Effect of genetic modifiers on cerebral lesions in Fabry disease

Abstract—Fabry disease is associated with increased risk of premature stroke and presumptive ischemic cerebral lesions. In 57 consecutive patients, 35% of whom had lesions on brain MRI, the authors found that genotypes of polymorphisms G-174C of interleukin-6, G894T of endothelial nitric oxide synthase, factor V G1691A mutation, and the A-13G and G79A of protein Z were all significantly associated with cerebral lesions. These findings suggest that these proteins modulate Fabry cerebral vasculopathy.

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Fabry disease, an X-linked systemic disorder caused by a deficiency of the lysosomal enzyme α-galactosidase A, results in a failure to metabolize α-D-galactosyl moieties. This defect causes accumulation of glycosphingolipids, particularly globotriaosylceramide within the heart, kidney, brain, skin, eyes, and vascular tissues.[1] As a consequence, renal failure and cardiac disease occur together with increased ischemic stroke incidence. The vasculopathy of Fabry disease has been well documented in the vertebrobasilar system and the carotid circulation.[2]

Presumptive small vessel disease brain lesions are easily observed on either T2-weighted or fluid-attenuated inversion recovery (FLAIR) MRI. Such lesions occur in less than 10% of the general population aged 20 to 55 years. These lesions are mostly in the white matter of the brain, predominate in the territory of the posterior circulation, and progress with age. Although these lesions are most often asymptomatic, virtually all the patients with symptomatic stroke have such lesions. Increasing evidence suggests that nonnitric oxide-related hyperactivity of endothelial nitric oxide synthase (eNOS) is associated with an increased production of reactive oxygen species such as superoxide ($O_2^{\bullet -}$). This is also associated with a significant elevation of blood myeloperoxidase levels.[2]

Because not all patients with Fabry disease develop high-signal deep white matter lesions, we set about to identify possible genetic markers that will predict the presence of these lesions on head MRI. We tested the hypothesis that the polymorphism of interleukin-6 (IL-6),[3] eNOS,[4] factor V,[5] prothrombin,[6] methylenetetrahydrofolate reductase (MTHFR),[7] and protein Z[8] are determinants of these abnormalities in hemizygous male patients. All these polymorphisms have previously been shown to be associated with increased risk of ischemic vascular disease and in particular stroke. We therefore assessed the likelihood of developing cerebral MRI lesions as a function of these polymorphisms controlling for other known risk factors for stroke in a well-characterized group of hemizygous patients with Fabry disease.

Methods. *Patients.* Fifty-seven consecutive male hemizygous patients with Fabry disease were studied. All the patients had typical Fabry disease. The age range of the NIH Fabry cohort was 12 to 64 years with a mean age of 36 ± 12 years. Four patients were Hispanic in origin, and the others were Caucasian. Only one patient was significantly overweight and one other was a smoker, with eight patients having a history of symptomatic stroke. Twenty percent of patients had a filial relation (sibling) in this cohort, but each patient was analyzed individually. The Institutional Review Board of the National Institute of Neurologic Disorders and Stroke approved the protocol, and all patients gave their written informed consent.

MRI FLAIR abnormalities. FLAIR images (TR 10,000, TE 148, TI 2,500 ms, slice thickness 5 mm, FOV 24.0 cm) acquired over the 5-year period from 1997 to 2001 were reviewed by one of the authors (DFM) and an experienced neuroradiologist, who was blinded to the genotypes of the patients, for the presence or absence of high signal intensity white matter lesions.

IL-6 polymorphism. DNA from patients and controls was extracted and amplified by PCR as previously described.[3,9] The 190-bp PCR product was digested overnight at 37°C with NlaIII (New England Biolabs, Stockholm, Sweden). The C allele in position -174 creates a cut site for restriction endonuclease NlaIII.

eNOS polymorphisms. The procedure for identifying the 894 G→T exon 7 polymorphism of eNOS consisted of PCR amplification of the G894T allelic variant using the following primers: forward: 5'-CATGAGGCTCAGCCCCAGAAC-3'; reverse: 5'-AGTCAATCCCTTTGGTGCTCAC-3'. The amplified 248-bp PCR product was digested with the restriction enzyme MboI (Promega Corp. Madison, WI). The G894T substitution of the eNOS gene produces a single cut site yielding two fragments of 158 bp and 90 bp. The T-786→C polymorphism analysis was performed as previously described.[4]

Protein Z polymorphisms. The G79A intron F and the A-13G polymorphisms were analyzed as previously described.[8]

Factor V Leiden mutation, the MTHFR C677T, and the prothrombin G20210A polymorphisms were genotyped as previously described.[5-7]

Statistics. Each locus was independently analyzed using a logistic regression model, with age, total cholesterol, the presence of hypertension and residual α-galactosidase A activity (<1.5% of normal vs ≥1.5%) included as covariates using R (http://www.r-project.org/). Comparison was made between the heterozygote (wild type/mutant) and the homozygote genotype (mutant/mutant) of the polymorphism. The wild type homozygote significance is implied. The polymorphisms were examined for a significant explanatory effect in relation to the presence of high-signal FLAIR lesions on brain MRI. The model was refined based on the Akaike information criterion (AIC) (Akaike H. A new look at statistical

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Table *The relationship of polymorphisms genotype and the presence of cerebral lesions on MRI*

Genotype	*p* Value in full model*	*p* Value in partial model†	*p* Value after adjustment for false discovery rate
eNOS 894 G/T‡	0.04	0.01	0.04
eNOS 894 T/T	0.49	0.49	0.86
IL-6 174 G/C	0.003	0.003	0.02
IL-6 174 C/C	0.03	0.01	0.04
Protein Z 79 G/A	0.03	0.01	0.04
Protein Z 79 A/A	0.99	0.99	0.99
Protein Z 13 A/G	0.02	0.01	0.04
Protein Z 13 G/G	0.99	0.99	0.99
Factor V Leiden G1691A G/A	0.001	0.0005	0.007
Factor V Leiden G1691A A/A	0.99	0.99	0.99
MTHFR 677 C/T	0.18	0.04	0.08
MTHFR 677 T/T	0.99	0.99	0.99
Prothrombin 20210 G/A	0.5	0.8	0.9
Prothrombin 20210 G/A	0.7	0.6	0.9

* Adjusted for age, blood pressure, cholesterol, and residual enzyme activity.
† Adjusted for age.
‡ T-786C endothelial nitric oxide synthase polymorphism was not significant (not shown).

ENOS = endothelial nitric oxide synthase; IL = interleukin; MTHFR = methylenetetrahydrofolate reductase.

model identification. IEEE Transactions on Automatic Control 1974;AU-19:716–722) with retention of only age as a significant covariate. Subsequently, a correction for Type I error using the false discovery rate correction of Reiner et al.[10] was made before reporting a particular polymorphism as significant.

Results. The details results are shown in the table. In all statistical logistic regression models, age was significantly associated with increased likelihood of developing abnormal high signal abnormalities on cerebral FLAIR imaging. Cholesterol, hypertension, and residual enzyme activity were not associated with brain lesions. The polymorphisms G-174C of IL-6 and G894T of eNOS, factor V G1691A mutation, and the A-13G and G79A of protein Z were all significantly associated with cerebral lesions but not prothrombin, MTHFR, or the T-786C polymorphism of eNOS (see table).

Discussion. Modification of the phenotype of mendelian traits by loci distinct from the disease locus is frequently evoked, but only a few such cases have been identified; none thus far have been found in lysosomal disorders. In the present study, we found that IL-6, eNOS, factor V, and protein Z polymorphisms significantly influence the likelihood that patients with Fabry disease will develop high-intensity signal MR-FLAIR brain lesions possibly related to small vessel disease. These proteins are associated with inflammation, vascular wall biology, and the clotting mechanism. These findings suggest a multifactorial mechanism for cerebral lesions in Fabry disease.

It has been previously established that the -174 GG genotype causes a higher level of IL-6 expression.[9] Some authors have found IL-6 to be neuroprotective.[3] Although we did not measure the circulating IL-6 levels in our patients, it is possible that this polymorphism protects the brain of patients with Fabry disease through a tendency to increase IL-6 expression, thereby decreasing the inflammatory component of Fabry vasculopathy or by enhancing neuroprotection of neurons and glia. IL-6 inhibits the production of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) and stimulates the production of receptor antagonists to TNF-α and IL-1. In culture, IL-6 protects neurons from NMDA toxicity. We have also found that in patients with normal head MRI, the cerebral areas most susceptible to develop lesions on MRI are hyperperfused and hypometabolic. It is possible therefore that IL-6 may have a pivotal role in both the vascular and neuronal aspects of cerebral white matter lesion pathology in Fabry disease.

eNOS 894 G→T exon 7 polymorphism causes a missense mutation Glu298Asp in the eNOS dimer localizing on the exterior of the dimer surface. This relatively conservative amino acid substitution might decrease the dimer stability and the interaction of eNOS with other proteins. This polymorphism is apparently more vulnerable to enzymatic cleavage in cell lysates in vitro compared with the wild type protein. It is not clear by what mechanism this polymorphism influences the cerebral disease in patients with Fabry disease. However, we have previously found possible abnormalities in the function of eNOS consistent with an increased production of reactive oxygen species.[2] On the other hand, the lack of association between the T-786→C polymorphism of the eNOS promoter and the presence of cerebral lesions suggests that the extent of expression of this protein is less important in generating these brain lesions in Fabry disease. We have indeed previously shown that eNOS expression in the blood vessels of the skin and brain of patients with Fabry disease is no different than that in controls.[1]

Our findings also suggest that a procoagulant state plays an important mechanistic role in these brain lesions, while homocysteine is likely less relevant. These data are the first indication of genetic modification of the phenotypic expression in this single gene disorder. Such an approach is important for understanding the mechanism and prognosis of the cerebral vasculopathy of Fabry disease. Genetic modifiers likely exist in other inborn errors of metabolism.

References

1. Moore DF, Scott LT, Gladwin MT, et al. Regional cerebral hyperperfusion and nitric oxide pathway dysregulation in Fabry disease: reversal by enzyme replacement therapy. Circulation 2001;104:1506–1512.
2. Moore DF, Ye F, Brennan ML, et al. Ascorbate decreases Fabry cerebral hyperperfusion suggesting a reactive oxygen species abnormality—an arterial spin tagging study. J Magn Reson Imaging 2004;20:674–683.
3. Revilla M, Obach V, Cervera A, Davalos A, Castillo J, Chamorro A. A -174G/C polymorphism of the interleukin-6 gene in patients with lacunar infarction. Neurosci Lett 2002;324:29–32.

4. Paradossi U, Ciofini E, Clerico A, Botto N, Biagini A, Colombo MG. Endothelial function and carotid intima-media thickness in young healthy subjects among endothelial nitric oxide synthase Glu298→Asp and T-786→C polymorphisms. Stroke 2004;35:1305–1309.
5. Bertina RM, Koeleman BP, Koster T, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994;369:64–67.
6. Longstreth WT Jr, Rosendaal FR, Siscovick DS, et al. Risk of stroke in young women and two prothrombotic mutations: factor V Leiden and prothrombin gene variant (G20210A). Stroke 1998;29:577–580.
7. Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995;10:111–113.
8. Lichy C, Kropp S, Dong-Si T, et al. A common polymorphism of the protein Z gene is associated with protein Z plasma levels and with risk of cerebral ischemia in the young. Stroke 2004;35:40–45.
9. Fishman D, Faulds G, Jeffery R, et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest 1998;102:1369–1376.
10. Reiner A, Yekutieli D, Benjamini Y. Identifying differentially expressed genes using false discovery rate controlling procedures. Bioinformatics 2003;19:368–375.

Giant arachnoid granulations

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A 43-year-old woman presented with new-onset right temporal headache associated with episodes of visual loss in her right eye for 3 months. Initial MRI showed a filling defect in the left transverse sinus, and subsequent contrast-enhanced MR venography (MRV) confirmed a filling defect in the sinus (figure, A). Laboratory results were negative for a hypercoagulable state. Central venous thrombosis was suspected.

Anticoagulation was commenced. The patient continued to complain of headache. A subsequent MRV done 3 months later showed the same finding. A cerebral angiogram showed a giant arachnoid granulation (figure, B). Arachnoid granulations can mimic venous sinus thrombosis.[1] Other considerations include thrombus, meningioma, extraaxial cavernous hemangioma, or artifact.[2]

1. Chin SC, Chen CY, Lee CC, et al. Giant arachnoid granulation mimicking dural sinus thrombosis in a boy with headache: MRI. Neuroradiology 1998;40:181–183.
2. Mamourian AC, Towfighi J. MR of giant arachnoid granulation, a normal variant presenting as a mass within the dural venous sinus. AJNR Am J Neuroradiol 1995;16:901–904.

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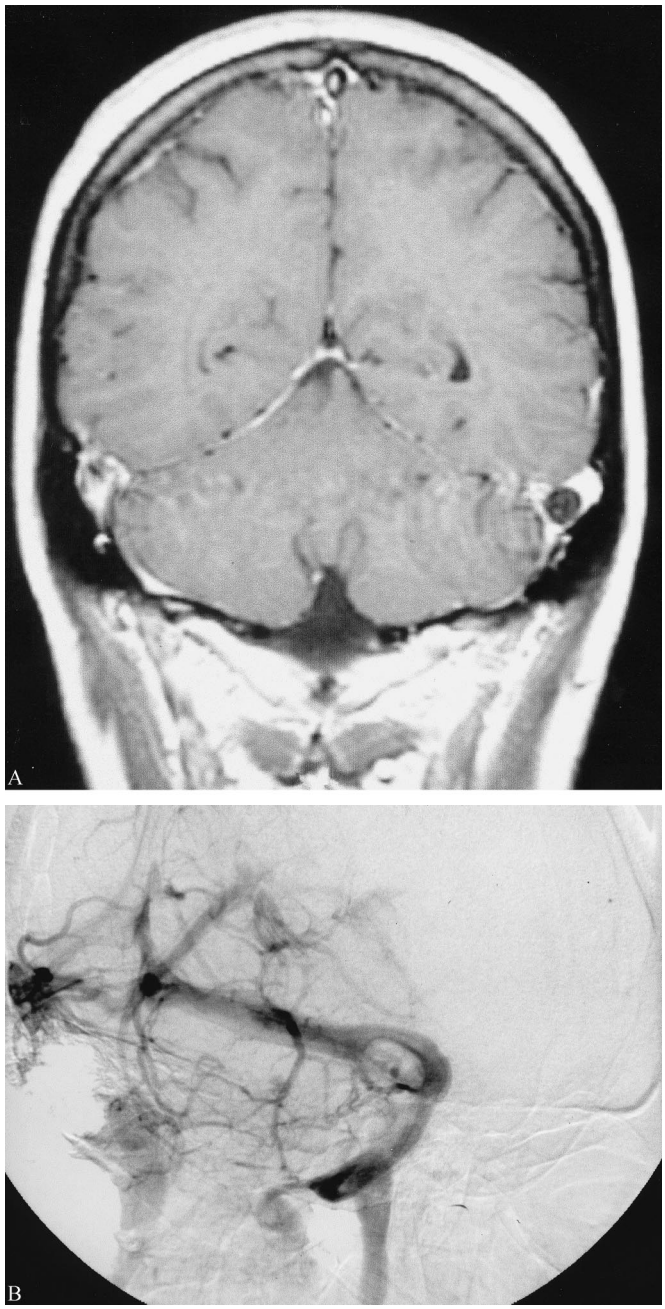

Figure. (A) Brain MRI with contrast shows unenhancing lesion in the left transverse sinus. (B) Cerebral angiogram shows a focal smooth filling defect in the left transverse/sigmoid sinus junction with no limitation of the outflow.

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