Increased plasma von Willebrand factor antigen levels but normal von Willebrand factor cleaving protease (ADAMTS13) activity in preeclampsia

Attila Molvarec1; János Rigó Jr.1; Tamás Bőze1; Zoltán Derzsy1; László Cervenak2; Veronika Makó3; Timea Gombos3; Miklós László Udvardy4; Jolán Hársfalvi4; Zoltán Prohászka2,3

11st Department of Obstetrics and Gynecology, Semmelweis University, Budapest, Hungary; 2Research Group of Inflammation Biology and Immunogenomics, Hungarian Academy of Sciences, Budapest, Hungary; 33rd Department of Internal Medicine and Szentágothai Knowledge Center, Semmelweis University, Budapest, Hungary; 4Clinical Research Center, University of Debrecen, Debrecen, Hungary

Summary
The activity of ADAMTS13, the von Willebrand factor (VWF) cleaving protease is low in several conditions, including HELLP (haemolysis, elevated liver enzymes, and low platelet count) syndrome. As HELLP syndrome develops in most cases on the basis of preeclampsia, our aim was to determine whether plasma ADAMTS13 activity is decreased in preeclampsia. Sixty-seven preeclamptic patients, 70 healthy pregnant women and 59 healthy non-pregnant women were involved in this case-control study. Plasma ADAMTS13 activity was determined with the FRETS-VWF73 assay, while VWF antigen (VWF:Ag) levels with an enzyme-linked immunosorbent assay. The multimeric pattern of VWF was analyzed by SDS-agarose gel electrophoresis.

There was no significant difference in plasma ADAMTS13 activity between the preeclamptic and the healthy pregnant and non-pregnant groups (median [25–75 percentile]: 98.8 [76.5–112.8] %, 96.3 [85.6–116.2] % and 91.6 [78.5–104.4] %, respectively; p>0.05). However, plasma VWF:Ag levels were significantly higher in preeclamptic patients than in healthy pregnant and non-pregnant women (187.1 [145.6–243.1] % versus 129.3 [105.1–182.8] % and 70.0 [60.2–87.3] %, respectively; p<0.001).

The multimeric pattern of VWF was normal in each group. Primiparas had lower plasma ADAMTS13 activity than multiparas (92.6 [75.8–110.6] % versus 104.2 [92.1–120.8] %; p=0.011). No other relationship was found between clinical characteristics, laboratory parameters and plasma ADAMTS13 activity in either study group. In conclusion, plasma ADAMTS13 activity is normal in preeclampsia despite the increased VWF:Ag levels. However, further studies are needed to determine whether a decrease in plasma ADAMTS13 activity could predispose preeclamptic patients to develop HELLP syndrome.

Keywords
ADAMTS13, HELLP syndrome, preeclampsia, pregnancy, von Willebrand factor

Introduction
Preeclampsia is a severe complication of human pregnancy with a worldwide incidence of 2–10% (1). It is one of the leading causes of maternal, as well as perinatal morbidity and mortality, even in developed countries. Despite intensive research efforts, the etiology and pathogenesis of preeclampsia are not fully understood. Increasing evidence suggests that an excessive maternal systemic inflammatory response to pregnancy with activation of endothelial cells plays a crucial role in the pathogenesis of the disease (2). The development of preeclampsia is influenced by both genetic and environmental risk factors, suggesting its multifactorial inheritance (3).

ADAMTS13 (A Disintegrin-like And Metalloprotease with Thrombospondin type 1 motif, member 13), a circulating zinc metalloprotease, cleaves von Willebrand factor (VWF) whenever its cleavage site at the peptide bond between tyrosine at position 1605 and methionine at position 1606 in the VWF A2 domain is exposed by shear stress. This proteolysis converts endothelial-derived, newly secreted ultralarge VWF multimers into smaller, less reactive derivatives (4, 5). ADAMTS13 is synthesized mainly in hepatic stellate cells, but variable expression was observed in other cell types, including endothelial cells and platelets (6–8).

A profound deficiency of ADAMTS13, due to genetic mutations or autoimmune inhibition, impairs VWF cleavage, lead-
ing in turn to the disseminated formation of platelet-rich thrombosis in the microcirculation and to symptoms of end-organ ischemia, characteristics of thrombotic thrombocytopenic purpura (TTP). However, a substantial proportion of patients with TTP, particularly of those with the acquired form, have detectable and even normal plasma ADAMTS13 activity (9, 10). On the other hand, the VWF-cleaving protease activity is also low in several physiological and pathological conditions, most of which are associated with an increased thrombotic tendency, organ involvement and/or acute phase reaction (11–21). Nevertheless, a severe deficiency in plasma ADAMTS13 activity (less than 5% of normal) seems to be a specific beacon of TTP (22).

Preeclampsia is also characterized by systemic inflammation with an acute phase reaction, multi-organ involvement and a pro-thrombotic state, as well as by increased production of the protease’s substrate, VWF by activated endothelial cells. In addition, reduced ADAMTS13 activity has been observed previously in pregnant women with HELLP (haemolysis, elevated liver enzymes, and low platelet count) syndrome (23, 24). HELLP syndrome develops in most cases on the basis of preeclampsia and is one of its most severe complications. However, ADAMTS13 activity has not been investigated yet in a large number of preeclamptic patients without HELLP syndrome. Therefore, our aim was to determine whether plasma ADAMTS13 activity is decreased in preeclampsia. We also measured VWF antigen (VWF:Ag) levels in the same samples and examined the relationship of clinical characteristics, standard laboratory parameters and plasma VWF:Ag levels to plasma ADAMTS13 activity. The multimeric pattern of VWF was also analyzed in our study groups.

Materials and methods

Study patients

Our study was designed employing a case-control approach. Sixty-seven preeclamptic patients, 70 healthy pregnant women with uncomplicated pregnancies and 59 healthy non-pregnant women were involved in the study. The study participants were enrolled in the 1st Department of Obstetrics and Gynecology and in the Department of Obstetrics and Gynecology of Kútöölgyi Clinical Center, at the Semmelweis University, Budapest, Hungary. All women were Caucasian and resided in the same geographic area in Hungary. Exclusion criteria were multifetal gestation, chronic hypertension, diabetes mellitus, autoimmune disease, angiopathy, renal disorder, maternal or fetal infection and fetal congenital anomaly. The women were fasting, none of the pregnant women were in active labor, and none had rupture of membranes. The healthy non-pregnant women were in the early follicular phase of the menstrual cycle (between cycle days 3 and 5), and none of them received hormonal contraception.

Preeclampsia was defined by increased blood pressure (≥140 mmHg systolic or ≥90 mmHg diastolic on ≥2 occasions at least 6 hours [h] apart) that occurred after 20 weeks of gestation in a woman with previously normal blood pressure, accompanied by proteinuria (≥0.3 g/24 h). Blood pressure returned to normal by 12 weeks postpartum in each preeclamptic study patient. Preeclampsia was regarded as severe if any of the following criteria was present: blood pressure ≥160 mmHg systolic or ≥110 mmHg diastolic, or proteinuria ≥5 g/24 h. Pregnant women with eclampsia or HELLP syndrome were not enrolled in this study. Early onset of preeclampsia was defined as onset of the disease before 34 weeks of gestation (between 20 and 33 completed gestational weeks). Fetal growth restriction was diagnosed if the fetal birth weight was below the 10th percentile for gestational age and gender, based on Hungarian birth weight percentiles (25).

The study protocol was approved by the Regional, Institutional Committee of Medical Ethics at the Semmelweis University, and written informed consent was obtained from each patient. The study was conducted in accordance with the Declaration of Helsinki.

Biological samples

Blood samples were obtained from an antecubital vein into, as well as EDTA- or sodium citrate anticoagulated tubes and centrifuged at room temperature with a relative centrifugal force of 3,000 g for 10 minutes (min). The aliquots of serum and plasma were stored at −80°C until the analyses were performed.

Determination of plasma ADAMTS13 activity

The fluorogenic substrate, FRET-S-VWF73, was purchased from Peptides International (Louisville, KY, USA) and applied for the determination of ADAMTS13 enzyme activity according to the protocol provided by the supplier with minor modifications (26). Briefly, the test citrated plasma were diluted 1:20 in assay buffer (5 mM Bis-Tris, 25 mM CaCl₂, 0.005% Tween 20, pH 6.0) and mixed with 5 µM FRET-S-VWF73 substrate solution (20 µl each), in white 384-well plates. Fluorescence was measured at 37°C every 2 min for 1 h in Chameleon microplate reader (Hidex, Turku, Finland) equipped with a 340 nm excitation and a 460 nm emission filter. The reaction rate was calculated by linear regression analysis of fluorescence over time. A two-fold dilution series of normal human plasma (mixed from citrated plasma samples of 10 healthy blood donors) was applied as standard curve, 100% ADAMTS13 activity was set at the reaction rate observed in the 1:20 diluted sample. The intra-assay coefficient of variation (CV) was <5%, the inter-assay CV was 6–9% (measured at 60 and 100% activity levels).

In six plasma samples, we also measured ADAMTS13 enzyme activity with the collagen-binding assay, as described by Gerritsen et al. (1999) (27). Pooled citrated plasma of 10 healthy blood donors was used as reference. The results of the FRET-S-VWF73 assay showed good agreement with those of the collagen-binding method (Spearman R=0.82; p<0.05).

Determination of plasma VWF antigen (VWF:Ag) levels

VWF antigen (VWF:Ag) levels were measured in citrated plasma by an enzyme-linked immunosorbent assay (ELISA) using commercially available antibodies (Dakopatts, Glostrup, Denmark). Microtiter plates (Immunoplate Maxisorb, Nunc, Roskilde, Denmark) were coated with polyclonal rabbit anti-human VWF antibody diluted 1:800 in sodium bicarbonate buffer, pH 9.6. After overnight incubation at 4°C, plates were washed in Tris-buffered saline containing 0.05% Tween 20 (TBS-T). The plates were incubated with plasma samples diluted 1:500 or standard control plasma (dilution range 1:250 to 1:8,000) for 2 h at
room temperature. After washes with TBS-T, horse radish peroxidase (HRP)-conjugated polyclonal rabbit anti-human VWF antibody was added to the plates and incubated for 1 h, followed by addition of ortho-phenylenediamine (OPD). The optical density was measured at 492 nm. Results are expressed as percentages of a standard composed of pooled human plasma of 10 healthy blood donors.

**Multimeric analysis of VWF**

The multimeric pattern of VWF was analyzed in EDTA plasma samples by sodium dodecyl sulphate (SDS)-agarose gel electrophoresis. Briefly, low resolution (0.8%) agarose (Seakem HGT, Lonza, Basel, Switzerland) gels were cast on glass plates, using Tris-Borate buffers as recommended by the manufacturer of the agarose. Electrophoresis was performed at constant current for 6 h at 20 mA/gel on a plate cooled to 18°C. Proteins were transferred to PVDF membranes (Immobilon-P, Millipore Corp., Bedford, MA, USA) by tank electro-blotting method. To enhance the transfer of the largest VWF molecules, VWF multimers were degraded by treating the gels for 10 min with 1 mM β-mercaptoethanol (mercaptolysis) before blotting (28). VWF was visualized by immunostaining using HRP-labelled polyclonal rabbit anti-human VWF antibody (DakoCytomation, Glostrup, Denmark) and DAB substrate.

**Quantitative multimer analysis** was performed similarly to Budde et al. (2008) (29). Briefly, membranes were digitalized by GS-800 (Bio-Rad Laboratories, Richmond, CA, USA) calibrated densitometer, and processed by the QuantityOne software (Bio-Rad Laboratories). The percentage of large multimers – defined as oligomers larger than the 20-mer (band 10) – was calculated using the reflective density against relative front data, and was used for the description of VWF multimer distribution.

**Determination of other laboratory parameters**

Standard laboratory parameters were measured by Roche Integra 800 autoanalyzer (clinical chemistry, C-reactive protein) or by Cell-Dyn 3500 hematology analyzer (platelet count).

**Statistical analysis**

The normality of continuous variables was assessed using the Shapiro-Wilk’s W-test. As the continuous variables were not normally distributed, non-parametric statistical methods were

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### Table 1: Clinical characteristics, standard laboratory parameters, plasma ADAMTS13 activity and von Willebrand factor antigen (VWF:Ag) levels of healthy non-pregnant and pregnant women and preeclamptic patients.

<table>
<thead>
<tr>
<th></th>
<th>Healthy non-pregnant women (n=59)</th>
<th>Healthy pregnant women (n=70)</th>
<th>Preeclamptic patients (n=67)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>28 (23–35)</td>
<td>30 (28–32)</td>
<td>29 (26–33)</td>
</tr>
<tr>
<td><strong>BMI at blood draw (kg/m²)</strong></td>
<td>20.8 (19.6–22.9)</td>
<td>25.9 (24.2–27.7)</td>
<td>30.0 (27.7–33.3)²⁺</td>
</tr>
<tr>
<td><strong>Smokers</strong></td>
<td>14 (23.7%)</td>
<td>0 (0%)</td>
<td>3 (4.5%)¹</td>
</tr>
<tr>
<td><strong>Primiparas</strong></td>
<td>n.a.</td>
<td>45 (64.3%)</td>
<td>43 (64.2%)</td>
</tr>
<tr>
<td><strong>Systolic blood pressure (mmHg)</strong></td>
<td>115 (110–120)</td>
<td>110 (105–120)</td>
<td>160 (154–180)¹</td>
</tr>
<tr>
<td><strong>Diastolic blood pressure (mmHg)</strong></td>
<td>80 (70–80)</td>
<td>70 (60–80)</td>
<td>100 (97–110)¹</td>
</tr>
<tr>
<td><strong>Gestational age at blood draw (weeks)</strong></td>
<td>n.a.</td>
<td>35 (30–36)</td>
<td>38 (36–39)²</td>
</tr>
<tr>
<td><strong>Gestational age at delivery (weeks)</strong></td>
<td>n.a.</td>
<td>39 (38–40)</td>
<td>38 (37–40)²</td>
</tr>
<tr>
<td><strong>Fetal birth weight (grams)</strong></td>
<td>n.a.</td>
<td>3500 (3200–3800)</td>
<td>3200 (2450–3600)⁴</td>
</tr>
<tr>
<td><strong>Fetal growth restriction</strong></td>
<td>n.a.</td>
<td>0 (0%)</td>
<td>11 (16%)⁴</td>
</tr>
<tr>
<td><strong>Platelet count (cells/µl)</strong></td>
<td>194 (170–225)</td>
<td>192 (188–221)</td>
<td>214 (186–254)²</td>
</tr>
<tr>
<td><strong>Serum BUN level (mM)</strong></td>
<td>4.1 (3.5–4.8)</td>
<td>2.7 (2.1–3.2)</td>
<td>3.4 (2.7–4.1)¹</td>
</tr>
<tr>
<td><strong>Serum creatinine level (µM)</strong></td>
<td>66 (61–72)</td>
<td>47 (41–51)²</td>
<td>63 (55–70)⁴</td>
</tr>
<tr>
<td><strong>Serum bilirubin level (µM)</strong></td>
<td>9.3 (6.6–12.4)</td>
<td>5.1 (3.8–6.6)</td>
<td>7.4 (5.8–9.3)²</td>
</tr>
<tr>
<td><strong>Serum AST activity (U/l)</strong></td>
<td>17 (15–20)</td>
<td>19 (16–21)</td>
<td>19 (15–24)</td>
</tr>
<tr>
<td><strong>Serum ALT activity (U/l)</strong></td>
<td>14 (12–17)</td>
<td>12 (10–16)</td>
<td>15 (11–21)³</td>
</tr>
<tr>
<td><strong>Serum LDH activity (U/l)</strong></td>
<td>154 (128–170)</td>
<td>159 (131–174)</td>
<td>192 (155–225)²</td>
</tr>
<tr>
<td><strong>Serum CRP level (mg/l)</strong></td>
<td>0.7 (0.5–1.8)</td>
<td>3.6 (1.7–7.3)</td>
<td>6.7 (3.0–12.1)⁵</td>
</tr>
<tr>
<td><strong>Plasma ADAMTS13 activity (%)</strong></td>
<td>91.6 (78.5–104.4)</td>
<td>96.3 (85.6–116.2)</td>
<td>98.8 (76.5–112.8)</td>
</tr>
<tr>
<td><strong>Plasma VWF:Ag level (%)</strong></td>
<td>70.0 (60.2–87.3)</td>
<td>129.3 (105.1–182.8)³</td>
<td>187.1 (145.6–243.1)³</td>
</tr>
</tbody>
</table>

Data are presented as median (25–75 percentile) for continuous variables and as number (percent) for categorical variables. n.a.: not applicable; ADAMTS13: A Disintegrin-like And Metalloprotease with ThromboSpondin type 1 motif, member 13; BMI: body mass index; BUN: blood urea nitrogen; AST: aspartate aminotransferase; ALT: alanine aminotransferase; LDH: lactate dehydrogenase; CRP: C-reactive protein; VWF:Ag: von Willebrand factor antigen. ¹ p<0.05 versus healthy non-pregnant women. ² p<0.001 versus healthy non-pregnant women. ³ p<0.05 versus healthy pregnant women. ⁴ p<0.001 preeclamptic patients versus healthy pregnant women. ⁵ p<0.001 preeclamptic patients versus healthy non-pregnant women.
used. To compare continuous variables between two groups, the Mann-Whitney U-test was applied, whereas to compare them among multiple groups, the Kruskal-Wallis analysis of variance (ANOVA) by ranks test was performed. Multiple comparisons of mean ranks for all groups were carried out as post-hoc tests. The Fisher exact and Pearson Chi² tests were used to compare categorical variables between groups. The Spearman rank order correlation was applied to calculate correlation coefficients. As plasma ADAMTS13 activity and VWF:Ag levels showed a skewed distribution, we performed analysis of covariance (ANCOVA) and multiple linear regression analysis with logarithmically transformed data.

Statistical analyses were carried out using the following software: STATISTICA (version 7.1; StatSoft, Inc., Tulsa, OK, USA) and Statistical Package for the Social Sciences (version 15.0 for Windows; SPSS, Inc., Chicago, IL, USA). For all statistical analyses, p<0.05 was considered statistically significant.

In the article, data are reported as median (25–75 percentile) for continuous variables and as number (percent) for categorical variables.

**Results**

**Patient characteristics and standard laboratory parameters**

The clinical characteristics and standard laboratory parameters of the study participants are described in Table 1. There was no statistically significant difference in age between preeclamptic patients and healthy pregnant and non-pregnant women. However, as shown in Table 1, most of the clinical features and measured laboratory parameters differed significantly among the three study groups. Nevertheless, no significant difference was observed in the percentage of patients with blood group 0 between the preeclamptic and the healthy pregnant and non-pregnant groups (data not shown).

**Plasma ADAMTS13 activity, VWF:Ag levels and VWF multimeric pattern**

The results of the ADAMTS13 and VWF:Ag assays are displayed in Table 1, Figure 1A and B. No significant difference was found in plasma ADAMTS13 activity among the three study groups.

![Figure 1: Plasma ADAMTS13 activity (A) and von Willebrand factor antigen (VWF:Ag) levels (B) of healthy non-pregnant and pregnant women and preeclamptic patients. Middle point: median; Box: interquartile range (25–75 percentile); Whisker: range.](image)
groups. However, plasma VWF antigen levels were significantly higher in preeclamptic patients than in healthy pregnant and non-pregnant women. Adjustment for age, body mass index (BMI) and gestational age at blood draw in analyses of covariance (ANCOVA) did not change these results. The preeclamptic and healthy pregnant groups were divided into gestational age subgroups, since gestational age at blood draw was significantly different between the two groups. Plasma ADAMTS13 activity and VWF:Ag levels were also compared between the two study groups in these gestational age categories separately. There were no significant differences in plasma ADAMTS13 activity between preeclamptic patients and healthy pregnant women in any of these categories. Nevertheless, preeclamptic patients had significantly higher plasma VWF:Ag levels than normotensive, healthy pregnant women in each gestational age category.

In the group of preeclamptic patients, no statistically significant differences were observed in plasma ADAMTS13 activity and VWF:Ag levels between patients with mild and severe preeclampsia, between patients with late and early onset of the disease, or between preeclamptic patients with and without fetal growth restriction (data not shown).

We determined the multimeric pattern of VWF in five preeclamptic patients, five healthy pregnant women and five healthy non-pregnant women, who were matched for age, gestational age and smoking status. The VWF multimeric pattern was found to be normal in each studied woman. The percentage of large multimers did not show significant difference between the preeclamptic and the healthy pregnant and non-pregnant women (median (range): 24.7 (18.5–33.7) %, 27.3 (22.2–30.2) % and 25.5 (20.2–28.8) %, respectively; p=0.45). Representative examples of the intact multimeric structure of VWF in our study groups are presented in Figure 2.

**Relationship of clinical characteristics, standard laboratory parameters and plasma VWF:Ag levels to plasma ADAMTS13 activity**

We also investigated whether clinical characteristics, standard laboratory parameters and plasma VWF:Ag levels are related to plasma ADAMTS13 activity in our study groups. There was a trend for primiparas to have lower plasma ADAMTS13 activity than multiparas both in the healthy pregnant and the preeclamptic groups, and this association reached statistical significance in the total group of pregnant women involved in the study (median 25–75 percentile): 92.6 (75.8–110.6) % versus 104.2 (92.1–120.8) %; p=0.011). There was a statistically significant negative correlation between plasma VWF:Ag levels and ADAMTS13 activity in the group of preeclamptic patients (Spearman R=–0.26; p=0.040), but the significance disappeared after adjustment for maternal age, primiparity, BMI and gestational age at blood draw in multiple linear regression analysis (standardized regression coefficient (β)=−0.11; p=0.48). Nevertheless, no significant correlations were found between these two variables either in the groups of healthy pregnant and non-pregnant women, or in the total group of study participants (Fig. 3). There was no other relationship between clinical features and measured laboratory parameters of the study subjects and plasma ADAMTS13 activity in either study group.

**Discussion**

In the present study, we did not find a significant difference in plasma ADAMTS13 activity between preeclamptic patients and healthy pregnant and non-pregnant women, whereas plasma VWF antigen levels were significantly higher in the preeclamptic group compared with the healthy pregnant and non-pregnant groups. The multimeric pattern of VWF was normal both in preeclamptic patients and healthy pregnant women.

Lattuada et al. (2003) reported significantly lower plasma ADAMTS13 activity in patients with HELLP syndrome com-
The mechanisms regulating ADAMTS13 activity are not completely understood at present, particularly in pregnancy, where the role of placental hormones needs also to be considered. According to our results, consumptive regulation by increased VWF:Ag levels seems to be a secondary phenomenon in preeclampsia, as suggested by the lack of correlation between VWF:Ag levels and ADAMTS13 activity in the adjusted model. Furthermore, liver and renal failure, as well as disseminated intravascular coagulation (DIC) were absent in our preeclamptic group, and serum C-reactive protein levels were only slightly increased, which are consistent with normal plasma ADAMTS13 activity. Additionally, ultralarge VWF multimers were not present in the plasma of our preeclamptic patients, which also supports the absence of a severe ADAMTS13 deficiency in preeclampsia. Taken together, our results indicate that ADAMTS13 activity is differentially regulated in preeclampsia from VWF:Ag level, the marker of endothelial activation/dysfunction (36).

A key issue is why certain patients with preeclampsia develop the serious, even life-threatening condition, the syndrome of haemolysis, elevated liver enzymes, and low platelet count. HELLP syndrome is a distinct entity and is characterized by a much more intense systemic inflammation than preeclampsia with more pronounced endothelial injury and signs of a compensated DIC, all of which are known to influence plasma ADAMTS13 activity (37, 38). Interestingly, an association has been observed previously between ADAMTS13 deficiency and the severity of inflammatory host response independently of its origin. Low ADAMTS13 activity was supposed to contribute to both activation of coagulation and platelets in patients with severe sepsis, resulting in consumptive thrombocytopenia (19).

VWF and its cleaving protease appear to interact to form a fine-tuned VWF/ADAMTS13 system. In patients developing HELLP syndrome, the transition from chronic endothelial activation—characteristic of preeclampsia—to acute activation could result in increased levels of newly released, active VWF (24). On the basis of our findings along with those of Lattuada et al. (2003) and Hulstein et al. (2006), we hypothesize that the combined...
In conclusion, plasma VWF cleaving protease (ADAMTS13) activity is normal in preeclampsia despite the increased VWF:Ag levels. However, further prospective studies are needed to determine whether a decrease in plasma ADAMTS13 activity precedes the development of HELLP syndrome in preeclamptic patients, and thus can help to predict this serious complication in preeclampsia.

Acknowledgements

The skilful technical assistance of Szijgiit-Antalné and Margit Kovács is acknowledged with many thanks. This work was supported by research grants from the Hungarian Scientific Research Fund (NF 72689) and the Faculty of Medicine of the Semmelweis University.

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