

Pharmacogenetics to prevent heparin-induced thrombocytopenia: what do we know?

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Heparin-induced thrombocytopenia (HIT) is a life-threatening, immune-mediated adverse reaction to heparin anticoagulants. The inability to predict HIT represents a considerable liability associated with heparin administration. Genetic studies of HIT are challenging due to the scarcity of true HIT cases, potential for misclassification, and many environmental risk factors. Genetic studies have not consistently identified risk alleles for HIT, the production of platelet factor 4/heparin antibodies or the thromboembolic complications of HIT. Genes implicated in HIT and platelet factor 4/heparin antibody levels include *FCGR2A*, *TDAG8*, *HLA-DR* and others. Compelling evidence also suggests that the *FCGR2A* H131R polymorphism is associated with HIT-related thrombosis. There is a need for well-powered, multiethnic studies with laboratory confirmation of HIT, detailed patient- and drug-specific data, and inclusion of both serologic and thromboembolic outcomes. Genomic biomarkers identified from such studies offer the possibility of shifting current clinical practice paradigms from early detection and treatment to prevention.

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Heparin-induced thrombocytopenia (HIT) is an antibody-mediated platelet activation condition in patients receiving unfractionated heparin (UFH) or low molecular weight heparin [1]. HIT develops in up to 2.4% of patients treated with heparin anticoagulants, has a >30% mortality rate and results in catastrophic thromboembolic complications, including life- and limb-threatening thrombosis [2–5]. Approximately 12 million individuals, or a third of all hospitalized patients, are exposed to heparins each year [6]. Although newer anticoagulants are available that carry little or no HIT risk, UFH is likely to remain a mainstay of prevention and treatment of thrombosis due to its immediate onset of action, short half-life, low cost, lack of renal adjustments, ease and inexpensiveness of reversal and laboratory monitoring, and a wealth of evidence supporting its use [7].

Despite the widespread use of heparin anticoagulants and high morbidity of HIT, clinical tools are not available to evaluate HIT risk prior to heparin administration. Several risk factors for HIT have been identified, including increased patient age, dose and duration of heparin, severity of trauma, intravenous versus subcutaneous administration, and UFH versus low molecular weight heparin formulations [8,9]. However, prevention of HIT-related thrombosis is only possible after manifestations of HIT are evident [6,10]. The inability to predict HIT represents a liability associated with heparin administration. Genomic biomarkers offer the potential to distinguish patients who are predisposed to HIT and could shift current clinical practice paradigms from early detection and treatment to prevention. Such biomarkers would enable clinicians to avoid heparin in at-risk patients, strategically monitor patients at high risk for HIT and prevent potentially catastrophic HIT-related thromboses. This special report will review the available evidence for genomic influences on HIT, evaluate the mechanistic insights that this evidence provides and provide a perspective on the potential for prevention of HIT using genomic biomarkers.

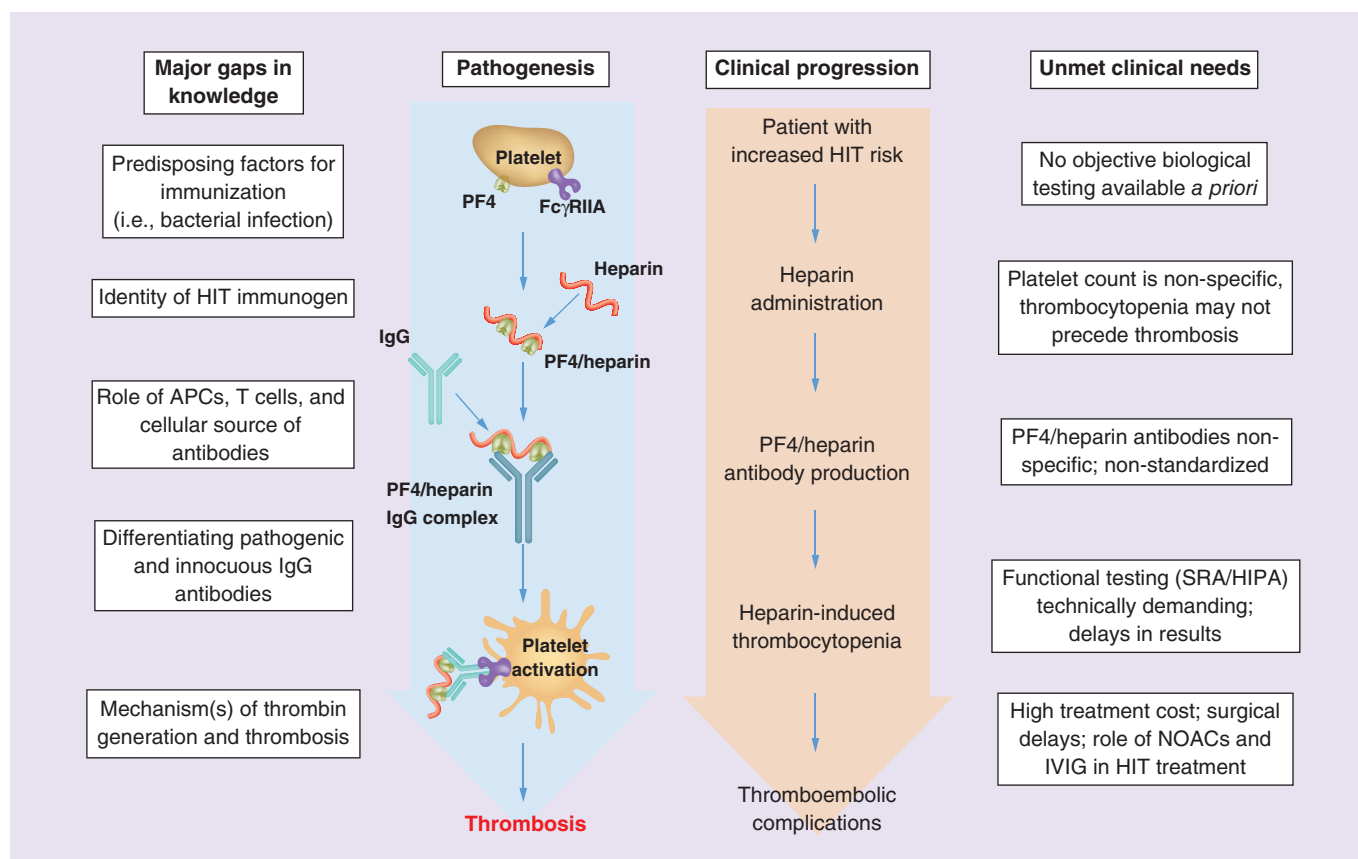


Figure 1. The pathogenesis of heparin-induced thrombocytopenia is characterized by gaps in our knowledge. Genomic biomarkers have the potential to answer critical questions at every stage of HIT pathogenesis, including the predisposing immunogen, the cellular source of antibodies, the identification of pathogenic IgG antibodies and the mechanisms of thrombosis. Similarly, the clinical progression of patient who will eventually develop HIT is characterized by various unmet clinical needs. Genomic biomarkers have the potential to meet many of the unmet clinical needs for HIT, including limitations in the clinical utility of platelet count monitoring, PF4/heparin antibody testing, functional assay testing and limited treatment options.

APC: Antigen-presenting cell; Fc γ RIIA: Platelet Fc γ RIIA receptor; HIPA: Heparin-induced platelet aggregation; HIT: Heparin-induced thrombocytopenia; IVIG: Intravenous immunoglobulin; NOAC: New oral anticoagulant; SRA: Serotonin release assay.

Unmet clinical needs for heparin-induced thrombocytopenia

Two major types of HIT exist. Type I HIT is a transient, non-immune complication of heparin treatment with few clinical consequences that is caused by agglutination effects of heparin on platelets [11]. In contrast to type I HIT, type II HIT is an antibody-mediated platelet activation condition associated with life- and limb-threatening thrombosis [2–5]. Classic type II HIT manifests as an approximate 50% fall in platelet count within 5–10 days of heparin administration. The timing and magnitude of platelet count decrease are central to the estimation of HIT likelihood using the 4Ts score [8,12]. This score also includes the presence of thrombosis and other potential causes of thrombocytopenia, and is used to estimate the likelihood of HIT when clinically suspected. Platelet count monitoring is the primary mode of detecting HIT, but can result in a number of false-positive diagnoses (Figure 1). Decreasing platelet counts can be due to a number of causes, especially for the post-surgical and intensive care populations in which HIT is common. In a substantial number of HIT patients, thrombocytopenia may not occur at all, or thrombotic events may occur before thrombocytopenia is evident [3].

After HIT is suspected based on decreasing platelet counts, clinical guidelines recommend that heparin be discontinued and an alternative anticoagulant started in patients with 4Ts scores greater than or equal to four [6]. Alternative anticoagulants options for HIT in the USA are few, including argatroban, bivalirudin and fondaparinux, and often carry significant additional drug and monitoring costs [13]. Argatroban is the only available US FDA-approved agent for prevention of thrombosis related to HIT. Danaparoid is a unique agent that suppresses platelet activation by replacing PF4/heparin antibody complexes on the platelet surface [14]. However, danaparoid is no

longer available in the USA and manufacturing shortages are common with this agent [15]. Direct oral anticoagulants (DOACs) such as rivaroxaban have an emerging role in the treatment of HIT and are an attractive option due to their oral administration and the limited treatment options for HIT. However, the use of DOACs to treat HIT is primarily backed by case studies and little prospective evidence is available to support DOAC use in HIT [16,17].

In suspected HIT patients, clinical guidelines recommend acquiring a platelet factor 4 (PF4)/heparin IgG immunoassay. Up to 50% of heparin-treated patients will develop PF4/heparin antibodies, but only a fraction of those patients will develop full-blown HIT and its thromboembolic complications [1,18,19]. Although PF4/heparin antibody tests have a near 100% negative predictive value, they are not able to differentiate pathogenic IgG antibodies from those without clinical consequences. PF4/heparin antibody tests are also not standardized across laboratories [20]. Finally, functional assay confirmation of HIT in patients with a positive HIT antibody test is needed to conclusively diagnose HIT. These tests are technically demanding, usually restricted to specialized laboratories, and have the potential to result in significant delays in diagnosis.

Genomic biomarkers have the potential to be used prior to heparin administration as a screening tool to reduce heparin administration to patients with a high HIT risk. Such biomarkers might also be helpful as a risk stratification tool, identifying patients who would benefit from additional monitoring and early treatment to combat thromboembolic complications. Such approaches would reduce over diagnosis and over treatment of HIT, and combat the need for high cost alternative anticoagulants while waiting for laboratory test results. Genomic studies may identify new drug targets, leading to an increase in the clinician's armamentarium for HIT treatment. RNA signatures such as proliferating T-cell receptor clonotypes might also address the critical unmet clinical need for high accuracy diagnostic tools that can confirm HIT early in the disease process.

Pathophysiology of heparin-induced thrombocytopenia

The pathogenesis of HIT begins when heparin, a linear polyanion, binds to the positively charged PF4, an endogenous chemokine stored in α granules of platelets. Neoepitopes on PF4/heparin complexes are recognized by IgG antibodies, forming PF4/heparin-IgG complexes [21]. These PF4/heparin antibodies activate platelets and monocytes via the platelet Fc γ -receptor (Fc γ RIIa), causing thrombin generation and HIT-associated thrombotic complications [22–24]. Interactions between PF4 and heparin are dependent on charge and stoichiometric ratios, such that an excess of either PF4 or heparin will prevent assembly of PF4/heparin antibody complexes [25,26]. This property of PF4/heparin antibody complexes is exploited in laboratory assays such as the serotonin release assay [27].

HIT has characteristics of both innate and adaptive immunity. IgG antibodies are typically produced within 5–10 days, suggesting a secondary immune response, and are required to elicit an immune response [28,29]. However, these antibodies are typically transient, disappearing after approximately 90 days [30]. Recently, a direct role of PF4/heparin complexes in innate immunity has been described [31]. Heterologous immunity models have also been described, in which immune cells are primed by a bacterial antigen to stimulate an immune response upon subsequent heparin exposure [24,32]. Interestingly, PF4 has a role in innate host defense through binding to negatively charged lipopolysaccharides on bacterial cell walls, a defense mechanism which may be misdirected at linear polyanions such as heparin [33,34]. A severe form of HIT, autoimmune HIT, occurs without prior heparin administration and commonly develops after major surgery or infection [35]. Autoimmune HIT may be the result of these misdirected bacterial defense mechanisms against endogenous linear polyanions such as DNA, RNA, polyphosphates or bacterial wall components [36,37].

Despite decades of research into the immunopathology of HIT, the HIT immunogen, the roles of antigen-presenting cells and T cells, and the B-cell subtypes that produce PF4/heparin antibodies are unknown [7,38]. The clinical significance of nonpathogenic PF4/heparin antibodies and the molecular basis that distinguishes them from pathogenic PF4/heparin antibodies remain unclear. Although informative, prior targeted molecular approaches have not fully identified the mechanisms of HIT, likely due to the complicated and unusual nature of the HIT immune response as well as the relatively narrow focus of these targeted approaches. Genetic studies such as genome-wide association studies (GWAS) have the potential to answer many of these questions and further inform the pathophysiology of this adverse drug reaction.

Genetic studies of HIT

Genetic studies of HIT are challenging due to the scarcity of true HIT cases, the potential for misclassification and the many known patient- and drug-specific environmental factors that affect HIT risk. As with other uncommon pharmacogenomic phenotypes, studies aimed at heritability or those require samples before and after

drug exposure are burdensome. This difficulty in sample collection and accurate phenotyping is reflected in the disparate associations and lack of replication observed in genetic studies of HIT. This work is largely limited by a small number of HIT cases and often by a lack of key considerations, such as confirmation of HIT cases by functional assay, rigorously defined thromboembolic outcomes, and a PF4/heparin antibody-positive group to differentiate associations with HIT from associations with seroconversion and antibody production. Furthermore, the evidence is almost entirely generated in individuals of European ancestry, inviting the potential for racial and ethnic disparities if translational tools are developed. There is an ongoing need for well-powered, multiethnic studies with laboratory confirmation of HIT, detailed patient- and drug-specific data, and inclusion of both PF4/heparin antibody test results and thromboembolic outcomes to investigate the genetic basis of HIT. Such studies have the potential to identify clinically implementable biomarkers for HIT and generate key mechanistic insights into HIT pathophysiology.

Genome-wide association studies

A recent GWAS identified a SNP in chromosome 5 in AC106799.2 as a risk allele for HIT (Table 1) [39]. This SNP was associated with HIT in both discovery and replication cohorts with a combined odds ratio (OR) of 2.77 (combined $p = 2.7 \times 10^{-8}$). However, there were only 96 suspected HIT cases, the authors used a liberal threshold for significance ($\alpha = 1 \times 10^{-4}$), and this SNP was not the most significantly associated SNP in the discovery cohort. This variant is located in the lincRNA AC106799.2, which does not have functional annotation, and is situated in an intergenic region without protein coding genes, making speculation on the pathophysiologic implications of this association difficult.

Another GWAS included 67 suspected HIT cases and observed significant SNP associations with HIT near the *TDAG8* (or *GPR65*) gene [40]. These associations included an intronic SNP rs1887289 (OR: 16.83 [5.90–47.97]; $p = 1.01 \times 10^{-8}$) in moderate linkage disequilibrium (LD) with a missense SNP rs3742704 (OR: 33.48 [8.01–140]; $p = 2.04 \times 10^{-7}$). Two replication cohorts were used, including a German cohort of PF4/heparin antibody-tested patients and a French cohort of patients with three groups: patients with HIT, patients testing positive for PF4/heparin antibodies but negative on functional assay (Ab^{POS} patients) and patients testing negative for PF4 antibodies (Ab^{NEG} patients). Whereas rs3742704 showed a significant association with PF4/heparin IgG titer levels in the German cohort ($\beta = 0.07$ [standard error: 0.03]; $p = 0.03$), this SNP did not show association with HIT in the French cohort (OR: 1.90 [0.16–22.76]; $p = 0.61$). This association of *TDAG8* SNPs implies an important mechanistic role of T cells in HIT. *TDAG8* SNPs might decrease *TDAG8* function or abundance, leading to overactive or unregulated T cells in HIT or PF4/heparin antibody production.

In both GWAS, a low number of HIT cases were available for genome-wide analysis. In addition, many of the HIT cases in both studies were determined through antibody testing and 4Ts scores, and were not functional assay confirmed. This approach invites the potential for both misclassification and false-positive associations. Furthermore, discovery cohorts in both studies did not include both HIT cases and Ab^{POS} patients. Inclusion of an antibody-positive group is important to distinguish genetic influences on HIT development versus PF4/heparin antibody production. The mechanistic insight of these studies is also rather unclear. Whereas the association of *TDAG8* SNPs suggests T-cell help as part of pathogenesis, the chromosome 5 association has an unclear biological role in HIT.

Candidate gene or candidate SNP studies

The majority of studies on the genetic determinants of HIT have investigated specific genes or SNPs with putative roles in HIT immunology. A number of early studies investigated the influence of genomic variation in *FCGR2A*. *FCGR2A* (also known as CD32) carries a functionally relevant H131R polymorphism (rs1801274) in the IgG binding region and this H131R polymorphism has been inconsistently associated with HIT in several populations of primarily European ancestry [43,44,52–55]. The inconsistent association results are summarized succinctly in a meta-analysis, which found no consistent effect of the SNP on HIT using a random effects model (OR: 1.11 [0.56–2.19]; $p = 0.77$) [56,57]. Several subsequent studies have found no association of *FCGR2A* H131R with HIT [42,58]. In one candidate SNP study, the influence of the *FCGR2A* H131R and the *FCGR3A* (also known as CD16) 158V/F (rs396991) polymorphisms was investigated. Although no differences in genotype frequency were found between HIT and Ab^{NEG} patients, rs396991 VV homozygotes were significantly higher in HIT patients versus Ab^{POS} patients (21.5 vs 9.5%, $p = 0.02$), a difference that was more pronounced among patients with high PF4/heparin antibody titers (optical density > 1.5) [42]. The association of *FCGR2A* and *FCGR3A* polymorphisms

Table 1. Published literature identifying significant genetic associations with heparin-induced thrombocytopenia.

Study	Locus	Phenotype(s)	Variant(s)	Effect size(s)	Reported p-value(s)	Study description and limitations	Ref.
Witten <i>et al.</i>	Chromosome 5 near AC106799.2	HIT	rs1433265	2.79 (1.69–4.65; discovery) 2.77 (1.64–4.68) (replication)	$p = 6.47 \times 10^{-5}$ (discovery) $p = 1.5 \times 10^{-4}$ (replication)	GWAS; 96 HIT (discovery) and 86 HIT (replication) and equal number heparin-treated controls; subset of cases HIPA confirmed; liberal discovery $\alpha = 1 \times 10^{-4}$	[39]
Karnes <i>et al.</i>	<i>TDAG8 (GPR65)</i>	HIT, Ab production	rs1887289	16.83 (5.90–48; discovery)	$p = 1.01 \times 10^{-8}$	GWAS; 67 HIT cases and 884 matched, heparin-treated controls (discovery); no functional assay; SNP replicated for Ab production but not HIT	[40]
			rs3742704	33.48 (8.01–140; discovery)	$p = 2.04 \times 10^{-7}$		
Rollin <i>et al.</i>	<i>PTPRJ (CD148)</i>	HIT, platelet activation	rs1503185	0.37 (0.20–0.68)	$p = 0.002$	179 Ab ^{neg} , 160 Ab ^{pos} and 97 HIT patients; SRA confirmation; no replication; functional data support effect of SNPs on platelet activation	[41]
			rs1566734	0.36 (0.20–0.67)	$p = 0.001$		
Gruel <i>et al.</i>	<i>FCGR3A (CD16A)</i>	HIT	rs396991	21.5% (HIT) vs 9.5% (Ab ^{pos})	$p = 0.02$	86 Ab ^{neg} , 84 Ab ^{pos} and 102 HIT patients; SRA confirmation; no replication	[42]
Burgess <i>et al.</i>	<i>FCGR2A (CD32A)</i>	HIT	H131R (rs1801274)	0/19 RR (HIT) 7/22 RR (healthy)	$p < 0.01$	20 HIT and 24 healthy control patients; SRA confirmation; opposite direction of previous association; no replication	[43]
Carlsson <i>et al.</i>	<i>FCGR2A</i>	HIT	H131R (rs1801274)	HIT vs Ab ^{neg} (χ^2)	$p < 0.001$	389 HIT and 351 Ab ^{neg} patients; HIPA confirmation; no replication	[44]
Karnes <i>et al.</i>	<i>HLA-DR</i>	HIT	<i>DRB3*01:01</i>	2.81 (1.57–5.02)	$p = 2.1 \times 10^{-4}$ ($q = 0.02$)	65 HIT and 350 heparin-treated, matched controls; no functional assay; no replication	[45]
Paparella <i>et al.</i>	<i>HLA-DR</i>	Ab production	HLA-DR3 serotype	Relative risk 5.3 for Ab ^{pos} vs Ab ^{neg}	$p = 0.01$	10 Ab ^{pos} and 59 Ab ^{neg} consecutive patients undergoing CPB; no replication	[46]
Pouplard <i>et al.</i>	<i>IL10</i>	Ab production	IL10G G20 microsatellite	0.29 (0.12–0.70)	$p = 0.006$	85 Ab ^{neg} , 84 Ab ^{pos} and 82 HIT patients; SRA confirmation; no replication; no multiple comparisons adjustment	[47]
Rollin <i>et al.</i>	<i>ACP1</i>	Ab production	Haplotype: rs11553742/rs11553746	1.8 (1.2–2.6)	$p = 0.005$	179 Ab ^{neg} , 160 Ab ^{pos} and 89 HIT patients; SRA confirmation; no replication; no multiple comparisons adjustment	[48]
Rollin <i>et al.</i>	<i>FCGR2A</i>	HITT, IgG binding	H131R (rs1801274)	5.9 (1.7–20)	$p = 0.008$	35 HITT, 54 HIT, 160 Ab ^{pos} and 174 Ab ^{neg} patients; SRA confirmation; no replication	[49]
Carlsson <i>et al.</i>	<i>FCGR2A</i>	HITT	H131R (rs1801274)	37% (HITT) vs 17% (HIT)	$p = 0.036$	68 HITT and 54 HIT patients, HIPA confirmation; no replication	[44]
Harris <i>et al.</i>	<i>ITGB3 (GPIIb)</i>	HITT	PI ^{A2}	4.68 (1.39–15.72)	$p = 0.009$	39 HIT and 27 HITT patients; no replication; no Ab ^{neg} or Ab ^{pos} groups; no functional assay	[50]
Pamela <i>et al.</i>	<i>FCGR2A, ITGB3 (GPIIb), PECAM1</i>	HITT	3 SNP risk score (H131R, PI ^{A2} , L125V)	8.00 (4.59–13.93)	$p = 0.012$	50 HIT, 53 HITT and 51 Ab ^{pos} patients; HIPA confirmation; no single SNP association; no replication	[51]

Ab: PF4/heparin antibody; Ab^{neg}: Patient with negative PF4/antibody test result; Ab^{pos}: Patient with positive PF4/antibody test result but negative functional assay result; CPB: Cardiopulmonary bypass; GWAS: Genome-wide association study; HIPA: Heparin-induced platelet aggregation assay; HIT: Heparin-induced thrombocytopenia; HITT: Heparin-induced thrombocytopenia with thrombosis; RR: Recessive genotype for H131R R allele; SRA: Serotonin release assay.

with HIT reinforces the role of platelet receptors in HIT pathogenesis. SNPs at critical residues in these receptors might affect binding or activation by PF4/heparin antibody complexes.

In the same cohort, three missense polymorphisms in *PTPRJ* (also known as CD148) were tested for association with HIT [41]. Two missense SNPs in strong LD were associated with HIT compared with both Ab^{pos} and Ab^{neg} patients (ORs for rs1566734 were 0.47 [0.25–0.89], $p = 0.03$, and 0.36 [0.20–0.67], $p = 0.001$, respectively). Although this study did not include a replication cohort, the authors performed several functional studies and observed associations between these SNPs and multiple indices of platelet activation. Yet another study in the same cohort found that an *IL10* promoter microsatellite polymorphism *IL10G* G20 was protective for production of PF4/heparin antibodies (OR: 0.29 [0.12–0.70]; $p = 0.006$) [47]. However, the authors did not adjust their significance level for multiple testing and, like many previous studies of HIT, the results have not been replicated in an independent cohort. Similarly, a haplotype in *ACP1* was found to associate with PF4/heparin antibody production (OR: 1.8 [1.2–2.6]; $p = 0.005$) in this cohort, which had been increased to 179 Ab^{neg}, 160 Ab^{pos} and 89 HIT patients [48]. This study also observed no association between the *PTPN22* SNP rs2476601 and HIT

or PF4/heparin antibody production. *PTPRJ*, *PTPN22* and *ACPI* code various protein tyrosine phosphatases, which are critical for immune response and platelet activation signaling pathways. Association of polymorphisms in protein tyrosine phosphatase genes as well as *IL10*, a key regulatory cytokine in antibody-mediated immunity, implicates variation in these genes in immune regulation of PF4/heparin antibody production. Variation in the human leukocyte antigen (HLA) region of the genome has a central role in T-cell-mediated secondary immune responses and has been strongly associated with immune-mediated disease and adverse drugs reactions [59]. The HLA-DR3 antigen was first associated with the presence of PF4/heparin antibodies in a smaller study with 10 Ab^{pos} and 59 Ab^{neg} patients (relative risk = 5.3, $p = 0.01$) [46]. The GWAS studies which identified a *TDAG8* SNP as a potential HIT susceptibility allele also interrogated the *HLA* region using a candidate gene approach [40]. The analysis identified rs4348358 upstream of *HLA-DRA* as the strongest association for the candidate gene study (OR: 0.25 [CI 0.15–0.44]; $p = 2.06 \times 10^{-6}$). In a subsequent study of the same cohort, sequenced HLA alleles and killer cell immunoglobulin-like receptors (KIR) types imputed from GWAS array data were tested for association with HIT [45]. The authors found that the *HLA-DRB3*01:01* allele was significantly associated with HIT (OR: 2.81 [1.57–5.02]; $p = 2.1 \times 10^{-4}$, $q = 0.02$). Although no KIR types were associated with HIT, a significant interaction was observed between KIR2DS5 and the HLA-C1 KIR binding group ($p = 0.03$). Together, these studies suggest a role of the HLA region in the pathogenesis of HIT, specifically an influence of class II HLA variation and CD4⁺ T cells in HIT pathogenesis. Because these cohorts did not include functional assay confirmation of HIT cases, it is difficult to discern whether variation in *HLA-DR* is associated with HIT or with the production of PF4/heparin antibodies.

Genetic studies of thrombosis in heparin-induced thrombocytopenia

Since thrombosis is the most clinically significant outcome related to HIT, there is much interest in identifying genomic predictors of HIT complicated by thrombosis (HITT). A candidate SNP study investigated the influence of selected SNPs in genes related to platelet glycoprotein function and clotting risk, finding no significant associations in a cohort of 63 patients with isolated HIT and 79 patients with HIT-related thromboembolic complications [60]. Similarly, factor V Leiden was not associated with thrombotic events in patients with HIT [61]. Gene polymorphisms that are well characterized long-term thrombosis risk factors may not be thrombosis risk factors in HIT and other relatively short-term clinical situations such as post-surgical thrombosis, cancer-associated hypercoagulability and HIT [60,62–64].

The *FCGR2A* H131R polymorphism has the most robust evidence for association with HITT. In a study of 35 HITT, 54 HIT, 160 Ab^{pos} and 174 Ab^{neg} patients, 131RR homozygous patients were at increased risk for thrombosis relative to HIT patients without thrombosis (OR: 5.9 [1.7–20]; $p = 0.008$) [49,65]. The authors provide an explanation for their findings with studies suggesting that plasma IgG plays a key role in modulating thrombosis in HIT. In another study of 68 HITT and 54 HIT patients, the RR genotype groups was more common in HITT patients versus HIT patients (37 vs 17%, $p = 0.036$) [44]. A review by Rollin *et al.* combined the evidence for the *FCGR2A* H131R RR genotype group and HITT based on five studies [44,49,51–53]. They observed an overall association between thrombosis and the RR genotype group among HIT patients (OR: 2.86 [1.7–4.8]; $p < 0.0001$) [66]. While there is moderate evidence for this association, more rigorous methods of combining these studies through meta-analysis is needed.

Other studies on the genetic determinants for HITT include investigations of the PI^{A2} polymorphism (also known as HPA-1) in *ITGB3* (also known as GPIIb), which was found to be associated with HITT in a small cohort of HIT patients (OR: 4.68 [1.39–15.72]; $p = 0.009$) [50]. In addition, a polygenic risk score incorporating three SNPs, including *FCGR2A* H131R, *ITGB3* HPA-1 (PI^{A2}) and the *PECAM1* SNP L125V, was modestly associated with HITT (OR: 8.00 [4.59–13.93]; $p = 0.012$), despite no single SNP associations with HITT [51]. Repeated association of the *FCGR2A* H131R polymorphism with HITT suggests that platelets with *FCGR2A* carrying 131R are more intensely activated by PF4/heparin antibody complexes, causing an increase in thrombotic risk. The associations of *PECAM1* and *ITGB3* SNPs underscore the importance of platelet activation in HIT and related thrombosis.

Conclusion

While variation in multiple genes has been implicated in the pathogenesis of HIT, genetic studies have not consistently identified risk alleles for HIT, the production of PF4/heparin antibodies, or the thromboembolic complications of HIT. The association of the *FCGR2A* H131R polymorphism with HITT is a possible exception.

The disparate associations from this body of literature are reflective of the difficulty in clinically diagnosing HIT and the inconsistency of study approaches. There is an ongoing need for well-powered studies that overcome the various limitations of previous literature. Such studies will be critical to assess whether genetic influences on HIT and HIT-related outcomes exist and whether variants can be identified of sufficient effect size to be clinically implementable biomarkers. Considering the widespread use of heparin and the high rate of morbidity and mortality, such biomarkers would be extremely valuable and could shift current clinical practice paradigms in HIT from early detection and treatment to prevention.

Future perspective

Sufficiently powered GWAS with functional assay confirmation of HIT cases are currently underway, which will likely identify moderate to high effect size variants associated with HIT. Such data will aid in developing pathogenic models of HIT and will likely indicate whether clinically implementable biomarkers for HIT and HIT-related outcomes exist. Considering the lack of identification of such biomarkers from the previous literature, it is doubtful that risk alleles with high enough sensitivity and specificity to warrant *a priori* screening will be present as with HLA screening for other immune-mediated adverse drug reactions [67–69]. HIT is a complex condition related to aberrant immunologic and coagulation pathways, and many genetic associations with small to moderate effects sizes are likely to cumulatively influence HIT risk.

Genetic risk alleles are likely to be identified through studies of gene by environment interactions. Such studies will introduce additional challenges with regard to capturing key variables but would likely identify predisposing factors for HIT related to the secondary immune response, such as bacterial infection or colonization. Genetic predisposition to HIT and PF4/heparin antibody production is likely dependent on environmental factors rather

Executive summary

Significance of heparin-induced thrombocytopenia

- Heparin-induced thrombocytopenia (HIT) is a potentially catastrophic immune-mediated adverse drug reaction that represents a clinical liability for treatment with heparin anticoagulants.
- Unfractionated heparin is likely to remain a mainstay of prevention and treatment of thrombosis due to its pharmacologic and cost benefits.
- Genomic biomarkers for HIT have the potential to prevent HIT through *a priori* genotyping and to generate mechanistic insights into the biological basis of HIT.

Unmet clinical needs for heparin-induced thrombocytopenia

- The inability to predict HIT represents a considerable liability associated with heparin administration.
- Genomic biomarkers have the potential to meet many of the unmet clinical needs for HIT, including limitations in the clinical utility of platelet count monitoring, platelet factor 4 (PF4)/heparin antibody testing, functional assay testing and limited treatment options.

Pathophysiology of heparin-induced thrombocytopenia

- Despite decades of research into the immunopathology of HIT, the exact cellular and molecular mechanisms underlying HIT have yet to be identified.
- HIT has characteristics of both innate and adaptive immunity, and may be a secondary immune response following exposure to a yet unknown antigen.

Genetic studies of heparin-induced thrombocytopenia

- Genetic studies of HIT are challenging due to the scarcity of true HIT cases, the potential for misclassification, and the many known environmental factors that affect HIT risk.
- Genetic studies have not consistently identified risk alleles for HIT, the production of PF4/heparin antibodies or the thromboembolic complications of HIT.
- The strongest evidence from this body of literature supports an association between the *FCGR2A* H131R polymorphism and thromboembolic complications of HIT.
- These studies have key limitations, including small numbers of HIT cases, a lack of functional assay confirmation of HIT and an absence of rigorously defined thromboembolic and serological outcomes.

Conclusion

- There is an ongoing need for well-powered, multiethnic studies with laboratory confirmation of HIT, detailed patient- and drug-specific data, and inclusion of serological and thromboembolic outcomes to investigate the genetic basis of HIT.
- Genomic biomarkers identified from such studies offer the possibility of shifting current clinical practice paradigms, enabling clinicians to avoid heparin in at-risk patients, strategically monitoring patients at high risk for HIT, and/or prevent potentially catastrophic HIT-related thromboses.

than an independent genetic effect. Other omic approaches such as single immune cell sequencing are also likely to generate valuable insights into the disease and better define HIT endophenotypes such as autoimmune HIT. Such discoveries would have widespread clinical implications and could shift clinical practice paradigms, enabling clinicians to avoid heparin in at-risk patients, strategically monitor patients at high risk for HIT and/or prevent potentially catastrophic thromboembolic complications.

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Papers of special note have been highlighted as: ● of interest; ●● of considerable interest

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