Soluble Glycoprotein VI Is Raised in the Plasma of Patients With Acute Ischemic Stroke

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Background and Purpose—Ischemic stroke induced by thrombosis may be triggered by atherosclerotic plaque rupture and collagen-induced platelet activation. Collagen induces glycoprotein VI shedding.

Methods—We measured plasma-soluble glycoprotein VI (sGPVI) by enzyme-linked immunosorbent assay in 159 patients with acute (<7-day) ischemic stroke and age/sex-matched community-based control subjects.

Results—sGPVI was elevated in stroke compared with controls ($P=0.0168$). ORs were higher in Quartile 4 for stroke cases ($P=0.0121$), and sGPVI was significantly elevated in stroke associated with large artery disease across Quartiles 2 to 4 and small artery disease in Quartile 4. sGPVI decreased 3 to 6 months after antiplatelet treatment, consistent with elevated sGPVI due to platelet activation during the thrombotic event. sGPVI correlated with P-selectin ($P=0.0007$) and was higher in individuals with the GPVI*a* haplotype ($P=0.024$).

Conclusion—Glycoprotein VI shedding is implicated in the pathology of acute ischemic stroke. **(*Stroke*. 2011;42: 498-500.)**

Key Words: GPVI ■ platelets ■ stroke

The relative contribution of platelets to ischemic stroke varies between stroke etiologic subtypes, potentially modulating effectiveness of antiplatelet treatment.[1,2] Atherosclerotic plaque rupture and platelet recruitment to exposed collagen triggers thrombotic stroke[2] and the platelet-specific collagen receptor, glycoprotein VI (GPVI), initiates thrombus formation at arterial shear rates. Elevated platelet GPVI was reported in ischemic stroke,[3] and GPVI inhibition provides protection in experimental stroke models.[4] Collagen induces metalloproteinase-mediated shedding of GPVI, releasing plasma-soluble GPVI (sGPVI).[5,6] We evaluated whether plasma sGPVI was a useful biomarker for stroke, how it compares with the platelet/endothelial activation marker P-selectin, and whether sGPVI is generated by acute stroke or is a general consequence of platelet activation in arterial disease. We also assessed whether sGPVI was correlated with GPVI Gln317/Leu polymorphisms.[7]

Subjects and Methods

The cohort of stroke patients and matched controls was collected with ethics approval (Royal Perth Hospital) and characterized for stroke/stroke subtypes.[8,9] Plasma sGPVI was measured (blind) by enzyme-linked immunosorbent assay.[10] The relationship between sGPVI and age/sex and Gln317 was assessed using multivariate linear regression using logged sGPVI to meet required regression assumptions. Age was centered to reduce multicollinearity. Association of sGPVI with stroke was assessed for paired cases/control subjects by multivariate logistic regression.

Results

Analysis of 159 stroke cases and 159 community-based control subjects matched for age/sex and postal code[8,9] showed no significant correlation of sGPVI with age (mean, 67 years; range, 65 to 69 years) or sex (59 of 159 female) in either group. In the control group, standardized β coefficients (95% CI) were 0.013 (−0.11 to 0.14) for sex ($P=0.836$) and 0.0024 (−0.003 to 0.008) for age ($P=0.368$). In the stroke group, β values (95% CI) were −0.011 (−0.13 to 0.11) for sex ($P=0.854$) and 0.003 (−0.002 to 0.008) for age ($P=0.297$). There was an increase in plasma sGPVI between all stroke cases (21.5±9.1 ng/mL) compared with control subjects (19.7±8.1; $P=0.0168$). When analyzed by quartiles (Table 1), ORs were higher in Quartile 4 for stroke cases compared with control subjects. sGPVI was significantly elevated in large artery disease with a gradual, independent increase across Quartiles 2 to 4. sGPVI was increased in Quartile 4

Received September 12, 2010; final revision received September 12, 2010; accepted September 24, 2010.

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Stroke **is available at http://stroke.ahajournals.org** **DOI: 10.1161/STROKEAHA.110.602532**

Table 1. Association Between Plasma sGPVI and Stroke

	OR (95%CI) *P**			
	Quartile 1 (<15.2 ng/mL)	Quartile 2 (15.2–18.7 ng/mL)	Quartile 3 (18.8–25.5 ng/mL)	Quartile 4 (>25.5 ng/mL)
All stroke (n=159)†	1	1.61 (0.86–3.04)	1.45 (0.80–2.67)	2.25 (1.20–4.29)
		0.1357	0.2267	0.0121
Large artery (n=47)†	1	3.22 (1.16–9.91)	3.23 (1.21–9.65)	3.59 (1.26–11.25)
		0.0303	0.0247	0.0205
Cardioembolic (n=34)†	1	1.19 (0.42–3.31)	0.97 (0.34–2.67)	1.43 (0.50–4.04)
		0.741	0.949	0.494
Small artery (n=56)†	1	1.73 (0.72–4.25)	1.31 (0.54–3.20)	2.54 (1.08–6.17)
		0.2217	0.5486	0.0352

*OR and 95% Cls compared with matched control subjects.
†Reference Quartile 1; significance, $P<0.05$.

of small artery disease but not in cardioembolic stroke. In 59 of 159 available cases, 3 to 6 months after initial stroke,[8] and treated with antiplatelet agents (aspirin), mean sGPVI (95% CI) decreased from 20.4 ng/mL (18.6 to 22.2) to 14.9 (13.5 to 16.3; paired *t* test $P=3.8\times10^{-8}$). P-selectin was higher in stroke compared with control ($P=0.0484$).[8] The Pearson correlation coefficient for the linear relationship between sGPVI and P-selectin was 0.1899 ($P=0.0007$; Figure). GPVI polymorphism, Gln317 (Q/Q; GPVI*a*) compared with heterozygote (Q/L) and Leu317 (L/L; GPVI*b*), was not associated with stroke[9]; however, elevated sGPVI significantly correlated with Gln317 (Table 2).

Discussion

Metalloproteinase-mediated shedding of human platelet GPVI potentially regulates GPVI-dependent platelet function during thrombus formation.[5,6] Our study showing elevated sGPVI in patients with acute stroke at the time of the event, decreasing 3 to 6 months after antiplatelet treatment, provides the first evidence in humans that GPVI shedding is associated with atherothrombotic disease in vivo, consistent with a functional role for GPVI in experimental stroke models.[4] GPVI shedding may decrease platelet reactivity to collagen.[5] Dimeric sGPVI-Fc inhibits collagen binding/thrombus formation supporting a functional role for sGPVI,[11] and a GPVI-Fc-based drug (Revacept) is in Phase I trials.

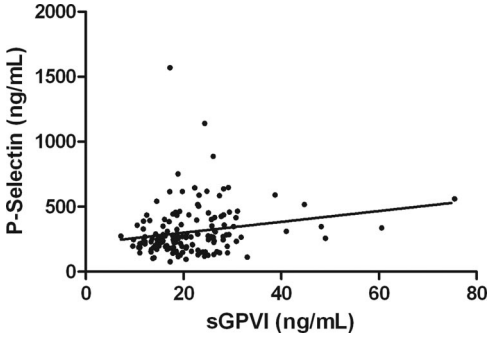

Figure. Correlation of sGPVI and P-selectin in stroke.

Etiologic subtypes of acute ischemic stroke can be caused by thrombosis characterized by platelet activation related to atherosclerotic plaque rupture causing collagen exposure ("platelet-rich thrombus") or cardioembolic stroke characterized by slow flow (stasis) associated with atrial fibrillation ("red cell-rich thrombus"). Our findings of increased sGPVI in large artery disease, and to a lesser extent small artery disease, but not cardioembolic stroke, might reflect the relative importance of platelets/platelet activation in stroke associated with vascular damage/collagen exposure and/or increased platelet GPVI expression.[3] The correlation between sGPVI and P-selectin is consistent with activation-dependent shedding of GPVI leading to elevated sGPVI. Highest P-selectin levels are associated with Quartiles 1 to 3 rather than Quartile 4 of sGPVI, which could reflect increased endothelial-derived rather than platelet P-selectin, whereas GPVI is platelet-specific; E-selectin and von Willebrand factor are not increased in Quartile 4 (not shown).[8]

Elevated sGPVI also correlated with the Gln317 polymorphism. GPVI*a* and *b* haplotypes involve 7 linked polymorphisms, 5 causing substitutions (Ser219Pro, Lys237Glu, Thr249Ala, Gln317Leu, His322Asn). Interestingly, Gln317Leu/His322Asn are proximal to a cytoplasmic calmodulin-binding site that regulates metalloproteinase-mediated shedding.[5] Compared with GPVI*a*, GPVI*b* exhibits normal ligand binding but disrupted ligand-induced signaling.[7] GPVI*a* shows lower-affinity calmodulin binding than GPVI*b*,[7] which could increase susceptibility to shedding.[5] Extracellular polymorphism Thr249Ala upstream of the cleavage site[5,6] could also influence metalloproteinase-mediated shedding.

Table 2. Association Between sGPVI and GPVI Genotype

Genotype	No. (%)	Mean sGPVI, ng/mL (Range)	*P**
Gln317	247 (70.4)	21.16 (20.01–22.31)	
Heterozygote and Leu317	104 (29.6)	19.27 (17.36–21.17)	0.0241*

**P* value compares heterozygote and Leu317 with Qln317. Significance, $P<0.05$.

Source of Funding

Supported by the National Health and Medical Research Council of Australia.

Disclosures

None.

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Stroke. 2011;42:498-500; originally published online December 30, 2010;
doi: 10.1161/STROKEAHA.110.602532
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0039-2499. Online ISSN: 1524-4628