ADAMTS13 activity and genetic mutations in Japan

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ADAMTS13, genetic mutation, thrombotic thrombocytopenic purpura, TTP

Summary
Thrombotic thrombocytopenic purpura (TTP), a life threatening disease, can be induced by congenital or acquired deficiency of plasma metalloprotease ADAMTS13. Since the publication of the first genetic analysis in patients with congenital ADAMTS13 deficiency in 2001, more than 100 genetic defects in the ADAMTS13 gene have been reported worldwide. Genetic analysis in patients with ADAMTS13 deficiency has greatly contributed to the understanding of the etiology of TTP. A rapid and quantitative assay method for the plasma ADAMTS13 activity was developed recently in 2005 and opened a new area of TTP research – namely genetic research using a general population to evaluate age and gender differences of ADAMTS13 activity as well as phenotype – genotype correlations of genetic polymorphisms and estimation of a homozygote or a compound heterozygote ADAMTS13 deficiency. The Japanese general population study included 3616 individuals with an age between 30 – 80 years confirming other studies that while ADAMTS13 activity decreased with age, VWF antigen increased and VWF antigen levels are lowest in blood group O individuals, whereas ADAMTS13 activity levels were not associated with the AB0 blood group. 25 polymorphisms with a minor allele frequency of more than 0.01 were found, among them 6 missense mutations and 19 synonymous mutations, except P475S missense polymorphisms that was only identified in an East Asian population, characterized by reduced ADAMTS13 activity. Prevalence of congenital ADAMTS13 deficiency in the Japanese population was estimated about one individual in 1.1 x 10^6 to be homozygote or compound heterozygote for ADAMTS13 deficiency. So far more than 40 mutations in Japanese congenital TTP patients were found, but R193W, Q449*, C754Afs*24 (c.2259delA) and C908Y were identified in more than four patients suggesting the precipitation of these mutations in the Japanese population.

Schlüsselwörter
ADAMTS13, Mutation, thrombotisch-thrombocytopenische Purpura, TTP

Zusammenfassung
VWF cleavage by ADAMTS13

A disintegrin-like and metalloprotease with thrombospondin type 1 motif-13 (ADAMTS13) is a plasma metalloprotease that cleaves a specific Tyr-Met bond in the A2 domain of von Willebrand factor (VWF). The gene, which consists of 29 exons, is located on chromosome 9q34.2, and is only 129 kb distant from the ABO blood group gene.

ADAMTS13 is 1427 amino acid residues in length and consists of a
- signal peptide, a propeptide,
- disintegrin-like domain,
- cysteine-rich domain,
- disulfide  bond that forms a link between the scissile bond, Tyr1605-Met1606, for ADAMTS13 is exposed (22).

The basic concept for the cleavage is that the scissile bond in the A2 domain is cryptic and sequestered and the shear stress partially unfolds the A2 domain, resulting in exposure of this bond (21, 22).

ADAMTS13 is constitutively active in plasma and the exposed scissile bond in VWF can be easily cleaved by ADAMTS13. Thus, the shear stress-dependent substrate-binding mechanism of ADAMTS13 in vivo is very unique.

VWF73 as a minimal substrate for ADAMTS13

Although identification of the proteolytic cleavage site, the Tyr1605-Met1606 bond, in VWF was reported more than two decades ago (23), a plasma assay for the ADAMTS13 activity was a laborious and time-consuming work (24, 25). In order to develop a fast, quantitative, and synthetic substrate for the VWF-cleaving activity of ADAMTS13, a minimal sequence specifically recognized and cleaved by ADAMTS13 should be determined in VWF.

To identify the region in VWF required for ADAMTS13 cleavage, we expressed a series of deletion mutants of the A2 domain and found that a 73-amino-acid fragment from Asp1596 to Arg1668 was essential for the cleavage of the Tyr1605-Met1606 bond by ADAMTS13 (26). We named this fragment VWF73. A 64-amino-acid fragment from Asp1596 to Arg1659 was not efficiently cleaved by ADAMTS13. We determined the solution structure of the 1H and 15N double-labeled substrates VWF73 and VWF64, each of which included a C-terminal 6xHis tag, by nuclear magnetic resonance. The results

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indicated an extended structure for both peptides, suggesting an induced-fit substrate recognition mechanism (27).

VWF73 has all the characteristics of an ADAMTS13 substrate. We developed a chemically modified VWF73 for use in a fluorescence resonance energy transfer (FRET) assay for ADAMTS13 activity. This substrate, FRETS-VWF73, quantitatively and reproducibly yielded the activity of plasma ADAMTS13 within one hour (24), which constitutes a remarkable improvement in rapidity and accuracy over the previous assays (28, 29). Using this assay, we were able to show that patients with congenital TTP exhibited severely decreased (<5% of the reference value) or undetectable ADAMTS13 activities. A slightly modified method using FRETS-VWF73 can quantitatively detect ADAMTS13 activity of less than 5% of the reference value (30).

An enzyme immunoassay of ADAMTS13 activity using a monoclonal antibody that specifically recognizes Tyr1605, the C-terminal residue of the cleaved A2 domain, has been developed and a chromogenic ADAMTS13-act-ELISA using a glutathione-conjugated VWF73 peptide as the substrate has also been reported (25).

These assays are utilized for the clinical diagnosis of TTP.

**ADAMTS13 activity in the Japanese population**

The Suita Study is an epidemiological study consisting of Japanese residents between the ages of 30 and 79 years who were randomly selected from the municipality population registry and stratified into groups by sex and age in 10-year increments. We have used FRETS-VWF73 to measure the plasma ADAMTS13 activity in 3616 individuals from this general population with age ranged from 30 to 80 years.

- When the mean of all plasma ADAMTS13 activity values was set at 100%, the mean activity of men (93 ± 24%, n = 1687) was significantly lower than that of women (106 ± 27%, n = 1929) (31).

- The plasma ADAMTS13 activity tended to decrease with age, especially after age 60, in both men and women. The mean ADAMTS13 activity value was
  - 110% for subjects in their 40s,
  - 109% for those in their 50s,
  - 101% for the 60s,
  - 93% for the 70s, and
  - 85% for the 80s.

We also measured the plasma VWF antigen level in this population. The VWF antigen level increased with age, as reported previously. Because of the combined effects of the increase in VWF antigen level and the decrease in ADAMTS13 activity, the ratio of VWF antigen-to-ADAMTS13 activity was dramatically increased with age (31). This may partly explain the prothrombotic state of elderly men and women. As the FRETS-VWF73 assay itself was not affected by VWF concentration in plasma samples (0–160 ug/ml) (31), the reduced ADAMTS13 activity in the plasma of elderly subjects was not considered to be due to the assay-dependent artifactual phenomenon. In fact, when age-adjusted VWF antigen level was compared among quartiles of ADAMTS13 activity in the population, no significant association between VWF antigen and ADAMTS13 activity levels was observed in men or in women.

The AB0 blood group is a well-known genetic determinant for plasma VWF antigen levels:

- Individuals with blood group 0 have a lower VWF level than those with non-0 groups (32).

The AB0 blood group gene is located approximately 129 kb from the ADAMTS13 gene, and this may suggest a possible correlation between the two genes. In our population, the individuals with blood group 0 exhibited a significantly lower VWF antigen level than those with non-0 groups, as shown in previous studies. In contrast, the plasma ADAMTS13 activity was not associated with the AB0 blood group (31). This was consistent with the observation that ADAMTS13 antigen levels were not associated with AB0 blood group in 387 male Dutch individuals (33). The results are also consistent with the fact that VWF (34), but not ADAMTS13 (35), contains AB0 blood group-related N-linked oligosaccharides.

**Control plasma for ADAMTS13 assay**

The level of ADAMTS13 activity in the general population varied widely, ranging from approximately 40% to 240% of the normal level (Fig. 1). In general, the plasma ADAMTS13 activity is expressed as a percentage of the activity in commercially available or locally prepared, pooled normal plasma (control plasma). Therefore, if there is a wide range of ADAMTS13 activity among the control plasma samples be-
fore pooling, this can create a serious problem for ADAMTS13 measurement. It is easy to assume that the control plasma samples prepared from a relatively small number of individuals would show large deviation. In order to estimate the ideal number of individuals for the preparation of the control plasma, we randomly selected the ADAMTS13 activity values from 10 individuals (5 men and 5 women) in the general population cohort consisting of 3616 individuals, and repeated this selection 10 times to obtain the mean ± 2 standard deviation. The results of the 10-times repeated selection showed that 80% was the value of the mean – 2 standard deviations and 125% was the value of the mean + 2 standard deviations. These results indicated that the control plasma randomly prepared from 10 individuals represented a wide variation of activity and thus was not suitable for use as a control.

When we selected the activities of 20 individuals (10 men and 10 women), the activities of the mean + and – 2 standard deviations were narrowed down to 89% and 113%, respectively, which might have been sufficient for diagnostic purposes. When we selected 40 individuals or 100 individuals, the activity ranges were reasonably narrowed and were useful for the rigorous analysis of the ADAMTS13 activity (40 individuals: mean ± 2 standard deviations, 91–108%; 100 individuals: mean ± 2 standard deviations, 94–104%). As described, sex and age influence plasma ADAMTS13 activity. Therefore, the control plasma for plasma ADAMTS13 activity can be prepared from at least 20 individual plasma samples in consideration of age and sex.

Currently, a new project, "Development of the WHO 1st International Standard for ADAMTS13 in Plasma" led by the VWF Subcommittee of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis has been initiated by Dr. Johanna Kremer Hovinga.

Genotype-phenotype correlation of polymorphisms

The ADAMTS13 gene contains several genetic missense polymorphisms (3, 36, 37) some of which may influence the VWF cleaving activity. Since low plasma ADAMTS13 and high VWF levels are related to ischaemic stroke and myocardial infarction (38–40), missense polymorphisms in the ADAMTS13 gene could be important. Twenty missense polymorphisms of the ADAMTS13 gene have been listed (37), and some of their possible structural defects have been examined in silico (41).

To identify genetic polymorphisms in the Japanese population, we sequenced the ADAMTS13 gene in 346 individuals and identified 25 polymorphisms with a minor allele frequency of >0.01 (42):

• 6 were missense polymorphisms and
• 19 were synonymous mutations.

We further genotyped six missense polymorphisms in a large Japanese cohort consisting of 3616 individuals whose plasma ADAMTS13 activities had been measured. We found that the minor allele frequencies were

• 0.192 for Q448E (c.1342C>G),
• 0.05 for P475S (c.1423C>T),
• 0.048 for S903L (c.2708C>T),
• 0.027 for P618A (c.1852C>G) and
• 0.022 for G1181R (c.3541G>A).

The T339R and P618A polymorphisms were in absolute linkage disequilibrium. When we examined the association of these polymorphisms with plasma ADAMTS13 activity, the ADAMTS13 activity of Q448E heterozygotes (QE) and minor allele homozygotes (EE) was significantly higher than that of major allele homozygotes (QQ):

• QQ: 97.6% ± 25.9%;
• QE: 104.2% ± 27.4%;
• EE: 105.7% ± 27.5%.

In contrast, the ADAMTS13 activity of P475S heterozygotes (PS) and minor allele homozygotes (SS) was significantly lower than that of major allele homozygotes (PP):

• PP: 101.4% ± 26.6%;
• PS: 87.2% ± 23.3%;
• SS: 73.3% ± 20.3% (42).

Four other missense polymorphisms did not affect the ADAMTS13 activity.

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Tab. 1 Non-synonymous mutations identified in four segregated groups with different ranges of ADAMTS13 activity

<table>
<thead>
<tr>
<th>group (average activity)</th>
<th>mutation</th>
<th>predicted damage</th>
<th>references</th>
</tr>
</thead>
<tbody>
<tr>
<td>maximum (183%)</td>
<td>L19F</td>
<td>benign</td>
<td>newly identified</td>
</tr>
<tr>
<td></td>
<td>R268Q</td>
<td></td>
<td></td>
</tr>
<tr>
<td>median (97.6%)</td>
<td>Q723K</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N1321S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd minimum (53.1%)</td>
<td>I380T</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Y1074Afs*46</td>
<td></td>
<td>causative for congenital ADAMTS13 deficiency (46)</td>
</tr>
<tr>
<td></td>
<td>R1274C</td>
<td>possibly damaging</td>
<td>newly identified</td>
</tr>
<tr>
<td>minimum (47.1%)</td>
<td>F324L</td>
<td>probably damaging</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F418L</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>I673F</td>
<td>possibly damaging</td>
<td>causative for congenital ADAMTS13 deficiency (47)</td>
</tr>
<tr>
<td></td>
<td>Q773*</td>
<td></td>
<td>newly identified</td>
</tr>
<tr>
<td></td>
<td>Y1074Afs*46</td>
<td></td>
<td>causative for congenital ADAMTS13 deficiency (46)</td>
</tr>
<tr>
<td></td>
<td>R1095Q</td>
<td>possibly damaging</td>
<td>newly identified</td>
</tr>
</tbody>
</table>

*stop codon
As described, the AB0 blood group gene is located near the ADAMTS13 gene. T339R and P618A were associated with the blood group A allele and P475S and S903L tended to be associated with the blood group 0 allele in our study (42).

The P475S missense polymorphism is ethnic specific, having only been identified in an East Asian population. The reduced plasma ADAMTS13 activity in individuals with the P475S mutation is consistent with the finding that the recombinant ADAMTS13-P475S mutant showed approximately 70% of the activity of the wild-type ADAMTS13 (43). To further elucidate the molecular basis of the reduced activity of the ADAMTS13-P475S mutant, we recently determined the enzymatic parameters of ADAMTS13-MDTCs (residues 75–685) and MDTCS-P475S and solved the crystal structure of the P475S mutant of the ADAMTS13-DTCS domain (44). MDTCS-P475S exhibited a reaction rate similar to that of wild-type MDTCS but showed twofold lower affinity for FRETS-VWF73, indicating that Pro475 is involved in formation of the substrate-binding exosite. The crystal structures showed that the conformation of the P475S-containing loop was significantly different between the mutant and the wild-type. This explains the higher susceptibility of the enzymatic activity of MDTCS-P475S to environmental conditions such as denaturants and high temperature. MDTCS-P475S can moderately cleave shear-treated VWF.

Incompatible evidences between in vivo and in vitro studies are accumulating on one of the missense polymorphisms, P618A. PolyPhen-2, an in silico tool which predicts the possible impact of an amino acid substitution on the structure and function of a human protein, predicted P618A as a damaging mutation. The crystal structure of the S domain of ADAMTS13 showed that Pro618 adopted the cis conformation (41), and the substitution of Pro618 with Ala, which cannot adopt the cis conformation, may cause structural distortion. Indeed, a transient expression study of the ADAMTS13-P618A mutant showed lower levels of activity and antigen in the conditioned media of HEK293 cells (45). However, as described, the P618A mutation was not associated with plasma ADAMTS13 activity in the general population. This inconsistent observation should be properly addressed in the future experiments.

**ADAMTS13 deficiency in a Japanese population**

The ADAMTS13 activity-genotype analysis based on ~3200 individuals enabled us to estimate the frequency of congenital ADAMTS13 deficiency. We selected 128 individuals according to their plasma ADAMTS13 activity:

- 32 individuals of the “minimum” activity group (average activity, 47.1%),
- 32 individuals of the “second minimum” activity group (average activity, 53.1%),
- 32 individuals of the “median” activity group (average activity, 97.6%), and
- 32 individuals of the “maximum” activity group (average activity, 183%).
Sequence analysis of the ADAMTS13 gene in these individuals showed that 14 individuals had rare non-synonymous mutations: seven individuals in the minimum activity group, three individuals in the second minimum activity group, two individuals in the median activity group, and two individuals in the maximum activity group (Table 1). In particular, three of the subjects had causative mutations for congenital ADAMTS13 deficiency, Y1074Af*46 (46) and I673F (47). These data indicated that 2 of every 32 individuals had a mutation that does not cause a functional defect of ADAMTS13. Therefore, it would be a reasonable assumption that five individuals in the minimum activity group and one individual in the second minimum activity group would be heterozygotes carrying a mutation with a functional defect. If this assumption is valid, 6 out of 3200 individuals would be heterozygotes for ADAMTS13 deficiency. This estimation suggested that ~1 individual in 1.1 × 106 should be a homozygote or a compound heterozygote for ADAMTS13 deficiency. If a part of homozygous/compound heterozygous mutation carriers would die during the neonatal period, the prevalence in the surviving population may be lowered. It has been reported that the E1382Rf*6 mutation (the E1382R frameshift mutation giving rise to the stop codon at six amino acid residues thereafter) due to the 4143insA mutation is frequent among patients with congenital ADAMTS13 deficiency in Northern and Central European countries (48). The estimation of the prevalence of patients with congenital ADAMTS13 deficiency may be biased due to insufficient sample sizes, ethnicity, lethality, and other factors.

ADAMTS13 mutations in congenital TTP

Since the publication of the first genetic analysis in patients with Upshaw-Schulman syndrome in 2001 (3), more than 100 genetic defects in the ADAMTS13 gene have been reported worldwide (36, 37, 49). The genetic variants that lead to TTP are very broadly distributed, occurring everywhere from the N-terminal signal peptide to the C-terminal CUB domain. The missense mutations are most frequent (about 60%), but other non-synonymous mutations such as frameshift mutations (small deletions or insertions), nonsense mutations, abnormal splicing, and insertions/deletions, are also detected (Fig. 2).

We have so far identified more than 40 genetic mutations in Japanese patients with congenital TTP.

Most of the mutations were found in a single patient, but four mutations, i.e., R193W, Q449*, C754Af*24 (c.2259delA), and C908Y, were identified in more than four patients, suggesting the accumulation of these mutations in a Japanese population (46).

Acknowledgments

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Conflict of interest

The National Cerebral and Cardiovascular Center where TM and KK (inventors) belong has an awarded patent on the use of reagent, FRETS-VWF73. MM is al clinical advisory board for Alexion Pharmaceuticals, and has a patent on the use of chimeric ADAMTS13 activity assay using the nonoclonal antibody, which specifically recognizes TYR1605 within VWF-A2 domain, exposed by ADAMTS13 cleavage. YF is a clinical advisory board for Baxter Bioscience and for Alexion Pharmaceuticals, and has a patent on the use of carboxyl-terminal domains of ADAMTS13 are bound to the surface of globular VWF. Blood 2009; 114: 2819–2828.

References


