ADAMTS13 mutations identified in familial TTP patients result in loss of VWF-cleaving protease activity

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Thrombotic thrombocytopenic purpura (TTP) is a life threatening systemic illness often associated with a decreased capacity of a proteolytic activity in plasma to cleave the blood clotting protein von Willebrand Factor (VWF). Recently the gene responsible for the familial form of TTP was identified as ADAMTS13, a novel member of the ADAMTS metalloproteinase family; and to date, 25 TTP-causing ADAMTS13 mutations have been found. Here we report 10 additional mutations, bringing the total number of TTP-causing ADAMTS13 mutations to 35. In order to explore the structure and function of ADAMTS13 we prepared full-length ADAMTS13 cDNA expression vectors. Additionally, site directed mutagenesis was used to introduce into the expression vectors each of nine missense mutations we identified previously in patients with familial TTP (H96D, R102C, T196I, R398H, R528G, R692C, C951G, C1213Y, and R1219W). These wild-type and mutant constructs were transiently transfected into CHO and COS-1 cells, and cell lysates and conditioned media were analyzed by SDS-PAGE. Western blotting was performed using antisera raised against a peptide derived from the predicted ADAMTS13 sequence. VWF-cleaving protease activity was measured using purified VWF as a substrate as described previously. Recombinant wild-type ADAMTS13 has an apparent molecular mass of 160-kDa to 200-kDa with similar electrophoretic mobilities observed for all nine mutants studied. Recombinant wild-type ADAMTS13 exhibits VWF-cleaving protease activity similar to that observed in normal human plasma. In contrast, all nine missense mutations associated with familial TTP exhibit markedly reduced activity ranging from 5 to 25% of wild-type. In conclusion these findings demonstrate the functional activity of recombinant wild-type ADAMTS13 and confirm its identity with the VWF-cleaving protease observed in normal plasma. These data also confirm the classification of all nine missense substitutions studied here as authentic familial TTP disease-causing mutations. Finally, some or all of these ADAMTS13 mutants may retain low or trace levels of VWF-cleaving activity, consistent with the possibility that complete ADAMTS13 deficiency may be lethal. Ongoing studies of mice genetically deficient for the ADAMTS13 gene will directly test this hypothesis and may provide further insight into the pathogenesis of TTP.

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