ELSEVIER

Investigation of the relationship between smoking and appendicitis in Australian twins

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PURPOSE: Appendicitis is an inflammation of the appendix, the etiology of which is still poorly understood. Previous studies have shown an increased risk for cigarette smokers but no accounts for the timing of exposure to smoking relative to appendectomy were made.
METHODS: Based on questionnaire data, both cohort and co-twin case-control analyses were conducted to assess the effect of active cigarette smoking on appendectomy in 3808 Australian twin pairs. Smoking status was defined as a time-dependent covariate to account for differences in timing of smoking initiation and onset of appendicitis.
RESULTS: The questionnaire had a 65% pairwise response rate. After controlling for sex, age, and year of birth, appendectomy risk in current smokers was statistically significantly increased by 65% relative to never-smokers. This was largely unchanged by the duration or intensity of smoking and was not affected by socioeconomic status or father's occupation. The effect was stronger in females. Among former smokers, increased time since quitting significantly reduced the odds ratio of appendectomy by 15% for every year since quitting.
CONCLUSION: After adjustment for age and other confounders, there was an increase in risk of appendectomy among current smokers relative to never-smokers, particularly in females. This study adds to the body of knowledge on the effects of tobacco smoking on the gastrointestinal tract.
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INTRODUCTION

Smoking has been variously associated with diseases of the gastrointestinal tract: increased risk of Crohn's disease (1) and peptic ulcer disease (2), as well as a decreased risk of ulcerative colitis (UC) (3). However, it has also been reported that appendectomy is protective against UC (4) and that this reduced risk is specific to appendectomies in persons younger than 20 years of age (5, 6), in particular when these are due to appendicitis (6). Since both smoking and appendectomy appear to be protective against UC, dependencies between the two factors might indicate that this protective effect is partly due to confounding.

An increased risk of appendectomy among active smokers and children of parents who smoke was reported by Montgomery, Pounder, and Wakefield (7). Although no claims of causation were made, it was suggested that smoking might disable an immune response associated with UC and leave the appendix vulnerable to inflammation. The study has been criticized for failing to account for the timing of exposure to smoking relative to appendectomy (8). Butland and Strachan (9) found further evidence for the association between passive smoking and appendectomy in children younger than 16 years of age.

The aim of this paper is to investigate the association between reported personal tobacco smoking and appendectomy in a large Australian twin cohort. Two statistical approaches were employed. The first was a cohort analysis using a time-dependent smoking status variable. The second was a co-twin case-control analysis in which the twin structure of the data afforded an assessment of whether dependencies between smoking and appendectomy are due to unique environment or common genetic influences.

METHODS

Sample

The Australian NHMRC Twin Registry (ATR) is a volunteer twin registry established in 1979. The data used in the present study are derived from a questionnaire mailed to 5,967 ATR twin pairs on November 14, 1980 and returned over a period of 2 years to 1,982 (10). Both members of 3,808 pairs completed and returned the questionnaire.

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Selected Abbreviations and Acronyms

UC = ulcerative colitis
ATR = Australian Twin Registry
OR = odds ratio
RR = relative risk
MZ = monozygous twin
DZ = dizygous twin
CI = confidence interval

The questionnaire included items on age, sex, zygosity, birth order, tobacco use, a disease checklist comprising some common medical procedures, whether the subject had undergone an appendectomy, and, if so, age at operation. These questionnaires are available to the readership on request to the corresponding author. A total of 1,718 appendectomies were recorded. Previously, analysis of these data showed appreciable genetic and common environment influences on risk of appendectomy (10).

To reduce bias due to poor recollection of early surgery and prophylactic or preventative procedures, the present analysis excluded 37 subjects who reported appendectomies that occurred in patients younger than 5 years old and after 55 years of age and 52 female subjects whose age at appendectomy coincided with age at hysterectomy.

Definition of Exposure

The analysis utilized information on participants' self-reported tobacco usage. Questionnaire items included lifetime cigarette smoking status (smoker or non-smoker), age started smoking, age stopped smoking, and the average number of cigarettes per day. We defined lifetime non-smokers as having smoked fewer than five cigarettes in total. Information on other smoking behaviors such as pipe and cigar usage was collected and used only to define a clean group of non-smokers. Potential recall bias was reduced by excluding 43 subjects who reported age at smoking initiation as younger than 10 years old.

To accommodate the ages of exposure to smoking initiation and onset of appendectomy, a time-dependent smoking status indicator was calculated for the cohort analysis and a variable describing smoking status prior to appendectomy was calculated for the case-control analysis. Both smoking status variables comprised the categories of smoker, former smoker and never-smoker. For the purposes of this study, former smokers were defined as having ceased smoking for 1 year or more.

Two approaches were adopted to accommodate former smokers. First, they were excluded from an analysis of current and never-smokers. Second, former and current (as of 1980) smokers were combined as "ever-smokers." A third analysis of the subpopulation of ever-smokers was undertaken to investigate the effect of reported length of time since quitting, duration of smoking, and intensity of smoking (average packs per day).

Statistical Analysis

Cohort analysis. Each individual's contribution of person-years at risk was calculated over 5-year age groups, from birth until either the age of appendectomy or the age at survey depending on their appendectomy status. Age-specific incidence rates were calculated by dividing the number of appendectomy cases by the person-years at risk for each age group.

A time-dependent smoking status was calculated for each individual. The non-smoker category included lifetime non-smokers and those who initiated smoking after the final year of the current age group. Smokers included those having an age at initiation less than or equal to the final year of the current age group and age at cessation greater than or equal to the first year of the current age group. Former smokers were defined as having age at smoking cessation less than the first year of the current age group.

Multivariate adjusted relative risks (RRs) for appendectomy associated with active smoking were obtained by using a Cox proportional hazards regression model. Time-independent covariates included gender and birth category (defined as year of birth from 1946 to 1962, 1926 to 1945, and before 1925). Time-dependent covariates consisted of age (in five-year categories) and smoking status. Dependencies associated with the twin structure of the data were accommodated by the inclusion of a familial frailty term in the regression. Age was included in two ways: first as a smoothed five-knot natural spline and, second, as three-level stratification by birth category.

Model adequacy was assessed by chi-square goodness-of-fit tests. Statistical significance of regression coefficients was tested using the Wald statistic. The proportional hazards assumption was tested by calculating the Pearson product-moment correlation between the scaled Schoenfeld residuals and log (time) for each covariate, with p values less than 0.05 indicating a violation of the proportionality assumption.

Record splitting, aggregation of person-years, statistical analysis, and diagnostics were undertaken in the R software package (11).

Case-control analysis. The associations between appendectomy and smoking status prior to appendectomy were assessed in a case-control analysis. To reduce the strong bias due to lack of opportunity to smoke in younger subjects, this study was restricted to subjects 20 years of age and older. Twins who had an appendectomy after age 20 were considered the cases and co-twins who had not had an appendectomy were the control subjects.

Smoking status prior to appendectomy was computed using the age-at-appendectomy and smoking initiation

age. Non-smokers were defined as those subjects with age of smoking initiation greater than age at appendectomy. Former smokers were defined as those with age of smoking cessation more than 1 year prior to age at appendectomy. Years since quitting smoking were calculated for each former smoker and smokers were assigned a value of zero.

Social class effects were modeled with the covariates highest education level and father's occupation. Other lifestyle activities, such as coffee and alcohol consumption, were also included as covariates.

Multivariate adjusted odds ratios (ORs) were calculated by using conditional logistic regression for all discordant twin pairs using each of the smoking models. The procedure was repeated on discordant monozygous (MZ) and dizygous (DZ) twin pairs.

Finally, a sensitivity analysis was conducted. The impact of removing subjects with smoking initiation age less than 10 years and those with appendectomies at less than 5 years or over 55 years was assessed by refitting the models using all of the data. To coincide with the removal of smokers under 10 years of age, we refit our models using a follow-up period to begin at age 10 years. To reduce misclassification bias, the model was repeatedly fit where a subset of the appendectomy cases (30%) was randomly misclassified. We explored another definition of lifetime non-smokers as individuals who gave up smoking the year they began for both the cohort and the case-control analyses.

RESULTS

Of the 3,080 pairs who responded to the survey (a 65% pairwise response rate), 7,460 twin individuals (4,814 females and 2,699 males) remained after cleaning. Approximately 21% of the respondents had undergone appendectomy by the time of survey (Table 1). Approximately 40% of subjects had ever been a smoker prior to appendectomy, with 14.1% of those having quit at least 1 year earlier. Ages of smoking initiation and appendectomy are summarized as cumulative proportions in Fig. 1. Almost 90% of lifetime smokers in this survey had initiated smoking by the age of 20. Smokers and non-smokers had the same age demography (mean age, 34 years; range, 17–90 years; interquartile range, 23–41 years for smokers, 23–43 years for non-smokers).

The numbers of appendectomies in each of the three birth categories are shown in Table 2. Appendectomies occurred in different proportions across the birth categories, with the lowest proportion seen in the most recent category most likely due to the age of respondents. Additional differences can be seen in the distributions of age-at-appendectomy and age-at-smoking initiation across the birth categories, shown in Table 3. Here, the first quartiles of age-at-appendectomy among those who had the disease monotonically decreased from 16 for males born during the period 1892–1925 to 11 for males born during the period 1946–1963. A similar pattern can be seen for females, but at slightly older ages.

Incidence rates of appendectomies decreased slightly overall between 1936 and 1980, but increased over time for females (Table 4). Similar trends showing an overall increase in incidence rates of smokers over time in both males and females can be seen.

The final model for the cohort data included sex, smoking status, an age-smoothed (five knot natural spline) term, an interaction term between sex and smoking status, and stratification by year of birth. Multivariate adjusted RR estimates of appendectomy show an elevated RR for current smokers (RR = 1.65; 95%CI: 1.41-1.93). There was no statistically significant effect for former smokers. An interaction between smoking and males was statistically significant (RR = 0.48, 95%CI: 0.37, 0.63), reducing the effect

TABLE 1. Summary of responses for 7,460 subjects in the Australian Twin Study included in analysis of the association between smoking and appendectomy

Variable	Level	No.	%
Appendectomy status	No	5,843	78.3
	Yes	1,617	21.7
Gender	Male	2,698	36.2
	Female	4,762	63.8
Year of birth	1892–1925	848	11.4
	1926–1945	2,030	27.2
	1946–1963	4,582	61.4
Smoking status	Never-smokers	4,138	55.5
	Ever-smokers	3,322	44.5
	Nonsmokers	4,497	60.3
Smoking status (prior to appendectomy)	Former smokers	1,060	14.2
	Current smokers	1,903	25.5
Smoking intensity (cigarettes/day)*	1–19	1,878	56.5
	20–30	1,225	36.9
	31–45	118	3.5
	>45	42	1.3
	NA	59	1.8
	<1	92	3.1
Duration of smoking (yr prior to appendectomy)†	1–5	918	30.7
	6–9	784	26.2
	10–20	709	23.7
	>20	485	16.2
Age of smoking initiation (yr)*	10–14	568	17.0
	15–20	2,347	70.1
	21–30	378	11.3
	>30	55	1.6
Time since quitting smoking (yr prior)†	<1	1,921	64.3
	1–5	499	16.7
	6–10	236	7.9
	>10	332	11.1

*Expressed as fractions of current and former smokers (prior to appendectomy).
†Expressed as a fraction of ever-smokers prior to appendectomy.

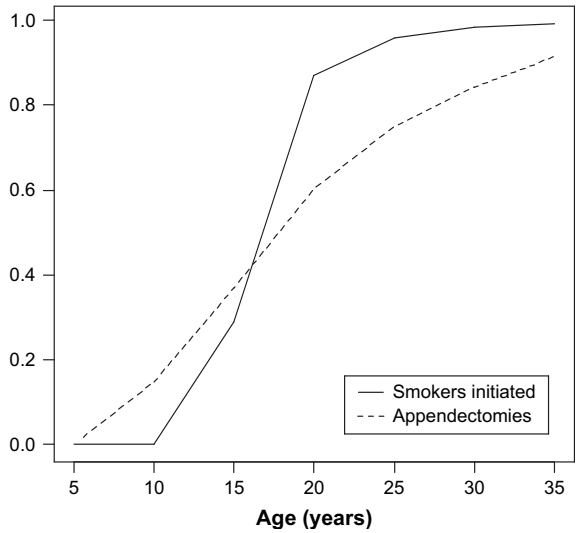

FIGURE 1. Cumulative proportions of initiation age of those who ever smoked and age of appendectomy for 7,460 subjects in the 1980 Australian twin study included in analysis of the association between smoking and appendectomy.

of smoking on appendectomy for males. These estimates were largely unchanged with the inclusion of former smokers as smokers (RR = 1.61, 95%CI: 1.37-1.89).

A frailty familial variance of 1.07 was estimated, which implies that individual families have a risk of appendectomy that is up to $\exp(\sqrt{1.07}) = 2.81$ times larger or smaller on average than the average risk. This variance was unchanged by the inclusion of smoking status covariates.

Sensitivity analysis, as described in the Methods section, found persisting smoking effects of similar magnitudes.

Case-Control Analysis

A total of 937 twin pairs were discordant in appendectomy, of which 380 pairs had an onset age older than 20 years. Multivariate adjusted ORs based on the subset of the data containing only current smokers and never-smokers prior to appendectomy are reported in Table 5. Discordant MZ twin smokers had an OR of 2.62 (95%CI: 1.16-5.93), compared with discordant DZ smokers (OR = 1.93, 95%CI: 1.04-3.61). Pooling former smokers with current smokers resulted in a dilution of the ORs for MZ twins (OR = 1.55, 95%CI: 0.86-2.81) and DZ twins (OR = 1.12, 95%CI: 0.70-1.89). There were noticeable gender differences, although the low counts of discordant males made comparison difficult. No statistically significant effects were found for coffee drinking, alcohol drinking, and highest education level or father's occupation.

TABLE 2. Appendectomy proportions by birth category for 7,460 subjects in the Canberra questionnaire (reported 1980–1982)

Birth year	No. of subjects	No. of appendectomies	Percentage of appendectomies
1892–1925	848	257	30.3
1926–1945	2,030	672	33.0
1946–1963	4,582	688	15.0

TABLE 3. Minimum, first quartile, and median age of smoking initiation (age smoked) and age at appendectomy

	1892–1925			1926–1945			1946–1963			Combined
	Min	25%	50%	Min	25%	50%	Min	25%	50%	50%
Females										
Age smoked	12	18	20	10	17	18	10	15	17	18
AAA	7	18	25	5	15	22	5	12	16	19
Males										
Age smoked	10	16	20	12	16	17	10	14	16	17
AAA	6	16	24	5	12	16	5	11	14	15

Min = minimum; AAA = age at appendectomy.

Duration and intensity of smoking effects on the subpopulation of ever-smokers, summarized in Table 6, were not statistically significant at the 5% level. The time from smoking cessation (years quit) was found to be protective against appendectomy for both MZ and DZ twins (OR = 0.85, 95%CI: 0.75-0.93 and OR = 0.83, 95%CI: 0.71-0.98) giving an approximate 15% reduction in the OR of appendectomy for every year since quitting.

DISCUSSION

This study has provided further evidence for an association between active tobacco usage and onset of appendectomy. The analysis accounted for gender, year of birth and the difference between the age of exposure to smoking initiation and the age onset of appendectomy. The findings indicate that smoking is a risk for appendectomy, especially in those who are currently smoking, irrespective of the intensity or duration of their habit. Sensitivity analysis revealed indifference to definitions of nonsmokers. The evidence suggests that cessation of smoking relates to a decrease in risk of the disease of 15% per year since quitting.

The rates of smokers in our sample are consistent with rates in the general population of Australia in 1980 (12). Secular trends were found in appendectomy and smoking rates, consistent with findings of an association between smoking and appendectomy. Differences between males and females in these results could be due to gender but could also be explained by other factors, for example, inclusion of a number of prophylactic appendectomies escaped detection. However, given the study design and the active exclusion of known non-appendicitis cases, this is unlikely to account for the entire observed effect.

TABLE 4. Appendectomy and smoking incidence rates (per 1000 person-years) between 1890 and 1980 from the Australian Twin Registry survey respondents

	Period					
	1890–1905	1906–1920	1921–1935	1936–1950	1951–1965	1966–1980
Females						
Appendectomy rate	0	1.26	4.94	8.63	8.39	9.22
Incidence rate	267.40	269.30	265.60	287.31	290.52	336.91
Person-years	172	2,373	10,334	23,752	50,649	57,687
Males						
Appendectomy rate	0	0.74	4.73	6.88	6.05	5.14
Incidence rate	341.77	274.81	294.18	329.95	310.91	352
Person-years	79	1,350	4,861	11,341	28,084	34,989

The results from the co-twin control study agree with those of the cohort study and show a strong effect of personal smoking on late-onset appendectomy (>20 years old). This effect was strongest among MZ twins, indicating that genetic factors may be contributing to the association.

The finding of a moderate familial effect was consistent with the findings of Duffy, Martin, and Matthews (10). Given that this effect was unchanged with the inclusion of smoking status as a covariate, it is unlikely that the familial clustering of appendectomy is explained entirely through smoking behavior. Since no significant social class effects were found, the strength of this familial effect could perhaps be explained by passive smoking in childhood along with possible genetic factors, but no information on passive smoking behavior was available to support this proposition. We acknowledge that there are other factors that have been shown to affect other gastrointestinal diseases. An example of this is the relationship between Crohn's disease and ethnicity (13). In a follow-up study, approximately half of the individuals participating in this study were asked the ancestry of all four grandparents, and these were overwhelmingly (95%) of northern European ancestry, mainly the British Isles. These data would therefore be unable to support any link between appendicitis and ethnicity.

The lack of a statistically significant relationship between smoking pack-years and appendectomy reported herein does not necessarily negate a causal relationship. The increased relative risk could be simply associated with any smoking but not significantly increased thereafter. Alternatively, the relationship could be nonlinear or influenced by other unmeasured factors. It is also acknowledged that recall bias and misclassification are potentially greater for smoking pack-years than for smoking/nonsmoking and that this may obscure a dose-response pattern. Although these questions could not be resolved in the present study, this motivates further research to confirm a causal association and the nature of the dose-response relationship.

The strengths of this study center on the twin study design. The advantage of this design is that MZ twins are perfectly matched, sharing an early environment and their genes. Through the co-twin case-control study, it was possible to eliminate confounding covariates, as any discordance between genetically identical relatives must depend on environmental factors. The question of causation was directly addressed in this study by examining the time-dependent

TABLE 5. Case-control odds ratios for the twin pairs discordant in appendectomy

Subpopulation	n_{pairs}	n_{+-}	n_{-+}	OR	95%CI
Ex-smokers as smokers					
MZ twins	192	28	18	1.55	0.86, 2.81
MZ males	31	5	5	1.00	0.29, 3.45
MZ females	161	23	13	1.77	0.90, 2.49
DZ twins	188	37	33	1.22	0.70, 1.79
DZ males	15	3	3	1.00	0.20, 4.95
DZ females	97	24	12	2.00	1.00, 4.00
Ex-smokers removed					
MZ twins	144	21	8	2.62	1.16, 5.93
MZ males	16	4	1	4.00	0.45, 35.80
MZ females	128	17	7	2.43	1.01, 5.86
DZ twins	140	20	15	1.93	1.04, 3.61
DZ males	10	3	1	3.00	0.31, 21,80
DZ females	76	18	7	2.57	1.07, 6.16

OR = odds ratio; CI = confidence interval; MZ = monozygous; DZ = dizygous.
n_{+-} = Case was a smoker prior to appendectomy.
n_{-+} = Control was a smoker prior to appendectomy.

TABLE 6. Multivariate adjusted odds ratios for appendectomy for duration of quitting (years since quit) and pack-years among the ever-smoker subpopulation

	OR	95%CI
MZ ever-smokers		
Years quit	0.85	0.75, 0.93
Pack-years	0.98	0.93, 1.03
DZ ever-smokers		
Years	0.83	0.71, 0.98
Pack-years	0.94	0.89, 1.02

MZ = monozygous; DZ = dizygous.

effects of smoking cessation. It should be noted that the time-dependent measure of smoking status is based on recollection of events.

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