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# Commentary: Does mortality from smoking have implications for future Mendelian randomization studies?

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The evidence for a causal association between tobacco consumption and mortality presented in the Mendelian randomization (MR) study by Rode and colleagues[1] is not unexpected. Nevertheless, it is the first time that the smoking-mortality relationship has been demonstrated using these causal analysis methods, although studies of

 

monozygotic twins discordant for smoking have arrived at similar conclusions.[2] This is an important proof of principle of the MR technique for investigating the causal effects of smoking. However, this finding also raises a potentially important methodological issue for future MR studies using this variant.

The genetic variant used in this analysis, rs1051730, is a single nucleotide polymorphism (SNP) in perfect correlation with a variant in the *CHRNA5* gene (rs16969968) that leads to an amino acid change (D398N) in the nicotinic receptor alpha-5 subunit protein. This is by far the strongest genetic determinant of smoking behaviour identified in genome-wide association studies to date.[3] This variant is robustly associated with smoking heaviness among smokers and shows evidence in some populations of associations with smoking cessation.[4,5] However, associations with smoking initiation (i.e. being an ever rather than a never smoker) have not been clearly established.[6]

The lack of evidence for an association of this variant with ever smoking has been a useful feature of the

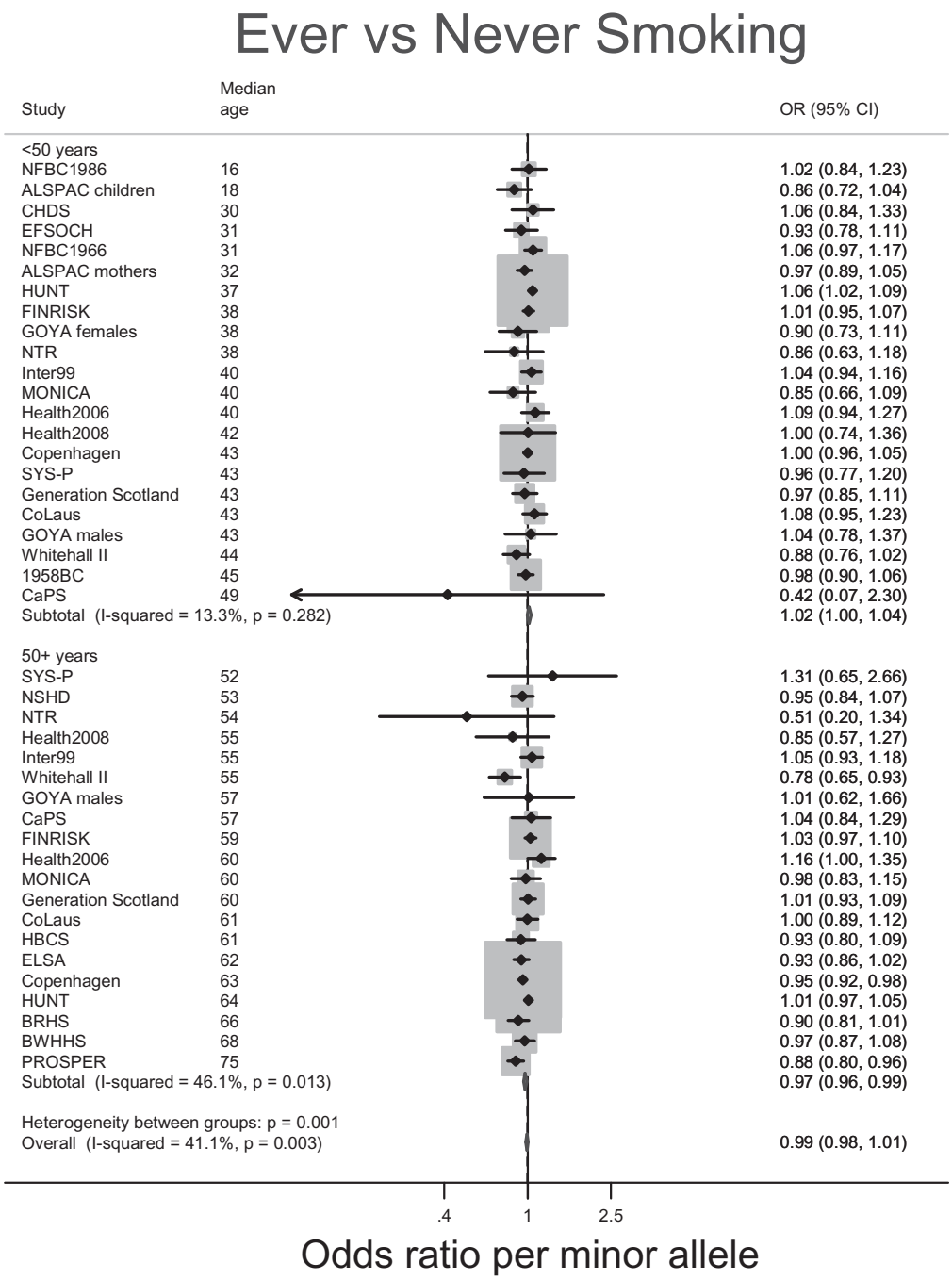


**Figure 1.** Association between rs16969968-rs1051730 and smoking initiation in the CARTA consortium and the Copenhagen General Population Study.

smoking MR analyses conducted to date. As the variant only associates with smoking heaviness among smokers, never smokers can be used as a control group to test the assumption of no pleiotropy. The lack of association between rs1051730 and mortality in never smokers in the analyses by Rode and colleagues is good evidence that these effects are due to tobacco consumption, and not to a pleiotropic effect of the gene.[1]

However, this in turn raises an interesting possibility. We would expect that higher rates of mortality among smokers carrying the smoking-increasing allele of this variant[1] would lead to an association between genotype and likelihood of being an ever smoker in older age. As smokers with the smoking-increasing allele are less likely to survive into old age, the smoking increasing allele will in turn become less prevalent among smokers as the age of the population increases. Some support for this was found by Rode and colleagues in the Copenhagen General Population Study, where there was some evidence that the smoking increasing allele of rs1051730 was weakly associated with lower age in ever smokers.[1] Furthermore, a negative association between the smoking-increasing allele and being an ever smoker, could potentially mask associations between the variant and smoking initiation in samples with a wide age range.

It is not currently clear to what extent this attrition by genotype impacts on associations with ever smoking. Figure 1 shows data from the Copenhagen General Population Study combined with data from the consortium for Causal Analysis Research in Tobacco and Alcohol (CARTA: http://www.bris.ac.uk/expsych/research/brain/targ/research/collaborations/carta), a collaboration of over 30 studies established to conduct MR analyses of the health and socioeconomic effects of smoking. We stratified analyses within each study by age (<50 years, ≥50 years), based on data suggesting that the effects of smoking on mortality are strongest after the age of 50 years.[7] There is suggestive evidence that the smoking increasing allele may be positively associated with smoking initiation (i.e. ever vs never) in the under-50 age group, but negatively associated with smoking initiation in the 50 and over age group (*P*-value for heterogeneity between age groups = 0.001). It

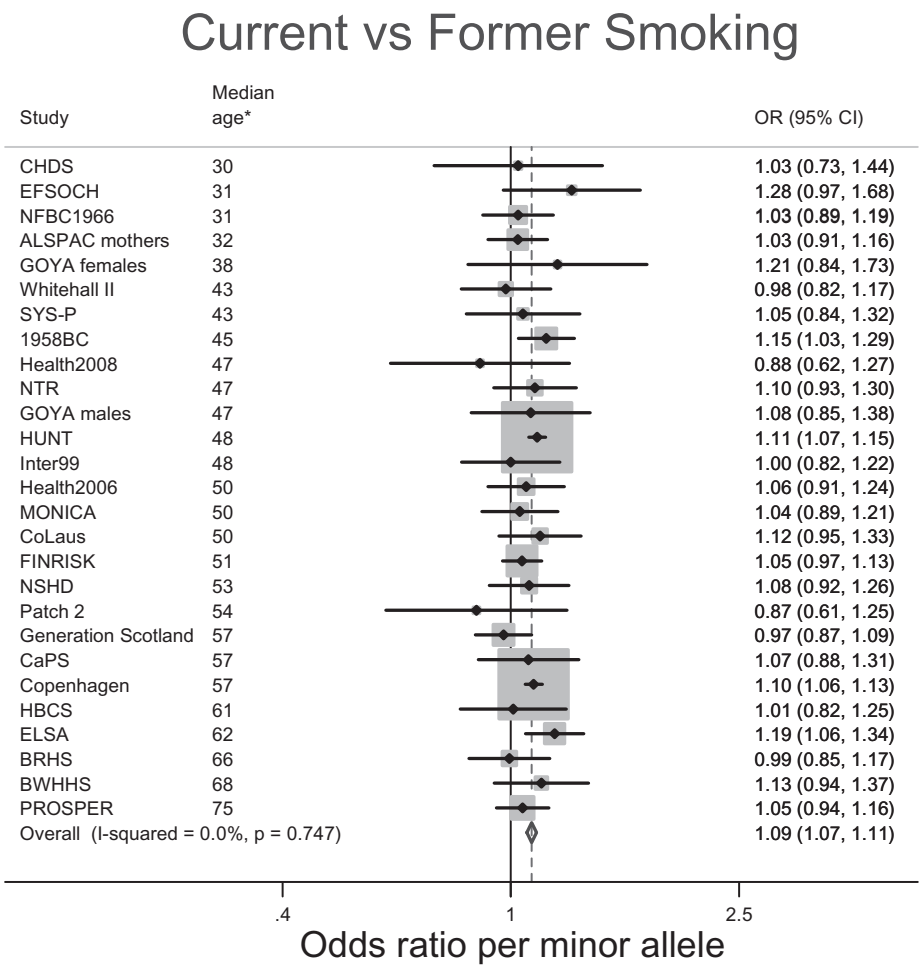


**Figure 2.** Association between rs16969968-rs1051730 and smoking cessation in the CARTA consortium and the Copenhagen General Population Study. *Median age is median age of total sample (including never smokers).

is important to note that these data are not conclusive, and more in-depth analyses of this possibility are required. For example, the crude age stratification we have employed may not be the most appropriate. Family studies may also be useful for investigating selection effects due to mortality, as genotypes of missing parents may be inferred from offspring genotypes. Within the CARTA and Copenhagen General Population Study samples, there is also clear evidence for an effect of rs16969968-rs1051730 genotype on smoking cessation, with the minor allele associated with an 9% increase in the odds of being a current rather than a former smoker [95% confidence interval (CI): 7% to 11%] (see Figure 2).

Associations between genotype and smoking status have potentially important implications for future MR studies of the causal effects of smoking. This is because stratification on a common effect of two variables can induce a phenomenon known as collider bias.[8] If smoking status is a common effect of both genotype and either the outcome of interest or confounders of the association between smoking status and the outcome of interest, this can lead to a statistical association between genotype and outcome measure in the absence of a true causal effect.[9] Researchers will therefore need to consider associations between rs16969968-rs1051730 and smoking initiation and cessation when conducting MR analyses. The association between this variant and smoking cessation may be less problematic if we are interested in the difference between ever and never smokers. One approach to overcome the problem of collider bias, which has been used in MR analyses of alcohol, is to stratify analyses by an exogenous variable, sex, rather than by drinking status.[10] This can only be done in samples where prevalence of alcohol consumption is strongly patterned by sex (e.g. in East Asian populations where alcohol consumption is very low among females[11]). This may not be possible for smoking MR due to the low allele frequencies of rs16969968-rs1051730 in many non-European populations.

MR is a potentially very useful tool for unravelling the causal effects of tobacco use and the pathways through which these operate; there are still many health outcomes which are strongly associated with smoking, but for which causal evidence is lacking. It is important that future MR studies of tobacco use take into account issues such as collider bias and other selection biases related to this variant, such as misreporting of smoking status, to prevent the reintroduction of confounding into analyses designed to minimize this problem.

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