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Elderly Patients With Minimal Common Carotid Atherosclerosis Not Infrequently Have Severe Coronary Atherosclerosis and Myocardial Infarction

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Background The presence of discordances between common carotid and coronary atherosclerosis in the same individual has not been previously reported.

Methods and Results The subjects of the present study were 1,518 consecutive autopsy cases at a general geriatric hospital. All were aged 60 years or older (821 men, 697 women) with an average age of 80 years. The atherosclerotic index of the common carotid artery (CC-AI) and coronary stenotic index (CSI) were semi-quantitatively evaluated. The simple correlation coefficient between the CC-AI and CSI was 0.456 ($p<0.0001$). Among 689 cases with minimal common carotid atherosclerosis (CC-AI ≤2), 74 (11%) had severe coronary atherosclerosis (CSI ≥12), 68 (10%) had coronary heart disease, and 80 (12%) had pathologically-verified myocardial infarction (MI). Among those with minimal common carotid atherosclerosis, the serum total cholesterol level, diabetes mellitus, and history of smoking were significantly higher or more frequent in cases with a CSI ≥12 than in the patients with a CSI <12.

Conclusions A considerable proportion of cases with minimal common carotid atherosclerosis had severe coronary atherosclerosis and MI. This discordance can potentially lead to an underestimation of coronary risks if normal common carotid morphology is obtained by ultrasound. (*Circ J* 2008; **72:** 1946–1952)

Key Words: Aging; Atherosclerosis; Carotid arteries; Coronary artery disease; Pathology

Atherosclerosis is a systemic disease affecting the intima of medium-sized or large arteries. Although the clinical evaluation of atherosclerosis is generally difficult, its superficial location beneath the skin of the carotid artery enables the use of carotid ultrasonography to estimate severity by evaluating luminal stenosis, intima–media thickness (IMT), and the presence and extent of plaque. Because the severity of carotid atherosclerosis supposedly reflects that of systemic atherosclerosis, a positive result on carotid ultrasonography is generally considered a risk factor for cardiovascular events, especially for coronary heart disease and stroke[1–3] Carotid IMT is even regarded as an indicator of generalized atherosclerosis[4]

The correlation between carotid and coronary atherosclerosis, however, remains unknown in the general population, because the assessment of coronary atherosclerosis is not as easy or as safe as carotid ultrasonography. Significant correlations have been reported among patients who underwent coronary angiography[5–16] Nevertheless those results can not be simply applied to the general population because of the apparent subject biases in the studies. Previous autopsy-based pathological reports have also described positive correlations between carotid and coronary atherosclerosis, but the correlations were relatively weak, with the correlation coefficients ranging from 0.4 to 0.6[17–21]

Consequently, the data suggest possible discordances between carotid and coronary atherosclerosis in the same individual. Thus, the simple assumption of a good correlation in atherosclerotic severity among the different arterial beds could lead to an under- or overestimation of coronary atherosclerosis in discordant cases. Recently, Bots et al addressed this issue of weak correlation between the carotid IMT and coronary atherosclerosis in an excellent review[22]

The present study attempted to identify discordances between carotid and coronary atherosclerosis by examining a large number of consecutive autopsy cases. We focused on cases with minimal carotid atherosclerosis, but with severe coronary atherosclerosis, in which the risk of coronary atherosclerosis would be underestimated based on the findings of common carotid ultrasonography alone.

Methods

Subjects

The subjects comprised 1,518 consecutive autopsy cases, performed at the Tokyo Metropolitan Geriatric Hospital (Tokyo, Japan) between 1995 and 2003, excluding 30 cases of regional autopsies or with insufficient descriptions of atherosclerosis. All the patients had been aged 60 years or older at the time of their death. No medicolegal cases or cases of community death were included in the series. The patients' characteristics are shown in Table 1. The male-to-female ratio was 1.2. The average age of the women at the time of death was 82.2 years, which was significantly higher

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Table 1 Clinical Summary of the Patients

	Total	Men	Women	p value
No. of cases	1,518	821	697	
Age at death (years)				
Mean±SD	80.6±8.4	79.2±7.9	82.2±8.6	<0.0001*
Range	60–104	60–102	60–104	
Lifestyle				
History of smoking	51.4%	73.6%	24.0%	<0.0001*
History of drinking	35.9%	55.7%	11.3%	<0.0001*
Clinical diagnosis				
Hypertension	29.3%	27.6%	31.3%	0.127
Diabetes mellitus	15.0%	15.0%	15.1%	1.000
Cerebrovascular disease	29.5%	30.5%	28.4%	0.397
Coronary heart disease	16.3%	15.6%	17.2%	0.404
Aneurysm	4.7%	5.6%	3.6%	0.068
Peripheral arterial disease	4.0%	4.6%	3.3%	0.238
Serum lipid profile				
Total cholesterol (g/L)	1.66±0.44	1.58±0.42	1.76±0.45	<0.0001*
Total cholesterol ≥2.2	11.2%	7.5%	16.2%	<0.0001*
HDL-cholesterol (g/L)	0.43±0.16	0.42±0.15	0.44±0.16	0.005*
HDL-cholesterol <0.4	44.9%	48.6%	40.1%	0.003*
Direct cause of death				
Malignancy	32.5%	33.7%	31.0%	0.271
Pneumonia	13.5%	14.9%	12.0%	0.098
Heart disease	12.2%	10.2%	14.6%	0.012*
Cerebrovascular disease	5.6%	4.4%	6.9%	0.042*
Miscellaneous	36.2%	36.8%	35.5%	0.629

*$p<0.05$.
HDL, high-density lipoprotein.

than that of the men (79.2 years). The average autopsy rate during this time period was 40%. We have been posting our autopsy cases on an Internet-based database known as "The Japanese SNP database for geriatric research (JG-SNP)" (http://www.tmgh.metro.tokyo.jp/jg-snp/english/E_top.html) since April 2003, as previously reported[23]

Clinical Information

The subjects' histories of smoking and drinking, the presence of various underlying or complicating diseases, and their serum lipid values were retrieved from their clinical charts. Information on smoking and drinking habits were available in 1,387 cases (91.4%) and 1,383 cases (91.1%), respectively. Smoking and drinking habits were significantly more common among the men than among the women.

The mean of all available values measured in either the outpatient clinic or during admission represented each patient's serum lipid value. Serum total cholesterol and high-density lipoprotein-cholesterol (HDL-C) data were available in 1,192 cases (78.5%). The data collection of lipid measurement was reviewed in the first 100 cases. The number of measurements of total cholesterol ranged from 0 to 89 times, with a mean (±SD) and median of 16.6 (±19.5) and 9 times, respectively. The number of measurements of HDL-C ranged from 0 to 82 times, with a mean (±SD) and median of 10.8 (±15.0) and 4.5 times, respectively. The duration of observation reached 5,733 days with a mean (±SD) and median of 1,972 (±1,716) and 1,540 days, respectively. The average total cholesterol and HDL-C values were relatively low, indicating malnutrition and a decrease in the daily activity levels of the subjects prior to death. The average total cholesterol and HDL-C levels were both significantly higher in women than in men. Hypercholesterolemia (total cholesterol ≥2.2 g/L) was more prevalent in women, whereas hypo-HDL-cholesterolemia (HDL-C <0.4 g/L) was predominant in men.

The direct causes of death were determined according to the death certificate issued after the autopsies. The major cause of death was malignant neoplasm, followed by pneumonia and heart diseases. The incidence of cerebrovascular disease was relatively low. Heart disease and cerebrovascular disease were significantly prevalent among the women in this elderly population.

Pathological Examination of Atherosclerosis

The atherosclerotic index of the common carotid artery (CC-AI) was evaluated by macroscopic examination of the luminal surface of formalin-fixed arteries, as previously reported[24] The degree of atherosclerosis was scored according to the ratio of the occupying atheroma to the entire intimal area: from 0 (absent, <1/20 of the intimal areas occupied by the atheroma), 2 (minimal, 1/20–1/6), 4 (mild, 1/6–1/3), 6 (moderate, 1/3–2/3), to 8 (severe, 2/3–1).

The coronary stenotic index (CSI) was determined according to a previously described method[25] The extent of coronary sclerosis was examined using transverse sections at 5-mm intervals. The degree of coronary stenosis was scored from 0 to 5: 0 for no sclerosis, 1 for slight stenosis, 2 for 25% stenosis, 3 for 50%, 4 for 75%, and 5 for 100% obstruction. The CSI was the sum of the stenotic scores in 3 branches: the left anterior descending branch, the left circumflex branch, and the right coronary artery. The calculation of the CSI was different from that used in angiographic evaluation, as shown in Fig 1.

The atherosclerotic stenosis at the origin of the internal carotid artery (ICA-S) was assessed in the most recent 210 cases (419 arteries). Three categories were defined as none (0%), mild stenosis (25% to 50%), and moderate to severe stenosis (≥75%).

Myocardial infarction (MI) was defined on a pathological basis as the presence of myocardial scars more than 1 cm in diameter and attributable to coronary stenosis.

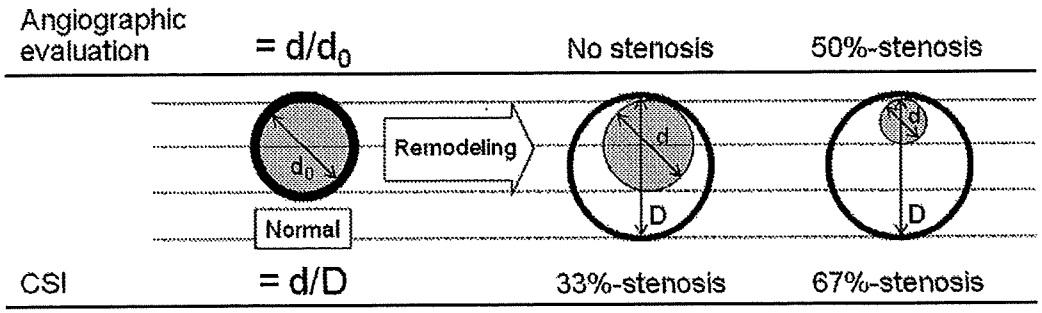

Fig 1. Methods of evaluation of coronary artery stenosis. Comparison between angiographic evaluation and coronary stenotic index (CSI). Coronary angiography evaluates the ratio of the diameters of the stenotic portion (d) to the normal portion (d_0). If coronary remodeling occurs and the luminal diameter is retained, imaging will not detect plaque burden. In contrast, CSI is defined as the ratio of luminal diameter (d) to vascular (intimal) diameter (D), so our assessment method could detect plaque burden even if coronary remodeling completely retained the vascular lumen.

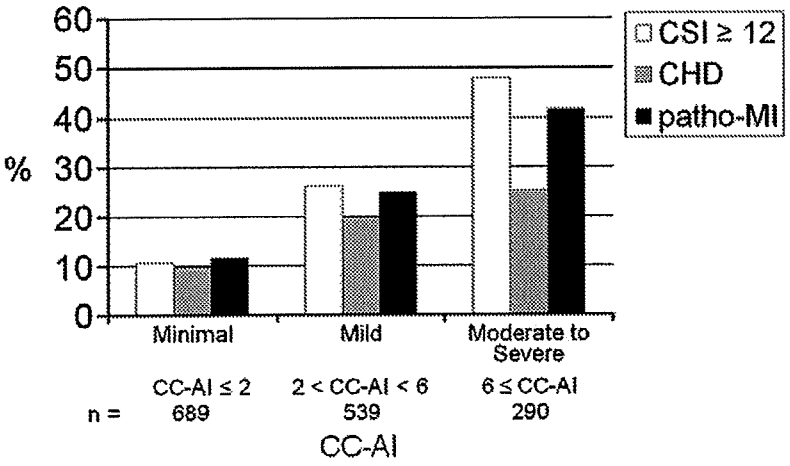

Fig 2. Prevalence of severe coronary atherosclerosis, coronary heart disease (CHD) and pathologically-verified myocardial infarction (patho-MI). Among the cases with minimal common carotid atherosclerosis (CC-AI ≤2), severe coronary atherosclerosis (CSI ≥12), CHD and patho-MI were not infrequent (≈10%). CC-AI, common carotid atherosclerotic index; CSI, coronary stenotic index.

Statistical Analysis

The t-test and Fisher exact test were used to compare background between the sexes and between cases with or without severe coronary atherosclerosis. The fitness to a normal distribution of the CC-AI and CSI values was examined using the Shapiro-Wilk W test. Multiple regression analysis was performed to examine the contributions of the pathobiological determinants for the CC-AI and CSI values. Independent variables for this analysis included the age at death; the presence of hypercholesterolemia, hypertension or diabetes mellitus; and smoking status. A multiple logistic regression analysis was also performed to examine the risk factors for coronary heart disease and pathologically determined MI. The statistical significance level was set at 0.05. The SAS system for Windows (ver. 8.1) and JMP (ver. 6.0) (SAS Institute Inc, Cary, NC, USA) were used for the statistical analyses.

Ethical Considerations

Written informed consent was obtained from the families prior to the autopsy examination. The use of autopsy materials for medical education and research is generally permitted by the Act of Postmortem Examinations of Japan.

Results

Severity and Correlation of Common Carotid and Coronary Atherosclerosis

The CC-AI and CSI scores had a normal distribution ($W=0.907$, $p<0.0001$; $W=0.972$, $p<0.0001$, respectively). The median and mean (±SD) of the CC-AI scores were 4 and 3.5 (±1.9), respectively, while those of the CSI scores were 8 and 8.3 (±3.7), respectively. The simple correlation coefficient was 0.456 ($p<0.0001$). The partial correlation coefficient was 0.38 ($p<0.0001$), when adjusted by sex, age, and presence of hyperlipidemia, hypertension, diabetes mellitus and a smoking history. As shown in Fig 2, severe coronary atherosclerosis (CSI ≥12), coronary heart disease and pathologically-verified MI were prevalent among the cases with moderate to severe common carotid atherosclerosis (CC-AI ≥6), but were not infrequent among 689 cases with minimal common carotid atherosclerosis (CC-AI ≤2): severe coronary atherosclerosis in 74 (10.7%), coronary heart disease in 68 (9.9%), and pathologically-verified MI in 80 (11.6%).

Discordance Between Common Carotid and Coronary Atherosclerosis

Among the 689 cases with minimal common carotid atherosclerosis (CC-AI ≤2), those with severe coronary atherosclerosis (CSI ≥12) were compared with those with less severe coronary atherosclerosis (CSI <12), as shown in

Table 2 Clinicopathological Comparison of Cases With or Without Severe Coronary Atherosclerosis Among Those With Minimal Common Carotid Atherosclerosis (CC-AI ≤2)

	Discordant cases (CSI ≥12)	Concordant cases (CSI <12)	p value
No. of cases (%)	74 (11%)	615 (89%)	
Coronary risk factors			
Male sex	58.1%	48.1%	0.1110
Age at death (years)	78.4±1.0	78.9±0.3	0.6016
Total cholesterol (g/L)	1.736±0.054	1.573±0.020	0.0054*
HDL-cholesterol (g/L)	0.464±0.022	0.420±0.008	0.0574
Hypertension	28.4%	18.5%	0.0613
Diabetes mellitus	23.0%	10.6%	0.0039*
History of smoking	60.0%	41.8%	0.0048*
Coronary disease			
CSI	12.8±0.3	6.0±0.1	<0.0001*
Coronary heart disease	33.8%	7.0%	<0.0001*
Pathologically-verified myocardial infarction	43.2%	7.8%	<0.0001*

Data are percentage or average ± SD.
CC-AI, atherosclerotic index of the common carotid artery; CSI, coronary stenotic index. Other abbreviation see in Table 1.
*p<0.05.

Table 3 Pathobiological Determinants of Common Carotid and Coronary Atherosclerosis in the Elderly

	Men			Women		
	Estimates	SE	p value	Estimates	SE	p value
CC-AI						
n, R^2	647, 0.124			486, 0.226		
Age at death (years)	0.043	0.009	<0.0001*	0.066	0.009	<0.0001*
Hypercholesterolemia#	0.506	0.140	0.0003*	0.259	0.104	0.0135*
Hypertension	0.513	0.082	<0.0001*	0.568	0.085	<0.0001*
Diabetes mellitus	0.134	0.102	0.1910	0.236	0.106	0.0275*
Smoking	0.369	0.086	<0.0001*	0.437	0.094	<0.0001*
CSI						
n, R^2	647, 0.056			486, 0.167		
Age at death (years)	0.052	0.018	0.0043*	0.085	0.020	<0.0001*
Hypercholesterolemia*	0.491	0.258	0.0575	1.061	0.004	<0.0001*
Hypertension	0.497	0.150	0.0010*	0.905	0.181	<0.0001*
Diabetes mellitus	0.632	0.188	0.0008*	0.856	0.223	0.0002*
Smoking	0.369	0.158	0.0202*	0.192	0.198	0.3404

R^2, coefficient of determination; SE, standard error. Other abbreviations see in Table 2.
Estimates are regression coefficients.
*p<0.05.
#Hypercholesterolemia diagnosed when the average total cholesterol >2.2 g/L.

Table 4 Risk Factors for Coronary Heart Disease and Myocardial Infarction in the Elderly

	Men		Women	
	OR (95%CI)	p value	OR (95%CI)	p value
Coronary heart disease				
n, R^2	647, 0.065		486, 0.105	
Age at death (years)	2.03 (0.65–6.41)	0.2226	4.25 (1.12–16.7)	0.0350*
Hypercholesterolemia#	1.51 (0.69–3.07)	0.2728	1.52 (0.82–2.72)	0.1698
Hypertension	2.42 (1.55–3.76)	<0.0001*	2.69 (1.65–4.40)	<0.0001*
Diabetes mellitus	2.99 (1.79–4.93)	<0.0001*	3.38 (1.93–5.91)	<0.0001*
Smoking	0.68 (0.42–1.11)	0.1169	0.65 (0.34–1.18)	0.1707
Pathologically-verified myocardial infarction				
n, R^2	647, 0.035		486, 0.088	
Age at death (years)	1.79 (0.64–4.86)	0.2490	3.60 (1.06–12.5)	0.0416*
Hypercholesterolemia#	1.06 (0.51–2.06)	0.8784	1.51 (0.86–2.61)	0.1476
Hypertension	1.94 (1.31–2.87)	0.0009*	2.84 (1.81–4.46)	<0.0001*
Diabetes mellitus	2.20 (1.37–3.49)	0.0009*	2.40 (1.40–4.11)	0.0014*
Smoking	0.78 (0.52–1.20)	0.2595	0.65 (0.36–1.13)	0.1401

CI, confidence interval; OR, odds ratio. Other abbreviation see in Table 3.
*p<0.05.
#Hypercholesterolemia diagnosed when the average total cholesterol >2.2 g/L.

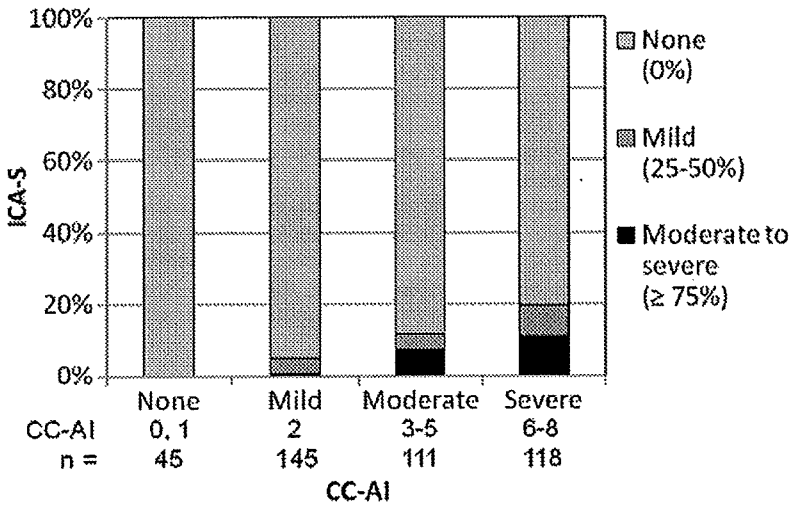

Fig 3. Association between common carotid atherosclerosis and internal carotid arterial stenosis. A significant linear association was present between common carotid atherosclerotic index (CC-AI) and internal carotid artery stenosis (ICA-S) ($p<0.0001$). Internal carotid arterial stenosis was noted in only 19.5% (8.5% with mild stenosis and 11.0% with moderate to severe stenosis) of the cases with severe common carotid atherosclerosis (CC-AI 6–8).

Table 2. The total cholesterol level was significantly higher, and diabetes mellitus and positive smoking history were significantly more prevalent among those with severe coronary atherosclerosis. Clinical diagnosis of coronary heart disease and pathological diagnosis of MI were made in 33.8% and 43.2%, respectively, of the cases with severe coronary atherosclerosis.

Pathobiological Determinants of Common Carotid and Coronary Atherosclerosis

The age at death and the hypercholesterolemia, hypertension and smoking status were selected as significant determinants of the common carotid atherosclerosis (CC-AI), as shown in Table 3. Diabetes mellitus was significant only in women. Hypercholesterolemia in men and hypertension in both sexes had relatively higher regression coefficients.

The age at death and the hypertension and diabetic statuses were significant determinants of coronary atherosclerosis (CSI) in both sexes. Hypercholesterolemia in women and smoking in men were also significant. Hypertension and diabetes in both sexes and hypercholesterolemia in women had relatively higher standard regression coefficients.

Risks of Coronary Heart Disease and Pathologically-Verified MI

Table 4 shows the risk factors for coronary heart disease and pathologically-verified MI. Hypertension, diabetes mellitus in both genders and age at death in women were significant risk factors for both coronary heart disease and MI.

Association Between Common Carotid Atherosclerosis and ICA-S

The number of arteries in the lowest, intermediate, and highest tertiles of ICA-S was as follows: 376 (90%; none), 21 (5%; mild stenosis), and 22 (5%; moderate to severe stenosis). As shown in Fig 3, a significant linear association was present between CC-AI and ICA-S ($p<0.0001$). Among the cases with severe common carotid atherosclerosis (CC-AI, 6 to 8), ICA-S was noted in only 19.5% (8.5% with mild stenosis and 11.0% with moderate to severe stenosis).

Discussion

The present study confirms the presence of different degrees of common carotid and coronary atherosclerosis in the same individual. More precisely, 11% of the present cases with minimal common carotid atherosclerosis had severe coronary atherosclerosis; these cases could be called "discordant cases". The discordant cases had higher serum cholesterol levels and higher prevalences of diabetes mellitus and a history of smoking.

Postmortem Study

Previous autopsy studies reported significant positive correlations between carotid and coronary atherosclerosis;[17-21] although those correlations were not strong, with correlation coefficients ranging from 0.4 to 0.6. Young et al examined the correlations among the cerebral arteries or among the coronary arterial branches and between the 2 arterial beds and reported a weaker correlation between carotid and coronary atherosclerosis ($r=0.57$) than among the cerebral branches ($r=0.89$–0.96) or among the coronary arterial branches ($r=0.74$–0.82).[17] Solberg et al also reported a weaker correlation between carotid and coronary atherosclerosis than between aortic and coronary atherosclerosis.[19] Pasterkamp et al reported no significant correlations between plaque accumulation or luminal stenosis in the common carotid artery and luminal stenosis of other peripheral arteries.[26]

Imaging Study

Several epidemiological studies (ARIC study, Rotterdam study and CHS) reported increased IMT of the carotid artery as a risk factor for cardiovascular events.[1-3] Those studies also showed that some participants with the lowest rank of carotid IMT suffered from cardiovascular events during the observation, which indirectly supports the presence of discordant cases of carotid and coronary atherosclerosis.[1,3]

Coronary angiography studies have yielded conflicting results regarding the association between coronary angiography findings and the carotid IMT. Several studies have reported significant associations between B-mode findings and the severity of coronary angiography findings.[5-16] In contrast, Adams et al studied 350 cases with an average age of 60 years and reported a weak correlation between the

carotid IMT and the extent of coronary atherosclerosis or stenosis (r=0.23 and 0.26, respectively), indicating a need for caution when using carotid IMT as a surrogate marker for coronary atherosclerosis[8] Sakaguchi et al reported that coronary artery lesions were present among 33.8% and 51.4% of the patients with the lowest quartile of CCA-IMT and combined IMT, respectively[27] Recently, Gongora-Rivera et al analyzed the degree of coronary atherosclerosis in patients with stroke and reported that coronary atherosclerosis and MI were frequent, not only in stroke patients with significant carotid and cerebral atherosclerosis, but also in stroke patients without it[28]

Even after determining the ideal carotid IMT thresholds, the negative predictive value of the examination was low, ranging from 0.41 to 0.58[9,11] Thus, a considerable number of cases with a normal carotid IMT can be expected to have severe coronary atherosclerosis.

Risk Factors and Causes of Discordance

All the previous studies have focused on the overall correlation between carotid and coronary atherosclerosis. The present study is the first to deal with discordant cases exhibiting conflicting severities of common carotid and coronary atherosclerosis. Although atherosclerosis is definitely a systemic disease, its severity in 1 arterial bed often differs from that in another arterial bed for unexplained reasons. Different risk factors may affect the arterial beds in different ways. The present study showed that not only common determinants of common carotid and coronary atherosclerosis, such as age, hypertension and hypercholesterolemia, but also artery-specific determinants, including smoking for common carotid atherosclerosis and diabetes mellitus for coronary atherosclerosis, were present. These findings agree with previous results[29–31] Thus, further studies are needed to clarify the pathological mechanisms of artery-specific risk factors. Otherwise, this discordance can be explained by differences in the temporal order of atherosclerotic involvement. The coronary artery is affected by atherosclerosis early in life, whereas the carotid artery is involved later[24,32] This time lag might be responsible for a number of discordant cases with severe coronary atherosclerosis and mild common carotid atherosclerosis.

Interpretation of Our Data

The atheroma-occupying ratio of the intima, not the IMT, of the artery was measured in this study. Pignoli et al first reported that the IMT, as measured by carotid ultrasonography, represented the histologically determined combined thickness of the intima and media[33] Thus, the CC-AI scores determined in the present study may parallel the plaque score of the common carotid artery rather than CCA-IMT values.

Sakaguchi et al reported that the plaque score, IMT of the bulb to internal carotid artery and the combined IMT measurement could be better than the IMT of common carotid artery for predicting coronary lesions in a population with cardiovascular risks[27] Hulthe et al stressed the importance of measuring the IMT not only in the common carotid artery, but also in the carotid bulb for coronary assessment[34] Other studies also showed that the carotid plaque burden, especially in the bifurcation and bulb, are important for predicting coronary events[35,36]

Because the study of internal carotid atherosclerosis was limited to small numbers of autopsy samples, we could not address the site-specific association with coronary atherosclerosis in the carotid arterial tree. Our data were consistent with those of clinical reports that showed that common carotid atherosclerosis was not a suitable site for ultrasound evaluation of coronary atherosclerosis. A detailed pathological analysis and comparison of internal carotid atherosclerosis and coronary atherosclerosis is warranted in future research.

Early studies by the International Atherosclerosis Project reported marked geographic, racial and gender-related variations of the degree and distribution of atherosclerosis[37] Because nearly all subjects in this study were Japanese, replication of our results in other populations are necessary.

Conclusion

We have confirmed that discordance between common carotid and coronary atherosclerosis can exist in the same person. The concept of atherosclerosis as a systemic disease could lead to an impression that a strong correlation exists between common carotid and coronary atherosclerosis. This could, in turn, lead to an underestimation of coronary atherosclerosis and coronary event risk in patients with normal carotid imaging. As several clinical studies indicate, ultrasound assessment of the carotid bifurcation and bulbs is necessary, in addition to that of the common carotid artery, to predict coronary events.

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Zonal Gene Expression of Chondrocytes in Osteoarthritic Cartilage

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Objective. **To determine the chondrocyte metabolism in respective zones of osteoarthritic (OA) cartilage.**

Methods. **OA cartilage was obtained from macroscopically intact areas of 4 knee joints with end-stage OA. The cartilage was divided into 3 zones, and gene expression profiles were determined in the respective zones by a custom-designed microarray that focused on chondrocyte-related genes. For the genes whose expression was significantly different among the zones, the expression was compared between OA and control cartilage in the respective zones by an analysis using laser capture microdissection and real-time polymerase chain reaction (PCR). For some genes, the correlation of expression was investigated in specific cartilage zones.**

Results. **A total of 198 genes (~40% of those investigated) were found to be expressed at significantly different levels among the zones. Expression of 26 of those genes was evaluated by laser capture microdissection and real-time PCR, which confirmed the validity of microarray analysis. The expression of cartilage matrix genes was mostly enhanced in OA cartilage, at similar levels across the zones but at different magnitudes among the genes. The expression of bone-related genes was induced either in the superficial zone or in the deep zone, and positive correlations were found among their expression in the respective zones. The expression of 5 proteinase genes was most enhanced in the superficial zone, where their expression was correlated, suggesting the presence of a common regulatory mechanism(s) for their expression.**

Conclusion. **In OA cartilage, the metabolic activity of chondrocytes differed considerably among zones. Characteristic changes were observed in the superficial and deep zones.**

Osteoarthritis (OA) is the most prevalent joint disease in developed countries that primarily affects articular cartilage. In OA, cartilage matrix is lost gradually, which eventually devastates functional joints. Chondrocytes are the sole type of cells that reside in articular cartilage. Currently, they are considered to play a pivotal role in progression of OA. Chondrocytes express catabolic cytokines and proteinases that promote loss of cartilage matrix (1). Chondrocytes undergo phenotypic changes and come to express matrix genes that are little expressed in normal articular cartilage (2–4). This induction of noncartilaginous matrices could facilitate the loss of cartilage matrix by altering the composition and properties of the matrix (5). Thus, in order to understand the pathology of OA, it is crucially important to know how the chondrocyte metabolism is altered within OA cartilage.

Articular cartilage is a highly organized tissue. Based on histologic features, the articular cartilage

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above the tidemark is divided into 3 distinct layers, the superficial, middle, and deep zones (6,7), in which the morphology and density of chondrocytes differ (8). The composition of cartilage matrix also differs among the zones. While the superficial zone is rich in collagens, the middle and deep zones contain more proteoglycan than the superficial zone (9). Corresponding to these differences, the chondrocyte metabolism differs considerably among the zones (2). The regional difference in chondrocyte metabolism may be even more important in the analysis of OA pathology. In the early stage of OA, chondrocytes in the superficial zone could be catabolically more active, which initiates cartilage degeneration at the surface areas (10–12).

In the present study, we attempted to determine the metabolic activity of chondrocytes in the respective zones of OA cartilage. Cartilage samples were obtained from macroscopically intact areas, and gene expression profiles of chondrocytes were determined by complementary DNA (cDNA) microarray that focused on chondrocyte-related genes. Comparison of gene expression profiles among the zones clarified the difference in cellular metabolism among the zones. Based on this result, gene expression was investigated in the respective zones of OA and control cartilage by an analysis using laser capture microdissection and real-time polymerase chain reaction (PCR) (2,13). The results of these analyses revealed previously unrecognized features of the chondrocyte metabolism in OA cartilage.

MATERIALS AND METHODS

Cartilage. The study was performed with the approval of the Human Ethics Review Committees of the institutes that participated in the study, and informed consent was obtained in writing from each subject or family of the donor before material collection. All OA cartilage was obtained from knees with end-stage OA at prosthetic surgery. The diagnosis of OA was based on the criteria for knee OA of the American College of Rheumatology (14). The cartilage was obtained from knees with medial involvement, and cartilage from laterally or bilaterally involved knees was not used for this study. For microarray analysis, OA cartilage was obtained from 4 OA knee joints in 4 patients (mean age 73.2 years, range 62–82 years). The cartilage was taken from the weight-bearing area of a lateral femoral condyle, where cartilage presented few signs of macroscopic degeneration. Cartilage was harvested in a square of 15–20 mm on a side, in full thickness above the tidemark. After harvest, the cartilage was separated into superficial, middle, and deep zones with a scalpel under a dissection microscope. The separated cartilages were each embedded in OCT compound (Sakura Finetek, Tokyo, Japan), snap-frozen in liquid nitrogen, and stored at −80°C until analysis. This processing of cartilage was completed within 4 hours after the acquisition.

Table 1. Abundance of gene expression in the superficial zone compared with that in the middle zone*

Gene symbol	Accession no.	Gene name	Signal intensity		Signal ratio†
			Superficial zone	Middle zone	
Greater expression in superficial zone					
CRABP2	NM_001878.2	Cellular retinoic acid binding protein 2	4.230×10^{1}	7.578×10^{0}	5.582
TNFAIP6	NM_007115.2	Tumor necrosis factor α–induced protein 6	8.398×10^{2}	2.370×10^{2}	3.543
MATN4	NM_003833.2	Matrilin 4, transcript variant 1	8.317×10^{2}	2.401×10^{2}	3.464
TNA	NM_003278.1	Tetranectin	3.155×10^{3}	1.032×10^{3}	3.057
WNT5B	NM_030775.2	Wingless-type MMTV integration site family, member 5B, transcript variant 2	3.733×10^{1}	1.340×10^{1}	2.786
MMP13	NM_002427.2	Matrix metalloproteinase 13	5.177×10^{2}	1.901×10^{2}	2.724
POSTN	NM_006475.1	Periostin, osteoblast-specific factor	7.608×10^{2}	3.156×10^{2}	2.411
DAF	NM_000574.2	Decay-accelerating factor for complement (CD55)	2.276×10^{3}	1.098×10^{3}	2.072
TIMP3	NM_000362.3	Tissue inhibitor of metalloproteinase 3	1.745×10^{3}	8.546×10^{2}	2.042
Greater expression in middle zone					
COL10A1	NM_000493.2	Collagen, type X, α1	4.840×10^{0}	5.309×10^{1}	0.091
LECT1	NM_007015.1	Leukocyte cell–derived chemotaxin 1	9.141×10^{1}	4.027×10^{2}	0.227
FGF13	NM_004114.2	Fibroblast growth factor 13, transcript variant 1A	4.701×10^{1}	1.458×10^{2}	0.322
CHAD	NM_001267.1	Chondroadherin	2.837×10^{3}	8.609×10^{3}	0.330
MATN3	NM_002381.3	Matrilin 3	6.803×10^{1}	1.725×10^{2}	0.394
ITM2A	NM_004867.2	Integral membrane protein 2A	6.745×10^{2}	1.566×10^{3}	0.431
MMP9	NM_004994.1	Matrix metalloproteinase 9	3.995×10^{1}	8.900×10^{1}	0.449

* Genes whose signals in the superficial zone were ≥2 times those in the middle zone are shown in the order of signal intensity ratio. Genes whose signals in the superficial zone were ≤0.5 times those in the middle zone are shown in the order of signal intensity ratio.
† Signal intensity of the superficial zone relative to that of the middle zone.

Table 2. Abundance of gene expression in the middle zone compared with that in the deep zone*

Gene symbol	Accession no.	Gene name	Signal intensity		Signal ratio†
			Middle zone	Deep zone	
Greater expression in middle zone					
MMP13	NM_002427.2	Matrix metalloproteinase 13	1.889×10^2	2.852×10^1	6.625
C3	NM_000064.1	Complement component 3	1.209×10^2	1.950×10^1	6.199
FGF7	NM_002009.2	Fibroblast growth factor 7	2.553×10^1	7.617×10^0	3.352
COL12A1	NM_004370.4	Collagen, type XII, α1, transcript variant long	9.605×10^2	3.355×10^2	2.863
CHI3L1	NM_001276.1	Chitinase 3-like 1 (cartilage glycoprotein 39)	2.895×10^4	1.046×10^4	2.768
LAMA4	NM_002290.2	Laminin, α4	2.492×10^2	9.907×10^1	2.515
MMP2	NM_004530.1	Matrix metalloproteinase 2	7.357×10^2	3.005×10^2	2.448
CHI3L2	NM_004000.1	Chitinase 3-like 2	2.582×10^4	1.063×10^4	2.429
FAP	NM_004460.2	Fibroblast activation protein, α	7.107×10^2	2.932×10^2	2.424
TIMP1	NM_003254.1	Tissue inhibitor of metalloproteinase 1	2.664×10^4	1.114×10^4	2.392
GRM1	NM_000838.2	Glutamate receptor, metabotropic 1	4.149×10^1	1.736×10^1	2.390
MMP3	NM_002422.2	Matrix metalloproteinase 3	9.857×10^3	4.692×10^3	2.101
Greater expression in deep zone					
WNT5B	NM_030775.2	Wingless-type MMTV integration site family, member 5B, transcript variant 2	1.358×10^1	8.472×10^1	0.160
SPP1	NM_000582.2	Secreted phosphoprotein 1 (osteopontin, bone sialoprotein 1)	9.187×10^2	4.301×10^3	0.214
IBSP	NM_004967.2	Integrin-binding sialoprotein (bone sialoprotein, bone sialoprotein II)	1.791×10^2	6.609×10^2	0.271
TNFRSF11B	NM_002546.2	Tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin)	3.485×10^2	1.081×10^3	0.322
MATN3	NM_002381.3	Matrilin 3	1.715×10^2	5.162×10^2	0.332
COL10A1	NM_000493.2	Collagen, type X, α1	5.325×10^1	1.422×10^2	0.374
SLC26A2	NM_000112.2	Solute carrier family 26, member 2	2.118×10^2	5.389×10^2	0.393
TNFAIP6	NM_007115.2	Tumor necrosis factor α-induced protein 6	2.352×10^2	5.207×10^2	0.452
PLOD2	NM_000935.1	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2, transcript variant 2	1.005×10^3	2.100×10^3	0.479

* Genes whose signals in the middle zone were ≥2 times those in the deep zone are shown in the order of signal intensity ratio. Genes whose signals in the middle zone were ≤0.5 times those in the deep zone are shown in the order of signal intensity ratio.
† Signal intensity of the middle zone relative to that of the deep zone.

For laser capture microdissection analysis, OA cartilage was obtained from another 42 knees with end-stage OA in 41 patients (from both knees in the case of 1 patient) (mean age 67.2 years, range 62–77 years) in the manner described for the microarray analysis. Control cartilage was harvested from 13 non-OA knees of 10 donors (from both knees in the case of 3 donors) (mean age 78.6 years, range 68–93 years) at the weight-bearing areas in lateral femoral condyles. The donors had no known history of joint disease or trauma, and normality of the joint was confirmed macroscopically at material collection. Control cartilage was obtained even when some signs of degeneration were noticed as long as the degeneration was limited (superficial degeneration in <20% of total cartilage area) and the lateral femoral condyle was spared from degeneration. After harvest, cartilages were immediately embedded in OCT compound and stored at −80°C until use.

RNA extraction. RNA for microarray analysis was extracted from the cartilage tissues following a previously described method (15) with some modifications. Briefly, 20-μm–thick cryosections were made from cartilage in OCT compound, and these were placed immediately into TRIzol reagent (Invitrogen, Carlsbad, CA). During this process, 1 of

Table 3. Abundance of gene expression in the superficial zone compared with that in the deep zone*

Gene symbol	Accession no.	Gene name	Signal intensity		Signal ratio†
			Superficial zone	Deep zone	
Greater expression in superficial zone					
MMP13	NM_002427.2	Matrix metalloproteinase 13	5.098×10^2	2.852×10^1	17.878
MMP2	NM_004530.1	Matrix metalloproteinase 2	1.369×10^3	3.005×10^2	4.554
POSTN	NM_006475.1	Periostin, osteoblast-specific factor	7.476×10^2	2.455×10^2	3.046
COL12A1	NM_004370.4	Collagen, type XII, α1, transcript variant long	9.530×10^2	3.355×10^2	2.840
FAP	NM_004460.2	Fibroblast activation protein, α	8.224×10^2	2.932×10^2	2.805
GRM1	NM_000838.2	Glutamate receptor, metabotropic 1	4.839×10^1	1.736×10^1	2.788
MATN4	NM_003833.2	Matrilin 4, transcript variant 1	8.168×10^2	3.037×10^2	2.689
TMSB4X	NM_021109.2	Thymosin, β4, X-linked	3.945×10^3	1.509×10^3	2.614
CHI3L2	NM_004000.1	Chitinase 3–like 2	2.693×10^4	1.063×10^4	2.534
LAMA4	NM_002290.2	Laminin, α4	2.482×10^2	9.907×10^1	2.505
FGF7	NM_002009.2	Fibroblast growth factor 7	1.883×10^1	7.617×10^0	2.473
ECM1	NM_004425.2	Extracellular matrix protein 1, transcript variant 1	5.263×10^2	2.130×10^2	2.471
ADAMTS5	NM_007038.1	A disintegrin-like and metalloproteinase with thrombospondin type 1 motif, 5 (aggrecanase 2)	1.497×10^2	6.068×10^1	2.466
TNA	NM_003278.1	Tetranectin	3.076×10^3	1.261×10^3	2.439
CHI3L1	NM_001276.1	Chitinase 3–like 1 (cartilage glycoprotein 39)	2.539×10^4	1.046×10^4	2.427
ITGB5	NM_002213.3	Integrin, β5	3.480×10^3	1.447×10^3	2.406
THBS2	NM_003247.2	Thrombospondin 2	3.281×10^2	1.426×10^2	2.300
MMP11	NM_005940.2	Matrix metalloproteinase 11	5.007×10^2	2.262×10^2	2.214
TIMP1	NM_003254.1	Tissue inhibitor of metalloproteinase 1	2.454×10^4	1.114×10^4	2.203
COL1A2	NM_000089.3	Collagen, type I, α2	8.784×10^3	4.019×10^3	2.186
MMP3	NM_002422.2	Matrix metalloproteinase 3	1.012×10^4	4.692×10^3	2.157
TNC	NM_002160.1	Tenascin C	4.803×10^3	2.271×10^3	2.115
IGFBP5	NM_000599.2	Insulin-like growth factor binding protein 5	8.847×10^2	4.183×10^2	2.115
GADD45A	NM_001924.2	Growth arrest and DNA damage–inducible, α	6.081×10^2	2.925×10^2	2.079
PLTP	NM_006227.2	Phospholipid transfer protein, transcript variant 1	5.329×10^2	2.617×10^2	2.036

every 40–50 sections was picked up on glass slides and stained with HistoGene stain (Arcturus, Mountain View, CA), and the appropriateness of zonal separation was confirmed under a light microscope by histology (6,7) (examples of histologic staining are available at http://www.hosp.go.jp/~sagami/rinken/crc/index.html). For the sections made from the deep zone cartilage, contamination of the calcified zone was also ruled out. RNA was first recovered from the TRIzol reagent in the aqueous phase, then purified using the RNeasy Micro kit (Qiagen, Hilden, Germany). The quality and quantity of RNA were routinely evaluated on a spectrophotometer (SmartSpec Plus; Bio-Rad, Hercules, CA) and a Bioanalyzer 2100 using RNA 6000 Nano Chips (Agilent, Santa Clara, CA). The RNA samples were used for the microarray analysis with the conditions that the A260 nm:A280 nm ratio was ≥1.8 and the 28S:18S ratio was ≥1.4.

Microarray analysis. Microarray analysis was performed using a custom-designed oligonucleotide microarray carrying 527 chondrocyte-related genes and 11 housekeeping genes (see Supplementary Table 1, available in the online version of this article at http://www3.interscience.wiley.com/journal/76509746/home). The chondrocyte-related genes were selected based upon previously reported data (16,17). They included genes encoding various matrix components, catabolic proteinases, cytokines, chemokines, growth factors, and their related molecules. The microarray was prepared by GeneFrontier (Tokyo, Japan) following a previously described method (18). In detail, 12 perfect-match probes and 12 mismatch probes, each 24 bases long, were designed for each gene and were synthesized on a microarray platform by NimbleGen's photolithography technology (NimbleGen Systems, Madison, WI) (19). The perfect-match probes had the sequences that exactly matched the gene, while the mismatch probes contained 2 mismatch bases in the 24 bases (6th and 12th bases from the 5′ end).

The RNA was reverse-transcribed, labeled with Cy3 fluorescent dye, and used for hybridization. The hybridization was performed using a 12-well array system (Nimble Screen 12; NimbleGen Systems) following the manufacturer's protocol. This system allowed simultaneous hybridization of 12 different samples on a single glass slide. The hybridization signal for each probe set was determined by the difference in signal intensities between the perfect-match and mismatch probes, using NimbleScan software (NimbleGen Systems). Obtained

Table 3. (*Cont'd*)

Gene symbol	Accession no.	Gene name	Signal intensity		Signal ratio†
			Superficial zone	Deep zone	
Greater expression in deep zone					
COL10A1	NM_000493.2	Collagen, type X, α1	4.892×10^{0}	1.422×10^{2}	0.034
LECT1	NM_007015.1	Leukocyte cell–derived chemotaxin 1	9.089×10^{1}	7.028×10^{2}	0.129
MATN3	NM_002381.3	Matrilin 3	6.775×10^{1}	5.162×10^{2}	0.131
IBSP	NM_004967.2	Integrin-binding sialoprotein (bone sialoprotein, bone sialoprotein II)	1.010×10^{2}	6.609×10^{2}	0.153
SPP1	NM_000582.2	Secreted phosphoprotein 1 (osteopontin, bone sialoprotein I)	7.099×10^{2}	4.301×10^{3}	0.165
CHAD	NM_001267.1	Chondroadherin	2.767×10^{3}	1.167×10^{4}	0.237
SLC26A2	NM_000112.2	Solute carrier family 26, member 2	1.343×10^{2}	5.389×10^{2}	0.249
GPC5	NM_004466.3	Glypican 5	1.783×10^{1}	7.109×10^{1}	0.251
ITM2A	NM_004867.2	Integral membrane protein 2A	6.632×10^{2}	2.353×10^{3}	0.282
TNFRSF11B	NM_002546.2	Tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin)	3.403×10^{2}	1.081×10^{3}	0.315
PLOD2	NM_000935.1	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2, transcript variant 2	7.420×10^{2}	2.100×10^{3}	0.353
CILP	NM_003613.2	Cartilage intermediate-layer protein, nucleotide pyrophosphohydrolase	9.287×10^{3}	2.553×10^{4}	0.364
HAPLN1	NM_001884.2	Hyaluronan and proteoglycan link protein 1	1.604×10^{3}	4.162×10^{3}	0.385
GREM1	NM_013372.4	Gremlin 1 homolog, cysteine knot superfamily	9.244×10^{2}	2.340×10^{3}	0.395
FRZB	NM_001463.2	Frizzled-related protein	2.664×10^{3}	6.556×10^{3}	0.406
S100A1	NM_006271.1	S100 calcium binding protein A1	1.798×10^{3}	4.104×10^{3}	0.438
COL2A1	NM_033150.1	Collagen, type II, α1, transcript variant 2	1.811×10^{4}	4.077×10^{4}	0.444
COL11A1	NM_001854.2	Collagen, type XI, α1, transcript variant A	2.684×10^{3}	5.936×10^{3}	0.452
AGC1	NM_001135.1	Aggrecan 1, transcript variant 1	4.317×10^{3}	9.068×10^{3}	0.476
COL2A1	NM_001844.3	Collagen type II, α1, transcript variant 1	1.959×10^{4}	4.110×10^{4}	0.477
COL9A2	NM_001852.3	Collagen, type IX, α2	1.123×10^{3}	2.354×10^{3}	0.477
CPE	NM_001873.1	Carboxypeptidase E	3.402×10^{2}	6.999×10^{2}	0.486
SPARC	NM_003118.2	Secreted protein, acidic, cysteine-rich	1.985×10^{3}	4.071×10^{3}	0.488

* Genes whose signals in the superficial zone were ≥2 times those in the deep zone are shown in the order of signal intensity ratio. Genes whose signals in the deep zone were ≥2 times those in the superficial zone are shown in the order of singal intensity ratio.
† Signal intensity of the superficial zone relative to that of the deep zone.

data were normalized by Microarray Analysis Suite 5.0 software (Affymetrix, Santa Clara, CA).

Laser capture microdissection. Laser capture microdissection was performed by a previously described method (2). Briefly, frozen sections of 10–20-μm thickness were prepared in the plane perpendicular to the joint surface from the cartilage samples embedded in the OCT compound. For laser capture microdissection, the sections were first treated with 0.5*M* EDTA (pH 8.0) for 3 minutes, then dehydrated with graded concentrations of ethanol and clarified with xylene. All reagents were prepared RNase free, and the entire process was completed within 30 minutes to minimize RNA degradation.

Under a laser capture microdissection device (PixCell IIe; Arcturus), each frozen section was divided into cartilage zones based on histologic features (2,6,7) (an example of histologic staining is available at http://www.hosp.go.jp/~sagami/rinken/crc/index.html). At each tissue procurement, the appropriateness of zone isolation was confirmed on the laser capture microdissection device.

Analysis of gene expression by real-time quantitative PCR. The obtained tissue was immediately placed in the RNA extraction buffer contained in an RNeasy Micro kit, and RNA was extracted by the kit with a routine use of DNase I (Qiagen). Complementary DNA was synthesized using Sensiscript reverse transcriptase (Qiagen). Gene expression was evaluated quantitatively by real-time PCR on an ABI PRISM 7700 (Applied Biosystems, Foster City, CA) or a LightCycler (Roche Diagnostics, Basel, Switzerland). Gene-specific primers were prepared (see Supplementary Table 2, available in the online version of this article at http://www3.interscience.wiley.com/journal/76509746/home), and the PCR reaction was performed using QuantiTect SYBR Green PCR (Qiagen). During the reaction, the amount of PCR product was monitored by the fluorescence from SYBR Green dye that bound to the product.

The PCR protocol was common for all genes: 94°C for 15 minutes to activate *Taq* polymerase, then 40–50 cycles of 94°C for 15 seconds, melting temperature of the primers for 30