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Increased Genetic Vulnerability to Smoking at *CHRNA5* in Early-Onset Smokers

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Author Contributions: Dr Bierut had full access to all the data in the meta-analysis and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Online-Only Material: The eAppendixes, eTables, and eFigures are available at http://www.archgenpsychiatry.com.

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Abstract

Context—Recent studies have shown an association between cigarettes per day (CPD) and a nonsynonymous single-nucleotide polymorphism in *CHRNA5*, rs16969968.

Objective—To determine whether the association between rs16969968 and smoking is modified by age at onset of regular smoking.

Data Sources—Primary data.

Study Selection—Available genetic studies containing measures of CPD and the genotype of rs16969968 or its proxy.

Data Extraction—Uniform statistical analysis scripts were run locally. Starting with 94 050 ever-smokers from 43 studies, we extracted the heavy smokers (CPD >20) and light smokers (CPD ≤10) with age-at-onset information, reducing the sample size to 33 348. Each study was stratified into early-onset smokers (age at onset ≤16 years) and late-onset smokers (age at onset >16 years), and a logistic regression of heavy vs light smoking with the rs16969968 genotype was computed for each stratum. Meta-analysis was performed within each age-at-onset stratum.

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Data Synthesis—Individuals with 1 risk allele at rs16969968 who were early-onset smokers were significantly more likely to be heavy smokers in adulthood (odds ratio [OR]=1.45; 95% CI, 1.36–1.55; n=13 843) than were carriers of the risk allele who were late-onset smokers (OR = 1.27; 95% CI, 1.21–1.33, n = 19 505) (P = .01).

Conclusion—These results highlight an increased genetic vulnerability to smoking in early-onset smokers.

Tobacco use is the leading cause of preventable death worldwide, killing more than 5 million people annually.[1,2] Smoking behaviors, including nicotine dependence, cluster in families,[3] and large twin studies,[4–9] indicate that this clustering reflects genetic factors. Completion of the Human Genome Project, coupled with rapid advances in genotyping technology, has led to association studies that harness natural genetic variation to gain insight into the genetic basis for smoking behaviors.

Numerous studies[10–19] have demonstrated an association between the nonsynonymous chromosome 15 single-nucleotide polymorphism (SNP) rs16969968, in the α5 nicotinic receptor subunit gene *CHRNA5* (often tagged by rs1051730), and smoking behavior, as measured by cigarettes per day (CPD) and nicotine dependence. This association is consistent with biological evidence suggesting the risk variant alters conductance of the α5-containing nicotinic receptors.[20,21] Of note, the same locus is associated with risk of lung cancer and chronic obstructive pulmonary disease in several genome-wide association studies.[14,18,22,23]

Because rs16969968 has a significant genetic effect on smoking behavior, it is important to understand how this genetic variant interacts with various factors, including potentially modifiable risk factors. In particular, age at onset of regular smoking is of interest because of the well-established association between early age at onset of smoking and higher risk of heavier smoking and nicotine dependence.[9,24–26]

An unresolved issue is whether rs16969968 plays a role in the heightened susceptibility to nicotine dependence in early-onset smokers.[10,27,28] One study[10] reported an increased risk of nicotine dependence at this locus in individuals who started smoking regularly at or before age 16 years. In contrast, a second study[27] found a stronger association with this locus in smokers with age at onset of 16 years or older compared with smokers with an earlier age at onset. The task of interpreting and reconciling published results is further complicated because the definition of nicotine dependence and age at onset of smoking varied across studies.

Multiple investigators collaborated on this meta-analysis of the effect of the interaction between age at onset and rs16969968 on the risk of heavy smoking, contributing 43 individual data sets for a total of 94 050 past or current smokers. In addition to age at onset and rs16969968, we evaluated sex, birth cohort, and educational attainment in these analyses, variables that are related to smoking and easily harmonized across studies. To our knowledge, this study is the first large-scale systematic investigation of variation in the association between rs16969968 and CPD across strata of age at onset of smoking and other individual characteristics, a significant step in deepening our understanding of how we can modify genetic associations for smoking behaviors.

METHODS

STUDY PARTICIPANTS

This study is a meta-analysis of 94 050 individuals from 43 data sets. Written informed consent was obtained from all the participants. Inclusion in the study required (1) reported

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number of CPD and (2) the availability of genotype data for rs16969968 or its proxy (rs1051730). All the participants were ever-smokers of European descent aged 25 years or older; within each sample, only unrelated individuals were included. The sample sizes for each of the studies included in this meta-analysis are listed in the Table. A brief description of each sample is provided in eAppendix 1 (http://www.archgenpsychiatry.com).

ETHICS STATEMENT

This study was conducted according to the principles expressed in the Declaration of Helsinki and obtained informed consent from participants and approval from the appropriate institutional review boards.

VARIABLES

The primary variables examined were smoking quantity (CPD) and age at onset of regular smoking. Secondary variables were sex, birth cohort, and educational attainment. The text used to ascertain these variables for each study is given in eTable 1.

Previously published meta-analyses of smoking behavior have used 2 different codings of CPD: (1) heavy smoking (CPD >20) vs light smoking (CPD ≤10), excluding the intermediate group,[19] and (2) CPD as a linear variable coded 0 (CPD ≤10), 1 (CPD of 11–20), 2 (CPD of 21–30), and 3 (CPD >30).[16–18] We used both variables for these meta-analyses. The 2 variables gave similar beta values and statistically similar results. For ease of interpretation, results are reported for heavy vs light smoking. The results using CPD as a linear variable are given in eTable 2.

Age at onset of regular smoking was dichotomized as 16 years and younger or older than 16 years, representing the median of smoking onset for smokers of all ages surveyed between 1992 and 2002 in the United States (http://riskfactor.cancer.gov/studies/tuscps/info.html). This definition corresponds to the dichotomization of age at onset of smoking used by Weiss and colleagues.[10] Of the 94 050 current or former smokers in the samples, 61 128 had age at onset of smoking reported, and 33 348 of these were either heavy smokers (CPD >20) or light smokers (CPD ≤10). There were current and former smokers in both groups. These 33 348 individuals, analyzed separately at each site, represent the individuals included in the primary meta-analysis.

The SNP of interest is rs16969968. However, the most frequently genotyped tag SNP for rs16969968 is rs1051730, which has an r^2= 1.00 with rs16969968 based on the CEU 1000 Genomes sample.[29,30] Therefore, rs1051730 was used in analyses when rs16969968 was unavailable. We report the results as an association with rs16969968 because (1) they are statistically equivalent results and (2) there is biological evidence that rs16969968 alters receptor function.[21] The minor allele frequency for each sample in the meta-analysis is given in the Table.

QUALITY CONTROL

Genotyping of each data set was performed locally. Strict quality control measures were implemented at each site and are detailed in eAppendix 1. For the meta-analysis, we implemented quality control procedures to ensure that data from individual studies were analyzed correctly. Specifically, we examined univariate and multivariate distributions of all the variables in each study to ensure consistent coding within and across studies.

STATISTICAL ANALYSES

To ensure uniform analyses, scripts in a commercially available software program (SAS; SAS Institute, Inc) and in the freeware program R (http://www.r-project.org) for genetic

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association analyses were developed centrally and were distributed following protocols similar to those of Saccone et al.[19] The scripts were executed by each participating group, and the results were returned to the coordinating group, where the meta-analysis was performed.

The diversity of individual data sets did not allow for a single reference group for age at onset of smoking across all studies. Accordingly, each site performed logistic regressions with heavy vs light smoking as the dependent variable (modeling the probability of heavy smoking) and sex and rs16969968 (coded additively) as the independent variables, stratified by age at onset of smoking (in individuals with age at onset of smoking ≤16 years and >16 years). Heterogeneity in the meta-analysis was assessed using the goodness-of-fit statistic Q', detailed in eAppendix 2.

To evaluate the robustness of these findings, we explored the sensitivity of the results to alternative codings of CPD and age at onset. The 2 alternative CPD codings used included (1) heavy smoking (CPD >20) vs light smoking (CPD ≤10), excluding the intermediate group, and (2) CPD as an ordinal variable coded 0 (CPD ≤10), 1 (CPD of 11–20), 2 (CPD of 21–30), and 3 (CPD >30), as described previously herein. The 3 alternative codings of age at onset included (1) dichotomized as 16 years or younger or older than 16 years (primary variable); (2) categorized as younger than 15, 15 to 16, 17 to 18, and older than 18 years; and (3) categorized by within-study quartile. The results of the secondary analyses are presented in detail in eAppendix 3.

The interaction between the secondary variables (sex, birth cohort, and educational attainment) and rs16969968 on smoking behavior was evaluated by a stratified meta-analysis of CPD (coded as a 4-level trait). These results are presented in eFigures 1 to 4.

RESULTS

Figure 1 shows the meta-analysis forest plots for the association between heavy vs light smoking and the rs16969968 *A* allele, stratified by early- and late-onset smoking. Specifically, this meta-analysis tests whether the genetic risk for heavy smoking, based on the rs16969968 *A* allele, is different between early- and late-onset smokers (n=33 348). Smokers in the intermediate smoking level were excluded (CPD of 11–20). Compared with individuals with the rs16969968 *GG* genotype (wild-type allele), the odds ratio (OR) in early-onset smokers was 1.45 (95% CI, 1.36–1.55) for individuals with *AG* genotypes and 2.10 (95% CI, 1.97–2.25) for individuals with *AA* genotypes. In late-onset smokers, the OR is 1.27 (95% CI, 1.21–1.33) for individuals with *AG* genotypes and 1.61 (95% CI, 1.54–1.69) for individuals with *GG* genotypes. The difference between the OR in early-onset smokers and the OR in late-onset smokers is significant (P=.01). However, the current model does not adequately capture the heterogeneity among data sets: the heterogeneity χ^2 value (Q') was calculated to be 117 (P<.001, 69 *df*).

The relationship among smoking, genotype, and age at onset of smoking is detailed in Figure 2. Consistent with previous work, early onset of smoking and rs16969968 contribute to the risk of heavy smoking. Initiating smoking earlier than 16 years of age is significantly associated with higher risk of heavy smoking (OR = 2.63, unadjusted for genotype; 95% CI, 2.49–2.78; P < .001). The *A* allele at rs16969968 is associated with a higher risk of heavy smoking (OR=1.28 per *A* allele, unadjusted for age at onset; 95% CI, 1.25–1.32; P < .001, modeled additively). The interaction between early-onset smoking and the *A* allele at rs16969968 is 1.16 (P=.01), with OR=1.27 in late-onset smokers compared with OR = 1.46 in early-onset smokers. In Figure 2, the interaction is illustrated by the larger differences

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between bar heights in the early-onset data compared with the late-onset data, corresponding to a larger genetic effect in the early-onset group.

Because early-onset smoking is a strong risk factor for heavy smoking in adulthood and age at onset of smoking is a heritable characteristic, we must consider the possibility that a shared genetic factor could lead to early-onset smoking and heavy smoking in adulthood. However, rs16969968 does not seem to be a shared genetic factor between early-onset smoking and heavy smoking. Specifically, no association was seen between dichotomized age at onset and rs16969968 in this sample of 67 128 smokers ($P = .77$). This suggests that a direct relationship between rs16969968 and age at onset is unlikely to explain the observed association between smoking quantity and rs16969968 in early-onset smokers.

The robustness of the statistical interaction was evaluated by varying the way in which CPD and age at onset were modeled. Finding an increased genetic association in early-onset smokers seems robust across CPD phenotypes and definitions of early onset. There was no statistical interaction observed between rs16969968 and sex, birth cohort, or educational attainment. The results of these analyses are detailed in eFigures 1 to 4 and eTables 1 and 2.

COMMENT

Once genetic factors contributing to disease susceptibility have been identified, the next goal is to find factors that can reduce an individual's genetic risk. We designed this large meta-analysis to determine whether age at onset modifies a known genetic association with smoking behavior. We found that the genetic risk of heavy smoking (as measured by the rs16969968 *A* allele) is greater in early-onset smokers (onset of smoking at ≤16 years) compared with later-onset smokers. These results are consistent with the findings of Weiss and colleagues[10,28] and are supported by animal models in which the developing adolescent brain has been shown to be particularly vulnerable to addictive effects of nicotine[31–35] and by human studies suggesting that adolescent neurodevelopment is a particularly vulnerable period for the development of addiction.[36] In addition, the increased association in early-onset smokers is consistent with the epidemiologic observation of increased vulnerability to dependence in early-onset smokers.[9,24,26] With this large international collaborative analysis, we also demonstrated that the stronger association of rs16969968 in white early-onset smokers is consistent across different continents (North America, Europe, and Australia).

The finding of a stronger genetic risk in early-onset smokers supports public health interventions to reduce adolescent smoking. However, of the variables evaluated in this study, the strongest single risk factor of heavy smoking is early-onset smoking, highlighting the importance of a reduction in adolescent smoking across the entire population, not just in individuals with the risk allele of rs16969968. Nonetheless, there is a robust debate on whether policy and other interventions aimed at curbing early use would have significant effects on the development of dependence and related health problems over the long term. Although early use is associated with greater vulnerability to addiction,[9,24] early use is also associated with a variety of behaviors reflecting a vulnerability already in place before the onset of use.[37] Accordingly, early use may not cause greater vulnerability to addiction; instead, early use and vulnerability to addiction may have a shared etiology.

Age at onset of regular smoking is a heritable trait consistently documented by twin studies, and it is influenced by environmental exposures, such as parental smoking and peer smoking.[37] In addition, there is evidence from twin studies for a shared susceptibility to early-onset smoking and nicotine dependence.[37] Therefore, it is interesting that we did not observe a direct association between rs16969968 and age at onset. Further analyses suggest that this result is robust to adjustment for sex, age, and coding of age at onset. We cannot

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rule out the possibility that unmeasured variables suppressed a true association between rs16969968 and age at onset. Further study is warranted to investigate the relationship between early smoking behaviors and genes in this region to improve our understanding of the mechanism by which age at onset modifies the genetic association with heavy smoking.

It is somewhat sobering to note that despite (1) the strong effect of age at onset on smoking behavior, (2) the strong genetic effect of the rs16969968 genotype on smoking behavior, and (3) the subjectively meaningful interaction between the 2 effects (increasing the OR per genotype from 1.27 to 1.46), the large sample detected this interaction with a somewhat modest P=.01. This result highlights the power needed to detect gene-environment interactions in complex disease.

There are several limitations of this study. First, as in any meta-analysis, this study is based on heterogeneous samples with differential assessment of measures. In addition there was statistical evidence of heterogeneity. Heterogeneity likely contributes to a lack of precision in this analysis. However, because misclassification is independent of genotype, any bias under the alternative hypothesis would decrease the power of the study (leading to an underestimate of the interaction effect).[38] Since we had adequate power to detect an interaction on heavy vs light smoking between rs16969968 and age at onset of regular smoking, improved homogeneity of the studies should only strengthen this result.

Although rs16969968 is the most strongly associated SNP genome-wide, multiple associations in the region form haplotypes associated with varying risk of smoking behavior.[10,16–19] Furthermore, although this article references rs16969968 as the likely causal association, more than half of the studies used the SNP rs1051730 to tag rs16969968, and there are multiple other SNPs spanning the *CHRNA5-CHRNA3-CHRNB4* cluster that are indistinguishable from rs16969968 in populations of European descent. Therefore, the SNP analysis is a simplification of the true genetic model and does not represent the full complexity of the relationship of age at onset with the association between genotype and smoking in this region. In addition, further study is required to better understand other modifiers of the association between CPD and rs16969968. For example, parental monitoring and peer smoking have been shown to modify this genetic association.[39,40]

A potential source of bias in the data is early death due to smoking in early-onset smokers. This could artificially cause an interaction between rs16969968 and early smoking onset if the early deaths were preferentially in individuals who lack the risk allele. However, if this were the case, we would expect to see a stronger interaction among individuals born in the 1920–1939 birth cohort compared with in the 1960–1979 birth cohort, which is not the case.

In summary, in a large meta-analysis, we found an increased association between CPD and rs16969968 in early-onset smokers, which helps explain the epidemiologic observation of increased rates of nicotine dependence in early-onset smokers. These results provide further compelling evidence in support of public health interventions targeting adolescent smoking.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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U01HG004446, UL1RR024992, NIH DA12854, DA019951, R21DA033827, HHSN268200782096C, U01 HG004738, U01DA02830, R01 MH67257, R01 MH59588, R01 MH59565, R01 MH59587, R01 MH60870, R01 MH59566, R01 MH59586, R01 MH60879, R01 MH81800, U01 MH46276, MH46289, MH46318, U54 RR020278, R01 DA017932, and R01 DA03706 from the National Institutes of Health; the Intramural Research Program of the National Institutes of Health, National Institute on Aging; grant 7PT2000-2004 from the University of California Tobacco-Related Disease Research Program; Academy of Finland (project grants 104781 and 120315 and the Center of Excellence in Complex Disease Genetics); University Hospital Oulu; the European Commission; the European Commission's Sixth Framework Program, Integrated Project GENADDICT (LSHM-CT-2004-005166), and the Seventh Framework Program, Integrated Project ENGAGE (HEALTH-F4-2007-201413); Academy of Finland Center of Excellence in Complex Disease Genetics, Global Research Awards for Nicotine Dependence; SALVE program grant 129494; The Finnish Foundation for Cardiovascular Diseases; Alfried Krupp von Bohlen und Halbach-Stiftung; German Federal Ministry of Education and Research grants 01ZZ9603, 01ZZ0103, 01ZZ0403, NGNF-2, NGFNplus, IG MooDS: 01GS08144, and 01GS08147; Geestkracht programme of the Dutch Scientific Organization (ZON-MW grant 10-000-1002); matching funds from participating institutes; Center for Molecular and Systems Biology; the Genetic Association Information Network of the Foundation for the US National Institutes of Health, German Research Foundation (DFG: GR 1912/5-1); University of Helsinki Biomedical Graduate School; Medical Research Fund of Tampere University Hospital; National Institute for Health Research Academic Clinical Fellowship at the Division of Mental Health, St George's, University of London; NARSAD Young Investigator Award; Well-come Trust; The Paul Michael Donovan Charitable Foundation; The Andrew W. Mellon Foundation; and The Mary Beryl Patch Turnbull Scholar Program.

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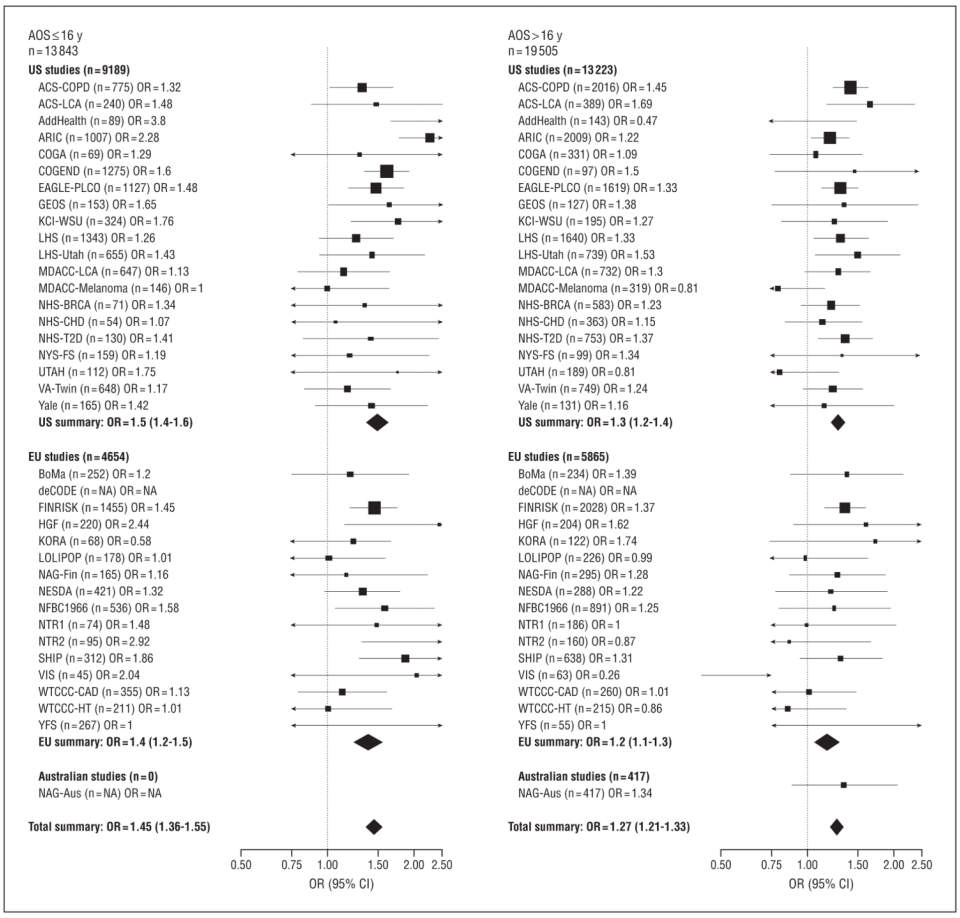

Figure 1.

Odds ratios (ORs) for heavy vs light smoking and rs16969968 *A* allele across studies where individuals are stratified by age at onset of regular smoking (AOS) of 16 years or younger vs older than 16 years. The *P* value for the difference between early onset and late onset is *P* = .01, based on 33 348 heavy and light smokers. The studies are grouped by continent to allow for cross-cultural comparisons. NA indicates not available.

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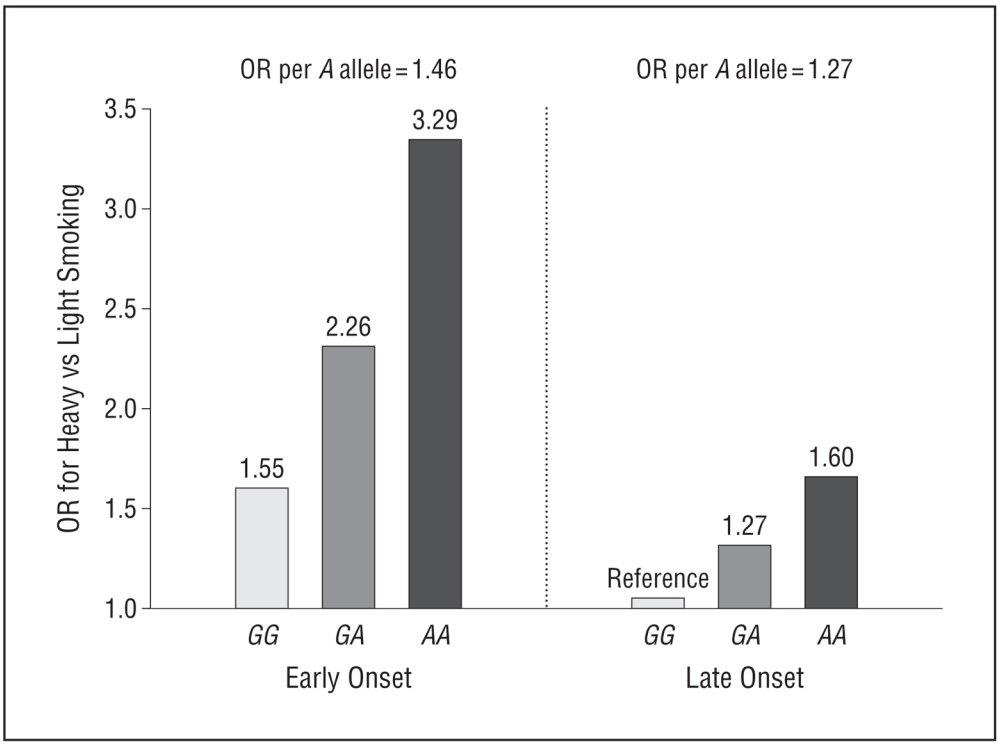

Figure 2.

Meta-analysis of the association between the rs16969968 genotype and heavy (cigarettes per day [CPD] >20) vs light (CPD ≤10) smoking, stratified by early-onset (age at onset ≤16 years) and late-onset (onset >16 years) smoking. Odds ratios (ORs) are given relative to late-onset smokers with the *GG* genotype. Effect of the interaction between the rs16969968 *A* allele and early-onset smoking on risk of heavy smoking: OR = 1.16, n = 36 936, *P*= .01.

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Table 1

Table Sample Sizes and *A* Allele Frequencies of rs16969968 for Studies Included in This Meta-analysis

Study	Participants, No. (n = 94 050)	Allele Frequency of rs16969968-*A*
US Data Set		
AddHealth	469	0.31
ACS-COPD	2775	0.35
ACS-LCA	1005	0.38
ARIC	9713	0.34
COGA	1704	0.34
COGEND	2073	0.35
Dental caries[a]	639	0.36
EAGLE-PLCO[a]	5955	0.40
GEOS	477	0.37
KCI-WSU	979	0.38
LHS	1943	0.39
LHS-Utah	1943	0.39
MDACC-LCA[a]	2291	0.37
MDACC-Melanoma[a]	847	0.33
MGS	2988	0.34
NHS-BRCA[a]	1198	0.35
NHS-CHD[a]	724	0.35
NHS-T2D[a]	1590	0.34
NYS-FS	751	0.34
SMOFAM[a]	146	0.34
UTAH[a]	484	0.39
VA-Twin	2768	0.33
Yale	1300	0.35
Australian Data Set		
NAG AUS[a]	1329	0.36
European Data Set		
BOMA	1050	0.36
deCODE[a]	16 404	0.35
DNBC[a]	370	0.34
FINRISK	7864	0.33
GenMetS[a]	648	0.34
HGF	719	0.42
KORA	1319	0.34
KORCULA[a]	384	0.39
LOLIPOP	646	0.31

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Study	Participants, No. (n = 94 050)	Allele Frequency of rs16969968-*A*
	US Data Set	
MUC12 cases[a]	421	0.36
MUC12 controls[a]	235	0.34
MUCMD cases[a]	641	0.37
MUCMD controls[a]	1052	0.35
NAG-Fin[a]	733	0.37
NESDA	1248	0.29
NFBC1966[a]	3067	0.32
NTR1[a]	1777	0.39
NTR2[a]	1173	0.31
SardiNIA	531	0.35
SHIP	4081	0.34
VIS[a]	436	0.39
WTCCC-CAD	1221	0.32
WTCCC-HT	801	0.30
YFS[a]	694	0.32

[a]Used rs1051730 instead of rs16969968 (r^2 = 1.00, based on CEU 1000 Genomes data).