Cigarette Smoking and Risk of Clinically Overt Thyroid Disease

A Population-Based Twin Case-Control Study

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Background: The effects of cigarette smoking on the thyroid gland have been studied for years. However, the effect of smoking on thyroid function and size is still controversial.

Objective: To determine the impact of cigarette smoking on the development of clinically overt thyroid disease.

Methods: Matched case-control study of 132 same-sex twin pairs (264 individuals) discordant for clinically overt thyroid disease, ascertained from a population-based nationwide twin register. Information on thyroid disease and smoking habits was gathered by questionnaire, and the patients’ endocrinologist or general practitioner verified the diagnosis.

Results: Overall, smoking was associated with an increased risk of developing clinically overt thyroid disease (odds ratio, 3.0; 95% confidence interval, 1.4-6.6; P = .003). This association remained statistically significant in monozygotic and dizygotic disease-discordant pairs. The effect of smoking was more pronounced in monozygotic vs dizygotic pairs (odds ratio, 5.0 vs 2.5; P = .04 for both). Essentially similar results were obtained after subdividing the twin pairs into groups discordant for clinically overt autoimmune (49 pairs) and nonautoimmune (83 pairs) thyroid disease. Among twin pairs concordant for smoking, probands with clinically overt autoimmune thyroid disease smoked significantly more than did their healthy co-twins (17 pairs; P = .03), whereas no difference was found between probands with nonautoimmune thyroid disease and their healthy co-twins (34 pairs; P = .20).

Conclusions: Smoking is associated with an increased risk of developing clinically overt thyroid disease. Furthermore, our data suggest that cumulative cigarette consumption is a risk factor, most pronounced in autoimmune thyroid disease.

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AUTOIMMUNE thyroid disease (Graves disease and autoimmune thyroiditis) and simple goiter are among the most common thyroid disorders, with a prevalence of about 10% in iodine-replete areas. Although common, the etiology of these diseases is still incompletely understood. Autoimmune thyroid disease and simple goiter are believed to be multifactorial in origin, and genetic and environmental factors are thought to play a role in disease development.

During the past decade, several articles have dealt with the possible effects of cigarette smoking on the thyroid gland. However, the effect of smoking on thyroid function and size is still controversial. In some articles, smoking has been identified as a risk factor for Graves disease, autoimmune thyroiditis, and goiter, whereas other studies have not demonstrated such a relationship for autoimmune thyroiditis and goiter. These discrepancies are probably partly related to differences in size of study populations, definitions of smokers and non-smokers, iodine intake, and methods of evaluating thyroid size and function. On the other hand, some of these differences might also reflect that none of the previous studies took the presence of a possible genetic liability to thyroid disease into consideration. Indeed, conventional case-control studies (such as all those cited previously) of environmental effects in diseases with genetic determinants tend to dilute the magnitude of any effect (in this case of smoking) because of the lack of genetic susceptibility among some individuals in the control group.

A study of twin pairs discordant for thyroid disease provides an ideal method of avoiding the possible difficulties arising from differences in genetic risk among pro-
PATIENTS AND METHODS

PATIENTS

This study was based on the young part of the Danish Twin Register, which was established in 1991 and comprises 20,888 twin pairs born between January 1, 1953, and December 31, 1982. The ascertainment procedure and characteristics of the cohort have been described in detail elsewhere.\(^1\) The present study was restricted to 5479 same-sex twin pairs (10,958 individuals) born between January 1, 1953, and December 31, 1972, in which both members of a pair had participated in a nationwide questionnaire survey in 1994. The questionnaire was extensive and contained questions about health, social, and physical characteristics.

Screening questions for thyroid disease were as follows: Do you have or have you ever had hyperthyroidism? Hypothyroidism? Or goiter? Only twin pairs in which 1 or both patients responded positively to at least 1 of the 3 screening questions for thyroid disease were included in this study. In 502 twin pairs, 1 or both individuals indicated that they had or had had a thyroid disease. These pairs were sent a second, more detailed questionnaire in 1996 containing questions about the general signs and symptoms of thyroid disease. The names and addresses of the general practitioner, specialists, and hospitals attended by the twin because of thyroid disorders were also requested, as were the twins’ telephone numbers. After 1 reminder, 385 complete pairs (76.7%) had returned the 1996 questionnaire. All respondents were residents of Denmark, which is a non-endemic goiter area with borderline iodine deficiency and average urinary iodine excretion of 70 to 100 \(\mu g/24\) h.\(^1\) There were no demographic differences (evaluated by the distribution of the patients’ ZIP codes) between those who responded to the 1996 questionnaire and those who did not.

To verify or exclude the presence of self-reported thyroid disease, information from hospitals, outpatient clinics, specialists, and general practitioners was collected and reviewed by 2 of us (T.H.B. and L.H.) who were unaware of the zygosity of the twins. On the basis of this review, with emphasis on blood test results and clinical criteria, we identified 132 same-sex twin pairs (121 female and 11 male pairs) discordant for thyroid disease (Table 1). Because we only confirmed the absence of thyroid disease in screen-negative persons whose co-twins screened positive, we cannot exactly assess the extent of possible misclassification. However, we performed a record linkage between the 4977 screen-negative pairs (9954 individuals) and the National Discharge Register to study whether any of these patients had been discharged from a hospital with a thyroid-specific diagnosis. Only 8 (0.08%) of these patients were recorded in the register with a thyroid disease. Thus, bias originating from the selection procedure is highly unlikely.

Informed consent was obtained from all participants. The study was approved by all the regional scientific-ethical committees in Denmark and was conducted according to the principles of the Helsinki Declaration.

CASE-CONTROL TWIN METHOD

The case-control twin method, or the co-twin control method, is based on the classic matched case-control design. In this approach, twin pairs who are discordant for a disease (or other risk factors) are considered as matched pairs.\(^3\) The case-control twin method has an additional advantage over other matched pair designs in that twin pairs are also genetically matched: MZ twins share 100% of their genes and DZ twins share 50%, on average. In addition, twins are of the same age (in this study, also of the same sex), and because monozygotic (MZ) twins carry identical genes, the effect of smoking, if any, would be expected to be more apparent in disease-discordant MZ than in disease-discordant dizygotic (DZ) twins who, as ordinary siblings, share half their genes.

In the present study, we investigated the impact of smoking on clinically overt thyroid disease by comparing smoking habits among 132 same-sex twin pairs discordant for clinically overt thyroid disease.

### RESULTS

#### THYROID DISEASE OVERALL

Irrespective of zygosity and phenotype, there was a significant association between smoking and clinically overt thyroid disease (see Table 1 for the clinical spectrum) (OR, 3.0; 95% confidence interval, 1.4-6.6; \(P = .003\)). When twin pairs were stratified according to zygosity (MZ and DZ), the association remained statistically significant (\(P = .04\)) in both MZ and DZ pairs (Table 2). Moreover, the effect of smoking was more pronounced in MZ (OR, 5.0) than in DZ (OR, 2.5) pairs.

In 51 twin pairs who were discordant for smoking (both the proband and the healthy co-twin were smokers), cumulative tobacco consumption, counted as pack-years, was significantly higher in twins with overt thyroid disease than in their healthy co-twins (Figure). Restricting the sample to female pairs (data not shown) did not change any result.

#### AUTOIMMUNE THYROID DISEASE

In twin pairs discordant for autoimmune thyroid disease (49 pairs), there was, irrespective of zygosity, a significant association between smoking and clinical autoimmune thyroid disease (OR, 3.5; \(P = .03\)) (Table 2). In addition, among pairs discordant for smoking, probands smoked significantly more than their co-twins (\(P = .03\)) (Figure).

When twin pairs were stratified according to zygosity (MZ = 17 pairs and DZ = 32 pairs) or clinical disease (Graves disease = 35 pairs and autoimmune thyroiditis = 14), the association did not reach statistical significance (Table 2). However, even in the small number of pairs discordant for Graves disease \((n = 35)\), there was a trend toward an increased risk associated with smoking (OR, 3.7; \(P = .06\)). Moreover, cumulative tobacco consumption was highest among probands (Figure). In 8 pairs, the proband had Graves ophthalmopathy. Of these,
and usually share their early life environment, such as home location, nutrition, in-house toxicant exposures, and socioeconomic background. However, matched twin comparison can be inefficient because of overmatching,23 which is present when the matched variable (being a co-twin) is related to the exposure (smoking). That is, the co-twins' smoking status is not independent of the matched proband. The extent to which information is lost due to overmatching depends on the degree of association between the matched variable and the exposure. A strong association will lead to relatively few discordant pairs (on which the odds ratio [OR] is calculated) and hence, the comparison will be of low power. The strength of such a possible association between the matched variable and exposure can be estimated by κ statistics (see the “Statistical Analysis” subsection).

ZYGOSITY

Determination of zygosity was based on self-reported answers to specific questions about similarity and mistaken identity, which is a well-established and valid method in large twin populations.22 A comparison22 of this method with laboratory methods (serologic markers) has shown that misclassification occurs in less than 3%. Evaluation of zygosity was made by 1 of us (K.O.K.) who was unaware of the thyroid status of the twins.

SMOKING HISTORY

Information about smoking habits was obtained from the initial nationwide questionnaire survey in 1994. The participants were asked, “Do you or have you ever smoked daily?” All who answered yes were classified as “smokers,” and were asked to answer further questions about what (cigarettes, cheroots, cigars, or pipes) and when they started to smoke, whether and when they stopped smoking, and how much they smoked (number of cigarettes, cheroots, or cigars per day or amount of pipe tobacco per week). In the present study, all smokers smoked cigarettes. As a quantitative estimate of smoking history, we used pack-years, ie, the number of cigarettes smoked per day times the number of years smoked, divided by 20 (number of cigarettes in a pack).

For assessment of quantitative tobacco consumption in the healthy co-twin, we used the date until disease onset in the corresponding proband.

STATISTICAL ANALYSIS

Categorical data (smoking vs nonsmoking) were analyzed using 2 × 2 tables for paired observations and tested using the McNemar test, including a continuity correction for small numbers.23 In this approach, the OR is given by the ratio of pairs in which exposure differs (number of pairs in which the proband smokes and the healthy co-twin does not, divided by the number of pairs in which the healthy co-twin smokes and the proband does not). Noncategorical data were analyzed using the Wilcoxon signed rank test for paired data (within–twin pair differences) and the Mann-Whitney test for unpaired data (between–twin pair differences).

Extent of overmatching was estimated by κ statistics.24 The κ statistic is usually interpreted as a measure of the chance-corrected proportional agreement within matched sets (in this case being a co-twin and smoking status). A κ value of zero indicates that the exposure status (smoking or not) of the co-twins is independent of that of the probands, whereas a κ value of 1 indicates a complete association. The κ values for smoking within the twin pairs discordant for clinically overt thyroid disease are given in Table 1.

All tests applied were 2-tailed, and P≤.05 was considered significant.

6 (75%) were smokers compared with 3 (38%) of the corresponding healthy co-twins (OR, 4.0; 95% confidence interval, 0.4-94.0).

NONAUTOIMMUNE THYROID DISEASE

In twin pairs discordant for a nonautoimmune thyroid disease (83 pairs), an increased risk associated with smoking was suggested (OR, 2.7; P = .06). Again, the effect of smoking seemed more apparent in MZ than in DZ pairs, although the effect estimates were imprecise (Table 2). Finally, there was no difference in number of pack-years between probands with nonautoimmune thyroid disease and their healthy co-twins in pairs discordant for smoking (Figure). Restricting the sample to pairs discordant for simple goiter (79 pairs) did not change the results.

COMMENT

In this study, we investigated the relationship between smoking and clinically overt thyroid disease among disease-discordant same-sex twin pairs. We found that smoking was associated with an increased risk of developing clinically overt thyroid disease (OR, 3.0). Most important, this association remained significant in disease-discordant MZ pairs (OR, 5.0), which eliminates the effect of genetic factors in the development of thyroid disease because MZ twins are genetically identical, yet by definition, only the proband has been exposed to the potential risk factor (in this case, smoking). Furthermore, we found that probands smoked more heavily than their healthy co-twins. This indicates that cumulative cigarette consumption might also have an effect on the development of clinically overt thyroid disease in genetically susceptible individuals.

We also tested the specificity of the relationship between smoking and clinically overt thyroid disease by subdividing the twin pairs into groups discordant for clinically overt autoimmune and nonautoimmune thyroid disease. Using this approach, smoking was associated with autoimmune and nonautoimmune thyroid disease, although the association with the latter was not, strictly speaking, statistically significant (P = .06). The presence of overmatching, which was clearly the case in our study, may well explain why no statistically significant association was found in this group, despite the relatively large number of pairs studied. This limitation of the co-twin case-control method may serve as a
reminder that no method is ideal for all circumstances. Overmatching might also at least partially explain why further stratification into MZ and DZ twins—among pairs discordant for autoimmune and nonautoimmune thyroid disease—was characterized by few twin pairs being discordant for smoking. However, although the effect estimates were imprecise, the effect of smoking seems to be more apparent in MZ than in DZ pairs.

The most striking effect of smoking on the thyroid is its strong association with Graves ophthalmopathy and Graves disease without ophthalmopathy, although the latter association is weaker.6,25 Although previous studies have repeatedly shown a clear association between smoking and Graves disease, with and without ophthalmopathy, they have come to somewhat different conclusions regarding a possible correlation between smoking severity and the prevalence or severity of Graves disease.6 In the present study, we found that, among twin pairs concordant for smoking, probands with clinically overt Graves disease smoked significantly more than their healthy co-twins. This finding is consistent with results from other studies6,26 demonstrating a positive correlation between cumulative cigarette consumption (counted in pack-years) and the development of Graves disease in genetically susceptible individuals. Such a correlation could not be demonstrated in clinically overt nonautoimmune thyroid disease, indicating that a dose effect of smoking is a risk factor in autoimmune thyroid disease, but not in thyroid disease in general.

Our results should be interpreted in the context of several potential limitations. First, data in this study were obtained from twins, and whether these data can be generalized to the singleton population depends on whether twins can be considered to be no different from non-twin individuals in the background population with respect to the prevalence of clinical thyroid disease. Unfortunately, the prevalence of clinical thyroid disease in Denmark among men and women aged 20 to 40 years is unknown. However, in our study, the prevalence of confirmed clinically overt thyroid disease was 3% in females and 0.3% in males (based on both concordant and discordant pairs), which is comparable with reported1 prevalences in other nonendemic areas. Thus, there is no evidence of any major differences between twins and singletons in the prevalences of thy-

Table 1. Characteristics and  for 132 Disease-Discordant Twin Pairs by Phenotype and Zygosity*  

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Twin Pairs, No.</th>
<th>Age at Diagnosis, Mean (Range), y</th>
<th>k</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MZ</td>
<td>DZ</td>
<td>MZ</td>
</tr>
<tr>
<td>Autoimmune thyroid disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Graves disease</td>
<td>12</td>
<td>23</td>
<td>26.6 (19.0-36.0)‡</td>
</tr>
<tr>
<td>Autoimmune thyroiditis†</td>
<td>5</td>
<td>9</td>
<td>30.8 (25.0-34.0)‡</td>
</tr>
<tr>
<td>Nonautoimmune thyroid disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simple goiter</td>
<td>22</td>
<td>57</td>
<td>26.2 (15.0-40.0)‡</td>
</tr>
<tr>
<td>Nodular toxic goiter</td>
<td>1</td>
<td>3</td>
<td>25.0‡</td>
</tr>
</tbody>
</table>

* MZ indicates monozygotic; DZ, dizygotic.
† Includes Hashimoto thyroiditis and primary myxedema and atrophic thyroiditis.
‡ MZ vs DZ, P > .30 (Mann-Whitney, 2-tailed).
§ Includes Graves disease and autoimmune thyroiditis.
|| Includes simple goiter and nodular toxic goiter.

Table 2. Number of Disease-Discordant Twin Pairs by Smoking Status of the Proband and the Healthy Co-Twin, by Phenotype and Zygosity*  

<table>
<thead>
<tr>
<th>Phenotype and Zygosity</th>
<th>Smoking Status of Proband and Healthy Co-Twin</th>
<th>Odds Ratio (95% Confidence Interval)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid disease</td>
<td>Smoker and Nonsmoker</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MZ</td>
<td>18 33 51</td>
<td>5.0 (1.0-33.0) .04</td>
<td></td>
</tr>
<tr>
<td>DZ</td>
<td></td>
<td>2.5 (1.1-6.2) .04</td>
<td></td>
</tr>
<tr>
<td>MZ + DZ</td>
<td></td>
<td>3.0 (1.4-6.6) .003</td>
<td></td>
</tr>
<tr>
<td>Autoimmune thyroid disease</td>
<td>Smoker and Nonsmoker</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonautoimmune thyroid disease</td>
<td>Smoker and Nonsmoker</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MZ</td>
<td>12 22 34</td>
<td>4.0 (0.4-93.9) .37</td>
<td></td>
</tr>
<tr>
<td>DZ</td>
<td></td>
<td>2.4 (0.8-7.6) .15</td>
<td></td>
</tr>
<tr>
<td>MZ + DZ</td>
<td></td>
<td>2.7 (1.0-7.6) .06</td>
<td></td>
</tr>
</tbody>
</table>

* MZ indicates monozygotic; DZ, dizygotic. Number of pairs discordant for Graves disease is given in square brackets (odds ratio MZ+DZ, 3.7; 95% confidence interval, 1.0-16.5; P = .06).

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necessarily matched for variables connected with life that even MZ smoking-discordant twin pairs are not precipitating. Indeed, it has repeatedly been reported28,29 from nonsmokers in several characteristics other areas. Finally, one might argue that smokers tend to differ from nonsmokers in several characteristics other than smoking, 1 or more of which might be disease precipitating. Indeed, it has repeatedly been reported28,29 that even MZ smoking-discordant twin pairs are not necessarily matched for variables connected with life events, education, occupation, or drinking and food habits. Thus, smoking might simply be an innocent bystander hiding the real causative factor(s). Recent reviews3-4 point toward certain infectious agents, iodine intake, and stressful life events as possible environmental factors. However, most of the background population is also exposed to 1 or more of these various environmental determinants, including smoking. Yet, only a small fraction actually develop clinically overt autoimmune thyroid disease or simple goiter. Thus, it seems apparent that if any of these exposures play a causative role in the development of these thyroid abnormalities, none can cause them on their own.

Despite these limitations, we believe that our study provides irrefutable evidence of an association between smoking, or a factor related to smoking, and an increased risk of developing clinically overt thyroid disease in genetically predisposed individuals, at least in areas with borderline iodine insufficiency.

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