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Contribution: U.L. and H.K. designed experiments, analyzed data, and wrote the manuscript; U.L., S.B., B.H., and R.G. conducted the experiments and analyzed the molecular data; J.S., G.B., and K.H. rendered clinical and histopathological data, and critically reviewed the manuscript.

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To the editor:

Complement factor H mutations are present in ADAMTS13-deficient, ticlopidine-associated thrombotic microangiopathies

The antiplatelet agent ticlopidine is associated with the rapid onset of a thrombotic microangiopathy (TMA) resembling thrombotic thrombocytopenic purpura (TTP) and atypical hemolytic-uremic syndrome (aHUS). These disorders occur in 0.06% of individuals exposed to ticlopidine, usually within 1.5 to 6 weeks of exposure.[1,2] The vast majority of ticlopidine TMAs are accompanied by antibody-mediated inhibition of the protease ADAMTS13, making them similar to idiopathic TTP.[3,4] However, ADAMTS13 deficiency alone is not sufficient for the development of TMAs. Our group has shown that exposure to both the pharmacologic levels of ticlopidine and plasma from patients with ticlopidine TMAs induces apoptosis in primary human microvascular endothelial cells, suggesting that ticlopidine-induced endothelial cell apoptosis is a provoking factor in ticlopidine TMAs.[5] In other TMAs such as aHUS, complement regulatory protein mutations represent another provoking factor for the development of these diseases.[6] Complement mutations have not been studied in ticlopidine TMAs.

We obtained plasma samples from 4 consecutive patients with TMAs that occurred within 2.5 to 4 weeks of ticlopidine exposure (Table 1).[7] ADAMTS13 activity and inhibitor titers were determined, as previously described by Bennett et al.[8] All patients had thrombocytopenia, schistocytosis, markedly elevated levels of lactate dehydrogenase (LDH), renal impairment, and significantly decreased ADAMTS13 activity. Three of 4 had ADAMTS13 inhibitors. Plasma levels of C5a and C5b-9 (membrane attack complex) were measured by enzyme-linked immunosorbent assay

Table 1. Clinical characteristics of 4 patients with ticlopidine TMAs

Patient code	Age	Sex	Duration of ticlopidine (wk)	Creatinine (μmol/L)	Platelets pretherapy	Platelets posttherapy	LDH pretherapy (U/L)	LDH posttherapy (U/L)	Outcome	PEX sessions (N)	ADAMTS13 Activity (%)*
010	84	M	3	110	40	235	2555	NA	Death	8	<5
022	77	M	4	150	5	131	1084	214	Survival	10	<5
003	78	F	3.5	260	33	93	1005	1736	Death	3	<5
012	42	F	2.5	110	13	323	790	170	Survival	30	<5

Platelet values expressed as $\times 10^3$.
F, female; M, male; NA, not available; PEX, plasma exchange.
*ADAMTS13 was assessed by both FRET-VWF assay (<5%) and immunoblot activity (<10%).

Table 2. Complement levels of 4 patients with ticlopidine TMAs and corresponding mutations

Patient code	C5a (ng/mL) (normal range, 0.3-70)	sC5b-9 (ng/mL) (normal range, 100-300)	CFH polymorphism
010	32.08	4862	Homozygous exon 18 E936D
022	51.21	6023	Heterozygous exon 18 Q950H
003	27.74	5904	Heterozygous exon 18 E936D
012	50.96	6229	Heterozygous exon 19 N1050Y

Primers used are exon 18 first step TAGACAGACAGACACCAGAAGG (forward), GGTACCACTTACACTTTGAATGAAGA (reverse); exon 18 second step AATTTATGAGTTAGTGAAACCTGAAT (forward), GGTACCACTTACACTTTG AATGAAGA (reverse); exon 19 first step TGTGTAATCTCAATTGCTACGGCT (forward), GGCTGGGCCCACACATTA (reverse); and exon 19 second step ACAAAATGGCTAATATATTTTCTCAAG (forward), GGCTGGGCCCACACATTA (reverse).

CFH, complement factor H.

(Quidel). Genomic DNA was isolated from plasma using the QIAamp kit (Qiagen). To determine the presence of complement mutations, we selected primers to soluble complement factor H (CFH), complement factor I, and membrane-linked membrane cofactor protein, 3 complement regulatory factors with mutations that have been identified with high frequency in aHUS.[4,5] We found substantially elevated levels of membrane attack complex despite normal C5a levels, and all 4 patients had CFH genetic abnormalities (Table 2). Two of the 3 polymorphisms are of known functional significance, and even heterozygous CFH mutations are sufficient to develop aHUS.[7,9]

This is the first report of CFH mutations in ticlopidine TMAs. These mutations are otherwise uncommon in healthy individuals and have a background mutation rate of <5% in northern Europeans.[10,11] ADAMTS13 deficiency has previously been considered to be pathognomonic of TTP. However, complement regulatory factor mutations represent another independent susceptibility factor in many types of TMAs, and their presence in addition to ADAMTS13 deficiency may be required for the onset of disease. If a complement mutation is required for TMA development after ticlopidine exposure, it could explain the rarity of ticlopidine TMAs despite the fact that the drug causes microvascular endothelial cell injury in vitro.[5] Unlike idiopathic TTP, both aHUS and ticlopidine TMAs respond poorly to plasma exchange, even though hematologic parameters may normalize. Renal disease and overall mortality are not affected, and LDH levels do not return to normal, which suggests ongoing endothelial injury.[9] We hypothesize that ticlopidine TMAs occur as a result of a failure to regulate complement on endothelial cell surfaces after cell injury, leading to ongoing microvascular damage. This defect may be present in other TMAs. We recently illustrated the role of complement in a patient with severe refractory idiopathic TTP with ADAMTS13 <5% and a high inhibitor titer. He rapidly responded to anti-C5 therapy after other treatment failures.[12] No complement mutations were detected utilizing a commercially available platform. Subsequent analysis, however, did reveal a CFH mutation (unpublished data).[12] In conclusion, complement regulatory protein mutations may form the basis for TMA susceptibility and should be further studied.

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Contribution: J.C. performed DNA extraction and collected clinical information; H.-M.T. performed ADAMTS13 testing; S.E. and R.S. performed semi-nested PCR on all DNA samples; J.L. and J.C. analyzed the data and authored the manuscript; and all authors contributed equally to the editing and revision of this work.

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