Clinical Investigation

Carotid Arteriosclerosis in Identical Twins Discordant for Cigarette Smoking

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Y. Antero Kesäniemi, MD, and Kauko Heikkilä, LicPhil

From a nationwide twin panel, identical twin pairs with highest discordance in cigarette smoking were selected for a study of arteriosclerosis (49 pairs with a mean age of 52 years). Smoking history was obtained in 1975, 1981, and 1986. The mean life-long smoking dose of the smoking cotwins was 20 package-years. The smoking and nonsmoking cotwins had similar systolic and diastolic blood pressures, total plasma cholesterol level, body mass index, and some psychosocial factors; the only difference was found in use of alcohol, which was greater among smoking cotwins. Duplex sonography of carotid arteries was performed. Carotid artery stenoses (narrowing of area of the lumen with 15–60%) were found in nine pairs: in nine smoking twins and in two of their nonsmoking cotwins (p = 0.036). The total area of carotid plaques was 3.2 times larger in smoking cotwins (p < 0.001). The thickness of the inner layer of carotid arteries was more marked in smoking cotwins (p < 0.001). The size of plaques and the degree of inner layer thickening correlated with the dose of smoking (NS). The association of smoking with carotid arteriosclerosis was highly significant even after the adjustment for age, total plasma cholesterol level, diastolic blood pressure, and body mass index in multiple logistic regression analyses. (Circulation 1989;80:10–16)

Smoking is associated with arteriosclerotic lesions. The relation has been most consistently shown in the abdominal aorta and in major arteries of the lower limbs,1-8 but in coronary arteries, the relation has been less consistently shown.4,9-11 There are only a few reports on the relation between smoking and atherosclerosis in cerebral arteries.12 In contrast to other epidemiologic evidence, studies of smoking-discordant twin pairs have not shown differences in manifest coronary heart disease in the smoking and nonsmoking cotwins.13-19 These findings have suggested to critics that the association of smoking and coronary heart disease is noncausal.20-22 Studies of mortality among smoking-discordant pairs found only a slightly increased risk of death in the smoking cotwin compared with the nonsmoking cotwin.2,23 Therefore a study of the incidence of ischemic heart disease in smoking-discordant twin pairs was undertaken in Finland in 1975; from 208 ischemic heart disease–free identical male pairs discordant for cigarette smoking, during a 6-year follow-up, 16 smoking cotwins and three nonsmoking cotwins died or were hospitalized because of ischemic heart disease.24 This result raised the question, “Was the increased ischemic heart disease risk associated with arteriosclerosis among the smoking cotwins?” Therefore, we decided to study noninvasively the carotid arteriosclerosis of identical twin pairs discordant for smoking.

Methods

Subjects

The Finnish Twin Cohort comprises all Finnish sex-matched twin pairs born before 1958 and alive in 1967. A questionnaire was mailed in 1975 to determine zygosity and to obtain data on health-related variables. The overall response rate was 89%. A second questionnaire study was carried out in 1981, and the response rate was 84%. The validity of zygosity was studied in a subsample of 104 pairs. The agreement of diagnosis from the questionnaires and 11 blood markers was 100%, and the estimated probability of misclassification was 1.7%.25

Smoking was defined by the following criteria. 1) Nonsmokers. Never smokers: life-long dose of cig-

The questionnaire included questions on whether diabetes mellitus had been diagnosed by a physician. The reported use of alcohol (amount of beer, wine, and hard liquor consumed separately each month) was converted to grams of absolute alcohol. Questions were included on the use of coffee, physical activity, marital status, education, and hostility.26

In 1986, 78 identical pairs (61 male and 17 female pairs) with the highest discordance in the life-long dose of cigarette smoking were invited to Seinäjoki Central Hospital. Smoking history was obtained from the 1975 and 1981 questionnaires and checked by the interview in 1986, and the life-long smoking dose of smokers was calculated by multiplying the mean number of cigarettes smoked daily and the number of years of smoking (by using the age of starting, possible quitting, and years without smoking). The life-long dose of smoking was calculated as package-years (i.e., smoking of one package each day during a year).

Twenty-eight (36%) pairs refused or did not respond, 16 (26%) male and 12 (71%) female pairs. The mean age of the participants was 52 years, and that of the nonparticipants was 58 years. The participant and nonparticipant male pairs did not differ in the life-long dose of smoking, but the participant female smokers had smoked more than the nonparticipants. For the nonparticipants, the distances of their homes from the Seinäjoki hospital were more than 200 miles in 19 pairs. Forty-five male and five female pairs were examined. One male pair was excluded from the analyses because a heavy smoking history was found in both cotwins. The mean age was 52 years (range, 31–77 years). The distribution of the smoking cotwins by the life-long smoking dose was >29 package-years (n = 7), 20–29 (n = 16), 10–19 (n = 16), and <10 (n = 10). The mean package-years of smoking cotwins was 19.7. In the nonsmoking cotwin group, 47 were nonsmokers, and two were exsmokers. The smoking doses of the two exsmokers were 7 and 1 package-years. The discordance in smoking of these two pairs was 19 and 17 package-years. Urine cotinine was measured in 10 pairs: all smoking cotwins were positive, but their nonsmoking cotwins showed no trace of smoking.27

Blood pressure was measured in the sitting position from the left arm with a standard cuff and a mercury manometer. Total plasma cholesterol level was determined from fasting blood samples.28 No statistical differences were found between smoking and nonsmoking cotwins in the following variables: body mass index, systolic and diastolic blood pressures, total plasma cholesterol level, prevalence of diabetes mellitus, use of coffee, physical activity, marital status, education, and self-rating of hostility (Table 1). The reported use of alcohol consumption was almost two times higher among smoking cotwins (p <0.05) (Table 1).

Duplex sonography was performed in all cases by one of the authors (A.H.). Carotid variables were estimated without the knowledge of smoking history. A Technicare Autoscan (Englewood, Colorado) with a 7.5-MHz real time transducer and a pulsed Doppler was used. Hard copies of real time images were made for longitudinal sections of common carotid arteries and transverse sections of carotid bifurcations. If the bifurcation had less than 15% stenosis (an arbitrary limit of decrease of lumen area), the condition was classified as a “nonstenosis,” and in case the decrease was more than 15%, it was classified as a “stenosis.” The decrease of lumen area was computer-calculated from the “intact” and stenosed intimal lines in the transverse section. Doppler spectra from common and internal carotid arteries were additionally used,29,30 but no significant discrepancy between real time image and Doppler spectra was observed in any of the cases. The dimensions of the plaques and the inner surface thicknesses were measured with a ruler on the hard copy (by attempting to use 0.25-mm increments) 2 months after the clinical examination by one of the authors (A.H.) without knowing the smoking status: 1) the size and the number

<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-smoking cotwins</th>
<th>Smoking cotwins</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>137±16.3</td>
<td>132±16.4</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>94±11.3</td>
<td>92±11.9</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.4±3.2</td>
<td>25.5±3.1</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.88±1.13</td>
<td>5.95±1.32</td>
<td>NS</td>
</tr>
<tr>
<td>Reported diabetes diagnosed by a physician (%)</td>
<td>4.3</td>
<td>2.1</td>
<td>NS</td>
</tr>
<tr>
<td>Blue collar work (%)</td>
<td>45.8</td>
<td>54.3</td>
<td>NS</td>
</tr>
<tr>
<td>Sport training (yr)</td>
<td>1.7±2.1</td>
<td>1.2±1.8</td>
<td>NS</td>
</tr>
<tr>
<td>Education (yr)</td>
<td>8.1±2.7</td>
<td>8.0±2.0</td>
<td>NS</td>
</tr>
<tr>
<td>Married (%)</td>
<td>93.6</td>
<td>89.4</td>
<td>NS</td>
</tr>
<tr>
<td>Reported use of coffee (cups/day)</td>
<td>5.4±3.7</td>
<td>5.6±2.7</td>
<td>NS</td>
</tr>
<tr>
<td>Reported use of alcohol (g/mo as absolute alcohol)</td>
<td>258±413</td>
<td>460±405</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Hostility score (range, 3–15)</td>
<td>6.6±2.8</td>
<td>6.6±2.7</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean±SD. p values by intrapair difference and its SD. NS is p>0.05.
of the echogenic plaques from the transverse section of both carotid bifurcations and 2) the thickness of the inner surface of the vessel wall at the thickest site of both common carotid arteries from the longitudinal section. The bright line along the inner margin of the artery wall and the sonoluent line beneath this echogenic line were included in this measurement. For clarification of intraobserver and interobserver variation of the measurements, we have conducted a separate study of 26 individuals (52 carotid arteries) in the same manner as in the study of twin pairs. The assessments were made on the same hard copies by two independent observers. The interobserver agreement of the assessments of inner layer thickening was 73% when classified with accuracy of 0.25 mm and was 83% when classified with accuracy of 0.5 mm. The agreement of the assessments of the summed area of echogenic plaques was 88% when classified with an accuracy of 5 mm². The corresponding values for the intraobserver agreement were 83%, 96%, and 88%.

The area of echogenic carotid plaques correlated highly significantly ($p<0.001$) with age ($r=0.36$), with the degree of carotid stenosis ($r=0.89$), and with the inner layer thickening ($r=0.59$).

**Statistical Analysis**

Data were missing for cholesterol levels in two smokers and for inner layer thickening in one non-smoker. Statistical difference between smokers and nonsmokers was tested with Student’s $t$ test and the intrapair difference and its standard deviation. Confidence limits of 95% for ratios were calculated by Fisher’s method. Conditional logistic regression models were used in multivariate analysis; relative risks and their confidence limits were calculated using the $\beta$-coefficient and its SEM.

**Results**

Carotid artery stenosis (narrowing of area of the lumen of 15% or more) was found in nine pairs: in nine smoking cotwins and in two nonsmoking cotwins ($p=0.036$) (Table 2). An example of carotid stenosis is shown in Figure 1. In addition, minor plaques in the carotid bifurcation were found in 38 of 49 smoking cotwins and in 38 of 49 nonsmoking cotwins. The mean number of echogenic plaques for each person (excluding persons with carotid stenosis) was 1.90 (SD=0.70) for smokers and 1.23 (SD=0.85) for nonsmokers ($p<0.001$). The mean area of all carotid plaques was 3.2 times larger in smoking cotwins than in nonsmoking cotwins ($p<0.001$) (Table 2). The thickness of the inner layer of carotid arteries was more marked in smoking cotwins ($p<0.001$) (Table 2). An example of inner layer thickening is shown in Figure 2.

The area of carotid plaques of the smoking cotwins compared with that of the nonsmoking cotwins had the following ratios with an increase in smoking dose: 2.66 (for the subgroup in which the smoking cotwins had smoked less than 10 package-years), 3.15 (10–19 package-years), 3.49 (20–29 package-years), and 3.57 (>29 package-years). Correspondingly, the ratios for the degree of inner layer thickening were 1.25, 1.27, 1.40, and 1.46. Those two trends, however, were statistically nonsignificant.

The distribution of individuals by the total area of carotid plaques, smoking, and plasma cholesterol level is given in Table 3, and the distribution of carotid arteries by inner layer thickening, smoking, and serum cholesterol level is given in Table 4.
Inner layer thickening was more marked among smokers than among nonsmokers in both subgroups of individuals: those with serum cholesterol levels above 6.0 mmol/l and those below it. The corresponding difference for the size of echogenic plaques was significant only in individuals with cholesterol levels above 6.0 mmol/l.

The association of smoking and carotid arteriosclerosis was also tested by a conditional logistic model with dichotomized variables. Age-adjusted relative risk (odds ratio calculated from the regression coefficient of the logistic model) of all indicators of carotid arteriosclerosis was significantly increased among smoking cotwins even after the adjustment of total plasma cholesterol level, diastolic blood pressure, and body mass index (Table 5).

**Discussion**

The validity of zygosity diagnosis was satisfactory for epidemiologic purposes because the overall probability of misclassification was 1.7% by blood tests in a subsample.25 A high proportion (89%) of the twins responded to questionnaire studies. Fifty of seventy-eight selected pairs (64%) participated in
the clinical examination. The relatively low participation rate was not surprising because the study involved long travel in most cases. The nonparticipants were mainly twin pairs over 60 years old. Therefore, arteriosclerotic changes were probably more frequent among nonparticipants than among participants. The mean life-long smoking dose of smoking cotwins corresponded to smoking one package each day for 20 years. The corresponding dose of the same-aged Finnish current male smokers was 28 package-years. The life-long dose of smoking of the current sample was clearly lower than that of the age- and sex-matched Finnish population. Thus, our results probably underestimate the real effect of smoking on carotid arteriosclerosis in the age- and sex-matched Finnish population.

Duplex carotid ultrasonography has been used for the screening of carotid arteriosclerosis. The duplex sonography of carotid arteries is an operator-dependent examination. In carotid stenosis over 50%, the sensitivity of duplex sonography was 91–94%, and the specificity was 85–89% according to previous studies. Recently, a Finnish population study that used high-resolution ultrasonography to assess carotid atherosclerosis as in our study reported that the agreement of reassessment was 89.8% when carotid arteriosclerosis was classified into four groups. In our study, the reliability of intraobserver and interobserver variation was clarified by the high correlation between reassessments and the assessments made by two independent observers and by the highly significant intercorrelations of the three different measurements of carotid arteriosclerosis.

The so-called "echogenic line" is a frequent finding in the real time image of carotid arteries in persons over 30 years of age, and it seems to correspond to the surface of the intima. The sonolucent line typically seen beneath the echogenic

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**Table 3. Distribution of Individuals by the Size of Carotid Plaques, Cigarette Smoking, and Plasma Cholesterol**

<table>
<thead>
<tr>
<th>Total size of echogenic carotid plaques (both sides) (mm²)</th>
<th>Cholesterol (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Smokers</td>
</tr>
<tr>
<td></td>
<td>&gt;6.0</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>0&lt;1</td>
<td>2</td>
</tr>
<tr>
<td>1&lt;5</td>
<td>3</td>
</tr>
<tr>
<td>5&lt;10</td>
<td>5</td>
</tr>
<tr>
<td>10&lt;15</td>
<td>6</td>
</tr>
<tr>
<td>15&lt;20</td>
<td>1</td>
</tr>
<tr>
<td>20&lt;30</td>
<td>1</td>
</tr>
<tr>
<td>30&lt;40</td>
<td>2</td>
</tr>
<tr>
<td>40&lt;60</td>
<td>2</td>
</tr>
<tr>
<td>80&lt;90</td>
<td>1</td>
</tr>
<tr>
<td>n</td>
<td>21</td>
</tr>
</tbody>
</table>

Mean (mm²) 9.7 5.4 2.4 2.1
95% confidence limits 5.0–14.4 2.2–8.6 1.1–3.8 1.1–3.1
Proportion of those with plaque size ≥10 mm² (%) 57 27 18 11

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**Table 4. Distribution of Carotid Arteries by Inner Layer Thickening, Cigarette Smoking, and Plasma Cholesterol**

<table>
<thead>
<tr>
<th>Degree of carotid inner layer thickening (0.5 mm)</th>
<th>Cholesterol (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Smokers</td>
</tr>
<tr>
<td></td>
<td>&gt;6.0</td>
</tr>
<tr>
<td>0&lt;0.5</td>
<td>5</td>
</tr>
<tr>
<td>0.5&lt;1.0</td>
<td>10</td>
</tr>
<tr>
<td>1.0&lt;1.5</td>
<td>19</td>
</tr>
<tr>
<td>1.5&lt;2.0</td>
<td>6</td>
</tr>
<tr>
<td>≥2.0</td>
<td>2</td>
</tr>
<tr>
<td>n</td>
<td>42</td>
</tr>
</tbody>
</table>

Mean (mm) 0.99 0.84 0.71 0.67
95% confidence limits 0.85–1.13 0.75–0.93 0.62–0.80 0.61–0.73

Proportion of those with inner layer thickening ≥1.5 mm (%) 36 19 11 2

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**Table 5. Age-Adjusted Odds Ratios (and 95% Confidence Limits) for Carotid Arteriosclerosis by Cigarette Smoking in Univariate Models and in Multivariate Models Adjusted for Plasma Cholesterol, Diastolic Blood Pressure, and Body Mass Index in 49 Identical Smoking-Discordant Twin Pairs**

<table>
<thead>
<tr>
<th>Arteriosclerotic endpoint</th>
<th>Carotid stenosis &gt;15% (n=11/98)</th>
<th>Total area of carotid plaques ≥10 mm² (n=26/98)</th>
<th>Carotid inner layer thickening ≥1.2 mm (n=21/97)</th>
<th>Either of those changes (n=34/98)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Univariate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current and exsmokers (vs. nonsmokers)</td>
<td>5.99</td>
<td>3.97</td>
<td>5.91</td>
<td>6.21</td>
</tr>
<tr>
<td>95% confidence limit</td>
<td>(1.19–30.3)</td>
<td>(1.56–11.6)</td>
<td>(1.76–19.8)</td>
<td>(2.13–18.1)</td>
</tr>
<tr>
<td>Multivariate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current and exsmokers (vs. nonsmokers)</td>
<td>6.24</td>
<td>3.91</td>
<td>6.96</td>
<td>6.37</td>
</tr>
<tr>
<td>95% confidence limit</td>
<td>(1.15–33.8)</td>
<td>(1.29–11.9)</td>
<td>(1.79–27.1)</td>
<td>(2.08–19.5)</td>
</tr>
</tbody>
</table>

Four univariate and four multivariate logistic regression models with dichotomized variables; cut-off points: 6.0 mmol/l for total cholesterol, 100 mm Hg for diastolic blood pressure, and 26 for body mass index.
intimal reflection probably represents the composite of intima and media sandwiched between the strong reflections from the intimal surface and the adventitial layer.40

A great deal of data of the causes of arteriosclerosis has been accumulated, but direct information is lacking on how smoking and other risk factors are related to the cellular and molecular factors.41-45 Smoking is associated with development of arteriosclerotic lesions in abdominal aorta and in major arteries of the lower limb1-8 and in coronary arteries.4,9-11 The relation between smoking and cerebral atherosclerosis has not been so widely studied, and the reported results have shown only a weak association.12 Smoking seems to be a significant risk factor for ischemic heart disease but not a strong and consistent risk factor for stroke.46-48

Smoking cotwins were slightly leaner than their nonsmoking twin partners, but the smoking and nonsmoking cotwins had similar blood pressures, serum cholesterol levels, prevalence of diabetes mellitus, use of coffee, physical activity, education, marital status, and aggressive behavior. Smoking cotwins consumed more alcohol than nonsmoking cotwins. Numerous epidemiologic and biochemical reports, however, suggest that the use of alcohol has an antiatherogenic effect. The higher consumption of alcohol of the smoking cotwins possibly masks the atherogenic effect of smoking found in our sample. In other studies on identical twin pairs discordant for smoking, smoking and nonsmoking cotwins also had similar serum cholesterol levels49 and psychosocial characteristics.50 Some nontwin studies of smoking and serum lipids, however, have shown that plasma high-density lipid cholesterol levels tend to be lower in smokers than in nonsmokers.51-54 The discrepancy between the results of studies of twins and nontwins may be explained in two ways: 1) twins are “overmatched”; that is, a smoker and his nonsmoking cotwin are more similar than an average smoker and nonsmoker with respect to the factors affecting serum high-density lipid cholesterol; 2) the proportion of heavy smokers was low in identical twins discordant for smoking in reported samples.

The association of smoking with carotid arteriosclerosis was significant also after adjustment for age, total plasma cholesterol level, diastolic blood pressure, and body mass index. The sample was small for subgrouping by the smoking dose, but the results showed a nonsignificant trend between smoking dose and degree of carotid arteriosclerosis.

Conclusions

Our results seem to indicate that smoking is a strong factor in the development of carotid arteriosclerosis independent of genetic factors, blood pressure, serum cholesterol level, body mass index, and a number of other potential confounding factors (diabetes, use of coffee and alcohol, physical activity, education, marital status, and hostility) in a population with a high serum cholesterol level.

Earlier studies of twins on smoking and coronary heart disease13-19 showed no or little difference between smoking and nonsmoking cotwins. These unexpected findings supported proponents20-22 of a noncausal hypothesis for the association between smoking and atherosclerosis and coronary heart disease. Recent data on the 21-year follow-up of the Swedish smoking-discordant twin pairs55 and the 12-year follow-up of the Finnish sample56 show that the smoking twins are at a significantly higher risk of coronary heart disease than their nonsmoking cotwins. The earlier negative results were probably due to the low level of smoking discordance and the small power of the studies. These new mortality studies and the results of our present study strongly support a causal role of smoking in the development of atherosclerosis.

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**KEY WORDS**
- carotid inner layer thickening
- duplex sonography
- serum cholesterol
- diastolic blood pressure
- carotid arteriosclerosis
- identical twins
- cigarette smoking
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