

Short communication

Smoking and the development of allergic sensitization to aeroallergens in adults: a prospective population-based study. The Copenhagen Allergy Study

Background: Several cross-sectional population-based studies have reported a negative association between smoking and allergic sensitization to aeroallergens. In a prospective study, we investigated the association between smoking and the development (incidence) of allergic sensitization as reflected by skin prick test (SPT) positivity and specific IgE positivity.

Methods: Participants in a population-based study of 15–69-year-olds in 1990 were invited to a follow-up in 1998. Thus, SPT positivity and specific IgE positivity to common aeroallergens were assessed in 734 subjects (participation rate: 69.0%) on two occasions 8 years apart. The effect of smoking on the development of allergic sensitization was adjusted for potential confounders such as age, sex, family history of hay fever, educational level, and total IgE.

Results: During the follow-up period, 58 and 33 subjects developed SPT positivity and specific IgE positivity, respectively. The risk of developing SPT positivity (adjusted odds ratio: 0.45, 95% CI 0.21–0.98) and specific IgE positivity (adjusted odds ratio: 0.62, 95% CI 0.26–1.49) was lower among sustained smokers than never-smokers.

Conclusions: In this adult population, sustained smoking was negatively associated with the development of allergic sensitization to aeroallergens during an 8-year follow-up. This negative association, if real, might be due to an immunosuppressive effect of smoking.

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Key words: adult; epidemiology; respiratory hypersensitivity; skin prick test; smoking; specific IgE.

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Accepted for publication 23 November 2000

In 1990, we conducted a cross-sectional study in an adult general population in order to assess the prevalence of allergic diseases and to identify potential risk factors (1). In this cross-sectional study, we found that allergic sensitization to aeroallergens was less prevalent among current smokers than among never-smokers (2). However, it is difficult to assess from cross-sectional data the temporal relationship between potential risk factors and development of disease. Thus, we cannot be certain whether smoking prevents the development of sensitization or whether sensitized subjects tend to quit smoking (the healthy-smoker effect). In the present paper, we report a follow-up of the participants in the above study after 8 years. We aimed to investigate whether smoking is likely to prevent or promote the development of allergic sensitization in adults as reflected by incidence of skin prick test (SPT) positivity or specific IgE positivity to common aeroallergens.

Material and methods

Baseline study

The baseline study was conducted according to a two-stage protocol.

In the first stage, a screening questionnaire on respiratory symptoms was mailed to a sample of 15–69-year-olds ($n=8000$), living in the western part of Copenhagen, the capital of Denmark. The sample was drawn randomly from the Civil Registration System. A total of 6998, or 87.5%, responded to the screening questionnaire. In the second stage, a random group and a symptom group selected among the respondents were invited to a health examination. The random group comprised 793 subjects randomly selected from among the respondents. The symptom group comprised 788 subjects selected from among those respondents who had reported respiratory symptoms on exposure to either pollen or furry animals in the screening questionnaire. A total of 146 subjects were included in both groups. Subsequently, 599 (participation rate: 75.5%) and 635 (participation rate: 80.6%) subjects were examined in the random and the symptom groups, respectively. A total of 122 subjects were included in both groups. Thus, a total of 1112 subjects (overall participation rate: 77.5%) were examined. Examinations took place between February 1990 and January 1991. The invitational procedure and characteristics of both participants and nonparticipants have previously been described (1).

Follow-up study

At the time of follow-up, 28 subjects had died, eight had emigrated, and 12 could not be located. Thus, a total of 1064 of the participants from the baseline study were invited to a re-examination; 734 subjects were examined (participation rate: 69.0%). We invited eligible subjects in the same month as they had been examined in the

baseline study to avoid potential seasonal differences. Hence, a total of 63.9% (469/734) of the participants in the follow-up study were examined on a date within 2 months (62 days) of the date of examination in the baseline study. The median follow-up time was 7 years and 10 months (range: 6 years and 10 months – 8 years and 8 months). The participants were examined between October 1997 and November 1998. The study was approved by the ethics committee of Copenhagen County.

SPTs

All subjects participating in the follow-up study had SPTs performed both at the baseline and the follow-up study. The Phazet® system (Pharmacia & Upjohn Diagnostics AB, Sweden) was used for the SPTs in the baseline study. The Phazet system was not available in 1997, and the Soluprick SQ® system (ALK Abelló, Denmark) was used in the follow-up study. Both methods used lancets and standardized allergen extracts (100 000 BU for Phazet and 10 HEP for Soluprick). The allergen extracts and the positive control were in a dried form on the tip of the Phazet lancets, and in a liquid form for the Soluprick SQ system. The Phazet and the Soluprick SPT systems previously have been compared, and the Phazet system tended to induce a slightly higher overall number of positive reactions (3). The panel of allergens comprised birch, grass (timothy), mugwort, horse, cat, dog, two species of house-dust mites (*Dermatophagoides pteronyssinus* and *D. farinae*), and two molds (*Cladosporium* and *Alternaria*). A negative control and a positive control (10 mg/ml histamine in both studies) were included. The test sites were placed at intervals of 20 mm on the volar side of the forearm in a straight line from approximately 5 cm below the elbow flexure to about 10 cm above the wrist. The skin reactions were read after 15 min by drawing the perimeter of the wheal with a fine-point, felt-tip pen, and transferred to the data-collection sheet. The largest and the smallest diameters (at the widest point and at the perpendicular bisector) of the wheals were measured with a transparent ruler. SPT reactivity was expressed as the allergen histamine wheal ratio, i.e., the mean wheal diameter at the allergen site divided by the mean wheal diameter at the histamine site. A positive SPT was defined as an allergen histamine wheal ratio of 0.5 or more, corresponding to grade 2+ according to Aas & Belin (4). SPT positivity was defined as a positive SPT to one or more of 10 allergens. The participants were asked to abstain from antihistamines for 72 h (astemizole for 8 weeks) before the examination. The same technician performed all SPTs in both the baseline and the follow-up study. The reproducibility of the SPT in the hands of the technician was established by performing two histamine SPTs on 20 volunteers. The coefficients of variation were 0.14 and 0.10 in 1990 and 1998, respectively.

Specific IgE measurements

In both the baseline and the follow-up study, venous blood was taken on the day of examination, and the blood samples were left to coagulate for 2 h. The serum was then separated by centrifugation at 3000 rpm for 10 min and frozen immediately afterward. The serum samples used for specific IgE analysis were kept at –20°C without thawing until the analysis in 1999. Serum samples were available for analysis from 98.7% (1098/1112) and 99.5% (730/734) of the subjects examined at baseline and follow-up, respectively. The serum samples were analyzed for specific IgE to birch, grass (timothy), mugwort, dog and cat dander, and house-dust mite (*D. pteronyssinus*) with the ADVIA® Centaur® specific IgE assay system (Bayer Corporation) (5, 6). The analysis for specific IgE was judged to be positive if the measurement was in excess of 0.35 kU/l. Specific IgE positivity was defined as a positive test for specific IgE to one or more of six allergens.

Total IgE measurements

The serum samples obtained in the baseline study had been analyzed for total IgE in 1991 by using the Pharmacia CAP System (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden). Analyses were performed in samples from 99.2% (1103/1112) of the participants.

Definition of variables

Three age groups were defined (15–34 years, 35–49 years, and 50–69 years) by the age of the participants at the time of the baseline study. Thus, the age of participants when presented in tables was indexed to age at baseline. In both studies, information on health, lifestyle, and socioeconomic variables was obtained in a detailed questionnaire, which was completed at home. The questionnaire was collected and checked by one of the authors N.H.N. (1990) or A.L. (1998) on the day of the examination before SPTs were performed. A smoking variable (smoking status) was defined under four categories: never-smoker (never-smoker at baseline and follow-up), past smoker (past smoker at baseline and follow-up), quitted smoker (current smoker at baseline and past smoker at follow-up), and sustained smoker (current smoker at baseline and follow-up). The number of subjects who took up smoking during follow-up was small ($n=26$), and these subjects were categorized as sustained smokers. Furthermore, additional potential determinants of allergic sensitization were defined with baseline information: educational level in years (grouped in quartiles), family history of hay fever (parent, sibling, or offspring), and total IgE measured at baseline (grouped in quintiles). A group variable defined whether subjects were in the random or the symptom group. Subjects were included in the analyses in the random group if they, by selection, had been included in both groups.

Allergic sensitization was expressed as either SPT positivity or specific IgE positivity. New (incident) cases of allergic sensitization were defined as subjects with a negative test at baseline and a positive test at follow-up (conversion). Analysis was also performed with reversion of allergic sensitization as the dependent variable (positive at baseline, negative at follow-up).

Statistical analysis

Differences in baseline characteristics between participants and nonparticipants in the follow-up study were evaluated by the chi-square test or the Mann–Whitney U-test. The association between smoking status and the development of allergic sensitization was analyzed by multivariate logistic regression with incidence of allergic sensitization as the dependent variable and smoking status as the exposure variable. Subjects who were sensitized at baseline were excluded from the analysis. Additional potential confounding variables were included in the model as explanatory variables. Potential interactions between smoking status and other explanatory variables were investigated. All data were analyzed with the Statistical Products and Service Solutions package (SPSS) for Windows (Release 9.0), and 95% confidence intervals were used.

Results

Table 1 shows the distribution of baseline characteristics among the participants and the nonparticipants in the follow-up. Male sex and a high educational level at baseline were significantly associated with participation in the follow-up study. No other significant differences in baseline characteristics between participants and nonparticipants were observed.

A total of 58 incident cases of SPT positivity and 33

Table 1. Comparison of baseline characteristics between participants and nonparticipants in follow-up study

	Participants in follow-up (n=734) Prevalence in %	Nonparticipants in follow-up (n=330) Prevalence in %	P value†
Female sex	52.0 (382/734)	58.8 (194/330)	0.041*
Age (years) (at baseline)			
15–34	40.3 (296/734)	37.9 (125/330)	0.126
35–49	35.8 (263/734)	32.4 (107/330)	
50–69	23.8 (175/734)	29.7 (98/330)	
Total	100.0	100.0	
Group			
Random	52.6 (386/734)	56.1 (185/330)	0.293
Symptom	47.4 (348/734)	43.9 (145/330)	
Total	100.0	100.0	
Educational level in years (mean)	11.5	10.8	0.001*
Total IgE (geometric mean)	33.0	29.8	0.212
Skin prick test positivity	40.1 (294/733)	35.1 (115/328)	0.118
Specific IgE positivity	38.2 (278/727)	33.7 (110/326)	0.162
Physician-diagnosed asthma ever	9.4 (69/734)	7.6 (25/330)	0.332
Physician-diagnosed hay fever ever	22.1 (162/734)	17.9 (59/330)	0.119
Family history of asthma	15.9 (116/731)	12.6 (41/326)	0.165
Family history of hay fever	29.4 (215/731)	26.7 (87/326)	0.365
Smoking status			
Never-smoker	34.3 (252/734)	31.8 (105/330)	0.239
Past smoker	21.1 (155/734)	18.2 (60/330)	
Current smoker	44.6 (327/734)	50.0 (165/330)	
Total	100.0	100.0	

† Comparison of participants and nonparticipants (chi-square test or Mann–Whitney U-test).

* Significant.

incident cases of specific IgE positivity were observed. Reversion of SPT positivity and specific IgE positivity occurred in 38 and 25 subjects, respectively. Table 2 shows that the incidence of SPT positivity was significantly lower among sustained smokers than never-smokers. Accordingly, the incidence of specific IgE positivity was lower among sustained smokers than never-smokers, although this was not statistically significant. None of the other smoking categories were significantly associated with the incidence of allergic sensitization (Table 2). In the above models, none of the tested interactions between smoking status and any of the other explanatory variables were statistically significant. Essentially similar results were obtained when a positive SPT was defined as a mean wheal diameter of 3 mm or more. Reversion of allergic

sensitization was not significantly associated with smoking status (Table 3). This was also the case when past, quitted, and sustained smokers were analyzed as one category.

A total of 63 (18.5% of current smokers at baseline) subjects quit smoking during the follow-up period. There was no statistically significant difference in sensitization status at baseline between sustained smokers and quitted smokers. Thus, 16.2% (18/111) of SPT-positive smokers and 20.2% (43/212) of SPT-negative smokers gave up smoking, whereas 20.4% (20/98) of specific IgE-positive smokers and 19.0% (42/221) of specific IgE-negative smokers gave up smoking. Nor was there a significant difference in sensitization status at follow-up between sustained smokers and quitted smokers.

Table 2. Results of logistic regression analysis with incidence of allergic sensitization expressed as skin prick test (SPT) positivity or specific IgE positivity as dependent variable, and smoking status as exposure variable (subjects positive at baseline were excluded from analysis)

	Dependent variable			
	Incidence of SPT positivity (n=58)		Incidence of specific IgE positivity (n=33)	
	Unadjusted (n=437 in analysis) Odds ratio (95% CI)	Adjusted† (n=433 in analysis) Odds ratio (95% CI)	Unadjusted (n=444 in analysis) Odds ratio (95% CI)	Adjusted † (n=441 in analysis) Odds ratio (95% CI)
Smoking status				
Never-smoker	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Past smoker	0.53 (0.23–1.21)	0.65 (0.25–1.67)	0.42 (0.13–1.35)	0.45 (0.13–1.57)
Quitted smoker	1.32 (0.57–3.07)	1.27 (0.47–3.43)	0.69 (0.18–2.56)	0.63 (0.15–2.55)
Sustained smoker	0.45 (0.23–0.89)*	0.45 (0.21–0.98)*	0.69 (0.31–1.55)	0.62 (0.26–1.49)

† Adjusted for group, sex, age, family history of hay fever, educational level, and total IgE.

* $P < 0.05$.

Table 3. Results of logistic regression analysis with reversion of allergic sensitization expressed as skin prick test (SPT) positivity or specific IgE positivity as dependent variable and smoking status as exposure variable (subjects negative at baseline were excluded from analysis)

Smoking status	Dependent variable			
	Reversion of SPT positivity (n=38)		Reversion of specific IgE positivity (n=25)	
	Unadjusted (n=294 in analysis) Odds ratio (95% CI)	Adjusted† (n=292 in analysis) Odds ratio (95% CI)	Unadjusted (n=277 in analysis) Odds ratio (95% CI)	Adjusted † (n=277 in analysis) Odds ratio (95% CI)
Never-smoker	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Past smoker	1.38 (0.51–3.69)	1.24 (0.41–3.74)	2.26 (0.72–7.12)	2.47 (0.73–8.41)
Quitted smoker	1.06 (0.22–5.13)	1.05 (0.19–5.85)	2.49 (0.55–11.33)	2.89 (0.56–14.94)
Sustained smoker	1.56 (0.71–3.41)	1.31 (0.54–3.22)	1.14 (0.36–3.57)	1.67 (0.49–5.74)

† Adjusted for group, sex, age, family history of hay fever, educational level, and total IgE.

Discussion

We found that sustained smoking was negatively associated with the incidence of allergic sensitization as reflected by SPT positivity. This is consistent with another prospective population-based study that showed that consistent smokers were less likely to develop sensitization during an 8-year follow-up period (7). Furthermore, this is consistent with the findings of several cross-sectional population-based studies that have reported a lower prevalence of sensitization to common aeroallergens among current smokers than never-smokers (8–12). One of these, however, reported that the association between smoking and sensitization varied with the allergen; i.e., smoking was negatively associated with sensitization to grass and cat, but positively associated with sensitization to house-dust mite (8). In the present study, there were no major differences in the effect of smoking on the incidence of sensitization to the different groups of allergens (pollens, dander, and mites; data not shown). In contrast, some occupational studies have found an increased risk of smoking associated with allergic sensitization to occupational allergens (13–15), although others did not find such an association (16, 17).

There was a greater likelihood of participation in follow-up for subjects sensitized at baseline. However, this effect was not greater for never-smokers than current smokers (data not shown). Therefore, selection bias due to nonparticipation in the follow-up study does not seem to be a likely explanation for the negative association between smoking and the incidence of sensitization.

Several interpretations of the observed negative association between sustained smoking and the development of sensitization are possible. Firstly, the healthy-smoker effect, i.e., smokers who develop allergic symptoms tend to quit smoking, has been put forward to explain the somewhat surprising negative association between smoking and allergic sensitization found in population-based studies. In several cross-sectional studies, however, past smokers were less likely to be sensitized than never-smokers (2, 8, 9, 12), a finding which does not indicate that smokers who develop

sensitization tend to quit smoking. Furthermore, in our study population, sensitized smokers did not quit smoking more often than did nonsensitized smokers. Moreover, the negative association was similar among subjects who reported respiratory symptoms on exposure to either pollen or furry animals in the screening questionnaire and among those who did not (data not shown). Nevertheless, if subjects prone to developing allergic sensitization decide early in life not to take up smoking, this could account, at least in part, for the lower incidence of sensitization among smokers and past smokers.

Secondly, the effect of smoking could merely reflect an effect on the *in vivo* measurement of SPT reactivity. To our knowledge, there are no published data on this issue. However, sustained smoking was also negatively associated with sensitization when ascertained by the *in vitro* measurement of specific IgE. This association was not statistically significant, a finding which may be explained by the lower number of incident cases of specific IgE positivity.

Thirdly, the effect of smoking could reflect a causal association. Smoking increases the risk of various diseases including infections; cardiovascular disease; cancer at various sites, particularly the lungs; asthma; and chronic pulmonary lung disease. The contribution of smoking to the development of these diseases may, in part, be facilitated through a suppressive effect of smoking on the immune system. For example, smoking has been reported to decrease the activity of natural killer cells and to induce T-cell unresponsiveness, i.e., failure to respond normally to antigens or mitogens (18, 19). Smoking also decreases serum levels of most of the immunoglobulin classes (18, 19), with the exception of IgE, which is significantly increased in smokers (8, 9, 12, 20, 21). Thus, it seems to be a paradox that allergic sensitization appears to be less prevalent in smokers despite increased levels of IgE. However, the mechanisms by which smoking exerts its immunosuppressive effect are not fully understood. The elucidation of these mechanisms and their possible interaction with allergic immune responses may also provide new insights into the immunology of allergic diseases. It may be of interest that

the prevalence of colitis ulceritis, a disease which is characterized by eosinophilic inflammation in the mucosa of the colon, also seems to be less prevalent among smokers in comparison with nonsmokers (22).

In conclusion, in this adult population, sustained smoking was negatively associated with the development of allergic sensitization to aeroallergens during an 8-year follow-up. Since only a few prospective studies have addressed this issue, and since selective mechanisms beyond our control could play a role, no firm conclusion can be drawn at present on the role of smoking in the development of allergic sensitization. The elucidation of the mechanisms by which smoking impairs the immune system may provide a clue to the possible immunologic link between smoking and allergic sensitization.

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Acknowledgments

We thank the staff at the Centre for Preventive Medicine for their assistance; the Product Development staff at ALK-Abelló A/S, Hørsholm (Denmark), for performing serologic analyses; and Allen Sawitz for help with translation. The Copenhagen Allergy Study is supported by grants from the Danish Medical Research Council, the Danish Health Insurance Fund, the Danish Ministry of Health (the National Health Fund for Research and Development), the Danish Medical Research Council and the Danish Ministry of Health (Research Centre for Environmental Health: Environmental Health Research Programme 1997), and ALK – Abelló A/S, Denmark.