The causal role of smoking on the risk of headache. A Mendelian randomization analysis in the HUNT Study

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ABSTRACT

Background: Headache has been associated with various lifestyle- and psychosocial factors, one of which is smoking. The aim of the present study was to investigate whether the association between smoking intensity and headache is likely to be causal.

Method: 58 316 participants from the Nord-Trøndelag Health Study (HUNT) with information on headache status were genotyped for the rs1051730 C>T single-nucleotide polymorphism (SNP). The SNP was used as an instrument for smoking intensity in a Mendelian randomization (MR) analysis. Association between rs1051730 T alleles and headache was estimated by odds ratios (OR) with 95% confidence intervals (CI). Additionally, we investigated the association between the SNP and migraine or non-migrainous headache vs. no headache. All analyses were adjusted for age and sex.

Results: There was no strong evidence that the rs1051730 T allele was associated with headache in ever smokers (OR 0.99, 95% CI 0.95-1.02). Similarly, there was no association between the rs1051730 T allele and migraine or non-migrainous headache vs. no headache.

Conclusion: Findings from this study do not support that there is a strong causal relationship between smoking intensity and any type of headache. Larger MR studies are required to examine whether higher smoking quantity can lead to a moderate increase in the risk of headache subtypes.

INTRODUCTION

Primary headache disorders are highly prevalent, with migraine and tension-type headache being the most common, affecting 14.7% and 20.8% of the world´s population respectively [1]. As much as 15% of the population report absence from work during the last year because of headache, and migraine alone is ranked as the 7th leading cause of disability worldwide [2, 3]. Even though headache has been linked to various lifestyle- and psychosocial factors [4-6], there is limited actionable knowledge about modifiable risk factors. Headache patients are often advised to change their lifestyle, including stopping smoking and increase their amount of physical exercise [5, 7]. However, the basis for such advice is largely based on epidemiological studies where definite conclusions about causal relationships cannot be drawn. Multiple studies have indicated that smoking is associated with headache with odds ratios from 1.3 to 1.5 [4, 5, 8]. The positive association seems to be true for both migraine and non-migrainous headache and is most evident among young adults [4]. Whether smoking causes headache or headache leads to altered smoking behavior is however uncertain.

Mendelian randomization (MR) offers an alternative approach to conventional observational studies, with the potential for stronger causal claims [9]. In MR, genetic variants are used as proxies for the exposure of interest. Since genetic variants are transmitted from parents to offspring at random during meiosis, they are not generally susceptible to confounding and reverse causation bias [9]. The single-nucleotide polymorphism (SNP) rs1051730 is the strongest genetic variant related to smoking behavior identified in genome-wide studies to date [10, 11]. In smokers, each additional T allele of the rs1051730 SNP is associated with an increased intensity of smoking corresponding to around one additional cigarette per day [11, 12]. Our aim was to explore whether smoking intensity has a casual effect on headache, using a MR approach.

MATERIAL AND METHODS

Study population

All inhabitants aged 20 years or older living in the county of Nord-Trøndelag, Norway, were invited to participate in the Nord-Trøndelag Health Study (HUNT) [13, 14]. For the present study, we included participants who had attended HUNT2 (1995-97) or HUNT3 (2006-08) (Figure 1 and Supplementary methods 1). All participants in HUNT have signed a written informed consent regarding the use of data from questionnaires and blood samples for research purposes [13, 14]. The current study was approved by the Regional Committee for Medical and Health Research Ethics (REK), 2012/2291/REK South-East C.

Genotyping

The HUNT sample was genotyped using one of three different Illumina HumanCoreExome arrays (HumanCoreExome12 v1.0, HumanCoreExome12 v.1.1 and HumanCoreExome-24+ v1.1 with custom content). Imputation was performed using Minimac3 (v2.0.1, http://genome.sph.umich.edu/wiki/Minimac3) [15] with default settings and a customized Haplotype Reference consortium release 1.1 (HRC v1.1) [16]. (Supplementary methods 1).

Smoking

Smoking status was self-reported and categorised as never, former and current daily smokers. Current smokers were asked to report the average number of cigarettes smoked per day (Supplementary methods 1).

Outcome

In the HUNT questionnaire, those who answered "yes" to "Have you suffered from headache during the last 12 months?" were classified as headache sufferers. Those who answered "no" were defined as headache-free controls. The headache sufferers were further classified as having

migraine or non-migrainous headache, based on a modified version of the International Classification of Headache Disorders (ICHD II) [17] (Supplementary methods 1).

Statistical methods

Descriptive statistics according to the number of rs1051730 T alleles were compared using χ^2 test for categorical variables and a linear regression for continuous variables. We assumed an additive genetic model, so risk estimates represent the odds ratios (ORs) per additional copy of the T allele.

SNP-exposure association:

We used linear regression to estimate the association between rs1051730 T alleles and smoking intensity.

Exposure-outcome association (observational analysis):

Logistic regression with ORs and 95% confidence intervals (CIs) was used to estimate the relationship between smoking intensity, or smoking status, and headache (Supplementary methods 1). In a sensitivity analysis, we restricted former smokers to those who reported smoking cessation > 1 year ago to avoid bias from current smoking at time of reporting headache.

SNP-outcome association (MR analysis):

Logistic regression was used to estimate the relationship between rs1051730 T alleles and headache (Supplementary methods 1). The main analysis was in strata of never vs. ever smokers (current and former smokers combined). Additionally, we stratified the SNP-headache association by current, former or never smoking. In a secondary analysis, we investigated the association between the SNP and migraine, or non-migrainous headache, vs. no headache, using a multinomial logistic regression. We also performed the MR analysis stratified by age < vs. ≥ 40

[4]. All analyses were adjusted for age and sex. Additionally, we adjusted for BMI as a potential confounder/mediator.

RESULTS

In total 58 316 participants were genotyped for rs1051730 and had information about headache. Of these, 25 666 (46.0%) were never smokers, 14 799 (26.5%) former smokers, 15 278 (27.4%) current smokers (Table 1). Among the 58 316 participants, 39.4% reported headache; 12.9% had migraine and 26.5% had non-migrainous headache (Figure 1). The probability of being a current smoker and the number of cigarettes smoked per day increased by number of rs1051730 T alleles. There was a slight decrease in age and BMI according to increasing number of T alleles. There was no difference in the prevalence of headache according to the number of T alleles (Table 1).

SNP-exposure association:

Among current smokers, each additional T allele was associated with 0.68, 95% CI 0.55-0.80 additional cigarettes smoked per day.

Observational analysis:

There was a positive association between smoking intensity and headache (Figure 2). The association (OR) between each additional cigarette smoked per day and headache was 1.02 (95% CI 1.01-1.02). According to smoking status, both current smoking (OR 1.30, 95% CI 1.25-1.36) and former smoking (OR 1.24, 95% CI 1.19-1.30), compared to never smoking, were positively associated with headache. Former smokers (cessation > 1 year ago) compared with never smokers also had increased odds of headache, OR 1.24 (95% CI 1.18-1.29).

MR analysis:

There was no association between rs1051730 T alleles and headache in ever smokers (OR 0.99, 95% CI 0.95-1.02) or in never smokers (OR 1.00, 95% CI 0.96-1.04) (Table 2). Moreover, there

was no association between the rs1051730 T allele and headache in current smokers (OR 1.00, 95% CI 0.96-1.06) (Table 3).

We found no association between the T allele and migraine or non-migrainous headache vs no headache (Table 2 and Table 3). Stratified analysis by age (< 40 or ≥ 40 years) showed no statistically significant associations between the rs1051730 T allele and headache vs no headache (Supplementary Table 1). Adjustments for BMI did not change the association between the T allele and any type of headache (data not shown).

DISCUSSION

We did not find evidence for a causal effect of smoking intensity on headache. While a positive association was observed between the number of cigarettes smoked per day and headache, there was no association between the rs1051730 T alleles and any type of headache. The results indicate that there is no strong causal effect of smoking intensity on headache overall.

The observational association between smoking status and headache in the present study is comparable to results from previous studies (ORs 1.3 to 1.6) [4, 5, 8]. Regarding smoking intensity, Gan et al. [18] found in a cross-sectional survey that the number of cigarettes smoked per day was associated with headache. Smoking between 11-18 cigarettes per day was associated with an OR of 1.53 for having headache, compared to never smokers [18]. In the observational analysis, we found the OR for headache to be 1.02 per cigarette, corresponding to an OR between 1.20-1.50 for headache if you smoked 10-20 cigarettes per day. The association between former smoking and headache was also present for former smokers who had quit smoking >1 year ago (OR 1.24), which confirmed that the observed association was not due to current smoking at time of reporting headache alone. However, the magnitude of the observational effect estimate should not be given too much emphasis. The current study was designed for MR analysis, where the aim was to determine whether the association observed between smoking and headache is likely to be causal, and not for determining the exact magnitude of the effect estimate. The significant association we found in the observational

analysis was, however, not replicated in the MR analysis. Still, we cannot refute by our MR analysis that a moderate causal association of smoking intensity on headache exists, as the 95% CI for our MR analysis included the magnitude of the observational association.

A former study on the same HUNT population found an association between current smoking (for those who smoked ≥ 10 cigarettes per day) and headache, in those younger than 40 years of age (OR 1.50), but not in those age ≥40 years [4]. However, the headache diagnoses in HUNT include only current headache (present within the last 12 months), which might partly explain this finding, since headache prevalence, and in particular migraine prevalence, is decreasing in the older age groups [19]. We therefore stratified by age 40 to investigate whether a causal effect of smoking might depend on age. Although the point estimate for the association was higher for those aged <40 (OR 1.07) compared to those aged ≥40 years (OR 0.97), there was no significant association in either group.

The large sample size and the use of a well-defined genetic instrument are strengths of this study. Using rs1051730 as a proxy for smoking intensity allowed us to examine the relationship between smoking and headache in a pseudo-randomized manner, avoiding confounding and reversed causation. Another strength is the use of validated headache diagnoses, but the use of questionnaire-based diagnoses will necessarily give some degree of misclassification, which could deflate our estimates. This deflation would probably have been most pronounced for non-migrainous headache, which had the lowest agreement between questionnaire-based and interview diagnoses [20, 21]. Another limitation is that we assessed current headache only, causing headache-sufferers who have become headache-free prior to baseline to be classified as controls. Such misclassification is likely to weaken any observed effect, in particular, in the older age groups where cessation rates are high [19].

Non-participation in HUNT has been associated with an unhealthier lifestyle with respect to tobacco smoking, physical inactivity and poor somatic health status [22]. Non-responders to the headache question in the current study were more often men, current smokers and younger

(data not shown). This is in line with the overall characteristics of non-responders to the headache questions in HUNT [23]. While we cannot rule out an effect of selection bias, it is unlikely that the association between rs1051730 and headache should be substantially different among participants versus non-participants.

MR studies rely on three main assumptions: 1) The genetic variant is associated with the exposure, 2) the genetic variant is associated with the outcome through the studied exposure only, and 3) the genetic variant is independent of other factors that affect the outcome [9]. Regarding assumption 1, the association between rs1051730 and smoking intensity has been confirmed in previous genome-wide association studies [10, 11] and the association was verified in the current data. The second assumption is violated if the genetic variant has multiple effects, or if the genetic variant is in linkage disequilibrium with another variant that affects the outcome in other ways than through the exposure of interest. If we found an association between the rs1051730 T allele and headache in never smokers, we might be suspicious of such a violation since the SNP is a proxy for smoking intensity. This can be considered a test of the second assumptions; that the genetic variant only operates on the outcome via its effect on the exposure (i.e. no pleiotropy) [9]. However, in our case, no association was seen in any of the strata of smoking status and the results did not change after adjustments for BMI.

Stratification of a MR analysis by smoking status groups may lead to collider bias by conditioning on a common observed measure [9], thus potentially introduce bias from confounders to the exposure-outcome association that may violate the third assumption. For this reason, we performed the MR analysis in broader strata of never vs ever smokers, and adjusted for BMI, but no association between the rs1051730 T alleles and headache was seen.

To conclude, this MR analysis suggests that there is no strong causal effect of smoking intensity on headache. However, larger MR studies are required to examine whether higher smoking quantity can lead to a moderate increase in the risk of headache subtypes.

Author contributions

All authors contributed to the conception and design of the study. MBJ, BSW, KS and JAZ were responsible for acquisition of the data. MBJ and GÅV performed the analyses of the data, while all authors contributed to the interpretation of data. MBJ, BSW and SB drafted the first version of the manuscript. All authors revised the manuscript and gave their final approval of the submitted version.

Disclosure of conflict of interest

None declared.

Table captions

Table 1 Baseline characteristics of the study participants.

Table 2 Association between rs1051730 T alleles and headache.

Table 3 Association between rs1051730 T alleles and headache according to smoking status.

Supplementary methods 1 Additional description of material and methods.

Supplementary Table 1 Association between rs1051730 T alleles and headache according to age.

Figure captions

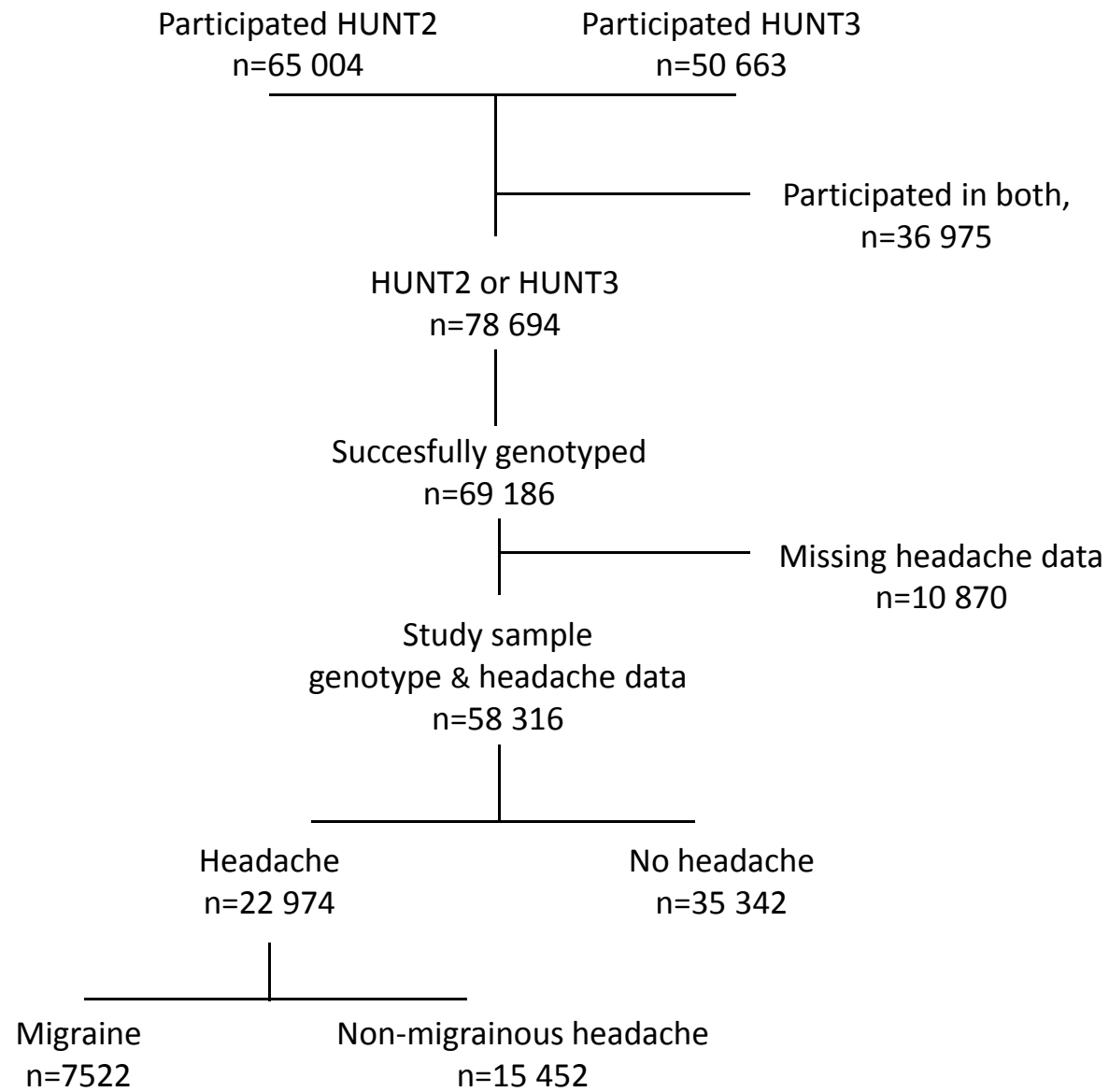**Figure 1** Flow chart of the study sample from the Nord-Trøndelag Health Study (HUNT).

Figure 2 Age- and sex-adjusted ORs for headache with 95% CI (grey) according to numbers of cigarettes smoked per day. Never and former smokers (zero cigarettes a day) are used as a reference group.

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Participated HUNT2
n=65 004

Participated HUNT3
n=50 663

Participated in both,
n=36 975

HUNT2 or HUNT3
n=78 694

Succesfully genotyped
n=69 186

Missing headache data
n=10 870

Study sample
genotype & headache data
n=58 316

Headache
n=22 974

No headache
n=35 342

Migraine
n=7522

Non-migrainous headache
n=15 452

Supplementary material 1 Additional description of the material and methods.

Study population

All inhabitants aged 20 years or older living in the county of Nord-Trøndelag, Norway, were invited to participate in the Nord-Trøndelag Health Study (HUNT). For the present study, we included participants who had attended HUNT2 (1995-97) or HUNT3 (2006-08) [1, 2]. We used HUNT2 as our main cohort and additionally included participants from HUNT3 who had not attended HUNT2 (Figure 1). This resulted in 78 694 participants, of which 69 186 had been successfully genotyped. After excluding 10 870 with missing headache data, our study sample comprised 58 316 participants.

Genotyping

DNA was extracted from blood samples collected at baseline in either HUNT2 or HUNT3 and stored at the HUNT Biobank. Genotyping was performed using one of three different Illumina HumanCoreExome arrays (HumanCoreExome12 v1.0, HumanCoreExome12 v.1.1 and HumanCoreExome-24+ v1.1 with custom content). Samples with call rate < 99%, or that failed other quality control criteria were excluded. Imputation was performed using Minimac3 (v2.0.1, http://genome.sph.umich.edu/wiki/Minimac3) [3] with default settings and a customized Haplotype Reference consortium release 1.1 (HRC v1.1) [4]. Imputed variants with R^2 < 0.3 or estimated minor allele frequency (MAF) below 0.05% were excluded. Imputation quality for the rs1051730 SNP was high with R^2=0.9999 and MAF=0.335, which was in agreement to HapMap-CEU data (MAF=0.389). There was no evidence of departure from the Hardy-Weinberg equilibrium (χ^2 test, p=0.20).

Smoking

Smoking status was self-reported in the questionnaire, categorised as never, former and current daily smokers. Current smokers were asked to report the average number of cigarettes smoked per day and former smokers to report the time since smoking cessation. Information about occasional smoking was only available from HUNT3. To obtain a homogenous exposure group of current daily smokers, we excluded those who reported to be occasional smokers only (n=1194 from HUNT3) from the analyses stratified by current, former and never smoking (presented in Table 3).

Outcome

In the questionnaire, those who answered "yes" to "Have you suffered from headache during the last 12 months?" were classified as headache sufferers. Those who answered "no" were defined as headache-free controls. The headache sufferers were further classified as having migraine or non-migrainous headache, based on a modified version of the International Classification of Headache Disorders (ICHD II) [5]. Based on the subsequent headache questions [6, 7], headache sufferers were classified as having migraine if they fulfilled all of the following criteria: (1) headache lasting from 4-72 hours (4 hours were accepted for those who reported commonly occurring visual disturbances before headache); (2) headache with at least one of the following characteristics: pulsating quality, unilateral location or aggravation by physical activity; (3) during headache, at least one of the following occurred: nausea, photophobia and phonophobia. In addition, the participants were asked if they suffered from migraine; those who responded positively to this question were also included in the migraine group. Headache sufferers who did not fulfill the criteria for migraine were classified as having non-migrainous headache. The headache diagnoses have previously been validated by clinical interviews performed by neurologists [6, 7], where the highest sensitivity and specificity were reported for headache (between 85-88% and 83-86%, respectively), then migraine (between 49-69% and 89-96%,

respectively) and lowest for non-migrainous headache (between 56-61% and 77-81%, respectively) [6, 7]. The main outcome in the MR analysis was headache vs no headache.

Statistical methods

Exposure-outcome association (observational analysis):

Logistic regression with ORs and 95% confidence intervals (CIs) was used to estimate the relationship between smoking intensity, or smoking status, and headache. To illustrate the association between the number of cigarettes smoked and the risk of headache, smoking intensity was expressed by a restricted cubic spline, which is a flexible description of a non-linear relationship, with knots at 0, 10, 20 and 30 cigarettes per day. Never and former smokers were assumed to smoke zero cigarettes and were used as the reference point. In a sensitivity analysis, we restricted former smokers to those who reported smoking cessation > 1 year ago to avoid bias from current smoking at time of reporting headache.

SNP-outcome association (MR analysis):

We performed the MR analysis, using logistic regression to estimate the relationship between rs1051730 T alleles and headache. Carriers of the rs1051730 T alleles may be less likely to quit smoking. Thus, stratification on current smoking may lead to collider bias by conditioning on an observed measure of exposure [8]. Therefore, the main analysis was in strata of never vs. ever smokers (current and former smokers combined). Additionally, we stratified the SNP-headache association by current, former or never smoking. In a secondary analysis, we investigated the association between the SNP and migraine, or non-migrainous headache, vs. no headache, using a multinomial logistic regression. We also performed the main MR analysis stratified by age < vs. ≥ 40, based on previous results of an interaction between smoking and age [4]. All analyses were adjusted for age and sex.

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Table 1. Baseline characteristics of the study participants.

	n^a	No. of rs1051730 T alleles			p-value[b]
		0	1	2	
Study sample* – %	58 316	44.4	44.3	11.3	
Smoking – %					
Never	25 666	46.4	45.7	45.6	
Former	14 799	27.2	26.2	25.1	
Current daily	15 287	26.3	28.1	29.3	<0.001
No. of cigarettes per day[c] – mean	15 003	10.7	11.4	12.1	0.008
Headache - %	22 974	39.3	39.4	39.5	0.96
Migraine	7522	12.9	13.0	12.7	
Non-migrainous headache	15 452	26.4	26.5	26.8	0.96
Age (years) – mean	58 313	48.1	47.9	47.6	0.03
BMI (kg/m^2) – mean	58 106	26.4	26.3	26.2	0.001
Women – %	31 446	53.5	54.2	54.8	0.08
Physical activity[d] – %					
Low	31 948	57.4	57.6	58.6	
High	23 509	42.6	42.4	41.4	0.21

*Participants who were genotyped and had information about headache.

[a]Total n varies from 55 457 to 58 316 due to missing data.

[b]χ^2 test for categorical variables and linear regression for linear associations according to number of T alleles.

[c] Among current smokers.

BMI = body mass index.

[d]Based on a weekly average of duration and intensity of physical activities during the last year; low=not being sweat or out of breath, high=being sweat or out of breath.
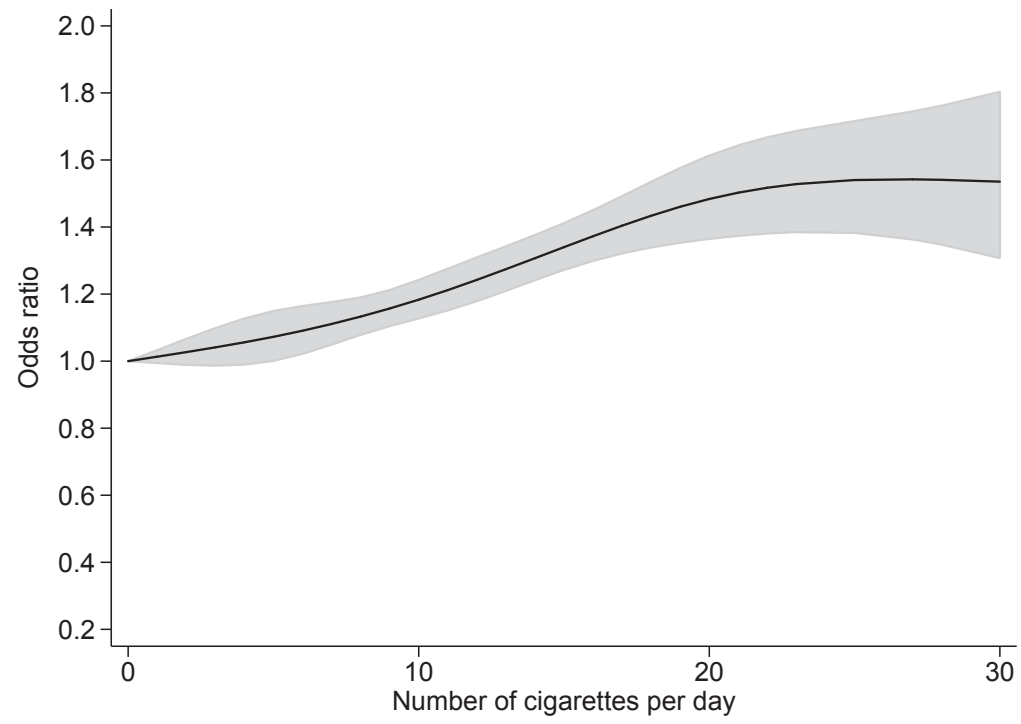
Table 2. Association between rs1051730 T alleles and headache.

Per T allele effect	All participants		Never smokers		Ever smokers	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Main analysis[a]						
Headache	0.99 (0.97-1.02)	0.61	1.00 (0.96-1.04)	0.91	0.99 (0.95-1.02)	0.41
Secondary analysis[b]						
Migraine	0.98 (0.95-1.02)	0.38	0.99 (0.93-1.04)	0.63	0.97 (0.92-1.03)	0.32
Non-migrainous headache	1.00 (0.97-1.03)	0.89	1.00 (0.96-1.05)	0.88	0.99 (0.95-1.03)	0.63

OR=odds ratio, CI=confidence interval.

[a]Headache vs no headache, adjusted for age and sex, n=58 313 (all), n=56 938 (according to never-ever smokers).

[b]Migraine or non-migrainous headache vs no headache, adjusted for age and sex, n=58 313 (all), n=56 938 (according to never-ever smokers).

Table 3. Association between rs1051730 T alleles and headache according to smoking status.

Per T allele effect	Never smokers		Former smokers		Current smokers	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Main analysis[a]						
Headache	1.00 (0.96-1.04)	0.89	0.97 (0.92-1.02)	0.24	1.00 (0.96-1.06)	0.87
Secondary analysis[b]						
Migraine	0.99 (0.93-1.04)	0.62	1.01 (0.93-1.09)	0.82	0.95 (0.89-1.03)	0.21
Non-migrainous headache	1.00 (0.96-1.05)	0.89	0.95 (0.90-1.01)	0.10	1.03 (0.97-1.08)	0.35

OR=odds ratio, CI=confidence interval.

[a]Headache vs no headache, adjusted for age and sex, n=55 749 (according to smoking status).

[b]Migraine or non-migrainous headache vs no headache, adjusted for age and sex, n=55 749 (according to smoking status).