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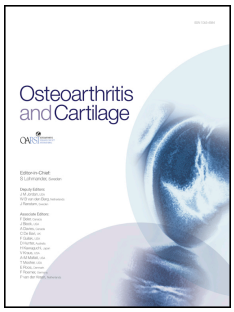

The causal role of smoking on the risk of hip or knee replacement due to primary osteoarthritis: a Mendelian randomisation analysis of the HUNT Study

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The causal role of smoking on the risk of hip or knee replacement due to primary osteoarthritis: a Mendelian randomisation analysis of the HUNT Study

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Running headline: The causal role of smoking on the risk of hip or knee replacement

Abstract

Objective: Smoking has been associated with a reduced risk of hip and knee osteoarthritis and subsequent joint replacement. The aim of the present study was to assess whether the observed association is likely to be causal.

Method: 55 745 participants of a population-based cohort were genotyped for the rs1051730 C>T single-nucleotide polymorphism, a proxy for smoking quantity among smokers.
A Mendelian randomization analysis was performed using rs1051730 as an instrument to evaluate the causal role of smoking on the risk of hip or knee replacement (combined as total joint replacement (TJR)). Association between rs1051730 T alleles and TJR was estimated by hazard ratios (HRs) and 95% confidence intervals (CIs). All analyses were adjusted for age and sex.

Results: Smoking quantity (no. of cigarettes) was inversely associated with TJR (HR 0.97, 95% CI 0.97-0-98). In the Mendelian randomization analysis, rs1051730 T alleles were associated with reduced risk of TJR among current smokers (HR 0.84, 95% CI 0.76 to 0.98, per T allele), however we found no evidence of association among former (HR 0.97, 95% CI 0.88 to 1.07) and never smokers (HR 0.97, 95% CI 0.89 to1.06). Neither adjusting for body mass index, cardiovascular disease nor accounting for the competing risk of mortality substantially changed the results.

Conclusion: This study suggests that smoking may be causally associated with the reduced risk of TJR. Our findings add support to the inverse association found in previous observational studies. More research is needed to further elucidate the underlying mechanisms of this causal association.

Keywords: smoking; osteoarthritis; genetic variants; epidemiology.

Introduction

Hip and knee osteoarthritis (OA) are one of the leading causes of global disability and the burden of OA is anticipated to increase due to an aging and more obese population [1]. No curative treatment for OA is available, which places emphasis on identifying modifiable risk factors for disease prevention and treatment of early OA [2]. The results of observational studies suggest that smoking could have a protective effect on the development of OA [3,4] and subsequent hip and knee replacement [5-7]. Although results from in vitro data have indicated a beneficial effect of nicotine on chondrocyte function, the mechanisms remain unclear [8,9]. The question remains; is there a causal effect of smoking on OA? Observational studies are prone to confounding and reverse causality; hence, it is difficult to infer causal links between smoking and OA or joint replacement using information from observational studies alone.

In Mendelian randomisation analysis, the causality of epidemiological relationships is investigated using genetic variants as proxies for the exposure of interest. Due to the random assortment of genetic variants at conception, genetic variants tend to be independent of potential confounders. Hence, genotypes associated with smoking are not likely to be associated with environmental factors that may confound conventional observational studies [10]. The C>T single-nucleotide polymorphism (SNP) rs1051730 in the CHRNA5- CHRNA3-CHRNB4 nicotinic acetylcholine receptor gene cluster on chromosome 15 is the strongest genetic contributor to smoking behavior identified in genome-wide association studies to date [11-13]. Each additional T allele at the rs1051730 SNP is associated with an increase in the number of cigarettes smoked per day and increased cotinine levels, a metabolite of nicotine, among current smokers [14]. The rs1051730 SNP has been used as an instrument for smoking intensity in former Mendelian randomisation studies investigating the causal effect of cigarette smoking on body mass index (BMI), anxiety and depression and cardiovascular risk factors [15-19].

We are not aware of studies using the rs1051730 SNP to study the association between smoking and OA, or hip or knee replacement. Thus, the aim of the present study was to investigate whether the association observed between smoking and hip or knee replacement is likely to be causal by using the rs1051730 SNP as an instrumental variable in a Mendelian randomisation analysis.

Method

Study population

The Nord-Trøndelag Health Study (HUNT) is a population-based study with data collected through three cross-sectional surveys; HUNT1 (1984-1986), HUNT2 (1995-1997) and HUNT3 (2006-2008). The surveys comprise data from questionnaires, interviews, clinical examinations and blood sampling. All residents of Nord-Trøndelag County, Norway aged 20 years and older were invited to participate. The HUNT study has been described in detail elsewhere [20,21]. For the present study, we included participants from HUNT2; in which 65 232 (69.5% of those invited) participated. Of these, 56 625 participants were successfully genotyped for the rs1051730 SNP. A total of 880 participants were excluded because of hip or knee replacement prior to baseline in HUNT2 (n=503), no date recorded for the primary hip or knee replacement (n=25), missing information on age at participation (n=3), or death/emigration before start of follow-up (n=2). Current smokers of only pipes and cigars, but not cigarettes, were also excluded (n=347). Our study sample therefore comprised 55 745 participants. The current study was approved by the Regional Committees for Medical and Health Research Ethics (REK), 2014/226/REK Central.

Genotyping

DNA was extracted from blood samples collected at baseline in HUNT2 and stored at the HUNT biobank. The rs1051730 SNP was genotyped at the HUNT biobank using a TaqMan assay (Assay ID: C_9510307_20, Applied Biosystems, USA) on an Applied Biosystems 7900HT Fast Real-Time PCR System, as described in former HUNT studies [15,16]. The call rate cut-off was set to 90%. The genotype was coded according to the number of T alleles (0=no T allele, 1=heterozygote for the T allele, 2=homozygote for the T allele). The genotyping success rate was 99.3% and the quality score for each individual genotype was > 90 (mean 99.6). There was no evidence of departure from the Hardy-Weinberg equilibrium (χ^2 test, p=0.26). The minor allele frequency was in agreement with HapMap-CEU data (MAF=0.335 and 0.389, respectively).

Smoking

Smoking status was self-reported in the HUNT2 questionnaire and categorised into never, former and current smokers. Current smokers were asked to report the average number of cigarettes smoked per day. Individuals, who reported being current smokers of pipes and cigars, but not cigarettes, were excluded from all analyses.

Covariates

Height and weight were measured by trained personnel. BMI is weight in kilograms divided by height in meters squared. Cardiovascular disease (CVD) was defined as a composite of myocardial infarction, angina or stroke [22].

Outcome

The outcome of interest was the first hip or knee replacement due to primary OA. To retain statistical power, hip and knee replacements were combined to one variable; total joint replacement (TJR). The unique 11-digit identity numbers of Norwegian citizens enabled us to link individuals' baseline data in HUNT2 with the corresponding prospective TJR data in the Norwegian Arthroplasty Register. The orthopaedic surgeon submits a standardized form to the register for each TJR performed, containing information about the diagnosis that lead to the TJR, any previous TJR or other operations performed in the joint, and the type of implant used. We censored TJRs secondary to injury (meniscal or ligamentous), rheumatoid arthritis, femoral neck fracture, congenital dysplasia, Perthes' disease, epiphysiolysis, ankylosing spondylitis and osteonecrosis of the femoral head, amongst others. The completeness of reporting hip and knee replacement in the register is high (>95%) [23,24].

Statistical analysis

Descriptive statistics according to the number of rs1051730 T alleles were compared using a Chi-square test for categorical variables and a linear regression for continuous variables. A Cox proportional hazards model was used both for the observational and Mendelian randomisation analyses. Estimates were given as hazard ratios (HRs) with 95% confidence intervals (CIs). Follow-up began on the day of inclusion in HUNT2 and ended at the date of TJR due to primary OA, date of TJR for conditions other than primary OA, date of death/emigration (available from Statistics Norway), or the end of follow-up (December 31, 2013), whichever came first. All analyses were adjusted for age as the time scale in addition to sex. The proportional hazards assumptions were tested by Schoenfeld residuals. No deviation from proportionality was detected. To illustrate the observational association between smoking quantity and risk of TJR, smoking quantity was expressed by a restricted

cubic spline with knots at 0, 10, 20, 30 and 40 cigarettes per day. Never and former smokers were assumed to smoke zero cigarettes a day and were used as the reference point.

We used a multinomial logistic regression and a linear regression to estimate the association between rs1051730 T alleles and smoking status and between rs1051730 T alleles and smoking quantity, respectively. Further, in the Mendelian randomisation analysis, the association between rs1051730 T alleles and risk of TJR was examined as an overall association as well as in the strata of never, former and current smokers. If smoking heaviness is causally associated with TJR, we would expect the association to be strongest among current smokers and absent among never smokers. To assess the statistical evidence that the association between rs1051730 T alleles and TJR was modified by smoking, we included interaction terms between the rs1051730 T alleles and smoking status (examining interaction across strata of never, former or current smoking) and between current vs. never and former smokers combined (examining interaction with current smoking). Models with and without the interaction terms were compared using the likelihood ratio test. Moreover, carriers of rs1051730 T alleles may be less likely to quit smoking. Stratifying on current and former smokers could therefore introduce bias, by conditioning on an observed measure of exposure. We therefore repeated the analysis between the SNP and TJR in strata of never vs. ever smokers (current and former smokers combined). We assumed an additive genetic model, so risk estimates represent the HRs per additional copy of the T allele.

In the sensitivity analyses, we adjusted for BMI (expressed as restricted cubic spline) as a possible mediator between smoking and TJR, and for CVD at baseline. Further, we calculated subhazard ratios (SHRs) according to the Fine and Grey model [25] to account for the competing risk of mortality, since smoking is strongly associated with mortality. The proportional subhazard assumption was tested by introducing a time-varying

coefficient/interaction with time (*tvc*) to the model. Data were analysed using Stata v.14.1 (StataCorp LP, USA).

Results

Descriptive statistics

In total, 54 898 participants were genotyped for rs1051730 and had data on smoking status. This group included 16 705 (30.4%) current smokers, 15 350 (28.0%) former smokers and 22 843 (41.6%) never smokers. In this sample, the number of TJRs during a follow-up 17.2 years (median) of was 2601 (4.7%). We found that as the number of T alleles increased, the participants tended to be slightly younger and have lower BMI. Among current smokers, rs1051730 T alleles were associated with a higher number of cigarettes smoked per day. Rs1051730 T alleles were not associated with any other characteristics (Table 1).

Observational analysis

We found an inverse association between smoking quantity and the risk of TJR (Fig. 1). The overall association (HR) between each additional cigarette smoked per day and TJR was 0.97 (95% CI 0.97 to 0.98), $p<0.001$.

Association of rs1051730 T alleles with smoking status and smoking quantity

The genetic variant was associated with current smoking (odds ratio (OR) 1.08, 95% CI 1.05-1.11) compared with never smoking, although there was no clear association with former smoking (OR 0.97, 95% CI 0.94-1.01) compared with never smoking. Similarly, in current

144 smokers, we found that each additional rs1051730 T allele was associated with an increase in
145 the number of cigarettes smoked per day (0.66, 95% CI 0.54-0.79).
146
147 *Mendelian randomisation analysis*
148 In regard to smoking status, we found an inverse association between rs1051730 T alleles and
149 TJR among current smokers, where each additional T allele was associated with a 16%
150 reduction in the risk of TJR (HR 0.84, 95% CI 0.74 to 0.96) (Table 2). In contrast, there was
151 no evidence of an association among never (HR 0.97, 95% CI 0.89 to 1.06) and former
152 smokers (HR 0.97, 95% CI 0.88 to 1.07). The overall age- and sex-adjusted association (HR)
153 between rs1051730 T alleles and risk of TJR was 0.93 (95% CI 0.89 to 1.00) (Table 2). There
154 was no statistical evidence for effect measure modification across all strata of current, former
155 and never smokers (p interaction=0.35), but there was indication of a greater effect per T
156 allele among current smokers when compared to never and former smokers combined (p
157 interaction=0.05). In the broader strata of never vs. ever smokers, rs1051730 T alleles were
158 associated with reduced risk of TJR among ever smokers (HR 0.91, 95% CI 0.84 to 0.99).
159 Adjustment for BMI had only a minor effect on the estimated association of
160 rs1051730 T alleles with the risk of TJR in current smokers (HR 0.87, 95% CI 0.77 to 0.99)
161 compared with the main result (Table 2). Adjustments for CVD did not change the results
162 from the main analysis. The competing risk analysis, including all-cause mortality as the
163 competing event to TJR, supported the main result of an inverse association between
164 rs1051730 T alleles and the risk of TJR in current smokers (SHR 0.82, 95% CI 0.73 to 0.93)
165 (Table 2).
166
167

Discussion

In this Mendelian randomisation analysis, we found support for a causal association between smoking and TJR. There was an inverse association between the number of cigarettes smoked per day and TJR, which was also evident between the rs1051730 SNP and TJR. We found that the risk of TJR among current smokers decreased with each additional copy of the T allele of the rs1051730. The lack of association among non-smokers offers further support to the notion that smoking is causally related to TJR. It indicates that, other than through smoking quantity, the rs1051730 T allele has no effect on the outcome [10].

Our data adds support to previous observational studies which have suggested a negative association between smoking and TJR [5-7]. In the Singapore Chinese Health Study current smokers compared with never smokers showed a dose-dependent inverse association between the numbers of cigarettes smoked and the risk of knee replacement [5]. Similarly, after accounting for comorbidities and the competing risk of death, an Australian prospective cohort study found that current smokers were less likely to undergo a TJR than non-smokers [6]. Current smoking also reduced the likelihood of receiving TKR in a former Norwegian cohort study, however only among women [7]. There is no clear biological explanation for an inverse association between smoking and OA, but one theory is related to the upregulation of glycosaminoglycan and collagen synthetic activity of articular chondrocytes as a direct effect of nicotine, something which has been shown in vitro [9]. These findings have been replicated in a study on articular chondrocytes from OA patients [8]. Another potential explanation is the indirect effect of smoking on lifestyle factors such as BMI. Increasing BMI is an established risk factor for OA and subsequent TJR [26,27] and there is evidence to suggest that smoking may lead to lower BMI [18,28,29], which could mediate the effect of smoking on TJR [30]. However, the association of rs1051730 T alleles with TJR was only slightly attenuated after statistical adjustment for BMI.

Smoking greatly contributes to the total burden of disease, including CVD and chronic respiratory diseases [31,32]. Moreover, smoking is an important risk factor for postoperative complications after TJR [33]. In addition to smoking, older age, high BMI, and comorbidities have been presented as possible contraindications that could result in a decision against performing TJR [34,35], and these contra-indicators may potentially mediate the effect of smoking on TJR. In our study, adjustments for CVD at baseline did not change the association we found between the rs1051730 T allele and TJR. However, we did not have information on incident CVD during follow-up, or on other smoking-related diseases. Further, it is known that high smoking quantity leads to increased all-cause mortality [36]. To account for the informative censoring of all-cause mortality, we performed a competing risk analysis to estimate the associations between rs1051730 T alleles given the outcomes; death, TJR, or end of follow-up, according to smoking status. The results were unchanged, which indicates that competing risk of all-cause mortality did not explain the association between rs1051730 T alleles and risk of TJR.

A key strength of this study is the large sample size and the robust instrumental variable (rs1051730) used as a proxy for smoking intensity among current smokers. Our longitudinal case ascertainment of TJR through linkage with the nationwide register ensured nearly complete data on hip and knee replacements [23,24]. A limitation of using TJR as the outcome is that it might not indicate the total burden of OA, as indications for replacement surgery depend not only on factors related to the disease itself, but also the general health status and requirements of the patient as well as the capacity of healthcare. Therefore, we acknowledge that the inverse association between smoking and TJR may both include a protective effect of smoking on OA as well as a reduced probability of TJR among smokers with OA. However, consensus on the diagnosis OA is lacking and only modest agreement has

been reported between radiographic, clinical and self-reported methods of diagnosing hip and knee OA [37,38]. Despite the potential limitation of using TJR as outcome, it does have the advantage of being an unambiguous indicator of the disease burden of OA [37].

Information on smoking status and smoking quantity in HUNT2 was self-reported, which makes this exposure prone to misclassification and reporting bias. Furthermore, smoking is likely to be associated with survival until time of HUNT2. We therefore cannot exclude the possibility of bias from non-participation due to increased mortality among smokers. Still, the mean age did not substantially differ by number of rs1051730 T alleles, suggesting that the SNP did not substantially affect risk of death prior to baseline. The rs1051730 SNP is an instrument of lifetime tobacco exposure that is not fully represented by cigarettes smoked per day [14,39]. The rs1051730 SNP has been shown to explain the variance in serum cotinine (4%), a biomarker of tobacco exposure, better than self-reported cigarettes per day (1%) [14], thus supporting the variant as a more accurate measure of smoking intensity. However, the rs1051730 SNP is still a valid instrument for smoking even if the self-reports do not capture every aspect of the exposure of smoking. We can still provide evidence of causality, although we cannot obtain an accurate measure of the effect size of the underlying causal exposure [39].

The strength of the Mendelian randomisation approach lies in the use of a randomly assigned genotype as a proxy for the modifiable exposure. This method allowed us to overcome many of the limitations of a conventional observational study, namely confounding and reversed causality [10]. However, the approach has its limitations and relies on certain assumptions that are only partly testable. First, the genetic variant should be reliably associated with exposure [40]. For rs1051730, the robust relationship with smoking intensity has been confirmed in previous genome-wide association studies [11-13] and the association was also substantiated in

the current study sample. Second, the genetic variant should only be associated with the outcome through the exposure of interest [40]. In our cohort, rs1051730 T alleles were only associated with TJR in current smokers, which indicates that the effect was mediated through smoking intensity. Analysis in broader strata of smoking (never vs. ever) supported the interpretation that the association between the rs1051730 T alleles and TJR in current smokers was not a result of collider/selection bias only, as the inverse association with TJR remained for ever smokers, although weakened. Third, the genetic variant should be independent of other factors affecting the outcome (measured and unmeasured confounders) [40]. The second and third assumptions are impossible to test completely. However, we performed additional analyses to assess the robustness of our findings. The results from these analyses supported an inverse association between rs1051730 T alleles and TJR among current smokers, independent of BMI, cardiovascular comorbidity, and competing risk of all-cause mortality.

To conclude, this Mendelian randomisation analysis indicates a causal role of smoking on the risk of TJR. Thus, our study corroborates the inverse association found in previous observational studies. The mechanisms underlying a causal association may be related both to a protective effect of smoking on OA, and to a reduced likelihood of receiving TJR among smokers with OA. The inverse association between smoking and TJR does not support smoking as a therapeutic treatment of OA due to the numerous other health hazards related to smoking. However, the current findings do emphasize the importance of finding the underlying mechanisms of the effects of smoking on the need for TJR.

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Contributions

MBJ, GÅV, BSW, JHB, BOÅ, LMP, GBF, KS, LN and JAZ were responsible for the study conception and design. MBJ, BSW, MEG, AIH, AL, OF, KS, LN and JAZ were responsible for acquisition of the data. MBJ, GÅV, JHB, BOÅ, MEG, FS and PRR were responsible for analysis and interpretation of the data. All authors, MBJ, GÅV, BSW, JHB, BOÅ, MEG, LMP, AIH, AL, OF, GBF, FS, PRR, KS, LN and JAZ, contributed to drafting and revising of the article, and all authors approved the final version to be published.

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Competing interests

None declared.

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Figure legend

Fig. 1. Age- and sex-adjusted hazard ratios for total joint replacement (hip or knee) with 95% confidence interval (grey) according to numbers of cigarettes smoked per day. Never and former smokers (zero cigarettes a day) are used as a reference group.

Table 2. Association between rs1051730 T alleles and joint replacement (TJR).

	All		Never smokers		Former smokers		Current smokers	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Main analysis								
TJR per T allele effect[a]	0.93 (0.89 to 1.00)	0.04	0.97 (0.89 to 1.06)	0.53	0.97 (0.88 to 1.07)	0.52	0.84 (0.74 to 0.96)	0.008
Sensitivity analyses								
TJR per T allele effect[b]	0.94 (0.89 to 1.00)	0.05	0.96 (0.88 to 1.05)	0.36	0.97 (0.88 to 1.08)	0.61	0.87 (0.77 to 0.99)	0.04
TJR per T allele effect[c]	0.94 (0.89 to 1.00)	0.04	0.97 (0.89 to 1.06)	0.54	0.97 (0.88 to 1.07)	0.55	0.84 (0.74 to 0.95)	0.007
TJR per T allele effect[d]	0.92[e] (0.87 to 0.98)	0.008	0.97[e] (0.89 to 1.06)	0.50	0.95[e] (0.86 to 1.05)	0.29	0.82[e] (0.73 to 0.93)	0.003

HR=hazard ratio, CI=confidence interval.

[a]adjusted for age and sex, n=55 745 (all), n=54 898 (according to smoking status).

[b]adjusted for age, sex and BMI, n=55 395 (all), n=54 561 (according to smoking status).

[c]adjusted for age, sex and CVD, n=55 629 (all), n=54 788 (according to smoking status).

[d]competing risk analysis accounting for mortality, adjusted for age and sex, no. of deaths among all (n=11 322 of 55 745), never smokers (n=4109 of 22 843), former smokers (n=3727 of 15 350) and current smokers (n=3145 of 16 705)

[e]estimates are given as subhazard ratios (SHRs).

Table 1. Baseline characteristics of the 55 745 study participants by numbers of rs1051730 T alleles.

		No. of rs1051730 T alleles			
	n[a]	0	1	2	p-value[b]
Study population – %	55 745	44.3	44.3	11.3	
Smoking – %					
Never	22 843	42.1	41.2	41.2	
Former	15 350	28.7	27.7	26.1	
Current	16 705	29.2	31.1	32.7	<0.001
No. of cigarettes per day[c] – mean	16 034	10.8	11.4	12.1	<0.001
Age (years) – mean	55 745	49.9	49.8	49.4	0.06
BMI (kg/m2) – mean	55 395	26.4	26.3	26.3	0.02
Women – %	29 252	52.3	52.6	53.0	0.52
Education – %					
<10 years	37 534	70.1	71.2	70.8	
10-12 years	4992	9.7	9.2	9.0	
≥13 years	10 575	20.2	19.6	20.2	0.08
Work status – %					
Unemployed	14 282	26.1	26.2	26.1	
Employed	40 355	73.9	73.8	73.9	0.90
Physical activity – %					
Inactive	4353	9.9	10.0	10.1	
Light	9134	20.5	21.1	21.1	
Moderate	16 898	38.7	38.4	38.8	
High	13 447	31.0	30.6	30.1	0.77
CVD – %	4335	7.8	7.8	7.4	0.50
Diabetes – %	1686	3.1	3.0	3.0	0.94

[a] Total n varies from 43,832 to 55,745 due to missing data.

[b] Chi-Square test for categorical variables and linear regression for linear associations according to number of T alleles.

[c] Among current smokers.

BMI = body mass index, CVD = cardiovascular disease.