Microarray Analysis Reveals Overexpression of CD163 and HO-1 in Symptomatic Carotid Plaques


Objective—We studied by microarray analysis whether symptomatic and asymptomatic carotid plaques from the same patient differ in gene expression and whether the same changes are present in an independent sample set.

Methods and Results—Carotid plaques from four patients with bilateral high-grade stenosis, one being symptomatic and the other asymptomatic, were analyzed on Affymetrix U95Av2 arrays. 33 genes showed >1.5-fold change between symptomatic and asymptomatic plaques in an intraindividual comparison with FDR ranging from 0.28 to 0.40. Three genes involved in iron-heme homeostasis, CD163, HO-1, and transferrin receptor, were further analyzed in 40 independent plaques. HO-1 (fold-change 1.93, 95%CI 1.04 to 3.94, P=0.040) and CD163 (1.58, 1.11 to 2.40, P=0.013) mRNAs were again induced, and also HO-1 protein was overexpressed in symptomatic plaques (4.38, 1.54 to 12.20, P=0.024). The expression of HO-1 and CD163 correlated with tissue iron content but iron itself was not associated with the symptom status.

Conclusions—Symptomatic plaques show overexpression of CD163 and HO-1 both in intraindividual and interindividual comparison. Their expression correlates with iron deposits but asymptomatic and symptomatic plaques from isolated patients do not differ in macroscopic hemorrhages or iron deposits. We suggest that symptomatic plaques show a more pronounced induction of CD163 and HO-1 in response to plaque hemorrhages. (Arterioscler Thromb Vasc Biol. 2007; 27:154-160.)

Key Words: atherosclerosis ■ carotid arteries ■ gene expression ■ microarray ■ stroke

Severe atherosclerotic narrowing of the internal carotid artery was found in 20% to 30% of patients with ischemic stroke in its supply territory.1 Features associated with a symptomatic plaque include the degree of vessel stenosis, prior symptoms, and plaque characteristics, such as ulceration, inflammatory cell infiltration, and a thin fibrous cap.2-4 However, these characteristics are poor predictors of the risk of thromboembolism and, as a result, 80% of patients undergoing carotid endarterectomy are needlessly exposed to surgical risks.1 Thus better markers for the symptom causing carotid disease are needed.

Microarray technology provides a rapid means to screen gene expression in the tissues of interest. Several efforts have been made to study large-scale gene expression in human atherosclerosis, for example by comparing gene expression in normal and atherosclerotic arteries.5,6 Changes involved in destabilization of the atherosclerotic plaque have been less in focus.7-9 Whereas symptomatic high-grade carotid plaque remains highly susceptible to recurrent ipsilateral symptoms, the risk of stroke from contralateral asymptomatic plaque is low, comparable to that of asymptomatic carotid stenosis in general.10 Thus intraindividual differences in the carotid stenoses exist in the same patient causing one plaque to become symptomatic and the other to remain silent. The causes of these differences are unknown. This led us to a microarray study on patients operated due to bilateral significant carotid stenosis, of which one is symptomatic and the other asymptomatic. These rare cases offer a valuable natural experiment, in which the symptom-causing unstable plaque can be compared with the stable one from the same patient, minimizing noise caused by interindividual differences in gene expression and giving insight into plaque-specific characteristics important in the destabilization. One main gene expression profile that appeared differentially regulated between symptomatic and asymptomatic plaques was genes involved in the metabolism of iron and heme. Three of these, CD163, HO-1, and transferrin receptor (TRFC), were further
analyzed at mRNA and protein level in 40 independent carotid plaques.

**Materials and Methods**

**Patients**

Patients were selected from a larger study population, the Helsinki Carotid Endarterectomy Study, HeCES, described elsewhere in detail. \(^{11-13}\) Briefly, the study included 97 consecutive patients subjected to endarterectomy because of high-grade carotid stenosis. Division into asymptomatic or symptomatic plaque phenotypes was based on prior clinical symptoms; carotid stenosis was considered symptomatic if a patient had suffered an ipsilateral stroke, transient ischemic attack of the carotid territory, or amaurosis fugax within 120 days before endarterectomy. All the patients gave informed consent, and the appropriate departmental Ethics Committees of Helsinki University Central Hospital approved the study protocol.

The present microarray study includes the bilateral cases, ie, the patients (n=4) who had significant carotid stenosis operated bilaterally, one side being asymptomatic and the other symptomatic. Three patients had an acute infarction in the supply territory of the symptomatic carotid artery and the fourth patient had a clinical ischemic stroke (left hemiparesis). None of the patients had ever experienced symptoms from the contralateral, asymptomatic side, and the brain imaging of the contralateral hemisphere was normal. The characteristics of the bilateral cases are detailed in supplemental Table I (available online at http://atvb.ahajournals.org).

For follow-up study by quantitative real-time RT-PCR we included carotid plaques from all HeCES patients with ipsilateral stroke symptoms (symptomatic specimen, n=22) and all patients without cerebrovascular symptoms (asymptomatic specimen, n=18), who in contrast to bilateral patients underwent endarterectomy for only one carotid artery stenosis, either symptomatic or asymptomatic. From these, carotid specimen from patients with radiologically confirmed ipsilateral ischemic stroke (n=13) and symptom-free patients with normal brain imaging (n=9) were used for the Western blotting analysis and immunohistochemistry. Clinical characteristics of all 40 patients are given in the Table.

**Tissue Sampling**

Carotid plaques were removed en bloc in longitudinal endarterectomy, drained with saline and graded macroscopically for smoothness, ulceration, hemorrhage, loose atheroma, calcification, and intramural thrombus by the same vascular surgeon (ES, Table 1 and supplemental Table I). All plaques were complicated lesions, ie, the AHA-class VI. \(^{14}\) Specimen were divided into longitudinal slices used each for specific purposes such as RNA-extraction, biochemical analyses, detection of infectious agents, standard histological examination, and immunohistochemical stainings.

**RNA Extraction and Microarray Analysis**

Total cellular RNA was extracted from each specimen with Trizol-reagent (Invitrogen Life Technologies) and purified with the RNeasy Total RNA Isolation Kit (Qiagen) according to the manufacturers’ recommendations.
Microarray experiments were performed using Affymetrix GeneChip U95Av2 arrays according to the manufacturer’s recommendations and the MIAME guidelines. Detailed descriptions of all data and protocols were submitted to a public repository, ArrayExpress (http://www.ebi.ac.uk/miamirexpress/login.htm, the accession number: E-MEXP-268). Each RNA sample was hybridized to its own microarray resulting in eight arrays from four patients. Hybridization data were analyzed using the BioC 1.8 Release of the Bioconductor packages. First, signal intensities were calculated and hybridization data were analyzed using the BioC 1.8 Release of the Bioconductor microarray resulting in eight arrays from four patients. Hybridization data were analyzed using the BioC 1.8 Release of the Bioconductor packages. Genes expressed at a reliable level and showing differential expression were identified by filtering, using the following criteria: (1) the raw signal >100 in either plaque from three patients and (2) 1.5-fold difference in the mean signal between symptomatic and asymptomatic plaques. Differential expression was tested by paired t test and Benjamini-Hochberg multiple testing correction was applied to obtain the false discovery rates. Hierarchical clustering was performed using the GeneSpring v. 7.1 software (Silicon Genetics) and gene-class testing for functional pathways and Gene Ontology (GO) categories by using DAVID software applying gene enrichment analysis.

Quantitative Real-Time RT-PCR for CD163, HO-1, and TRFC
Quantitative real-time RT-PCR was performed using Assays-on-Demand Gene Expression Products and ABI PRISM 7000 Sequence Detection System (Applied Biosystems) according to the manufacturer’s recommendations. Gene expression was determined by the comparative Ct method, normalizing expression to β-actin.

Protein Isolation and Western Blotting
Total cellular proteins were isolated from the phenol-chloroform phase left over from the RNA extraction with Trizol reagent (Invitrogen Life Technologies). Proteins were quantitated by the Bradford method and 10 µg was separated on 10% SDS-polyacrylamide gel electrophoresis followed by electroblotting to PVDF membranes (Hybond P, Amersham Biosciences). Membranes were blocked in 5% skimmed milk and 0.1% Tween 20 in tris-buffered saline. HO-1 was detected using rabbit polyclonal antibody (Stressgen, 1:2000) and β-actin using mouse monoclonal antibody (Sigma-Aldrich, 1:4800). Secondary antibodies were peroxidase-conjugated goat anti-rabbit IgG and anti-mouse IgG (Molecular Probes). Proteins were visualized using ECL Plus Western Blotting Detection Reagents and Typhoon Variable Mode Imager according to manufacturer’s recommendations, and HO-1 and β-actin bands were quantitated using ImageQuant TL 1D Gel Analysis v2003.1 software (all from Amersham Biosciences). The amount of HO-1 protein is given as the volume of HO-1 band divided by the volume of β-actin band.

Immunohistochemistry and Microscopy
Tissue slices embedded in paraffin were cut longitudinally into 5-µm sections, hence containing the region of highest stenosis. Sections were immunostained with primary antibodies: rabbit polyclonal antibody against HO-1 (Stressgen, 1:2000) and mouse monoclonal antibody against CD163 (Novocastra, 10D6, 1:100). Ferric iron was detected by Perl’s Prussian blue staining. One investigator (KN), blinded to the data, performed light microscopy (Axioplan 2, Carl Zeiss) and scored semiquantitatively the quantity of protein expression or iron staining in the whole section (0 = none, 1 = weak, 2 = moderate, 3 = strong).

Statistical Analysis
Statistical analyses, apart from microarray analyses, were performed using SPSS 10.0.7 for Windows (SPSS Inc). Pearson chi-square or Fisher exact test was used to evaluate the differences in noncontinuous and the independent-samples t test for continuous clinical characteristics shown in Table 1. Results from quantitative real-time RT-PCR and Western blotting were evaluated by independent-samples t test (Figures 1 to 3). Differences in protein and iron levels determined by immunohistochemistry were tested by the Mann-Whitney U test (Figure 3). Correlations were analyzed by the Spearman rank correlation. Results are given as an expression ratio and 95% confidence interval (CI). A two-tailed P < 0.05 was considered significant.

Results
Microarray Analysis in Patients With Bilateral Carotid Stenoses
Forty probes (33 genes) showed more than 1.5-fold change in expression in comparison between asymptomatic and symptomatic plaques (summarized in supplemental Table II). The expression of lysyl oxidase-like 1, was significantly different between symptomatic and asymptomatic samples and the expression of transferring receptor was borderline significant. However, if multiple testing corrections were applied, the false discovery rates were between 0.28 and 0.40, ie, the expected percent of false predictions in these 40 probes was determined by t test and Benjamin-Hochberg multiple testing correction.
The list of differentially regulated genes showed significant enrichment \((P<0.05)\) of 19 GO terms (please see http://atvb.ahajournals.org) with two main motifs: (1) a large immunoglobulin gene cluster down-regulated in the symptomatic samples and (2) a protease gene cluster (cathepsin L, matrix metalloproteinase [MMP] 7, 9, and 12) upregulated in the symptomatic plaques. There was no enrichment in pathways in the publicly available repositories (KEGG, Biocharta) but one of the most prominent functional clusters was genes involved in the tissue homeostasis of iron and heme, namely HO-1, hemoglobin scavenger receptor CD163, hemoglobins beta and gamma, and transferrin receptor (TFRC). Therefore we decided to pursue further the expression of genes known to be induced in tissue response to hemorrhages, namely CD163, HO-1, and TFRC, in an independent set of 40 carotid plaques.

**Overexpression of CD163 and HO-1 mRNAs in an Independent Set of Carotid Plaques**

CD163, HO-1, and TRFC were analyzed by quantitative real-time RT-PCR in the bilateral plaques as well as in the larger set of 40 carotid plaques. Microarray and quantitative real-time RT-PCR results of bilateral plaques showed a good correlation (data not shown). When analyzed in the larger set of carotid plaques, the mRNA expression of both CD163 and HO-1 was significantly higher in symptomatic compared with asymptomatic plaques, revealing 1.58-fold induction for CD163 \((95\% \text{ CI} 1.11 \text{ to } 2.40, P=0.013)\) and 1.93-fold induction for HO-1 \((95\% \text{ CI} 1.04 \text{ to } 3.94, P=0.040)\) (Figure 1). CD163 and HO-1 mRNA expressions were positively correlated with each other \((r_s=0.72, P<0.001)\). TRFC also showed a tendency toward higher expression in symptomatic than in asymptomatic plaques but this was not statistically significant. TRFC expression correlated with CD163 \((r_s=0.62, P<0.001)\) but not with HO-1 expression.

**Quantitation of HO-1 Protein Expression by Western Blotting**

HO-1 protein was quantitated by Western blotting in the set of 22 carotid plaques (asymptomatic \(n=9\) and symptomatic \(n=13\)). The expression of HO-1 protein relative to \(\beta\)-actin was significantly higher in the symptomatic than in the asymptomatic plaques (fold-change 4.38, 95% CI 1.54 to
12.20, \( P = 0.024 \); Figure 2). HO-1 protein levels correlated
with the HO-1 mRNA levels (\( r_s = 0.73, \ P = 0.001 \), see Figure
3B). CD163 protein was not quantitated because of incom-
patibilities related to the protein extraction method and
available antibodies.

**Immunohistochemistry of HO-1 and CD163**

To determine the localization of CD163 and HO-1 expres-
sion, the carotid plaques from 22 cases were studied by
immunohistochemistry. Representative stainings of CD163
and HO-1 are presented in Figure 4. CD163 expression was
abundant in the shoulder and cap regions of the atheroma
(Figure 4A, 4D, 4G, and 4J). Morphologically, the CD163
antibody stained a subpopulation of macrophages, which
were either scattered or assembled into infiltrates (Figure 4D,
4G, and 4J). Two different staining patterns were observed:
cytoplasmic and membranous patterns, the first being more
abundant (not shown). HO-1 expression was often bordering the
lipid core, mainly in macrophages but in some vascular smooth
muscle cells (VSMCs) as well (Figure 4B, 4E, 4H, and 4K).

Protein staining of CD163 and HO-1 was scored semiquan-
titatively. The amount of HO-1 and CD163 staining were
correlated with each other (\( r_s = 0.61, \ P = 0.002 \), and also
partially colocalized (Figure 4; compare the left and middle
columns). Both CD163 and HO-1 tended to be expressed
more intensively in symptomatic specimen in correlation with
the corresponding mRNA levels (Figure 3A and 3B).

**Correlations of CD163 and HO-1 Expression to
Clinical and Macroscopic Plaque Characteristics**

CD163 and HO-1 mRNA and protein levels showed association
to morphological features of symptom-causing carotid disease,
namely the degree of carotid stenosis (CD163 mRNA: \( r_s = 0.60, \ P = 0.004 \); CD163 protein (immunohistochemistry) \( r_s = 0.42, \ P = 0.042 \); HO-1 mRNA: \( r_s = 0.59, \ P = 0.005 \); HO-1 protein
(Western blotting): \( r_s = 0.37, \ P = 0.098 \) and plaque ulcer-
ation (CD163 mRNA: \( r_s = 0.70, \ P = 0.001 \); HO-1 mRNA: \( r_s = 0.47, \ P = 0.035 \); HO-1 protein: \( r_s = 0.49, \ P = 0.028 \). Both had a tendency
to associate directly to total serum total cholesterol and LDL levels
and indirectly to serum HDL and sensitized CRP.

**Staining of Iron Deposits**

Asymptomatic and symptomatic plaques did not differ in
the frequency of macroscopic hemorrhages (data not shown) but
HO-1 and CD163 mRNA as well as HO-1 protein level were
correlated to plaque hemorrhages (CD163 mRNA: \( r_s = 0.44, \ P = 0.005 \), HO-1 mRNA: \( r_s = 0.54, \ P < 0.001 \), HO-1 protein:
\( r_s = 0.52, \ P = 0.016 \). To reveal prior microhemorrhages, iron
deposits were stained (Figure 4C, 4F, 4I and 4L) with two
emerging patterns: both extracellular and cytoplasmic gran-
ular staining (Figure 4; panels I and L) and, on the other hand,
large confluent homogeneously stained areas (not shown).
Iron deposits were present in several locations, eg, near the
luminal surface, around the lipid core and in the intima-
media, but, interestingly, not always in the areas of past
rupture or erosion. With respect to the bilateral cases, each
symptomatic plaque contained more iron than the asymptom-
atic plaque from the same patient. However, the 22 indepen-
dent specimen showed no differences in the amount of iron
between symptomatic and asymptomatic plaques. Iron depos-
its correlated significantly with CD163 and HO-1 expression
both at mRNA (CD163: \( r_s = 0.47, \ P = 0.029 \), HO-1: \( r_s = 0.73, \ P < 0.001 \) and protein levels (CD163 immunohistochemistry:
Discussion

The present study started from a large-scale microarray analysis in four bilateral cases of carotid stenoses to screen genes that are differentially expressed between asymptomatic and symptomatic plaques within the same individual. One of the largest functional clusters included genes involved in the homeostasis of iron and heme and we could show that two of these, HO-1 and CD163, were induced both at the mRNA and protein level also in a larger set of carotid plaques (n = 40). In line, their expression correlated with traditional markers of unstable carotid disease, the degree of carotid stenosis and plaque ulcerations. Finally, we could show that both mRNA and protein levels of CD163 and HO-1 correlated strongly with iron deposits.

To our knowledge this is the first report comparing gene expression between symptomatic and asymptomatic carotid plaques from the same bilaterally operated patients, which are very rare. Our cohort of 98 patients including all consecutive patients admitted to our hospital for carotid endarterectomy during 1995 to 2000 had only four such patients. Consequently, the microarray study has not enough power to provide significant results at the genome-wide level without independent replication, for which we used a larger set of carotid specimen from isolated patients. The two sample sets and their analysis differ in some respects. In the microarray analysis of the bilateral cases the genetic background is identical and thus “the unique environment” of each plaque, which might be, eg, distinct anatomy, infections, hemorrhages, must underlie the differences in the expression profiles. In the case of the replication material, where we compared the groups of isolated asymptomatic and symptomatic plaques, similar kind of factors should also play a role but individual differences (eg, genetic factors, comorbidities, smoking, medication) may confound the effect. Thus even though replication in isolated single endarterectomies can be criticized it can also be used to confirm expression profiles that have such a large impact on plaque stability that their effect can also be detected at the population level.

Intuitively, the induction of CD163 and HO-1 would fit with more frequent intraplaque hemorrhages in the symptomatic plaques. This proved true in the bilateral cases where each symptomatic plaque contained more iron in comparison to the asymptomatic one from the same patient. This suggests that intraplaque hemorrhages might be an important incidental factor causing plaque destabilization. However, considering the whole material the situation was more complex since we did not find differences in macroscopic hemorrhages or long-term iron deposition between asymptomatic and symptomatic plaques. This might also explain why the differential expression of TRFC seen in the bilateral cases did not recur in the isolated cases; the expression of TRFC is more directly regulated by iron through the iron-responsive elements.

Our findings are in line with previous pathological studies showing that intraplaque hemorrhages are correlated with the degree of carotid stenosis rather than with symptom-producing plaques. Because symptomatic plaques did not show more iron deposition but showed higher expression of HO-1, this could imply that a stronger activation response to microhemorrhages and free iron takes place in symptomatic plaques, eg, attributable to differences in cellular composition or genetic background. In fact, regarding endothelial cells it has been shown that atherosclerosis-susceptible mouse strains demonstrate higher induction of HO-1 than atherosclerosis-resistant strains in response to modified LDL. We suggest that intraplaque hemorrhages have a deleterious effect on plaque stability, but this is strongly modulated by individual environmental and genetic factors. This might explain why pathological studies on the role of hemorrhages in plaque destabilization have remained inconclusive.

Protein staining of CD163 and HO-1 was strongest in areas of active inflammation characterized by various inflammatory cells, foam cells, extracellular lipids, cholesterol crystals, and red blood cells. In these areas, their expression also mainly colocalized (Figure 4), although not always in the same cells. CD163 is specifically expressed by monocyte-macrophage lineage and is commonly used as a macrophage marker. It is also regarded to mark “alternatively activated macrophages” that have a special regulatory role in immune responses. It could be argued that overexpression of CD163 in symptomatic plaques reflects differences in the cellular composition between plaques. We have previously studied macrophage density in this same set of carotid plaques using HAM56-staining and did not find increased density in symptomatic plaques. Still, it is possible that overexpression of CD163 indicates over-representation of a certain macrophage subtype in symptomatic plaques. HO-1, on the other hand, stained macrophages that had endocytosed red blood cells or hemosiderin and were surrounded by cholesterol crystals, which is in line with the results by others.

Especially HO-1 is regarded as an antiatherogenic agent during the early phases of atherosclerosis, and it is possible that the induction of HO-1 and CD163 purely reflects immune activation in response to intraplaque hemorrhages without being destabilizing phenomena per se. Yet, both HO-1 and CD163 have characteristics that might paradoxically turn harmful in advanced atheromas. HO-1 is known to prevent proliferation of VSMCs and endothelial cells after vascular injury, which can be important to sustain plaque integrity and stability. CD163, again, is linked to an increased risk of vascular complications via its different efficiencies to remove hemoglobin, depending on haptoglobin genotypes. Only one study has previously compared HO-1 or CD163 expression in asymptomatic and symptomatic atherothrombotic plaques. Ameriso and colleagues investigated HO-1 expression by immunohistochemistry in relation to Helicobacter pylori infection and concluded that HO-1 expression was more frequent in infected and asymptomatic carotid plaques. Unfortunately, many methodological differences, eg, in the patient selection and immunohistochemical methods, hinder direct comparison between their study and the present one.
It could be claimed that the induction of CD163 and HO-1 occurred “post-hoc” in response to thromboembolic events and associated surface bleeding into the plaque. We cannot entirely preclude this possibility, but several facts argue against it. First, asymptomatic and symptomatic plaques did not show differences in macroscopic hemorrhages or iron deposits, which suggests that symptomatic plaques have not suffered more abundant or more recent hemorrhages than asymptomatic ones. The majority of the HO-1 and CD163 expression occurred in the deeper layers of the plaques (Figure 4) rather than around the surface ulcerations/hemorrhages. Second, no correlation was found between CD163/HO-1 expression and time after onset of symptoms. Thus we suggest that the coinduction of CD163 and HO-1 does not merely reflect a surface hemorrhage associated with the latest thromboembolism but a more chronic process throughout the evolution of symptomatic plaques that could mark an increased risk for plaque destabilization and thromboembolic symptoms.

In conclusion, our study revealed several genes that are potentially important in the destabilization of atherosclerotic plaques and could all represent novel targets for development of plaque-stabilizing drugs. Especially, CD163 and HO-1 involved in the degradation of hemoglobin after intraplaque hemorrhage were induced in symptomatic plaques, warranting further studies on these signaling cascades in unstable atherosclerotic plaques.

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Disclosures

None.

REFERENCES

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### Table I. Characteristics of the patients with bilateral carotid stenoses

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<th>Patients</th>
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<td>M</td>
<td>F</td>
<td>F</td>
<td>F</td>
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<tr>
<td>Age, years</td>
<td>67</td>
<td>63</td>
<td>59</td>
<td>71</td>
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<tr>
<td>Degree of ICA stenosis*</td>
<td>75 / 71</td>
<td>75 / 50</td>
<td>75 / 83</td>
<td>83 / 70</td>
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<td>Cerebrovascular symptom</td>
<td>stroke</td>
<td>stroke</td>
<td>stroke</td>
<td>TIA</td>
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<td>(hemiparesis)</td>
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<td>(hemiparesis)</td>
<td>(hemiparesis)</td>
<td></td>
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<tr>
<td>Brain imaging†</td>
<td>MRI</td>
<td>CT</td>
<td>CT</td>
<td>MRI</td>
</tr>
<tr>
<td>- / -</td>
<td>infarct / -</td>
<td>infarct / -</td>
<td>- / infarct</td>
<td></td>
</tr>
<tr>
<td>Time, days ‡</td>
<td>24</td>
<td>46</td>
<td>111</td>
<td>11</td>
</tr>
<tr>
<td>Comorbidities§</td>
<td>acute bronchitis</td>
<td>CHD, DM, HA</td>
<td>HA, HC</td>
<td>CHD, DM, HA, HC, PAD</td>
</tr>
</tbody>
</table>

| Plaque characteristics|||
| Smoothness of surface | - / - | + / - | - / + | - / + |
| Ulceration | + / + | - / + | + / - | + / - |
| Loose atheroma | - / + | - / - | - / - | - / + |
| Intraplaque hemorrhage | + / - | + / - | - / - | + / - |
| Calcification | + / - | + / + | + / + | + / + |
| Intramural trombosis | + / - | - / - | + / - | - / - |

* The degree of internal carotid artery stenosis according to the NASCET criteria: the right / left internal carotid artery. The symptomatic side is underlined.
† Infarcts in the supply territory of the right / left internal carotid artery. – indicates no lesions.
‡ Time between symptom onset and carotid endarterectomy.
§ Abbreviations: CHD=coronary heart disease, DM=type II diabetes, HA=hypertension, HC=hypercholesterolemia, PAD=peripheral artery disease.
|| Presence of macroscopic plaque features: the right / left carotid artery.
Table II. Differentially expressed genes from the microarray analysis in the bilateral cases of carotid artery stenosis.

### A. Increased expression in symptomatic carotid plaques

<table>
<thead>
<tr>
<th>Probe</th>
<th>Gene name</th>
<th>Fold-Change</th>
<th>P-value</th>
<th>FDR</th>
<th>†</th>
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<tr>
<td>37324_at</td>
<td>transferrin receptor</td>
<td>1.8</td>
<td>0.077</td>
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<tr>
<td>34210_at</td>
<td>CD52 antigen (CAMPATH-1 antigen)</td>
<td>2.1</td>
<td>0.094</td>
<td>0.283</td>
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<td>37542_at</td>
<td>lipoma HMGIC fusion partner-like 2</td>
<td>1.5</td>
<td>0.100</td>
<td>0.283</td>
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<tr>
<td>41096_at</td>
<td><strong>S100 calcium binding protein A8 (calgranulin A)</strong></td>
<td>1.9</td>
<td>0.105</td>
<td>0.283</td>
<td></td>
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<tr>
<td>39994_at</td>
<td><em>chemokine (C-C motif) receptor 1</em></td>
<td>1.7</td>
<td>0.132</td>
<td>0.283</td>
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<tr>
<td>41209_at</td>
<td><em>lipoprotein lipase</em></td>
<td>1.6</td>
<td>0.154</td>
<td>0.283</td>
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<tr>
<td>32227_at</td>
<td>proteoglycan 1, secretory granule</td>
<td>1.7</td>
<td>0.172</td>
<td>0.283</td>
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<tr>
<td>36197_at</td>
<td>chitinase 3-like 1 (cartilage glycoprotein-39)</td>
<td>2.2</td>
<td>0.180</td>
<td>0.283</td>
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<tr>
<td>40522_at</td>
<td>glutamate-ammonia ligase (glutamine synthase)</td>
<td>1.6</td>
<td>0.206</td>
<td>0.300</td>
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<td>0.210</td>
<td>0.300</td>
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<tr>
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<td>1.7</td>
<td>0.228</td>
<td>0.314</td>
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<td>668_s_at</td>
<td>matrix metallopeptidase 7 (matrilysin, uterine)</td>
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<td>0.239</td>
<td>0.319</td>
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<td>38745_at</td>
<td>lipase A, lysosomal acid, cholesterol esterase</td>
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<td>0.262</td>
<td>0.322</td>
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<tr>
<td>31859_at</td>
<td><em>matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase)</em></td>
<td>2.0</td>
<td>0.265</td>
<td>0.322</td>
<td></td>
</tr>
<tr>
<td>31438_s_at</td>
<td>CD163 antigen</td>
<td>1.7</td>
<td>0.266</td>
<td>0.322</td>
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</tr>
<tr>
<td>32128_at</td>
<td><em>chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)</em></td>
<td>1.6</td>
<td>0.291</td>
<td>0.326</td>
<td></td>
</tr>
<tr>
<td>31525_s_at</td>
<td>hemoglobin, alpha 1</td>
<td>1.9</td>
<td>0.293</td>
<td>0.326</td>
<td></td>
</tr>
<tr>
<td>37603_at</td>
<td><em>interleukin 1 receptor antagonist</em></td>
<td>2.1</td>
<td>0.294</td>
<td>0.326</td>
<td></td>
</tr>
<tr>
<td>33802_at</td>
<td>heme oxygenase (decycling)</td>
<td>2.6</td>
<td>0.328</td>
<td>0.354</td>
<td></td>
</tr>
<tr>
<td>37391_at</td>
<td>cathepsin L</td>
<td>1.6</td>
<td>0.338</td>
<td>0.355</td>
<td></td>
</tr>
<tr>
<td>1481_at, 1482_g_at</td>
<td><em>matrix metallopeptidase 12 (macrophage elastase)</em></td>
<td>1.8</td>
<td>0.405</td>
<td>0.405</td>
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</tr>
</tbody>
</table>

### B. Decreased expression in symptomatic carotid plaques

<table>
<thead>
<tr>
<th>Probe</th>
<th>Gene name</th>
<th>Fold-Change</th>
<th>P-value</th>
<th>FDR</th>
<th>‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>36811_at</td>
<td>lysyl oxidase-like 1</td>
<td>-1.5</td>
<td>0.025</td>
<td>0.283</td>
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</tr>
<tr>
<td>35566_f_at</td>
<td>immunoglobulin heavy locus / immunoglobulin heavy constant mu / immunoglobulin heavy constant gamma 1 (G1M marker)</td>
<td>-1.8</td>
<td>0.094</td>
<td>0.283</td>
<td></td>
</tr>
<tr>
<td>37864_s_at</td>
<td>immunoglobulin heavy locus / immunoglobulin heavy constant gamma 1 (G1M marker) / immunoglobulin heavy constant gamma 2 (G2M marker) / immunoglobulin heavy constant gamma 3</td>
<td>-2.4</td>
<td>0.109</td>
<td>0.283</td>
<td></td>
</tr>
</tbody>
</table>
(G3M marker) / immunoglobulin heavy constant gamma 4 (G4M marker)

456_at  SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 3  -1.5  0.115  0.283

41827_f_at  similar to bK246H3.1 (immunoglobulin lambda-like polypeptide 1, pre-B-cell specific)  -2.4  0.117  0.283

37467_at  immunoglobulin heavy constant delta  -1.7  0.132  0.283

33273_f_at, 33274_f_at  lambda constant 1 (MCG marker) / immunoglobulin lambda variable 3 – 25 / immunoglobulin lambda joining 3  -2.8  0.136  0.283

35530_f_at  immunoglobulin lambda chain variable region / immunoglobulin lambda constant 1 (MCG marker) / Immunoglobulin lambda joining 3  -1.7  0.152  0.283

38194_s_at  immunoglobulin kappa constant / immunoglobulin kappa variable 1-5  -2.3  0.161  0.283

33499_s_at, 33500_i_at, 33501_r_at  immunoglobulin heavy constant alpha 1 / immunoglobulin heavy constant alpha 2 (A2M marker) / hypothetical protein MGC27165  -2.3  0.169  0.283

40161_at  cartilage oligomeric matrix protein  -2.1  0.169  0.283

34105_f_at, 41164_at, 41165_g_at  immunoglobulin heavy locus / immunoglobulin heavy constant mu  -1.9  0.184  0.283

* Genes previously suggested to have a role in the progression of atherosclerosis are bolded and the appropriate reference is given.
† Paired t-test, unadjusted p-values.
‡ FDR= the false discovery rate. P-values adjusted by Benjamin-Hoechberg multiple testing correction for the 40 probes tested. If p-values were corrected for all probes on the array (n=12625), the FDR is 0.98.
### Table III. Gene Ontology terms associated with the differentially regulated genes

<table>
<thead>
<tr>
<th>GO Term</th>
<th>Count*</th>
<th>%†</th>
<th>P-value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>immune response</td>
<td>17</td>
<td>42.5</td>
<td>1.77E-09</td>
</tr>
<tr>
<td>defense response</td>
<td>17</td>
<td>42.5</td>
<td>7.14E-09</td>
</tr>
<tr>
<td>MHC class I receptor activity</td>
<td>4</td>
<td>10</td>
<td>1.26E-04</td>
</tr>
<tr>
<td>antigen processing, endogenous antigen via MHC class I</td>
<td>4</td>
<td>10</td>
<td>1.89E-04</td>
</tr>
<tr>
<td>antigen presentation, endogenous antigen</td>
<td>4</td>
<td>10</td>
<td>1.89E-04</td>
</tr>
<tr>
<td>antigen processing</td>
<td>4</td>
<td>10</td>
<td>3.87E-04</td>
</tr>
<tr>
<td>antigen presentation</td>
<td>4</td>
<td>10</td>
<td>4.62E-04</td>
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<tr>
<td>peptidoglycan metabolism</td>
<td>3</td>
<td>7.5</td>
<td>2.75E-03</td>
</tr>
<tr>
<td>catabolism</td>
<td>7</td>
<td>17.5</td>
<td>7.98E-03</td>
</tr>
<tr>
<td>membrane fraction</td>
<td>8</td>
<td>20</td>
<td>1.26E-02</td>
</tr>
<tr>
<td>transmembrane receptor activity</td>
<td>7</td>
<td>17.5</td>
<td>1.43E-02</td>
</tr>
<tr>
<td>lipoprotein lipase activity</td>
<td>2</td>
<td>5</td>
<td>1.43E-02</td>
</tr>
<tr>
<td>opioid peptide activity</td>
<td>2</td>
<td>5</td>
<td>2.14E-02</td>
</tr>
<tr>
<td>metalloendopeptidase activity</td>
<td>3</td>
<td>7.5</td>
<td>2.38E-02</td>
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<tr>
<td>biopolymer catabolism</td>
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<td>10</td>
<td>2.91E-02</td>
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<tr>
<td>peptidase activity</td>
<td>5</td>
<td>12.5</td>
<td>3.34E-02</td>
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<tr>
<td>inflammatory response</td>
<td>4</td>
<td>10</td>
<td>3.42E-02</td>
</tr>
<tr>
<td>oxygen transport</td>
<td>2</td>
<td>5</td>
<td>3.47E-02</td>
</tr>
<tr>
<td>gas transport</td>
<td>2</td>
<td>5</td>
<td>3.47E-02</td>
</tr>
</tbody>
</table>

*Number of differentially expressed genes annotated under term.
†Percent of differentially expressed genes annotated under term.
‡Fisher’s exact test.
Figure I. Hierarchical cluster analysis of the differentially expressed genes. The dendrogram on the left indicates correlation between gene expression profiles. The columns in the middle show the normalized expression of each gene (the titles of which are given on the right) in pseudocolor scale (key in the upper right corner). If the gene title was abbreviated owing to space limitations the probe id is given in the parenthesis (see Table II for the full titles). Numbers above the gene expression columns refer to the bilateral carotid stenosis patients (see Table I).
References


