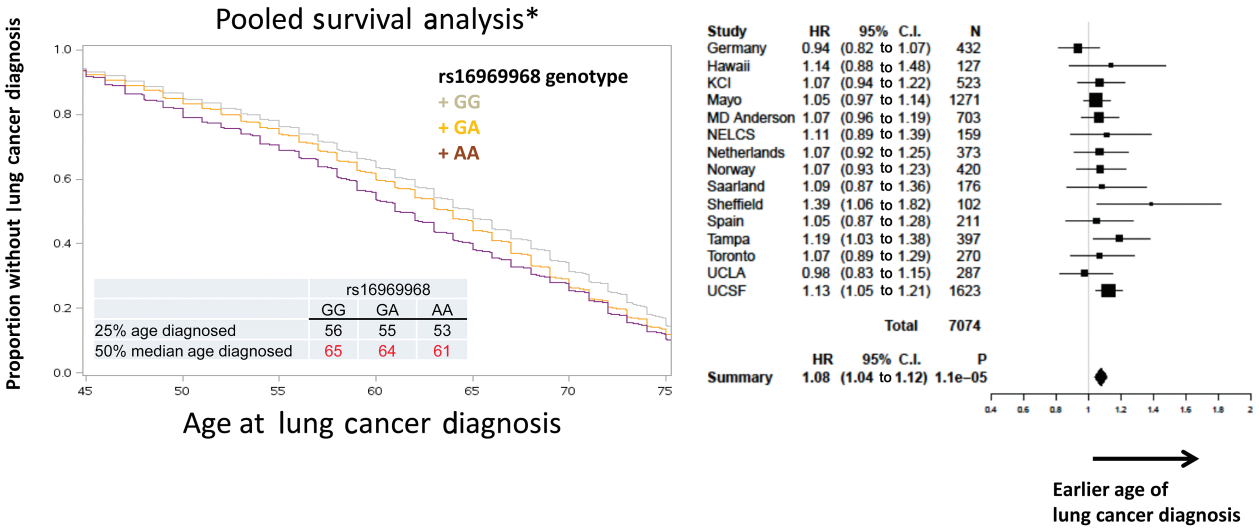

Figure 2. Effect of *CHRNA5* rs16969968 on diagnosis age among smokers with lung cancer. All models adjusted for sex and rs16969968. Random effect model is shown, $P_{heterogeneity}$ = .53. * Individual-level data for all studies are available and used for the pooled analysis (n = 7074). All statistical tests were two-sided.

lung cancer diagnosis that is four years earlier in smokers with the high-risk genotype (AA) (61 years of age), compared with those with the low-risk genotype (GG) (65 years of age), an association that remains after adjusting for smoking quantity. Lung cancer is the most common cancer in the United States and worldwide and responsible for 13% of all lung cancer incidence and 27% of all cancer deaths (29,30). The survival rate with a lung cancer diagnosis is low—50% die within a year of diagnosis and the five-year survival rate is 16.6% (31). Most cases (90%) of lung cancer are attributable to smoking. The acceleration of lung cancer diagnosis in those with AA genotypes (18% of ever smokers with lung cancer) by four years compared with those with GG genotypes (33%) can be clinically significant given the high mortality following a diagnosis of lung cancer. This finding is consistent with the previous reports of *CHRNA5* and earlier lung cancer diagnosis in a single study (by five years) (32), and a meta-analysis of five studies (by 1.1 years, n = 3898) by Lips et al. (33). The present study is a larger meta-analysis (15 studies, n = 7074), one overlapping study (Toronto study, n = 270) with the report by Lips et al.

The mechanisms underlying *CHRNA5*, age of smoking cessation, and age of lung cancer diagnosis are complex, and we attempted to summarize our results in Figure 3. The mechanisms through which *CHRNA5* accelerates the age of onset of lung cancer likely involve multiple pathways, including an increased number of cigarettes smoked (3–6), deeper inhalation of cigarettes leading to higher carcinogen exposure (34,35), and a delay in smoking cessation. This combination of risk factors could mediate the genetic effect, so that this one genetic variant, rs16969968, is then associated with earlier diagnosis of lung cancer by four years.

Evidence of the association between *CHRNA5* and smoking cessation has primarily been reported in smoking cessation trials (12,14,15,17,18) and less commonly in general population studies (5,18). However, our findings indicate that association between *CHRNA5* and smoking cessation may be generalizable to the general population of smokers. Examining age of quitting smoking, we confirmed the genetic association between *CHRNA5* and smoking cessation in smokers not ascertained with smoking-related disorders, a step forward in exploring the public health significance of this genetic marker.

These findings can help us understand discrepancies in existing reports of this genetic locus and smoking cessation. Some studies show a relation between *CHRNA5* and smoking cessation (12–16), whereas other studies do not (19–21). We hypothesize that the expression of this genetic risk on cessation varies with several factors, such as developing a smoking-related disease, use of cessation pharmacotherapy (18), and environmental influences on smoking cessation such as partner smoking (36). The effect of this genetic locus is seen most clearly in a sample where there is no smoking-related disorder, pharmacotherapy use is rare, and strong environmental push for smoking cessation is present. In addition, we posit that *CHRNA5* risk alleles dispose individuals to early development of smoking-related disease and that disease development encourages earlier quitting than would otherwise occur. This is why this genetic risk is more likely to be positively related to quitting age amongst individuals without smoking-related disease vs those with such disease; amongst the latter group, the effect of *CHRNA5* to delay quitting is offset by its effect of accelerating disease development, which in turn promotes quitting. Similarly, use of cessation pharmacotherapy may reduce the relation of *CHRNA5* with quitting age because pharmacotherapy appears to blunt this genetic risk (18). The heterogeneity among studies in this meta-analysis indicates the genetic risk association varies with potential moderators for the genetic risk such as the impact of disease/symptom, cessation, medication use, and environmental risks.

These results should be interpreted in the context of multiple limitations. First, smoking cessation in the samples was self-reported and not assessed using biochemical confirmation. However, research shows that self-report is a valid indicator of current smoking, especially when there are no strong incentives to deceive (37). Second, we examined age of smoking cessation as a primary phenotype. There is no evidence of association between rs16969968 and age of onset of smoking, and there is limited variation in age of onset for smoking (38,39). We also analyzed duration of smoking as the secondary outcome for a subset of studies with available information and found similar results. Third, we could not test the effect of cessation treatment on age of quitting, as this information was not available. We assume the use of medication is not common because the majority of smokers quit without medication in the general population (40). Fourth,

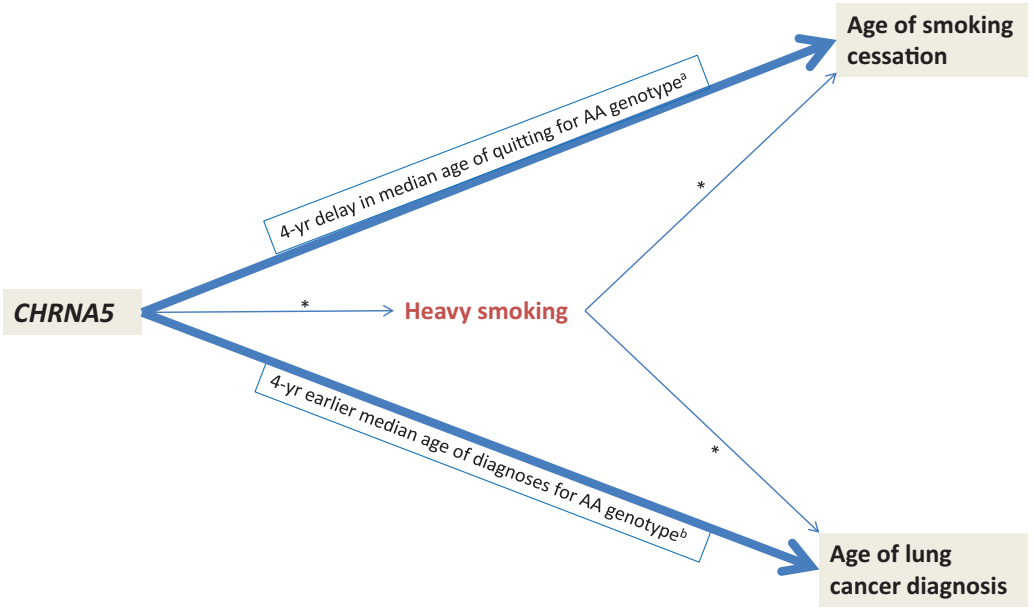

Figure 3. Summary diagram of *CHRNA5* rs16969968 genotype, age of smoking cessation, and age of lung cancer diagnosis. * Associations are supported by existing evidence (4–7,19,23). [a] No longer statistically significant after adjusting for heavy smoking. [b] Remains statistically significant after adjusting for heavy smoking.

this work analyzed only one genetic variant, and it is clear that multiple genes or variants contribute to smoking cessation (41). Fifth, we found the genetic effect of *CHRNA5* on earlier age of lung cancer diagnosis after adjusting for the effect of heavy smoking while there is potential residual confounding because of the imprecise nature of self-reported smoking quantity information. Finally, this study included only subjects of European ancestry.

Despite these limitations, this meta-analysis clarifies the clinical and public health significance of a robust common genetic marker identified in genetic studies of nicotine dependence. We demonstrate that the *CHRNA5* variant rs16969968 is a marker of delayed smoking cessation. The median age of smoking cessation for those with the high-risk variants (AA) at rs16969968 is 56 years vs 52 years for those with the low-risk variants (GG). Similarly, those with the high-risk variants have a four-year-earlier age of lung cancer diagnosis (61 years) compared with those with the low-risk variants (65 years). Recent research has revealed that two prominent smoking-related risk factors for lung cancer are heavy smoking quantity and late age of quitting smoking (28), and rs16969968 influences both risk factors. Moreover, the current research suggests the boundary conditions affecting the relation between rs16969968 and cessation, suggesting that development of smoking-related disease obscures the manifestation of this genetic risk. Early detection of lung cancer is important (42), and these results suggest the potential use of a common genetic variant such as *CHRNA5* rs16969968 as a susceptibility marker for earlier lung cancer diagnosis.

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