Role of the MMP9 Gene in Hemorrhagic Transformations After Tissue-Type Plasminogen Activator Treatment in Stroke Patients

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Background and Purpose—Despite the benefits of tissue-type plasminogen activator treatment, some stroke patients experience adverse hemorrhagic transformations (HT). Plasma protein levels of MMP9 have been associated with HT occurrence. We aimed to analyze the association of the *MMP9* gene with HT occurrence.

Methods—We analyzed the *MMP9* gene in blood samples from 885 stroke patients treated with tissue-type plasminogen activator by tag-SNP, imputed SNP, direct sequencing, and RNA expression.

Results—We did not observe any significant association between *MMP9* genetic variations or *MMP9* expression and HT occurrence. Moreover, no association was found between *MMP9* expression and *MMP9* polymorphisms.

Conclusions—Genetic variations in the *MMP9* gene are not associated with HT occurrence in tissue-type plasminogen activator-treated patients. **(*Stroke*. 2012;43:1398-1400.)**

Key Words: genetics ■ tissue-type plasminogen activator

Tissue-type plasminogen activator (tPA) is the only drug approved for the acute treatment of ischemic stroke, but its great benefits are counterbalanced by undesired effects such as symptomatic hemorrhagic transformations (HT), occurring in 1.5% to 4% of cases.[1]

Symptomatic HT are parenchymal hemorrhages associated with high mortality and neurological worsening. To minimize bleedings, a precise selection of ischemic stroke patients is mandatory, leading to only 14% of them being benefited by the use of the drug.[2]

Previous studies observed that increased metalloproteinase-9 (MMP9) levels measured in plasma samples of patients before tPA administration were associated with HT occurrence.[3] Our aim was to perform a thorough study of the *MMP9* gene to determine if genetic variations might be the reason for HT occurrence and increased plasma MMP9 levels in some tPA-treated patients.

Subjects and Methods

Study Population

Our target group consisted of consecutive white patients (n=885) experiencing an acute ischemic stroke who received tPA in a standard 0.9-mg/kg dose within the first 4.5 hours after symptoms onset. The mean age was 70.5±12.0 years, 54.8% were men, and 22% had development of an HT (9.6% had a parenchymal hemorrhage subtype).

HT presence was excluded before tPA infusion by MRI or CT scan. HT was evaluated in a follow-up CT scan performed 24 hours after symptoms onset or whenever neurological worsening occurred and was classified according to ECASS criteria.[4]

Complete clinical and methodological data are available in the online-only Supplemental Methods and in the online-only Supplemental Table I (http://stroke.ahajournals.org).

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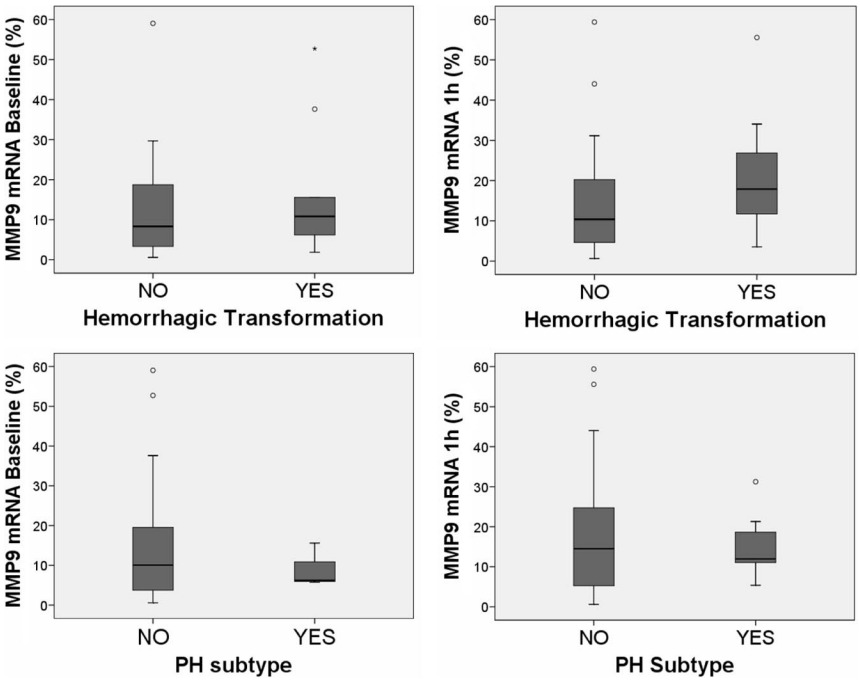

Figure. Baseline mRNA levels depending on hemorrhagic transformation or parenchymal hematoma (PH) subtype.

Genetic and Functional Analysis

We attempted to genotype 14 tag-SNP located in the *MMP9* gene in 885 samples using data from the Hapmap project (http://hapmap.ncbi.nlm.nih.gov/). Genotyping was conducted by SNPlex technology. The analysis of haplotypes was performed using the PHASE software. Imputation was performed using our genotyping data and according to the recommendations of the PLINK software (http://pngu.mgh.harvard.edu/~purcell/plink/).

We analyzed the $(CA)_n$ microsatellite, previously associated with *MMP9* expression in cell cultures,[5] in 885 samples. Primers were labeled with fluorescence, and the polymerase chain reaction amplicons were checked on an ABI 3730 DNA analyzer (Applied Biosystems).

We screened the promoter region of the *MMP9* gene by direct sequencing (2 Kb upstream of the transcription start site) in a subset of 10 HT patients and 10 non-HT patients matched for age and gender to find rare mutations. All the variations detected were compared with data integrated from the dbSNP database (37.1 built) to determine whether they were new mutations or previously described SNP.

Fifty-four subjects, including 41% with HT (20.9% with a parenchymal hemorrhage subtype), were included in the analysis of the association of genotyped SNP, imputed SNP, haplotypes, $(CA)_n$ microsatellite, and HT occurrence with *MMP9* gene expression. RNA was extracted from blood samples at baseline (before tPA administration) and 1 hour after tPA. The mRNA levels were measured by real-time polymerase chain reaction using TaqMan fluorogenic probes and a 7500 Real-Time PCR System (Applied Biosystems).

Statistical Analysis

Sample size calculation was performed using the Ene 2.0 software. The 14 SNP with frequencies higher than 0.35 in the experimental group (HT subjects) and lower than 0.20 in the reference group (non-HT subjects) would be found significantly associated with a power of 80% in a sample of 138 subjects, for unadjusted $P=0.05$. In addition, the analysis of the 14 SNP together and an adjusted $P=0.05$ (using Bonferroni correction) showed robust statistical power (online-only Supplemental Figure I).

The Hardy-Weinberg equilibrium was assessed using a χ^2 test with 1 degree of freedom. SPSS statistical package 15.0 was used for data analysis. When indicated, χ^2 or Fisher exact test for categorical variables and the analysis of variance, *t* test, Mann-Whitney *U*, or Kruskal-Wallis tests were used.

Results

Fourteen SNP were successfully genotyped in 836 DNA samples; all of them were in Hardy-Weinberg equilibrium. Additive and dominant/recessive models were used. We did not find any SNP significantly associated with HT occurrence (online-only Supplemental Table II and Table III).

After the imputation process and quality control tests, we analyzed 6 new SNP. We did not find any imputed SNP associated with HT occurrence (online-only Supplemental Table IV).

Using the PHASE software, we obtained 13 different haplotypes (online-only Supplemental Table V). The H1 haplotype was the most frequent (54.9%), followed by the H2 (22%). No significant association between the haplotypes and HT occurrence was observed. None of the $(CA)_n$ repetitions detected was associated with HT occurrence.

Last, we did not find any new mutation in HT (parenchymal hemorrhage subtype) or non-HT subjects when we screened the promoter region of the gene.

No significant difference in the mean relative levels of RNA expression was observed between subjects depending on the genotyped SNP, imputed SNP, haplotypes, or $(CA)_n$ microsatellite. Moreover, *MMP9* mRNA levels were not associated with HT occurrence (Figure).

Discussion

In our study, we found that the *MMP9* gene variants are not related to HT occurrence after tPA administration. We aimed to perform the first complete study of the *MMP9* gene because plasma levels of the protein play an important role in HT occurrence, and we hypothesized that genetic factors of *MMP9* could be associated with HT occurrence in some patients. However, after an extended analysis of the *MMP9*

gene, we did not find any genetic marker associated with the HT process.

The well-powered analysis by tag-SNP, imputed SNP, and haplotypes did not reveal any association between any polymorphism and HT. When we studied the presence of rare mutations, we did not find any new mutation that could modify the transcription process and increase the levels of the protein.

Moreover, when we analyzed the expression levels of the gene, we did not find any association with HT occurrence or with the SNP or haplotypes analyzed. Nevertheless, it should be noticed that we analyzed *MMP9* expression in blood and the *MMP9* expression could be different in other tissues, such as brain.

Several studies have revealed a genetic predisposition in tPA safety and efficacy. The Val34Leu SNP of the *FXIII* gene was associated with HT rates after tPA treatment.[6] Previously, we analyzed the role of a common polymorphism (C-1562T) in the *MMP9* gene.[7] This SNP was associated with MMP9 levels in vitro; however, we did not observe any association with HT rates.[7]

Altogether, our results suggest that the *MMP9* gene is not associated with HT. The increase in *MMP9* levels observed in HT patients might be caused by a different process in which the *MMP9* gene is not involved. The α-2-macroglobulin, low-density-related protein-1, and tissue inhibitors of metalloproteinases are molecules that inhibit *MMP9* and modulate its plasma levels.[8] Moreover, we have observed that neutrophils releasing *MMP9* are involved in the HT process,[9] and a great interindividual load difference of *MMP9* exists in the vesicles of human neutrophils that are degranulated after contacting with tPA.[10]

We conclude that the *MMP9* gene is not associated with HT occurrence and another process might be involved in HT after tPA administration. Our data suggest that preformed *MMP9* (ie, filling neutrophil vesicles) instead of newly synthesized protein might be more relevant in the HT process.

Disclosures

None.

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