Association of the Glutathione S-transferase Omega-1 Ala140Asp Polymorphism With Cerebrovascular Atherosclerosis and Plaque-Associated Interleukin-1α Expression

Heike Kölsch, PhD; Sergey Larionov, MD; Oliver Dedeck; Mario Orantes, MD; Gerd Birkenmeier, MD; W. Sue T. Griffin, PhD; Dietmar Rudolf Thal, MD

Background and Purpose—Glutathione S-transferase omega-1 is a multifunctional enzyme. The Asp/Asp genotype of the Ala140Asp polymorphism of the GSTO1 gene has been alleged to increase the risk of vascular dementia. The objective of this study is to address the question of whether common vessel disorders known to cause vascular dementia are modified in their severity by this polymorphism.

Methods—The severity and expansion of atherosclerosis in the circle of Willis vessels, cerebral small vessel disease, and cerebral amyloid angiopathy were studied in a sample of 79 autopsy cases. Genotyping of the GSTO1 Ala140Asp polymorphism as well as immunohistochemistry for glutathione S-transferase omega-1 was performed.

Results—Carriers of the GSTO1 Asp/Asp genotype presented with more severe and widespread atherosclerosis than noncarriers. However, there was no effect on small vessel disease expansion and cerebral amyloid angiopathy severity. Immunohistochemically, we detected interleukin-1α expressing macrophages in the lipid core of atherosclerosis plaques exhibiting glutathione S-transferase omega-1-positive material. GSTO1 Asp/Asp carriers showed larger areas of atherosclerosis plaques containing interleukin-1α-positive material than carriers of the GSTO1 Ala-allele.

Conclusions—The GSTO1 Asp/Asp genotype presumably modulates the severity and expansion of atherosclerosis in the circle of Willis. The cellular colocalization of glutathione S-transferase omega-1 and interleukin-1α suggests a functional interaction between both proteins which in part might explain the function of glutathione S-transferase omega-1 in the pathogenesis of cerebral atherosclerosis. (Stroke. 2007;38:2847-2850.)

Key Words: atherosclerosis ■ cerebral arteries ■ GSTO1-1

Glutathione S-transferase omega-1 (GSTO1-1) is a member of the glutathione S-transferases family of phase II enzymes that catalyze the glutathione dependent detoxification of, eg, oxidants. Recently, we have shown that the Asp/Asp genotype of the GSTO1 Ala140Asp polymorphism in exon 4 is associated with an increased risk for vascular dementia. However, the relationship between this GSTO1 polymorphism and common vessel diseases is not clear. Major vessel diseases causing vascular dementia include atherosclerosis (AS) of the arteries of the circle of Willis, small vessel disease, and cerebral amyloid angiopathy. Small vessel disease includes vascular lesions of arteriolosclerosis, arteriohyalinosclerosis, lipoamyloidosis, fibrohyalinosclerosis, and arteriosclerotic intimal proliferation and fibrosis of the media in small arteries but no AS plaques.

The objective of this study was to investigate whether one of these vessel disorders is influenced by the GSTO1 Ala140Asp polymorphism.

Materials and Methods
We analyzed a sample of 79 formalin-fixed human brains from continuously collected autopsy cases of 60 years of age and older without further selection criteria (Table). We counted the number of vessels with macroscopically detectable AS plaques in relation to the total number of vessels analyzed within the circle of Willis. Vessels included in this quantification were the major branches of the vertebral, basilar, anterior cerebral, middle cerebral, posterior cere-
bral, and internal carotid arteries. The expansion of AS was calculated as the percentage of vessels in the circle of Willis exhibiting AS plaques.

Histopathology confirmed the diagnosis of AS. The histopathological type of AS lesions of the most severely affected artery of the circle of Willis was determined. The extent of small vessel disease was determined in leptomeningeal, cortical, and subcortical vessels of the medial temporal lobe and the occipital lobe. The severity of cerebral amyloid angiopathy was analyzed. Frozen sections from AS plaques were stained with anti-GSTO1-1. Paraffin sections stained with anti-interleukin (IL)-1/H9251, anti-neutrophil elastase, and anti-2-macroglobulin were quantitatively analyzed as described previously to determine the percentage of the AS plaque area stained with a given antibody. Positive, negative, and blank controls were performed.

Genotyping of the GSTO1 (Ala140Asp, rs4925), IL1A (−889, C/T), IL1B (+3954, C/T), IL1B (−511, C/T) polymorphisms in genomic DNA was performed. Genotyping and histopathological observations were performed blind by different investigators. Logistic regression analysis controlled for age and gender and post hoc power analysis were performed.

Results

Logistic regression analysis revealed that the GSTO1 Asp/Asp genotype was associated with an increased expansion of AS (Figure 1A; power: 0.71) and an advanced histopathological type when compared with the GSTO1 Ala/Ala and Ala/Asp genotypes (Figure 1B; power: 0.8). There was no association of the GSTO1 Ala140Asp polymorphism with small vessel disease and cerebral amyloid angiopathy (Figure 1C–D).

Cryosections stained with an antibody directed against GSTO1-1 exhibited GSTO1-1 immunofluorescence in the peripheral part of the lipid core of the AS plaque (Figure 2). Double-label immunofluorescence showed colocalization of IL-1/H9251 and GSTO1-1 in macrophages (Figure 2).

Using logistic regression models controlled for age, gender, and IL1A, IL1B, and IL1B polymorphisms, GSTO1 Ala/Ala and Ala/Asp–individuals exhibited less IL-1α and GSTO1-1 in macrophages (Figure 2).

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Discussion

Our results revealed that carriers of the GSTO1 Asp/Asp genotype exhibited AS lesions of (1) a more advanced histopathological type and (2) a higher degree of expansion of AS plaques in the circle of Willis than carriers of GSTO1

Table. Clinical and Pathological Characteristics of the Human Autopsy Cases Analyzed

<table>
<thead>
<tr>
<th>General Clinical and Pathological Characteristics</th>
<th>Cause of Death</th>
<th>Dementia</th>
<th>AS Type</th>
<th>Degree of SVD</th>
<th>CAA Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean: 75 years</td>
<td>Cardiovascular diseases</td>
<td>41%</td>
<td>No dementia</td>
<td>73%</td>
<td>1/2</td>
</tr>
<tr>
<td>Range: 60–91 years</td>
<td>Malignant tumors</td>
<td>34%</td>
<td>AD</td>
<td>12%</td>
<td>3/4</td>
</tr>
<tr>
<td></td>
<td>Infectious disorders</td>
<td>13%</td>
<td>VD</td>
<td>5%</td>
<td>5/6</td>
</tr>
<tr>
<td></td>
<td>Metabolic disorders</td>
<td>6%</td>
<td>MD and OD</td>
<td>10%</td>
<td>7/8</td>
</tr>
<tr>
<td></td>
<td>Trauma</td>
<td>3%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Respiratory disorders</td>
<td>1%</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Anaphylactic shock</td>
<td>1%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stroke</td>
<td>1%</td>
<td></td>
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</table>

Genotype Distributions

<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>Genotype</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTO1 Ala140Asp</td>
<td>Asp/Asp</td>
<td>11 (13.9)</td>
</tr>
<tr>
<td>IL1A-889 (C/T)</td>
<td>C/C</td>
<td>42 (53.2)</td>
</tr>
<tr>
<td>IL1B-3954 (C/T)</td>
<td>C/C</td>
<td>35 (44.3)</td>
</tr>
<tr>
<td>IL1B-511 (C/T)</td>
<td>C/C</td>
<td>35 (44.3)</td>
</tr>
</tbody>
</table>

Clinical and Pathological Characteristics in GSTO1 Asp/Asp Individuals Compared with GSTO1 Ala/Ala and Ala/Asp Carriers

GSTM Genotype | Brain Infarction | Cerebral Hemorrhage | Arterial Hypertension | Diabetes Mellitus |
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp/Asp</td>
<td>3 (27%)</td>
<td>8 (73%)</td>
<td>0 (0%)</td>
<td>1 (9%)</td>
</tr>
<tr>
<td>Ala/Ala/Asp</td>
<td>19 (28%)</td>
<td>49 (72%)</td>
<td>9 (13%)</td>
<td>28 (41%)</td>
</tr>
</tbody>
</table>

Cardiovascular diseases include myocardial infarction, myocardial failure, vessel rupture, and thromboembolic events; metabolic disorders include diabetes mellitus, hepatic and renal dysfunction; infectious disorders include sepsis, pneumonia, and tuberculosis.

MD indicates mixed dementia, ie, combined AD (Alzheimer disease), VD (vascular dementia), and/or argyrophilic grain disease; OD, other types of dementia, including argyrophilic grain disease and progressive supranuclear palsy; SVD, small vessel disease; CAA, cerebral amyloid angiopathy.

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Ala/Ala and Ala/Asp genotypes. Despite the small number of cases studied, these associations showed a power of (1) 0.8 and (2) 0.71. The use of logistic regression models revealed that these effects were not caused by age or gender. A further argument for an important role of GSTO1-1 in AS was the detection of GSTO1-1 in AS plaques. An effect of GSTO1 Ala140Asp-polymorphism on either small vessel disease or cerebral amyloid angiopathy was not found within our sample. The small number of cases studied here did not allow confirmation of the reported clinicopathological correlation with vascular dementia.2

A possible link between GSTO1-1 and the expression of IL-1 has already been discussed.7 Our results support a functional link between GSTO1-1 and IL-1 expression. Arguments favoring this hypothesis are (1) the association of the GSTO1 Ala140Asp-polymorphism with AS and the IL-1α expression and (2) the colocalization of GSTO1-1 with IL-1α in macrophages of AS plaques. Thus, our findings extend the present knowledge insofar as an interaction of GSTO1-1 and IL-1 appears to be pathogenetically relevant for the inflammatory response in AS lesions.

In summary, our findings reveal an association between the GSTO1 Ala140Asp polymorphism and the expansion and severity of AS. Furthermore, they support the notion that GSTO1-1 plays an important role in AS lesions by modulating IL-1α expression in macrophages within AS plaques.
Acknowledgments
The skillful technical assistance of Mrs N. Kolosnjaji, Mrs A. Schulz, Mrs A. Hrychyk, and Mr H.-U. Klatt is gratefully acknowledged.

Sources of Funding
This work was supported by DFG-grant No. TH 624/4-1 (D.R.T.) and BONFOR-grant Nos. O-154.0041 (D.R.T.), O-154.0043 (D.R.T.), and O-128.0055 (H.K.), and NIH-NIA AG12411 (W.S.T.G.).

Disclosures
None.

References
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*Stroke*. 2007;38:2847-2850; originally published online August 23, 2007;
doi: 10.1161/STROKEAHA.107.484162

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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