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Variants in chondroitin sulfate metabolism genes in thrombotic storm

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ABSTRACT

Introduction: Thrombotic storm (TS) presents as a severe, acute thrombotic phenotype, characterized by multiple clotting events and frequently affecting younger adults. Understanding the extensive hypercoagulation of an extreme phenotype as TS will also provide insight into the pathogenesis of a wider spectrum of thrombotic disorders.

Material and methods: We completed whole exome sequencing on 26 TS patients, including 1 multiplex family, 13 trios and 12 isolated TS patients. We examined both dominant and recessive inheritance models for known thrombotic factors as well as performed a genome-wide screen. Identified genes of interest in the family and trios were screened in the remaining TS patients. Variants were filtered on frequency (< 5% in 1000 genomes), conservation and function in gene and were annotated for effect on protein and overall functionality.

Results: We observed an accumulation of variants in genes linked to chondroitin sulfate (CS), but not heparan sulfate metabolism. Sixteen conserved, rare missense and nonsense variants in genes involved in CS metabolism (*CHPF*, *CHPF2*, *CHST3*, *CHST12*, *CHST15*, *SLC26A2*, *PAPSS2*, *STAB2*) were identified in over one-third of the TS patients. In contrast, we identified only seven variants in known thrombosis genes (including FV Leiden).

Conclusions: As CS has multiple functions in the glycocalyx protecting the endothelial cells, reduced availability of CS could diminish the normal control mechanisms for blood coagulation, making these CS metabolism genes

Abbreviations: TS, thrombotic storm; CS, chondroitin sulfate; APS, antiphospholipid antibody syndrome; aPL, antiphospholipid antibodies; TM, thrombomodulin; PROC, protein C; PROS1, protein S; PLG, plasminogen; PLAT, plasminogen activator; WES, whole exome sequencing; CGT, Center for Genome Technology; HIHG, John P. Hussman Institute for Human Genomics; GATK, Genome Analysis Tool Kit; AD, allelic depth; KNG1, kininogen 1; FV, factor V; 1kGP, 1000 genomes project; CADD, Combined Annotation Dependent Depletion algorithm; MAF, minor allele frequency; SLC26A2, Solute Carrier Family 26, member 2; CHPF2, Chondroitin Polymerizing Factor 2; PAPSS2, phosphoadenosine phosphosulfate synthase 2; DS, dermatan sulfate; STAB2, stabilin-2; HMWK, high molecular weight kininogen deficiency; CHPF, Chondroitin Polymerizing Factor 1; CHSY1, Chondroitin sulfate synthase 1; CHSY3, Chondroitin sulfate synthase 3; CSGALNACT1, Chondroitin sulfate *N*-acetylgalactosaminyltransferase 1; CSGALNACT2, Chondroitin sulfate *N*-acetylgalactosaminyltransferase 2; CHST15, *N*-acetylgalactosamine 4-Sulfate 6-O-sulfotransferase; CHST12, Chondroitin 4-O-sulfotransferase 2; CHST3, Chondroitin 6-O-Sulfotransferase 1; CHST11, Chondroitin 4-O-Sulfotransferase 1; CHST13, Chondroitin 4-O-Sulfotransferase 3

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strong potential risk factors for TS. Overall, no single gene was identified with strong evidence for TS causality; however, our data suggest TS is mediated by an accumulation of rare pro-thrombotic risk factors.

1. Introduction

Thrombotic storm (TS) is a rare clinical phenotype characterized by rapidly progressive, recurrent thromboembolic events affecting multiple vascular beds. The term 'thrombotic storm' was first coined by Kitchens in 1998 [1], who described six patients with rapid development of multiple thrombotic events. Diagnostic criteria for TS were subsequently published by the Thrombotic Storm Study Group [2] (Table 1), with later reports describing additional patients [3]. Most patients are young adults or children. TS typically follows an initial "trigger" event (e.g. infection, surgery or injury) in the patient. Importantly, the triggering events associated with TS are not uncommon, but occur in thousands of individuals each day, who never develop TS or a significant coagulation problem. Prompt initiation and aggressive management of anticoagulant therapy is needed to halt the thrombotic process, but unfortunately, in some cases, is not successful. Most patients described so far present as sporadic cases, with only one multiplex family identified and presented in this study.

Catastrophic antiphospholipid syndrome (APS), occurring in about 1% of all patients with APS [4,5], falls under the umbrella of TS (Fig. 1). Previously the Thrombotic Storm Study Group noted that approximately 50% of known patients with TS either have a history of APS (consistent with a diagnosis of catastrophic APS) or had antiphospholipid antibodies (aPL) present just at the time of presentation [2]. Conversely, approximately 50% of TS patients previously described do not have detectable aPL. Therefore, it appears that additional factors are involved in the initiation of TS.

Such factors could be rare genetic variants occurring in TS patients. Rare genetic risk variants in genes encoding proteins involved in the regulation of the coagulation pathway [6] have been identified in patients with milder thrombotic phenotypes. These proteins play critical roles in the down-regulation of thrombin and other procoagulant proteinases (e.g. antithrombin, *SERPINC1*), inactivation of procoagulant cofactors Va and VIIIa (though protein C pathway including thrombomodulin (TM, *THBD* [7]), protein C (*PROC* [8]) and protein S (*PROS1* [9])) and degradation of fibrin by the fibrinolytic pathway (including plasminogen (*PLG*) and tissue plasminogen activator (*PLAT*)). Additionally, post-translational modifications, including glycosylation and sulfation, play an important role in the function and regulation of these proteins as well (review [10]). Conversely, other extreme phenotypes have been shown to be due to environmental stimuli, such as the triggering factors that occur in TS, interacting with rare genetic variation (s). For example, individuals with mutations in *CACNA1S or RYR1* can develop malignant hypothermia when exposed to routine anesthesia [11,12], while those with rare genetic variants in *CYP2D6* can develop severe respiratory distress when exposed to normal dosages of codeine [13]. Therefore, the concept of rare genetic variants leading to extreme phenotypes, when exposed to seemingly common triggers, is well established.

Thus we hypothesized that a portion of TS patients harbor an underlying susceptibility to a hypercoagulability state, due to the presence of underlying rare, previously undescribed genetic variants. Here we report additional TS patients and the first whole exome sequencing (WES) analyses in patients with TS. We find that rare variants in genes involved in metabolism of chondroitin sulfate, a major factor in coagulation/thrombosis regulation, are overrepresented in TS, particularly in those patients without aPL.

2. Material and methods

2.1. Patient population

Individuals with TS were identified from patients seen at the respective institutions of the Thrombotic Storm Study Group members, or by physician referral to a group member. Data collected on each case comprised demographics, detailed information related to the thrombotic events, prior medical history, and relevant clinical and laboratory data, including any data relevant to a diagnosis of APS. Each case was reviewed by the members of the Thrombotic Storm Study Group and assigned a likelihood of having TS using a predefined list of diagnostic criteria (Table 1) [1–3]. Blood samples for analysis were collected at the time of enrollment into the study, and whenever possible, additional family members were enrolled and ascertained for family-based analyses.

2.2. Standard protocol approvals, registrations, and patient consents

All subjects of the collaboration were collected with local IRB approval by each contributing center as well as the University of Miami's institutional review board, and provided written informed consent. Sequencing data was analyzed at University of Miami.

2.3. Laboratory serum/plasma testing

Serum and plasma samples were obtained at the time of enrollment into the study for antiphospholipid antibody testing. Laboratory testing for lupus anticoagulants, anticardiolipin antibodies, and anti-β_2-glycoprotein I antibodies was performed at Duke University according to published guidelines [14,15]. As samples were not ascertained at time of event and most patients are on lifetime anticoagulant therapy, no statements can be made on acquired deficiencies of protein S, C or antithrombin due to antibodies; genetic analyses of these factors is described below.

2.4. Sequence capture and whole exome sequencing

We performed whole exome sequencing (WES), targeted sequencing

Table 1
Diagnostic criteria for thrombotic storm.[a]

Inclusion criteria	
Criterion	**Score**
Acute onset of ≥ 2 arterial and/or venous thromboemboli and/or thrombotic microangiopathy	1
Unusual clot location (e.g., cerebral sinus; intra-abdominal)	1
Progressive or recent unexplained reoccurrence, generally within 1 week	1
Refractory to acute therapy and/or atypical response to therapy	1

Exclusion criteria
Active cancer (known or diagnosed at time of presentation)
Severe coronary artery disease
Complications associated with an intravascular device
Inadequate (or no) antithrombotic therapy
Severe/extensive trauma (e.g., affecting multiple limbs)

[a] A definite diagnosis of TS is made with a score of 2 or more of the inclusion criteria and none of the exclusion criteria.