A Novel Homozygous Missense ADAMTS13 Mutation Y658C in a Patient with Recurrent Thrombotic Thrombocytopenic Purpura

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Abstract. Thrombotic thrombocytopenic purpura (TTP) is a devastating systemic disorder that is characterized by microangiopathic hemolytic anemia, thrombocytopenia, neurological dysfunction, and renal failure. In the hereditary form of TTP, severe deficiency of ADAMTS13, a plasma metalloprotease that cleaves von Willebrand factor, is associated with the development of this disorder. A 34-year-old woman was diagnosed with TTP due to severely reduced ADAMTS13 activity; clinical manifestations resolved only by repeated total plasma exchanges or transfusion. Homozygous and heterozygous Y658C (c.1973A>G) alleles were detected in the patient and her child with severe and mild ADAMTS13 deficiencies, respectively. Herein, we report a novel missense mutation Y658C (c.1973A>G) on exon 17 of ADAMTS13 and discuss its clinical implications.

Keywords: ADAMTS13, thrombotic thrombocytopenic purpura, missense mutation, pregnancy

Introduction

Thrombotic thrombocytopenic purpura (TTP) is a life-threatening disease that is characterized by thrombocytopenia, neurological dysfunction, renal failure and thrombotic microangiopathy, and it can be classified into hereditary or acquired forms [1]. Hereditary TTP, also known as Upshaw-Schulman syndrome (USS, MIM# 274150) reveals a severe deficiency of ADAMTS13 activity caused by various kinds of mutations [2]. Mutations of the ADAMTS13 gene (a disintegrin-like and metalloprotease with thrombospondin type 1 motif, 13, MIM#604134), located at chromosome 9q34, have been suggested to result in deficiencies of this plasma zinc metalloprotease responsible for the cleavage of von Willebrand factor (vWF) [3-4]. To date, more than 70 mutations of the ADAMTS13 gene have been identified in hereditary TTP [5]. However, such mutations have not been characterized sufficiently to establish the genotype-phenotype correlation. Additionally, 20 missense, three frameshift, one nonsense and 16 synonymous SNPs are annotated in the coding regions of ADAMTS13 in dbSNP (http://www.ncbi.nlm.nih.gov/SNP/, accessed April 30, 2010). In this report, we present a novel homozygous missense mutation Y658C (c.1973A>G) on exon 17 of ADAMTS13 in a patient with recurrent TTP.

Methods and Results

Clinical presentation. In January 2008, a 34-year-old Vietnamese pregnant woman at 25 weeks gestation was admitted due to an abrupt onset of severe microangiopathic hemolytic anemia and thrombocytopenia. She had not been diagnosed with or treated for any hematologic disorders prior to this pregnancy. She first experienced anemia accompanied by nausea and vomiting at 12 weeks gestation in Vietnam, but because her manifestations had resolved, she emigrated from Vietnam to Korea without further investigation. On admission, her initial complete blood count (CBC) revealed a hemoglobin level of 7.0 g/dL.
with 19.99% reticulocytes, a platelet count of 12,000 /μL, and a white blood cell (WBC) count of 9,116 /μL (corrected for the presence of 12 nucleated red blood cells per 100 WBCs). Peripheral blood smear revealed schistocytes and polychromasia. Increased total and indirect bilirubin (3.0 and 2.4 mg/dL), lactate dehydrogenase (5,772 U/L) and D-dimer (6.46 μg/mL) were noted. A severe deficiency (<5%) of ADAMTS13 activity was detected, but ADAMTS13 inhibitor was negative (<0.4 inhibitor units). Finally, she was diagnosed with TTP due to severely low ADAMTS13.

After diagnosis, total plasma exchanges (TPE) were performed. Her symptoms resolved after eight sessions of TPE; however, TPE should have been performed repeatedly because her manifestations recurred. Following the resolution of her symptoms, she delivered a full-term male baby and now receives plasma transfusions on a regular basis. Initially, there were no signs or symptoms suggestive of TTP in her newborn.

**ADAMTS13 Mutations analysis.** Peripheral blood samples were obtained from the proband and her one-year-old child with informed consent. Genetic analysis by direct sequencing of all 29 exons of the ADAMTS13 gene and their flanking regions was carried out using the patient’s extracted product according to the published method [4]. Direct sequencing of the ADAMTS13 gene in this patient revealed a homozygous missense mutation Y658C (c.1973A>G) on exon 17, where the spacer domain of the ADAMTS13 protein is encoded. Along with this mutation, a homozygous P475S (c.1423C>T) polymorphism on exon 12 and three homozygous synonymous SNPs (c.420C>T, c.1716A>G, and c.2280C>T) were also identified. To confirm this novel sequence variation, exons 17 and 18 of ADAMTS13 were amplified repeatedly and sequenced from genomic DNA from a normal healthy individual, the patient, and her newborn (Figure 1). The sequences from the normal individual revealed wild-type (adenine at cDNA position 1973) alleles. In contrast, the sequences from the patient and her newborn contained homozygous and heterozygous missense mutations (c.1973A>G), respectively (GenBank Accession No. GU592206).

**In silico prediction of altered protein function.** To predict the deleterious effect of this sequence variation, substitution of the tyrosine at position 658 with cysteine (Y658C) and a polymorphism P475S were submitted to the web-based program Sorting Intolerant From Tolerant (SIFT, http://sift.jcvi.org) and Polyphen (http://genetics.bwh.harvard.edu/pph/) [6-7]. Y658C was predicted to affect the protein function with a score of 0.02 in SIFT (score <0.05: deleterious substitution) and to be probably damaging with a 2.616 Position-Specific Independent Counts (PSIC) score difference in Polyphen (PSIC score >2.0: probably damaging), while P475S was predicted to be tolerated (0.90) and benign (1.309).

**ADAMTS13 functional activity level in patient (homozygous) and patient’s child (heterozygous).** ADAMTS13 activity in the patient and her newborn was tested. Samples were transferred to the Blood Center of Wisconsin (Blood Center of Wisconsin, WI, USA) and measured directly using a fluorescence resonance energy transfer (FRET) assay, in which a fluorescent signal is detected when a synthetic substrate (FRETS-VWF73) is cleaved by ADAMTS13 [8-9]. The inhibitor activity was determined by mixing studies, where one inhibitor unit is defined as the concentration of inhibitor able to reduce the ADAMTS13 activity of an equal volume of normal pooled plasma by half. In this patient, markedly reduced ADAMTS13 activity was detected (<5%, reference range >66%) at the initial diagnosis. Her one-year-old child also exhibited reduced ADAMTS13 activity (48%).

**Discussion**

The plasma metalloproteinase ADAMTS13 is essential in modulating the vWF multimeric size, and
its mutation plays a critical role in the pathogenesis of TTP. The \textit{ADAMTS13} protein cleaves at a single site in the vWF A2 domain (AA1498-1665; UniProtKB/Swiss-Pro database; Accession: P04275) between Y1605 and M1606. As noted, the measurement of \textit{ADAMTS13} activity is important for the diagnosis and treatment of microangiopathies including TTP. Measurements of human \textit{ADAMTS13} activity and inhibitor levels have been proposed to predict clinical outcome and to assist in tailoring the treatment of TTP [10-11]. Of interest, recent literature reports have linked \textit{ADAMTS13} deficiency to inflammation [12-15], stroke [3], and cirrhosis [16]. In this study, the patient revealed decreased \textit{ADAMTS13} activity with a negative inhibitor level.

Mutations of the \textit{ADAMTS13} gene, which are distributed throughout the gene without a distinct hot spot, are known to be a cause of \textit{ADAMTS13} deficiency in hereditary TTP. Until now, the majority of known cases have exhibited compound heterozygous mutations. Homozygous mutations have been reported rarely and only in consanguineous families [2]. However, to reveal genotype-phenotype relationships, suitable cases with homozygous \textit{ADAMTS13} mutation need to be collected and characterized [5].

Even though defects in \textit{ADAMTS13} are present from birth, some patients with hereditary TTP experience their first episode in their second or third decade of life [17-19]. Delayed presentation of TTP and the heterogeneity of its manifestations suggest that another triggering factor is needed to induce overt TTP in individuals with reduced \textit{ADAMTS13} activity. Although its mechanism is not well understood, pregnancy has frequently been shown to induce thrombocytopenia and clinically overt TTP [20]. The patient in this study experienced her first TTP episode in her third decade, during the second trimester of pregnancy. After the first episode, she experienced TTP with a chronically relapsing course. As for the protein structure, \textit{ADAMTS13} consists of an N-terminal signal peptide metalloproteinase, a disintegrin-like and a thrombospondin type 1 (TSP1) motif, a cysteine-rich/spacer domain and additional TSP motifs, and CUB domains [3]. We found the missense mutation Y658C (c.1973A>G) allele on exon 17 of \textit{ADAMTS13}, which encodes the spacer domain. The cysteine-rich/spacer domain of \textit{ADAMTS13} has been suggested to be essential for its function and important in protein folding and maintaining its stability [21]. Therefore, the homozygous sequence variation of Y658C could be the cause of this patient’s \textit{ADAMTS13} deficiency and recurrent TTP. Using sequence homology-based bioinformatics tools, the amino acid substitution at position 658 from tyrosine to cysteine is highly suspected to have a damaging effect. Additionally, the patient’s child with heterozygosity of Y658C revealed approximately 50% \textit{ADAMTS13} activity.

In summary, we suggest that an Y658C mutation in the spacer domain has a deleterious effect on the activity of \textit{ADAMTS13}, based on clinical manifestation and in silico analysis. To our knowledge, this is a novel homozygous missense mutation in a patient with recurrent TTP. Both functional and population studies of this sequence variation should be investigated to reveal its biological role in the pathogenesis of TTP.

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