Biological Signatures of Asymptomatic Extra- and Intracranial Atherosclerosis

The Barcelona-AsIA (Asymptomatic Intracranial Atherosclerosis) Study

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Background and Purpose—Intracranial atherosclerotic disease (ICAD) remains a challenge for stroke primary and secondary prevention. Molecular pathways involved in the development of ICAD from its asymptomatic stages are largely unknown. In our population-based study, we aimed to compare the risk factor and biomarker profiles associated with intracranial and extracranial asymptomatic cerebral atherosclerosis.

Methods—The Asymptomatic Intracranial Atherosclerosis (AsIA) study cohort includes a random sample population of 933 white subjects >50 years with a moderate to high vascular risk (based on REGICOR score) and without a history of stroke (64% males; mean age, 66 years). Carotid and intracranial atherosclerosis were screened by cervical and transcranial color-coded Duplex ultrasound, being moderate to severe stenoses confirmed by MR angiography. We registered clinical and anthropometric data and created a biobank with blood samples at baseline. A panel of biomarkers involved in atherothrombogenesis was determined: C-reactive protein, asymmetric-dimethylarginine, resistin, and plasminogen activator inhibitor-1. Insulin resistance was quantified by Homeostasis Model Assessment index.

Results—After multinomial regression analyses, male sex, hypertension, smoking, and alcoholic habits were independent risk factors of isolated extracranial atherosclerotic disease. Diabetes and metabolic syndrome conferred a higher risk for ICAD than for extracranial atherosclerotic disease. Moreover, metabolic syndrome and insulin resistance were independent risk factors of moderate to severe ICAD but were not risk factors of moderate to severe extracranial atherosclerotic disease. Regarding biomarkers, asymmetric-dimethylarginine was independently associated with isolated ICAD and resistin with combined ICAD–extracranial atherosclerotic disease. Diabetes and metabolic syndrome conferred a higher risk for ICAD than for extracranial atherosclerotic disease. Moreover, metabolic syndrome and insulin resistance were independent risk factors of moderate to severe ICAD but were not risk factors of moderate to severe extracranial atherosclerotic disease. Regarding biomarkers, asymmetric-dimethylarginine was independently associated with isolated ICAD and resistin with combined ICAD–extracranial atherosclerotic disease.

Conclusions—Our findings show distinct clinical and biological profiles in subclinical ICAD and extracranial atherosclerotic disease. Insulin resistance emerged as an important molecular pathway involved in the development of ICAD from its asymptomatic stage. (*Stroke. 2012;43:2712-2719.*)

Key Words: asymptomatic ■ biomarkers ■ intracranial atherosclerosis ■ prevention ■ risk factors

Atherosclerosis is a multifactorial, progressive, and systemic disease considered the most important cause of morbidity and mortality worldwide, even in developing countries.1 Atherosclerosis usually attacks multiple arteries throughout the body, including the aorta, coronary arteries, limbs, and arteries supplying the brain. Although similar well-known vascular risk factors and molecular pathways are involved in the global process of atherogenesis, local expression and severity of atherosclerosis differ among vulnerable individuals. A paradigm of this disparity is the different ethnical–race cervicocephalic distribution of large-artery cerebral atherosclerosis.2 Whereas intracranial arteries are more frequently affected in Asians, blacks, and Hispanics, extracranial carotid atherosclerosis is more prevalent among whites.3–5 The origin of these differences is not explained alone by a different prevalence of conventional vascular risk factors among races, thus supporting the hypothesis that other underlying environmental, molecular, and genetic factors must play determinant roles in the distinct cerebral atherogenic process. Besides this, differences in hemodynamic, histological, or molecular characteristics between extra- and intracranial arteries

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2712
could lead to diverse responses to the same deleterious stimuli even in the same race–ethnic group.6–9

Intracranial atherosclerotic disease (ICAD) remains a challenge for stroke secondary prevention because even with aggressive medical treatment, stroke recurrence is high.10 Therefore, the study of risk factors and molecular pathways involved in ICAD from its asymptomatic stage is essential to improve preventive strategies and to find new therapeutic targets. Some risk factors such as diabetes, insulin resistance, and metabolic syndrome have been lately reported to be more strongly associated with intracranial than with extracranial atherothrombotic ischemic stroke.11–14 The study of circulating biomarkers related to atherosclerosis is a good tool to assess its physiopathology in vivo. Few retrospective studies have evaluated inflammatory and metabolic markers associated with the different location (extra- versus intracranial) of cerebral atherosclerosis,15–18 with only one study performed in asymptomatic subjects.19 A proinflammatory state and an impaired fibrinolysis have also been associated with progression of symptomatic intracranial atherosclerosis.20 Prospective studies on biomarkers associated with asymptomatic intracranial atherosclerosis are lacking.

The main objective of the present study is to investigate whether there is a differential profile of vascular risk factors and relevant biomarkers between asymptomatic intracranial atherosclerosis and cervical carotid atherosclerosis in a randomly recruited stroke-free white population. Novel vascular risk factors assessed included direct measures of insulin resistance. Several predefined biomarkers involved in atherogenesis were determined in the present study: C-reactive protein (CRP) and plasminogen activator inhibitor-1, previously reported as markers of progression of symptomatic intracranial atherosclerosis;20 resistin, previously reported as a marker of asymptomatic coronary and carotid atherosclerosis21,22; and the endogenous nitric oxide synthase inhibitor asymmetric-dimethylarginine (ADMA), previously associated with extracranial asymptomatic atherosclerosis.23

Methods

The Barcelona-AsIA study (Asymptomatic Intracranial Atherosclerosis study) is an ongoing population-based, cross-sectional, and longitudinal study that includes a random sample of 933 subjects >50 years with a moderate to high vascular risk and without a history of either stroke or coronary disease. The complete study protocol and first results on intracranial atherosclerosis prevalence have been reported in detail elsewhere.24,25 This study has been approved by the ethics committee of our institution.

Briefly, all subjects were prospectively visited at our public tertiary stroke center, the Germans Trias i Pujol Hospital (Badalona, Barcelona, Spain), with the only purpose of this study. Study sample selection is detailed in the online-only Data Supplement. At baseline visit and after signing written informed consent, a complete questionnaire on demographic and clinical data was obtained, anthropometric variables were measured (blood pressure, weight, height, waist circumference), and fasting blood samples were drawn, processed, and stored in a biobank at −80°C. All subjects underwent a complete color-coded duplex ultrasound study of the cervical and intracranial vessels to determine the presence of atherosclerotic lesions. Significant stenoses of the intra- and extracranial vessels were confirmed by MR angiography.

Traditional Vascular Risk Factors

Vascular risk was calculated using the REGICOR score. REGICOR is the Framingham function adapted and validated for the Spanish population and evaluates the risk (%) of having a coronary event in 10 years based on a compute of the following traditional risk factors: sex, age, diabetes, smoking, hypertension, and cholesterol levels. REGICOR <5% indicates low risk, 5% to 9% moderate risk, 10% to 14% high risk, and ≥15% very high risk.26,27

Hypertension, diabetes, and dyslipidemia were defined based on personal history of these diagnoses and on current diet or medical treatment intake for these disorders. Cholesterol, triglycerides, and fasting glucose determined in baseline samples were also evaluated separately as continuous variables. Smoking habit was considered to be present in current smokers or if the time interval since abstinence was <5 years. Alcohol consumption was considered moderate to severe if superior to 20 g/day.

Novel Metabolic Vascular Risk Factors

Metabolic syndrome was defined following the unified criteria of the last joint intermin statement28 when ≥3 of the following were present: abdominal obesity for European population (>94 cm in men and >80 cm in women), arterial blood pressure ≥130/≥85 mm Hg in baseline visit or specific medication, level of triglycerides ≥150 mg/dL or specific medication, low high-density lipoprotein cholesterol (in men <40 mg/dL and in women <50 mg/dL) or specific medication, and fasting plasma glucose ≥100 mg/dL or history of diabetes mellitus or taking anti diabetic medications.

Insulin resistance was evaluated using the homeostatic model assessment technique index (HOMA-IR) following the formula described by Matthews et al29: HOMA-IR = glucose mg/dL × insulin μU/mL/405. Insulin resistance was considered when HOMA-IR ≥3.2 based on the previously published 75th percentile of a general nondiabetic Spanish population.30 We also evaluated triglyceride/high-density lipoprotein ratio, also related to insulin resistance.31

Biomarkers

All blood samples were drawn at baseline visit after overnight fast of at least 12 hours. Immediately after extraction, blood samples were centrifuged at 3500 rpm and 4°C for 15 minutes, blind-coded, and stored at −80°C until analyzed. All biomarkers were assessed in the same laboratory. CRP measurement was carried out with a nephelometric method (Delta, Radim Iberica). Plasminogen activator inhibitor-1 levels were measured in citrated plasma using a sensitivity enzyme-linked immunosorbent assay method (TECHNOZYM plasminogen activator inhibitor-1 Actibind; Technoclone GmbH, Vienna, Austria). Serum resistin concentrations were determined using an enzyme-linked immunosorbent assay method (BioVendor, Czech Republic) with a coefficient of variation interassay of 6.8%. ADA was measured in serum with a competitive ADA enzyme-linked immunosorbent assay from DLD Diagnostika (reference EA201/06) with a coefficient of variation interassay of 8.3%. All 3 previous enzyme-linked immunosorbent assay analyses were performed in replicate in an automatic open system (Brio 2; Radim SpA, Pomezia, Italy). Mean coefficients of variation were <3% in all replicated samples. Insulin was measured using an enzyme-linked immunosorbent assay handily determination (Radim reference KP101W) with a coefficient of variation interassay <6%.

Evaluation of Extracranial and Intracranial Atherosclerosis

All cervical and transcranial color-coded duplex ultrasound studies were performed at baseline in the same laboratory by 2 certified neurologists using a General Electric Vivid/Pro equipment (Horten, Norway). Subjects with a significant extracranial carotid stenosis (>50%) and/or a moderate to severe intracranial stenosis in the baseline neurosonologic study underwent a confirmatory MR angiography study with 1.5-T equipment (Philips). Intracranial stenoses were assessed with a time-of-flight sequence and carotid stenoses with a contrast-enhanced angiography and maximum-intensity-
projection reconstruction sequence. Ultrasound protocol is detailed in the online-only Data Supplement.

Subject Grouping
According to location of atherosclerotic lesions, subjects were categorized in 4 exclusive groups: (1) nonatherosclerotic group, if neither carotid plaques nor intracranial stenoses were found in the baseline ultrasound examination. This group served as the reference group for all further analyses; (2) extracranial atherosclerosis disease (ECAD) group, if one or more carotid plaques were present in carotid arteries, regardless of stenosis degree, and with no concurrent intracranial arterial stenosis; (3) ICAD group, if at least one intracranial stenosis of any degree was present and without carotid atherosclerotic lesions; and (4) combined extracranial and intracranial group, if intracranial cerebral arteries and extracranial carotid arteries were affected simultaneously.

According to the severity of lesions, subjects were classified into moderate to severe ECAD if at least one extracranial carotid stenosis ≥50% or occlusion was present and into moderate to severe ICAD if at least one intracranial stenosis ≥50% was present. Subjects with moderate to severe lesions in both extra- and intracranial vasculatures were not considered for this classification due to low numbers.

Statistical Analysis
Statistics were performed with the SPSS 15.0 statistical package. The association of vascular risk factors and circulating biomarkers with each location of atherosclerosis was analyzed by Pearson χ² test for categorical variables and Mann-Whitney U test, Kruskal-Wallis test, Student t test, or analysis of variance test for continuous variables. Multinomial logistic regression analyses were performed to identify vascular risk factors and biomarkers independently associated with each location of atherosclerosis and with their severity, taking the nonatherosclerosis group as the reference for all analyses. For traditional vascular risk factors and circulating biomarkers, we included in the model all risk factors significantly associated with any location in the univariate analyses (P<0.05). For metabolic syndrome (which includes the individual risk factors diabetes, hypertension, and dyslipidemia), we included in the model age, sex, and REGICOR (Registro GIRon del COR Heart Registry) score. Insulin resistance (HOMA index ≥3.2) was evaluated in a model adjusted for the rest of components of metabolic syndrome. Regarding circulating biomarkers, we analyzed them as continuous variables due to the small size of some groups that did not allow reliable analyses by quartiles. The predictive capacity of a particular cutoff point to assess atherosclerosis location was also explored for each biomarker using receiver operating characteristic analyses. Statistical significance was defined as a probability value <0.05. Results were expressed as ORs and 95% CIs.

Results
Of the 933 subjects included at baseline (64.2% men; mean age, 66 years; 99% of white origin), 449 (48.2%) were classified in the nonatherosclerosis group (reference group), 404 (43%) in the ECAD group, 57 (6.1%) in the ICAD group, and 23 (2.5%) in the combined extracranial and intracranial group. Regarding stenosis severity, 29 subjects (3.1%) had a significant carotid stenosis (≥50%) and 31 subjects (3.3%) had at least one moderate to severe intracranial stenosis; 4 patients had both extra- and intracranial moderate to severe lesions and were excluded for the analyses on stenosis severity. Baseline characteristics according to the location and severity of atherosclerosis and comparisons with the nonatherosclerotic group are shown in Table 1.

Traditional Vascular Risk Factors and Location/Severity of Atherosclerosis
Subjects with asymptomatic atherosclerosis in extra- and/or intracranial vessels had a significantly higher REGICOR score when compared with control subjects (Table 1). Prevalence of atherosclerosis in each location according to REGICOR score classification in moderate, high, and very high vascular risk subjects is represented in Figure 1.

After multinomial regression analysis, male sex, smoking habit, alcohol abuse, and hypertension were independent predictors for isolated ECAD but not for ICAD. Age and diabetes were independent risk factors of both locations (Figure 2). Independent risk factors for combined ECAD–ICAD were age, male sex, diabetes, and hypertension (data not shown in Figure 2).

In the multinomial regression analysis for severity groups (Figure 3), age and diabetes were independent risk factors for the presence of moderate to severe lesions in both ECAD and ICAD groups. Diabetes increased 6.6-fold the odds of having moderate to severe intracranial atherosclerosis and 3.7-fold the odds of having moderate to severe extracranial atherosclerosis when compared with control subjects. Differentially, male sex was an independent risk factor only for moderate to severe ECAD.

Novel Metabolic Risk Factors and Location/Severity of Atherosclerosis
Metabolic syndrome was more prevalent in atherosclerosis groups than in control subjects (Table 1). After multinomial regression analysis, metabolic syndrome was an independent risk factor of both locations of atherosclerosis but with a higher OR for intracranial location (Figure 2). Regarding severity, metabolic syndrome was significantly associated with moderate to severe ICAD and was not with moderate to severe ECAD. Moreover, prevalence of moderate to severe ICAD was progressively higher with increasing number of metabolic syndrome criteria, whereas prevalence of moderate to severe ECAD was similar among these groups (Figure 4A). Metabolic syndrome emerged as an important predictor for moderate to severe ICAD after multinomial regression analysis (Figure 3).

Insulin resistance (HOMA-IR ≥3.2) was also more prevalent in all atherosclerotic groups, especially in moderate to severe ICAD (Table 1). Prevalence of moderate to severe ICAD was progressively higher with HOMA-IR quartiles, whereas prevalence of moderate to severe ECAD was similar among these groups (Figure 4B). In multinomial analysis adjusted for the rest of components of the metabolic syndrome, insulin resistance was an independent predictor for moderate to severe ICAD and was not a predictor for moderate to severe ECAD (Figure 3).

Triglyceride/high-density lipoprotein ratio was significantly associated with moderate to severe ICAD (Table 1) but was not an independent predictor after multinomial analysis.

Circulating Biomarkers and Location/Severity of Atherosclerosis
Regarding location groups, CRP was significantly associated with isolated ECAD and with combined ECAD–ICAD, high resistin levels were significantly associated with ICAD and with combined ECAD–ICAD, and high ADMA levels were significantly associated with isolated ICAD (Table 1; online-only Data Supplement Figure 1). In multinomial analyses, resistin remained independently associated with combined
Table 1. Baseline Characteristics According to Location and Severity of Atherosclerosis

<table>
<thead>
<tr>
<th>Baseline Characteristics</th>
<th>Control (n=449)</th>
<th>ECAD Group (n=404)</th>
<th>ICAD Group (n=57)</th>
<th>COMB Group (n=23)</th>
<th>ECAD Moderate to Severe (N=25)</th>
<th>ICAD Moderate to Severe (N=27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>REGICOR score</td>
<td>6 (5–8)</td>
<td>7 (6–11)†</td>
<td>9 (6.5–11)†</td>
<td>11 (7–17)†</td>
<td>10 (7–16)†</td>
<td>9 (7–11)†</td>
</tr>
<tr>
<td>Age, y</td>
<td>64.1±7.7†</td>
<td>67.9±7.9†</td>
<td>69.8±6.8†</td>
<td>71.9±7.5†</td>
<td>72.8±7.4†</td>
<td>69.6±10†</td>
</tr>
<tr>
<td>Sex, % male</td>
<td>56</td>
<td>72.5†</td>
<td>54.4</td>
<td>82.6*</td>
<td>84†</td>
<td>59.3</td>
</tr>
<tr>
<td>Smoking habit, %</td>
<td>21.4</td>
<td>28.2*</td>
<td>17.5</td>
<td>21.7</td>
<td>36</td>
<td>14.8</td>
</tr>
<tr>
<td>Alcohol abuse, %</td>
<td>8.5</td>
<td>16.6†</td>
<td>8.8</td>
<td>8.7</td>
<td>4</td>
<td>14.8</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>47.5</td>
<td>62.3†</td>
<td>70.2</td>
<td>91.3†</td>
<td>72*</td>
<td>77.8†</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>18.8</td>
<td>30.2†</td>
<td>50.9*</td>
<td>65.2</td>
<td>44†</td>
<td>63†</td>
</tr>
<tr>
<td>Dyslipidemia, %</td>
<td>50.4</td>
<td>56.7</td>
<td>64.9*</td>
<td>69.6</td>
<td>52</td>
<td>66.7</td>
</tr>
<tr>
<td>Metabolic syndrome, %</td>
<td>61.8</td>
<td>73.1†</td>
<td>82.5†</td>
<td>95.7†</td>
<td>64</td>
<td>88.9†</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>101 (94–106)</td>
<td>102 (92.6–111.2)</td>
<td>100 (93.2–104.7)</td>
<td>104 (101–110)</td>
<td>102 (94.5–112.2)</td>
<td>101 (94–105)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.7 (±4.3)†</td>
<td>28.7 (±4.4)†</td>
<td>28.1 (±3.5)</td>
<td>29.2 (±4.5)</td>
<td>28.8 (±5.3)</td>
<td>28.1 (±3.1)</td>
</tr>
<tr>
<td>HOMA-IR &gt;3.2, %</td>
<td>54.3</td>
<td>65†</td>
<td>66.7*</td>
<td>69.6</td>
<td>56</td>
<td>81.5†</td>
</tr>
<tr>
<td>TG/HDL ratio</td>
<td>2.48 (±1.47)</td>
<td>2.51 (±1.49)†</td>
<td>2.41 (±1.41)</td>
<td>2.98 (±2.41)</td>
<td>2.2 (±1)</td>
<td>3.1 (±2.25)*</td>
</tr>
<tr>
<td>Fasting glycemia, mg/dL</td>
<td>107.2 (±24.7)</td>
<td>119.4 (±36.1)†</td>
<td>126.2 (±45.5)†</td>
<td>128.8 (±45.5)†</td>
<td>109.7 (±29.1)</td>
<td>131.1 (±51.6)*</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>206.7 (±39.6)</td>
<td>204.2 (±40.6)</td>
<td>211.3 (±42)</td>
<td>188.7 (±42.8)†</td>
<td>201.2 (±23.4)</td>
<td>211.9 (±45.6)†</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>132.9 (±34.3)</td>
<td>129 (±35.6)</td>
<td>133.74 (±36.3)</td>
<td>116.4 (±37)*</td>
<td>129.3 (±29.9)</td>
<td>131.7 (±40.7)</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>53.6 (±11.1)</td>
<td>54.6 (±11.9)</td>
<td>56.8 (±12.9)</td>
<td>50.3 (±11.8)</td>
<td>53.9 (±10.9)</td>
<td>55.1 (±12.5)</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>125.6 (±63.8)</td>
<td>128.9 (±66.2)</td>
<td>129.6 (±69.5)</td>
<td>137.5 (±94.6)</td>
<td>112.6 (±44.1)</td>
<td>157.2 (±97.5)*</td>
</tr>
<tr>
<td>CRP, mg/dL</td>
<td>4.1 (±0.26)</td>
<td>5.64 (±0.65)*</td>
<td>4.07 (±0.53)</td>
<td>6.78 (±2.5)*</td>
<td>6.4 (±1.82)</td>
<td>4.38 (±0.87)</td>
</tr>
<tr>
<td>Resistin, ng/mL</td>
<td>4.35 (±0.08)</td>
<td>4.57 (±0.11)</td>
<td>5.09 (±0.59)*</td>
<td>6.53 (±1.83)*</td>
<td>5.23 (±0.47)*</td>
<td>4.57 (±0.38)</td>
</tr>
<tr>
<td>ADMA, μmol/L</td>
<td>0.71 (±0.01)</td>
<td>0.73 (±0.01)</td>
<td>0.80 (±0.04)*</td>
<td>0.67 (±0.07)</td>
<td>0.63 (±0.04)</td>
<td>0.73 (±0.04)</td>
</tr>
<tr>
<td>PAI-1, U/mL</td>
<td>6.08 (±0.43)</td>
<td>6.64 (±0.82)</td>
<td>6.34 (±1.35)</td>
<td>6.65 (±1.41)</td>
<td>5.5 (±2.11)</td>
<td>9.81 (±2.40)*</td>
</tr>
</tbody>
</table>

Values are provided as median (interquartile range), mean±SD (circulating biomarkers), or percentages when (%) is indicated in the first column. Each group was compared with the reference group (control) using χ², Student t test, or Mann-Whitney U tests.

Control indicates nonatherosclerosis group; ECAD, extracranial atherosclerotic disease; ICAD, intracranial atherosclerotic disease; COMB, combined extra- and intracranial atherosclerotic disease; BMI, body mass index; HOMA-IR, Homeostasis Model Assessment; REGICOR, Registro GIRoní del COR Heart Registry; TG/HDL ratio, triglyceride/high-density lipoprotein ratio; LDL, low-density lipoprotein; CRP, C-reactive protein; ADMA, asymmetric-dimethylarginine; PAI-1, plasminogen activator inhibitor-1.

*P<0.05. †P<0.01.

extra-/intra-atherosclerotic disease and ADMA remained independently associated with isolated ICAD (Table 2). Regarding severity of atherosclerosis, high plasminogen activator inhibitor-1 levels were significantly associated with moderate to severe ICAD and high resistin levels were significantly associated with moderate to severe ECAD (Table 1; online-only Data Supplemental Figure IVB), but these circulating biomarkers were not independent predictors after
multinomial analyses. There were no differences in ADMA or CRP levels between moderate to severe atherosclerosis groups and the control group.

Receiver operating characteristic analyses did not disclose any good cutoff point for biomarker levels for predicting capacity of atherosclerosis location (area under the curve. 0.57, \( P < 0.06 \) for ADMA and ICAD; 0.62, \( P = 0.06 \) for resistin and combined atherosclerosis).

**Discussion**

In our stroke-free white population, different traditional vascular risk factors are independently associated with the distinct cervicocephalic location and severity of asymptomatic atherosclerosis. Male sex, smoking habit, and alcohol abuse were independent predictors of isolated extracranial carotid atherosclerosis, with male sex independently associated with moderate to severe carotid stenoses. Age and diabetes were independent risk factors of atherosclerosis in both locations, but diabetes conferred a substantially higher risk for intracranial location of atherosclerosis and especially for the presence of moderate to severe intracranial atherostenoses. According to REGICOR score, prevalence of asymptomatic atherosclerosis was progressively higher across groups of moderate, high, and very high vascular risk (Figure 2).

![Multinomial regression analysis: independent risk factors of ECAD and ICAD](image1)

**Figure 2.** Multinomial regression analysis: independent risk factors of ECAD and ICAD. *Metabolic syndrome was adjusted by age, sex, and REGICOR score. The nonatherosclerosis group was used as the reference. Horizontal bars represent 95% CI. ECAD indicates extracranial atherosclerotic disease; ICAD, intracranial atherosclerotic disease; REGICOR, REgistro GIRoní del COR Heart Registry.

![Multinomial regression analysis: independent risk factors of moderate to severe ECAD and ICAD](image2)

**Figure 3.** Multinomial regression analysis: independent risk factors of moderate to severe ECAD and ICAD. *Metabolic syndrome was adjusted by age, sex, and REGICOR score. **HOMA-IR (insulin resistance) was adjusted for the rest of components of metabolic syndrome: waist circumference, blood pressure, triglyceride, and low-HDL components. The nonatherosclerosis group was used as the reference. Horizontal bars represent 95% CI. ECAD indicates extracranial atherosclerotic disease; ICAD, intracranial atherosclerotic disease; HOMA-IR, Homeostasis Model Assessment; HDL, high-density lipoprotein; REGICOR, REgistro GIRoní del COR Heart Registry.
1). Due to different sensitivities of transcranial color-coded duplex and carotid duplex in detecting very mild atherosclerosis, prevalence of asymptomatic ECAD of any degree (including carotid plaques without stenosis) is disproportionately higher than prevalence of ICAD (intracranial stenoses) in Figure 1.

Metabolic syndrome has received increased attention in the past few years because it seems to promote the development of atherosclerotic cardiovascular disease. In our population, metabolic syndrome was independently associated with both ECAD and ICAD, with this association especially high with moderate to severe intracranial lesions. Metabolic syndrome has been previously reported as a specific risk factor for intracranial atherosclerosis in stroke populations of different racial backgrounds, and the present study confirms this particular association in asymptomatic white subjects. The reasons that explain the different effect of vascular risk factors in the cervicocephalic distribution of atherosclerotic lesions are not well understood. In a recent study comparing stroke mechanisms, authors conclude that the impact of metabolic syndrome may differ by race–ethnicity, reporting that metabolic syndrome was associated with ECAD in whites and with ICAD in nonwhites (Hispanics, Asian Americans, and blacks). However, we found that metabolic syndrome is a strong independent risk factor of ICAD in our white population, supporting the hypothesis that other underlying factors have to determine this specific association in all ethnicities. Metabolic syndrome confers a proinflammatory and hypercoagulable state mainly mediated by insulin resistance. Accelerated atherosclerosis in metabolic syndrome has been related to defective insulin signaling pathways in humans and animal models. In the present study, insulin resistance measured by HOMA index was an independent marker of moderate to severe ICAD even after adjusting for the rest of components of metabolic syndrome. These findings support the idea that intracranial arteries are especially prone to suffer the effects of insulin resistance and impaired insulin signaling. In a previous autopsy study, authors showed that in younger ages, intracranial arteries contain a higher activity of antioxidant enzymes compared with extracranial arteries, protecting them from atherosclerosis. This antioxidant activity decreases with age, coinciding with a rapid acceleration of atherosclerosis in intracranial arteries. A previous work also showed that adiponectin levels were

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>ECAD</th>
<th>ICAD</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP, mg/dL</td>
<td>1.02 (0.99–1.04), P=0.06</td>
<td>0.99 (0.94–1.05), P=0.86</td>
<td>1.03 (0.99–1.06), P=0.15</td>
</tr>
<tr>
<td>Resistin, mg/dL</td>
<td>1.02 (0.95–1.09), P=0.62</td>
<td>1.06 (0.97–1.17), P=0.19</td>
<td>1.1 (1.01–1.20), P=0.04</td>
</tr>
<tr>
<td>ADMA, mg/dL</td>
<td>1.54 (0.93–2.53), P=0.09</td>
<td>2.91 (1.21–6.27), P=0.02</td>
<td>0.48 (0.07–3.35), P=0.46</td>
</tr>
</tbody>
</table>

OR and (95% CI) with nonatherosclerosis group taken as the reference. ECAD indicates extracranial atherosclerotic disease; ICAD, intracranial atherosclerotic disease; Combined, extracranial and intracranial atherosclerotic disease; CRP, C-reactive protein; ADMA, asymmetric-dimethylarginine.
lower in symptomatic ICAD when compared with other stroke subtypes. Adiponectin is an antiatherogenic protein that improves insulin sensitivity.\textsuperscript{18} In this line and supported by our findings, intracranial arteries seem to be more sensitive to the oxidative stress conferred by insulin resistance and related metabolic and molecular alterations, even from the early subclinical stages of atherosclerosis. This hypothesis needs to be confirmed by further basic research.

Circulating biomarkers may represent a powerful tool to assess the pathophysiology of atherosclerosis in vivo. We found that CRP levels were significantly higher in subjects with ECAD than in those with ICAD, similar to previously reported.\textsuperscript{15,19} Resistin is a protein secreted by adipocytes and related to insulin resistance in rodents, whereas in humans it is mainly secreted by inflammatory cells and has been reported as a marker of coronary and carotid atherosclerosis.\textsuperscript{21,22} In the present study, resistin was independently associated with atherosclerotic burden, especially with combined ECAD and ICAD. Intriguingly, we found that high levels of ADMA, an endogenous inhibitor of endothelial nitric oxide, were independently associated with isolated ICAD but were not with moderate to severe ICAD or with combined ECAD and ICAD. ADMA has previously been associated with carotid intima-media thickness but in a site-specific manner, being independently associated with increased intima-media thickness in the carotid bulb but not in the common carotid artery.\textsuperscript{23} In the present study, high circulating levels of ADMA may indicate a predominant role of endothelial dysfunction in early stages of intracranial atherosclerosis. This disparity in associated circulating biomarkers among the distinct location of atherosclerosis supports the hypothesis that intracranial and extracranial arteries respond differently from atherogenic stimuli.

Strengths of this study include the large random population-based sample, the determination of molecules blinded to atherosclerosis data, and determination of molecules not previously studied in asymptomatic intracranial atherosclerosis. Limitations include the cross-sectional design that does not allow us to establish causal inferences and the small size of some groups that could have affected the identification of statistically significant associations. As commented previously, sensitivity of carotid duplex and transcranial color-coded duplex in detecting mild degrees of atherosclerosis is different, leading to a high prevalence of ECAD. However, prevalence of moderate to severe stenoses was similar in extracranial and intracranial locations and analyses on associated risk factors revealed comparable results. We also regret not having data on intra- and interobserver reproducibility.

In conclusion, asymptomatic ICAD and ECAD seem to be characterized by a differential risk factor and molecular profile in our white population, thus suggesting a distinct vulnerability of intracranial and extracranial arteries to be affected by atherosclerosis. Our findings support a pre-eminent role of insulin resistance and related metabolic abnormalities in the development of ICAD from its early stages. This observation may have practical implications for the design of specific prevention strategies in ICAD and for the research with new therapies targeting insulin sensitivity. This study also warrants further study on genetic background explaining the differential profile in cerebral atherosclerosis.

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### Disclosures
None.

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Biological Signatures of Asymptomatic Extra- and Intracranial Atherosclerosis: The Barcelona-AsIA (Asymptomatic Intracranial Atherosclerosis) Study
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Supplemental Methods:

Study sample selection: National Health System in Spain universally covers any subject living in our country with an active legal work permit (or retired) and also covers all his direct relatives. Demographic and contact details of all covered subjects are registered in a main dataset (Primary Care Information Technology System). From our Hospital reference population of 600,000 inhabitants, a random sample of 3010 subjects older than 50 years was initially selected in 2007 in order to participate in an on-going primary prevention study on peripheral artery disease¹. From this sample, we selected all the 1503 subjects that met our inclusion criteria: no history of stroke or transient ischemic attack; no history of coronary disease; exposure to a moderate-high vascular risk assessed by REGICOR (which is the Spanish adaptation of Framingham score risk); and absence of institutionalization or severe disability. All subjects were contacted by phone from our tertiary stroke center offering them to participate in the AsIA study. After 5 phone calls 229 were not located, 316 declined to participate for several reasons, 20 died during the recruitment phase and 5 suffered a stroke. Finally, 933 subjects were enrolled between March 2007 and June 2010. There were no significant differences in age, gender and vascular risk scores between the final cohort and the group of subjects not located or who declined to participate.

Ultrasound protocol: Carotid atherosclerotic plaques were defined in B-mode as the presence of any focal structure encroaching into the arterial lumen of at least 0.5 mm and/or a thickness > 1.5 mm of the intima-media interface in the common carotid artery, bifurcation, internal or external carotid arteries². Significant internal carotid stenosis ≥ 50% was defined if systolic peak velocity was higher than 125 cm/s³. TCCD was performed following consensus recommendations⁴ and intracranial stenoses were defined based on previously published criteria⁵. Intracranial stenosis severity by TCCD was classified following validated cut-off values of systolic peak velocity for <50% (mild)/≥50% (moderate-severe) stenosis: ≥155/≥220
cm/s for middle cerebral artery; ≥120/≥155 cm/s for anterior cerebral artery; ≥100/≥145 cm/s for posterior cerebral and basilar arteries and ≥90/≥120 cm/s for vertebral artery. For intracranial carotid stenosis we used the cut-off values ≥120 cm/s/≥200 cm/s² and we also analyzed turbulent flux and repercussion on extracranial carotid flux and/or existence of compensatory fluxes. We used angle correction in straight vessel segments of at least 15 mm and we used contrast agents in cases with an insufficient acoustic window (25%).

Supplemental figure 1: Circulating biomarkers according to location and severity of cerebral atherosclerosis. Circulating biomarkers related to any degree of atherosclerosis (panel A) and to moderate-severe lesions (panel B). Control: non-atherosclerosis group. ECAD: extracranial atherosclerotic disease. ICAD: intracranial atherosclerotic disease. *p<0.05 and **p<0.01 compared to control group (non atherosclerosis group). Bars show 95% CI of the mean.
Supplemental references:


