Identification of Genes Potentially Involved in Rupture of Human Atherosclerotic Plaques


Abstract—Although rupture of an atherosclerotic plaque is the major cause of acute vascular occlusion, the exact molecular mechanisms underlying this process are still poorly understood. In this study, we used suppression subtractive hybridization to make an inventory of genes that are differentially expressed in whole-mount human stable and ruptured plaques. Two libraries were generated, one containing 3000 clones upregulated and one containing 2000 clones downregulated in ruptured plaques. Macroarray analysis of 500 randomly chosen clones showed differential expression of 45 clones. Among the 25 clones that showed at least a 2-fold difference in expression was the gene of perilipin, upregulated in ruptured plaques, and the genes coding for fibronectin and immunoglobulin λ chain, which were downregulated in ruptured plaques. Reverse transcriptase–polymerase chain reaction analysis on 10 individual ruptured and 10 individual stable plaques showed a striking consistency of expression for the clones SSH6, present in 8 ruptured and 2 stable plaques, and perilipin, expressed in 8 ruptured plaques and completely absent in stable plaques. Localization studies of both perilipin mRNA and protein revealed expression in cells surrounding the cholesterol clefts and in foam cells of ruptured atherosclerotic plaques. No expression was observed in nondiseased artery, and only a few cells in the shoulder region of stable plaques tested positive for perilipin. In conclusion, this study shows that it is possible to identify genes that are differentially expressed in whole-mount stable or ruptured atherosclerotic plaques. This approach may yield several potential regulators of plaque destabilization. (Circ Res. 2001;89:547-554.)

Key Words: atherosclerosis ■ plaque rupture ■ gene expression ■ humans

Rupture of atherosclerotic plaques is the predominant underlying process in the pathogenesis of acute coronary syndromes and peripheral vascular disease.1–3 Although the morphology of ruptured plaques is well described,4,5 specific markers to identify ruptured plaques or plaques prone to rupture in vivo are not available.6 One possible explanation for the lack of plaque-type–specific markers is the fact that the exact molecular mechanisms underlying the process of plaque rupture are still largely unknown. In an attempt to shed more light on the possible molecular mechanisms involved in the onset and progression of atherosclerosis, several studies compared the gene expression of activated human umbilical vein endothelial cells, vascular smooth muscle cells, and cholesterol-loaded macrophages with that of nonactivated cells.7–9 These studies in cell lines revealed differential regulation of genes involved in leukocyte trafficking, cell cycle control, and apoptosis. However, expression of these genes in vivo remains to be determined. Other groups focused on differences in gene expression between fatty streaks and advanced lesions10 and intima and media of human atherosclerotic plaques.11 Adams et al12 revealed different gene expression patterns between veins and arteries in macaques. However, in the present study, we focused on differential gene expression of morphologically advanced, but stable, human atherosclerotic lesions and ruptured human atherosclerotic lesions. Suppression subtractive hybridization (SSH)13 on whole-mount specimens was used to make an inventory of genes differentially expressed in both lesion types. The advantage of SSH is the isolation of low abundant sequences that might not be isolated by use of microarray technology. We identified several genes that were differentially expressed in a larger panel of individual ruptured and stable human atherosclerotic plaques. Our results show that it is possible to identify genes that are specifically and reproducibly associated with specific stages of atherosclerosis by using whole-mount human atherosclerotic specimens.

Materials and Methods

Experimental Procedures

SSH was performed on RNA pools of 3 ruptured and 3 stable human atherosclerotic plaques. Two libraries, one containing clones upregulated in ruptured plaques and the other containing clones downregu-

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lated in ruptured plaques, were constructed. Differential expression was confirmed by macroarray analysis. Clones showing at least a 2-fold difference in expression were sequenced. To validate the reproducibility of expression of these clones, reverse transcriptase–polymerase chain reaction (PCR) analysis was performed on a larger series of ruptured (n=10) and stable (n=10) plaques. Cellular distribution of the two clones with the most reproducible expression pattern was assessed by RNA in situ hybridization, whereas expression and localization of the protein of one of the two clones was determined by immunohistochemistry. Figure 1 schematically depicts the experimental design and the reduction of the number of candidate genes by the various screening steps.

**Tissue Sampling and RNA Isolation**

Plaques were obtained from patients undergoing vascular surgery (Department of General Surgery, Academic Hospital Maastricht). Patient characteristics are summarized in Table 1. Immediately after resection, the atherosclerotic specimen was divided into parallel parts of 5 mm for RNA isolation and histological analysis. Tissue destined for RNA isolation was immediately frozen in liquid nitrogen and stored at −80°C. Total RNA was isolated by using the guanidine isothiocyanate/CsCl method. Specimens for histological analysis were fixed in 10% phosphate-buffered formalin (pH 7.4), routinely processed, and embedded in paraffin. Sections were cut, stained with hematoxylin and eosin, and classified according to the morphological criteria of the American Heart Association (AHA). Stable lesions (types IV and V) are characterized by an intact fibrous cap that contains smooth muscle cells, fibroblasts, and connective tissue. These plaques contain either a large lipid core (type Va), calcification (type Vb), or fibrous tissue (type Vc). A disrupted fibrous cap and the presence of a thrombus characterize the ruptured lesion (type VI).

**Suppression Subtractive Hybridization**

The SSH procedure was performed by using the PCR-Select cDNA Subtraction Kit (Clontech) essentially according to the protocol of the manufacturer, with minor adjustments. Briefly, total RNA was isolated from whole-mount plaques of 6 age-matched male patients undergoing vascular surgery (Table 1). To correct for patient-based differences in gene expression, we generated two pools of total RNA.

<table>
<thead>
<tr>
<th>Plaque Type</th>
<th>Patient No.</th>
<th>Sex</th>
<th>Age, y</th>
<th>Artery</th>
<th>Analysis</th>
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<td>1</td>
<td>M</td>
<td>60</td>
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</tr>
<tr>
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<tr>
<td></td>
<td>3</td>
<td>M</td>
<td>72</td>
<td>Abdominal aorta</td>
<td>SSH/array/RT-PCR/ISH/ISH</td>
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<tr>
<td></td>
<td>4</td>
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<tr>
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<td>M</td>
<td>73</td>
<td>Abdominal aorta</td>
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<tr>
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<tr>
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<td>67</td>
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<td>M</td>
<td>70</td>
<td>Carotid artery</td>
<td>RT-PCR</td>
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</tbody>
</table>

M indicates male; F, female; array, custom-made macroarray; ISH, RNA in situ hybridization; and IHC, immunohistochemistry.
Pool 1 contained 1 μg of total RNA derived from 3 ruptured plaques of the abdominal artery of 3 individual patients. Pool 2 contained 1 μg of total RNA derived from 3 stable plaques of the iliac artery, femoral artery, and carotid artery, respectively, of 3 individual patients. The SMART PCR cDNA Synthesis Kit (Clontech) was used for the preparation and amplification of double-stranded cDNA. In the forward reaction, genes upregulated in ruptured plaques were isolated, whereas the reverse reaction resulted in the isolation of genes downregulated in ruptured plaques. Real-digested tester cDNA was ligated to two different adaptors and hybridized twice to a 4-fold excess of driver cDNA to enrich for differentially expressed genes. Differentially expressed genes were amplified by two rounds of PCR. The resulting fragments were gel-purified, cloned into the pGEMT-easy vector (Promega), and subsequently transformed to highly competent Escherichia coli JM109 cells (Promega).

**Analysis of Subtracted cDNA Libraries**

**Macroarray Analysis**

Clones derived by SSH were tested by macroarray analysis. Inserts were amplified by PCR with the use of the T7 (5'-TAAATAGCTCTACTATAGGG-3') and SP6 (5'-ATTTAGGTGACACTATA-3') primers under standard conditions. Briefly, 10 μL of PCR product was diluted in 190 μL of 6X SSC, heated to 95°C, and quenched on ice. Two identical macroarrays were made by transferring 100 μL of standard product of the sample to a nylon membrane (Nytran, Schleicher & Schuell) by using a standard procedure. The filters were hybridized at high stringency with [32P]labeled (High Prime, Boehringer-Mannheim) SMART cDNA of either stable or ruptured plaques by using standard procedures. Hybridization signals were normalized with the use of RNA-polymerase II and genomic DNA signals. Quantitative analysis was performed by phosphor image analysis (Quantity One, Bio-Rad).

**Sequencing**

Differentially expressed clones were sequenced by using the Thermo Sequenase fluorescently labeled primer (M13 reverse, 5'-TTTCCACACAGAAAACAGGAACAGCTATGAC-3'; M13 forward, 5'-GGCCACGGGTTTTCCAGTGAC-3') cycle sequencing kit (Amersham Pharmacia Biotechnology) and analyzed on an ALF-express automatic sequencer (Pharmacia Biotech). Homology searches were performed automatically by use of the advanced Blast Program on the combined GenBank/EMBL nonredundant expressed sequence tag (dEST), mouse EST, human EST, rat EST, Swiss protein, and human tagged genomic sequence (htgs) databases (National Center for Biotechnology Information, which can be accessed online at www.ncbi.nlm.nih.gov/).

**Reverse Transcriptase–Polymerase Chain Reaction**

Isolation of total RNA was carried out as described above. The SMART PCR cDNA Synthesis Kit (Clontech) was used for the preparation of double-stranded cDNA from 0.5 μg template RNA. cDNA was diluted to a total volume of 50 μL. PCR amplification of SSH6 (sense, 5'-GGCTAATTCCGGAGATAGCC-3', plus antisense, 5'-CAACACCTCAGGACAAGGAAACAGCTATGAC-3'; SSH11 (sense, 5'-CTTTACCAAACTTGTGGCC-3', plus antisense, 5'-TACTCAGAAAGTGACACTAG-3') was performed on 1 μL of first-strand cDNA by using standard conditions (30 cycles of denaturation for 1 minute at 94°C, annealing for 1 minute at 55°C, and extension for 1 minute at 72°C; reaction volume 25 μL). Resulting PCR products of ~300 bp were analyzed on a 1% agarose gel.

**RNA In Situ Hybridization**

Sense and antisense digoxigenin (DIG)-labeled RNA probes of perilipin (SSH1/SSH11) and SSH6 were generated on the EcoRI-EcoRI cDNA fragments, which were first recloned in pTZ18 (Pharmacia) in both orientations. After linearization with BamHI, DIG-labeled RNA was transcribed from the T7 promoter. Hybridization of 4-μm paraffin-embedded sections and visualization with alkaline phos-

**Immunohistochemistry**

Rabbit polyclonal antibody, raised against human perilipin, was a kind gift of Dr A.S. Greenberg (Jean Mayer Human Nutrition Research Center at the Tufts University, Boston, Mass). Paraffin sections (4 μm) were deparaffinized, hydrated, and pretreated by boiling in 0.01 mol/L citrate buffer (pH 6.0) for 10 minutes in a microwave oven (750 W). Sections were incubated with the polyclonal perilipin antibody (1:1000) for 30 minutes. Subsequently, the sections were incubated with a biotinylated swine anti-rabbit antibody (1:1000, Dako) for 30 minutes, followed by a 30-minute incubation with an alkaline phosphatase–coupled ABC reagent (1:200, Dako). Alkaline phosphatase activity was visualized by using the Alkaline Phosphatase Kit I (Vector), resulting in a red precipitate. The sections were counterstained with hematoxylin. Combined immunohistochemical staining was performed to identify the cell types expressing perilipin. Perilipin was first localized by using the above-described protocol, with minor adjustments. The ABC reagent was coupled to horseradish peroxidase (1:500, Dako), and the peroxidase activity was visualized by using diaminobenzidine, resulting in a brown precipitate. Subsequently, the sections were treated with 0.1% pepsin (Boehringer) in 0.1N HCl for 30 minutes and incubated with either monoclonal anti-CD68 (1:100, Dako) or polyclonal anti–factor VIII (1:2000, Biomakers) for 30 minutes. The respective sections were incubated with a biotinylated swine anti-mouse antibody (1:250, Amersham Life Sciences) or biotinylated swine anti-rabbit antibody (1:1000, Dako) for 30 minutes, followed by a 30-minute incubation with an alkaline phosphatase–coupled ABC reagent (1:200, Dako). Alkaline phosphatase activity was visualized by using the Alkaline Phosphatase Kit III (Vector), resulting in a blue precipitate.

**Results**

**Macroarray and Sequencing Analysis**

SSH resulted in a forward library containing 3000 clones upregulated in ruptured atherosclerotic plaques and a reverse library containing 2000 clones downregulated in ruptured plaques. Differential expression of the clones of both SSH libraries was verified by a second independent method, the macroarray analysis. Ten percent of the clones of both libraries, 300 of the forward subtracted library and 200 of the reverse library, were randomly chosen and screened for expression in ruptured and stable plaques. The macroarrays were performed in triplicate, and the variation was ~10%. Figure 2 shows a representative macroarray of clones selected by SSH, hybridized to the pool of stable plaque cDNA (Figure 2A) and to the pool of ruptured plaque cDNA (Figure 2B). Hybridization signals were normalized for the signals of RNA–polymerase II and human genomic DNA. Forty-five of the 500 clones tested showed a differential expression pattern by macroarray analysis. Three clones were uniquely expressed in ruptured plaques, and 4 clones were uniquely expressed in stable plaques. In addition, 18 of the 45 clones showed at least a 2-fold difference in expression between ruptured and stable plaques. These 25 clones were sequenced and compared with available data in GenBank. All 11 clones upregulated in ruptured plaques had different sequences; two of these clones were homologous to distinct parts of the gene encoding perilipin (SSH1 and SSH11). Furthermore, one clone was homologous to a serine/arginine-rich protein. Additional searches in the EST (human, mouse, and rat) and
htgs databases revealed homology to human ESTs for 4 clones, and 2 clones showed homology to distinct parts of the human chromosome 5. One clone (SSH6) shared high homology to a sequence encoding a putative protein; however, no information regarding its expression is available. Finally, one clone contained a previously unknown sequence. Homology data for the clones upregulated in ruptured plaques are shown in Table 2.

Alignment of the 14 clones downregulated in ruptured plaques revealed high homology of 3 clones to the known genes of β-actin, fibronectin, and an immunoglobulin λ light chain. Additional searches in the EST (human, mouse, and rat) and htgs database showed homology to human ESTs for 8 clones, and 2 clones were homologous to parts of chromosomes 5 and 17. Finally, one clone was homologous to a human genomic clone of a thus-far-unknown chromosomal localization. The results of the homology searches are shown in Table 3.

**RT-PCR Analysis**

To further validate the expression profile found in the macroarray, RT-PCR analysis on 10 ruptured and 10 stable plaques was performed. Because we were mostly interested in genes that were upregulated in ruptured plaques, we focused our further analysis on those genes that showed unique expression in ruptured plaques (SSH1/11 and SSH6) and on one (SSH42) of four clones that were uniquely expressed in stable plaques. To exclude patient- and artery-biased expression, plaques originated from different arteries of different patients (Table 1). Expression was normalized to the expression level of GAPDH, the expression level of which was comparable between the samples (Figure 3).

Expression of clone SSH6 was found in 8 of 10 ruptured plaques, whereas only 2 of 10 stable plaques tested positive. Perilipin (clones SSH1 and SSH11) was expressed in 8 of 10 ruptured plaques, whereas expression was completely absent in all 10 stable plaques tested (Figure 3). Although macroarray analysis showed the absence of clone SSH42 in ruptured plaques, RT-PCR analysis showed no qualitative difference in expression between stable and ruptured plaques (Figure 3).

**RNA In Situ Hybridization**

Because perilipin (SSH1 and SSH11) showed a unique expression pattern in ruptured plaques, RNA in situ hybridization was performed to localize expression of the perilipin gene within different lesion types. Specific expression of

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**Table 2. Summary of Characteristics of Genes Upregulated in Ruptured Plaques**

<table>
<thead>
<tr>
<th>cDNA Fragment</th>
<th>Ratio R/S</th>
<th>Size, bp</th>
<th>GenBank Match†</th>
<th>Accession No.‡</th>
<th>Homology, %</th>
</tr>
</thead>
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<td>SRProtein member</td>
<td>EmbZ85986.1</td>
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</tr>
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<td>SSH1</td>
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<td>Perilipin</td>
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<tr>
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<td>Perilipin</td>
<td>AB005293</td>
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<td>B/W§</td>
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<td>EST</td>
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<td>700</td>
<td>Chromosome 5</td>
<td>AC026775</td>
<td>97</td>
</tr>
</tbody>
</table>

SRProtein indicates serine/arginine-rich protein.

*Ratio of hybridization signal between ruptured human atherosclerotic plaques and stable human atherosclerotic plaques as determined by macroarray analysis.

†Homology of the cDNA clone to an annotation in the public database (GenBank; human, mouse, and rat EST and htgs).

‡Accession number in the public database.

§Black and white difference.
perilipin within ruptured plaques (n=2) was observed in the cytoplasm of several cells surrounding the cholesterol clefts (indicated with an arrow) and in cells (indicated with an arrowhead) most likely resembling foam cells (Figures 4A and 4B). Furthermore, positive staining was observed in endothelial cells of newly formed vessels (data not shown). However, no detectable signal was observed in nondiseased arteries (n=2) and stable plaques (n=2) (Figures 4E and 4F).

The sense perilipin riboprobe did not show any hybridization signal (Figure 4C). Unfortunately, thus far, we were not able to obtain a specific in situ hybridization signal for clone SSH6.

Immunohistochemistry
To further substantiate the perilipin mRNA expression data, immunohistochemistry was performed. Perilipin immunoreactivity in ruptured human atherosclerotic plaques (n=6) was observed in several cells surrounding cholesterol clefts (indicated with an arrow, Figure 5A), foam cells (indicated with an arrowhead, Figure 5A), and endothelial cells of newly formed small vessels (indicated with an arrow, Figure 5B). Combined staining for perilipin and antibodies directed toward CD68 or factor VIII confirmed that perilipin was expressed in foam cells (Figure 5C) and endothelial cells (Figure 5D) of ruptured plaques. No staining was observed in nondiseased arteries (n=3) (Figure 5E), whereas only few cells in the shoulder region of stable plaques (indicated with an arrow) (n=6) tested positive for perilipin (Figure 5F).

Discussion
In the present study, we show that it is possible to identify genes that are differentially and reproducibly expressed in whole-mount advanced human atherosclerotic plaques by using the SSH procedure. In the present study, we describe the identification of several genes differentially expressed between advanced stable and ruptured atherosclerotic plaques. Our stable plaques are classified according to the morphological criteria of the AHA and are characterized by a thick fibrous cap, high smooth muscle cell/collagen content, and a small lipid core, but also comprise rupture-prone lesions, with a thin fibrous cap and a large lipid core, but with no signs of intraplaque hemorrhage or a healed fibrous cap. According to the classification scheme described by Virmani et al.,5 our group of stable atherosclerotic plaques includes thin and thick fibrous cap atheromas and fibrocalcified plaques. Our group of ruptured plaques (type VI lesions according to the AHA classification) is defined by the presence of a ruptured fibrous cap and a luminal thrombus, and the plaques correspond to the fibrous cap atheromas with hemorrhage according to the Virmani classification.
Recently, several groups published data involving differential gene expression between activated and nonactivated human umbilical vein cells and vascular smooth muscle cells. \(^7\) \(^8\) Although cell lines do provide a reproducible source of RNA, it remains to be determined whether gene expression in vitro mimics gene expression in vivo. Hiltunen et al. \(^10\) also used whole-mount human atherosclerotic plaques to study differences in gene expression between fatty streaks and advanced lesions. However, they did not validate their findings on a large panel of individual patients and did not study the localization of differentially expressed genes. Two other groups used the whole-mount approach; McCaffrey et al. \(^11\) studied differences in gene expression between the intima to the media of an atherosclerotic plaque, and Adams et al. \(^12\) studied differential gene expression of veins and arteries in macaques. However, until now, data regarding differential gene expression profiles in human plaque rupture were lacking.

Because of differences in cellular composition between the different lesion types, a challenge of the whole-mount approach, as applied in the present study, is the isolation of plaque-type–specific genes rather than the isolation of cell-type–specific genes. \(^3\) To obtain plaque-type– rather than cell-type–specific genes, both pools contained comparable sets of morphologically diverse plaques regarding the presence of a lipid core, calcium deposition, and the amounts of inflammatory cells. To circumvent patient-based differences, the initial SSH was performed on two pools, with each containing 3 lesions. Our data indicate that we did indeed select for differences in gene expression rather than differences in cellular composition. We selected only one inflammatory cell–related gene (human immunoglobulin \(\lambda\) chain), which was even downregulated in ruptured plaques. Additionally, the RT-PCR analysis did show differential expression of two genes (SSH6 and perilipin [SSH1/SSH11]) in 10 different ruptured plaques compared with 10 different stable plaques, including some stable plaques that contained many inflammatory cells. Although heterogeneity in the types of arteries used for the initial SSH procedure does exist, the RT-PCR analysis illustrated that we did select for plaque-type–specific genes rather than artery-specific genes. This is illustrated by the expression profile of perilipin and SSH6, which was present in ruptured plaques derived from the abdominal aorta, femoral artery, and carotid artery. Additionally, SSH6 was expressed in stable plaques of the peripheral and carotid artery, and SSH42 was expressed at comparable levels in all specimens tested.

Forty-five (9%) of 500 randomly chosen clones showed differential expression between ruptured and stable plaques in macroarray analysis. The number of differentially expressed genes might even be higher, because most likely we missed the differential expression of some low-abundance genes, the expression level of which was below the detection level of the macroarray. However, differential expression of 9% of the clones recovered during the SSH procedure is in close agreement with previous studies investigating differential gene expression by this technique. \(^16\) \(^17\)

Sequence analysis of the 25 clones, which showed at least a 2-fold difference in expression, revealed homology of 6 clones (24%) to known genes; 12 clones were homologous to

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**Figure 4. Localization of perilipin mRNA expression within the different plaque types by RNA in situ hybridization.**

A, Representative ruptured plaque hybridized with an antisense DIG-labeled perilipin RNA probe. B, Section of the same ruptured plaque at a higher magnification. Dark purple staining indicates expression of perilipin mRNA. Arrow indicates a cholesterol cleft and arrowhead indicates a foam cell. C, Serial section of the ruptured plaque hybridized with the sense RNA probe. D, Hematoxylin and eosin staining of a serial section. E, Representative nondiseased artery and F, representative stable plaque both hybridized with an anti-sense DIG-labeled RNA probe. Bar=100 μm.
ESTs (48%), whereas 6 clones (24%) were homologous to parts of genomic sequences but did not have any functional annotation, and one clone contained a complete novel sequence. The distribution of known genes/EST fragments and novel sequences among the selected clones is comparable to the data derived from the human genome project.18–21

A very interesting finding of the present study is that differences in gene expression found in small pools (n = 3) of stable or ruptured plaques could be reproduced in a larger panel (n = 10 for both stable and ruptured plaques) of plaques derived from individual patients. RT-PCR analysis revealed mRNA expression of SSH6 in 80% of the ruptured plaques and in 20% of the stable plaques. Expression of SSH6 in stable plaque number 6 might be explained by the presence of a very thin fibrous cap, a known risk factor for plaque rupture. However, plaque morphology could not explain the expression of SSH6 within stable plaque number 9. Differential expression of perilipin (SSH1/SSH11) was even more striking, inasmuch as expression was completely absent in all 10 stable plaques, whereas 80% of the ruptured plaques tested positive. Although perilipin is known to be associated with lipid droplets,22-24 mRNA expression could not be explained by the presence of a lipid core or large amounts of foam cells, inasmuch as several stable plaques also shared these features. In addition, plaques 3 and 5, which showed high expression of perilipin, contained a very small lipid core. In contrast to perilipin and SSH6, expression of SSH42 showed no gross differences between stable and ruptured plaques in RT-PCR analysis.

The differential expression pattern of SSH6 and perilipin (SSH1/SSH11) suggests a possible role for these genes in plaque rupture. SSH6 is highly homologous to clone AL161991, which is present in the public domain and expressed in the amygdala. However, our clone contains an insertion of 122 nucleotides at position 1116 of the previously identified clone. Although clone AL161991 encodes a putative protein of 493 amino acids, nothing is known about the function of this protein.

The perilipin gene turned out to be exclusively expressed in ruptured atherosclerotic lesions. The present study is the first to link perilipin expression with atherosclerosis. Although human perilipin has recently been cloned,22 most studies to unravel its possible function are performed with the use of rat adipocytes. Perilipin is a phosphoprotein present on the surface layer of intracellular lipid droplets in adipocytes and steroidogenic cells.23,24 On lipolytic stimulation, perilipin is phosphorylated.23 Phosphorylation of perilipin causes an alteration in the lipid droplet surface that, in turn, may facilitate the actions of hormone-sensitive lipase in catalyzing the process of lipolysis.25 Furthermore, overexpression of perilipin in 3T3-L1 preadipocytes increases triacylglycerol storage by a reduction of triacylglycerol hydrolysis,26,27 suggesting that the nonphosphorylated form of perilipin provides a barrier for the enzymes involved in lipolysis.28 This idea is supported by the data obtained in mice carrying a defective perilipin gene.29,30 Because of the increased activity of hormone-sensitive lipase, these mice have smaller white adipocytes and show elevated basal lipolysis. Our finding of specific expression of perilipin in ruptured plaques, together with the localization of perilipin mRNA and protein in cells surrounding cholesterol crystals and in foam cells, might indicate a reduced lipolysis in these plaques. It is
attractive to speculate that this will result in increased lipid retention and plaque destabilization.

In conclusion, the present results show that it is possible to identify genes that are differentially expressed in a larger panel of whole-mount stable or ruptured human atherosclerosis plaques. A first analysis of differentially expressed clones revealed several interesting candidates that may be involved in destabilization of atherosclerotic plaques. Thus, this approach may lead to a better understanding of the molecular processes involved in plaque destabilization.

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