Received: 18 June 2024 | Revised: 16 December 2024 | Accepted: 31 December 2024
https://doi.org/10.1016/j.rpth.2025.102677

ORIGINAL ARTICLE

Beyond platelet activation: dysregulated lipid metabolism in defining risk and pathophysiology of VITT

Hannah Stevens[1,2,3] | James D. McFadyen[1,2,3,4] | Natalie A. Mellett[5] | David J. Lynn[6,7] | Thy Duong[5] | Corey Giles[3,5] | Jane James[6] | Rochelle Botten[6] | Georgina Eden[6] | Miriam Lynn[6,7] | Paul Monagle[8,9,10,11] | Peter J. Meikle[3,5,12] | Sanjeev Chunilal[13,14] | Karlheinz Peter[1,3,15] | Huyen Tran[2,4]

[1]Atherothrombosis and Vascular Biology Laboratory, Baker Heart and Diabetes Institute, Melbourne, Victoria, Australia
[2]Department of Haematology, Alfred Hospital, Melbourne, Victoria, Australia
[3]Baker Department of Cardiometabolic Health, University of Melbourne, Parkville, Victoria, Australia
[4]Australian Centre for Blood Diseases, Central Clinical School, Monash University, Melbourne, Victoria, Australia
[5]Metabolomics Laboratory, Baker Heart and Diabetes Institute, Melbourne, Victoria, Australia
[6]South Australian Health and Medical Research Institute, Adelaide, South Australia, Australia
[7]Flinders Health and Medical Research Institute, Flinders University, Bedford Park, South Australia, Australia
[8]Department of Paediatrics, University of Melbourne, Parkville, Victoria, Australia
[9]Haematology Research, Murdoch Children's Research Institute, Parkville, Victoria, Australia
[10]Department of Haematology, Royal Children's Hospital, Melbourne, Victoria, Australia
[11]Kids Cancer Centre, Sydney Children's Hospital, Randwick, New South Wales, Australia
[12]Baker Department of Cardiovascular Research, Translation and Implementation, La Trobe University, Bundoora, Victoria, Australia
[13]Department of Haematology, Monash Health, Clayton, Victoria, Australia
[14]School of Clinical Sciences, Monash Health, Monash University, Clayton, Victoria, Australia
[15]Department of Cardiology, Alfred Hospital, Melbourne, Victoria, Australia

Correspondence
Hannah Stevens, Alfred Hospital, Baker Heart and Diabetes Institute, 99 Commercial Road, Melbourne, VIC 3004, Australia.
Email: hannah.stevens@baker.edu.au

Handling Editor: Dr Michael Makris

Abstract

Background: VITT has emerged as a rare but serious adverse event linked primarily to adenoviral vector COVID-19 vaccinations, such as ChAdOx1-S (Oxford/AstraZeneca) vaccination. The syndrome is characterized by thrombosis with thrombocytopenia, elevated D-dimer, and pathologic platelet factor 4 antibodies within 42 days of vaccination.

Objectives: Despite dysregulated lipid metabolism underpinning many thrombotic conditions, the role of lipid alterations in VITT remains unexplored. Here, we examined the plasma lipidome of patients with VITT and compared it with those following ChAdOx1-S vaccination and with unprovoked venous thromboembolism (VTE) to understand the role of lipids in VITT pathophysiology.

Methods: This was a multicenter, prospective cohort study evaluating plasma lipidomics in newly diagnosed VITT samples, which were compared with both healthy controls following ChAdOx1-S vaccination and with unprovoked VTE.

Results: Comparison with ChAdOx1-S controls reveals a distinct lipid signature in VITT, characterized by elevations in phosphatidylserine and ceramide species, alongside reductions in several plasmalogens and acylcarnitine species. Notably, similarities between VITT lipid profiles and insulin resistance phenotypes suggest potential metabolic susceptibility. While few significant associations were found between VITT and VTE, an inverse correlation with several acylcarnitine species was demonstrated. Given the known anticoagulant role of acylcarnitine species, these findings suggest a plausible mechanistic pathway elevating the thrombotic potential of VITT above that of standard VTE.

Conclusion: These findings underscore the important role of lipid metabolism in VITT pathophysiology and highlight the complex interplay between lipids, coagulation, and pathologic thrombosis.

KEYWORDS
lipidomics, mass spectrometry, platelet activation, thrombosis

Essentials

- VITT is a rare thrombotic disorder.
- We compared lipid profiles in VITT with postvaccine controls and standard venous thromboembolism.
- Phosphatidylserine and ceramides were higher in VITT, consistent with elevated prothrombotic risk.
- Anticoagulant acylcarnitine lipids were reduced in VITT, resulting in increased thrombotic risk.

1 | INTRODUCTION

The COVID-19 pandemic has resulted in an estimated 14 million excess deaths and an unprecedented burden on healthcare systems globally [1,2]. In response to escalating SARS-CoV-2 morbidity and mortality, vaccines against the virus were rapidly developed and deployed on a global scale. During this time, a rare but life-threatening adverse event termed VITT emerged and was linked primarily to adenoviral vector COVID-19 vaccinations, such as ChAdOx1-S (Oxford/AstraZeneca) and the Ad26.COV2.S COVID-19 (Janssen/Johnson & Johnson) vaccine [3–6].

VITT is characterized by thrombosis in conjunction with thrombocytopenia, elevated D-dimer, and the presence of pathologic antibodies against platelet factor 4 (PF4). Clinically, VITT manifests as a spectrum of thrombotic events occurring 4 to 42 days after vaccination, including cerebral venous sinus thrombosis, splanchnic vein thrombosis, deep vein thrombosis, pulmonary embolism (PE), and arterial thrombosis [7]. With similarities to heparin-induced thrombocytopenia, the prothrombotic nature of VITT appears to develop from the VITT antibody/PF4 complex binding to platelet FcγIIa receptors, resulting in strong platelet activation and downstream recruitment and activation of leukocytes [7–9].

The interplay between lipid metabolism and thrombotic diseases is well-recognized, with elevated cholesterol and triglyceride levels found to be causally associated with atherothrombosis and cardiovascular disease (CVD) [10–13]. Lipid species, such as cholesterols, are known to contribute to thrombosis by altering endothelial function, modulating coagulation factors, and inducing platelet activation [11]. In addition to these clinical lipids, high-throughput lipidomic approaches now enable the analysis of hundreds of lipid species, which have both procoagulant and anticoagulant properties [14]. Given the established role of lipids in the pathogenesis of arterial and venous thromboembolism (VTE), it is important to understand the role of lipid abnormalities in VITT, which may provide valuable insights into disease mechanisms.

In this context, our study performs comprehensive plasma lipidomics on patients with newly diagnosed VITT to elucidate the role of lipid metabolism. We compared lipid profiles from VITT samples with healthy controls after ChAdOx1-S vaccination and with patients with standard, unprovoked VTE to understand how lipid profiles in VITT differ from unprovoked venous thrombotic events. We aimed to identify unique lipid signatures that may offer novel insights into the pathophysiology of VITT.

2 | METHODS

2.1 | Study design

This was a multicenter, prospective cohort study using plasma samples from patients with VITT (17 samples) and comparing them with plasma from patients following first dose of ChAdOx1-S (14 samples) and plasma from patients with newly diagnosed, unprovoked VTE (19 samples).

Suspected VITT samples were collected from individuals with clinical signs and symptoms suggestive of VITT presenting to emergency departments across Victoria, Australia, following any vaccination against COVID-19. Recruitment occurred between April 2021 and December 31, 2021. Suspected cases were evaluated according to the Thrombosis and Haemostasis Society of Australia and New Zealand VITT guideline [15] using the Brighton criteria [16]. Criteria for confirmed VITT cases and study inclusion included the occurrence of a new venous and/or arterial thrombotic event following the first dose of ChAdOx1-S, accompanied by a platelet count below 150×10^9 and D-dimer >8 times upper limit of normal. The diagnosis was confirmed by testing for anti-PF4 antibodies by enzyme-linked immunosorbent assay or functional assays. The adjudication of all cases was undertaken by the Thrombosis and Haemostasis Society of Australia and New Zealand VITT working group, who met weekly during the study period. Ethics approval was sought from the local ethics review board (approval number 63960).

The non-VITT controls were recruited as part of the COVID-19 Vaccine Immune Responses Study (COVIRS), which longitudinally profiled immune responses following BNT162b2 (Pfizer-BioNTech) or ChAdOx1-S vaccinations in the absence of community transmission of SARS-CoV-2, and recruitment has been described previously [17]. Briefly, healthy adult participants were recruited in Adelaide, South Australia, between April 8 and November 1, 2021, under protocols approved by the Central Adelaide Local Health Network Human Research Ethics Committee, Adelaide, Australia (approval number 14778). Participants provided blood samples before and after their first, second, and third vaccinations against COVID-19. Importantly, an analysis of the immune response, including lipidomics, before and after BNT162b2 and ChAdOx1-S vaccinations has previously been published [17]. As such, this study focused on post–ChAdOx1-S vaccination samples. Specifically, we utilized plasma samples collected approximately 6 days after the first dose of ChAdOx1-S.

The samples from individuals with unprovoked VTE were collected between May 2019 and December 2021, with ethics approval from local hospital ethics review board (approval number 635/18). Samples were collected within 72 hours of diagnosis of PE or proximal deep vein thrombosis.

No patients in any of the 3 cohorts had been infected with SARs-CoV-2 prior to sample collection, ensuring that the findings were not influenced by prior COVID-19 infection.

2.2 | Venous blood samples

All samples were collected in 3.2% sodium citrate in a 1:9 ratio and centrifuged at $800 \times g$ for 15 minutes at room temperature within 4 hours of collection. The plasma supernatant was transferred into Eppendorf tubes and centrifuged ($16{,}000 \times g$ for 2 minutes at room temperature) before being aliquoted for storage at −80 °C until batch analysis.

2.3 | Lipidomic analysis

Lipid extraction was performed as previously described [18]. Briefly, 10 μL of plasma was mixed with 100 μL of butanol:methanol (1:1) with 10 mM ammonium formate, which contained a mixture of internal standards (Supplementary Table S1). Samples were then vortexed and set in a sonicator bath for 1 hour at room temperature. Samples were centrifuged ($14{,}000 \times g$ for 10 minutes at 20 °C) before transferring into the sample vials for analysis. Analysis of plasma extracts was performed on an Agilent 6495C QQQ mass spectrometer with an Agilent 1290 series HPLC system and a ZORBAX Eclipse Plus C18 column (2.1 × 100 mm, 1.8 mm, Agilent) using mass spectrometry and chromatographic conditions described in Supplementary Tables S2 and S3. Mass spectrometry analysis was performed with dynamic scheduled multiple reaction monitoring, and a full list of transitions is listed in Supplementary Table S4. Chromatogram integration was performed using Agilent MassHunter v10.0, and quantification of lipid species was determined by comparison with the relevant internal standard. Lipid species were evaluated individually and as lipid classes, which represent the sum of species within a class.

2.4 | Statistical analysis

Linear regression was performed comparing VITT samples with ChAdOx1-S vaccination or with unprovoked VTE. Regression was adjusted for age and sex. Lipids were log-transformed and scaled prior to analysis. Within the VITT cohort, Spearman correlation analysis was performed for platelet-related endpoints, including platelet count and PF4 enzyme-linked immunosorbent assay optical density. The Spearman rank coefficient (ρ) and corresponding P values were calculated. The Benjamini–Hochberg method was used to adjust for multiple comparisons, and an adjusted P value <.05 was considered statistically significant.

3 | RESULTS

Overall, 50 participants were included in the analysis: 17 with VITT, 14 ChAdOx1-S controls, and 19 with VTE. Among the VITT cohort, 41% were female, with a median age of 70 years (IQR, 57-74 years). In the ChAdOx1-S control group, 57% were female, with a median age of 38 years (IQR, 32-54.5 years). For the VTE cohort, 47% were female, with a median age of 60 years (IQR, 51.5-78 years). Clinical and laboratory details for the VITT cases are outlined in the Table.

3.1 | Lipid associations with VITT compared with ChAdOx1-S vaccination

When evaluating lipid species, 163 lipid species were found to be significantly associated with VITT, with 30 lipid species upregulated

TABLE Clinical and laboratory data of participants with VITT.

Subject no.	Age (y)	Sex	Time after vaccination (d)	Platelet count (10^9/L)	D-dimer (× ULN)	Type of thrombosis
1	79	M	13	37	40	PE
2	58	M	7	30	126	DVT
3	44	M	8	70	228	Splanchnic vein thrombosis
4	74	M	14	89	29	PE/DVT
5	80	M	9	140	40	CVST/PE/DVT
6	75	M	14	41	25	Renal artery thrombosis
7	55	M	20	107	40	PE
8	74	F	4	31	40	Splanchnic vein
9	70	M	10	100	22	DVT
10	74	F	12	62	20	PE
11	70	F	5	16	40	DVT
12	67	F	7	34	40	IJV thrombus
13	74	F	25	136	14	PE/DVT
14	48	F	12	18	58	CVST
15	59	M	14	94	14	PE
16	57	F	10	13	20	Severe headache
17	25	M	15	50	40	DVT

CVST, cerebral venous sinus thrombosis; DVT, deep vein thrombosis; F, female; IJV, internal jugular vein; M, male; PE, pulmonary embolism; ULN, upper limit of normal.

and 133 species downregulated (Figures 1 and 2, and Supplementary Tables S5 and S6). When compared with ChAdOx1-S controls, the strongest positive associations with VITT were seen with several ceramide (Cer) species (Cer[d18:1/18:0], Cer[d19:1/18:0], Cer[d19:1/20:0], and Cer[d19:1/24:1]), several diacylglycerol (DG) species (DG [16:0_16:1], DG[16:1_18:1], and DG[16:0_18:1]), and phosphatidylserine (PS 40:5). The most marked negative associations in the plasma were with GM1 ganglioside (GM1[d18:1/16:0]), several plasmalogen species (alkenylphosphatidylcholine plasmalogen [PC{P}]-16:0/18:2], PC[P-16:0/22:6], and PC[P-18:0/18:2]), dihexosylceramide (Hex2Cer[d18:2/18:0]), and sulfatide (SHexCer[d18:1/24:0]; Figures 1 and 2, and Supplementary Tables S5 and S6).

At a lipid class level, VITT was associated with 16 lipid classes, with 2 positive associations and 14 negative associations (Figure 2 and Supplementry Table S6). The lipid classes most strongly associated with VITT included GM1 ganglioside, PC(P), and alkylphosphatidylcholine (PC[O]), all of which showed a negative association with VITT compared with ChAdOx1-S vaccination.

3.2 | Lipid associations with VITT compared with unprovoked VTE

When evaluating lipid species, there were 12 lipid species that showed an association with VITT, and all showed a negative association (Figures 3 and 4, Supplementary Table S7). These lipids included 5 acylcarnitine (AC) species (AC[17:0], AC[18:2], AC[18:3], AC[20:4], and AC[22:5]), 2 PC(O) species (PC[O-42:5] and PC[O-42:6]), sphingomyelin (sphingomyelin [44:3]), and PS(38:5) (Figures 3 and 4, Supplementary Table S7).

At a lipid class level, VITT showed associations with 3 lipid classes, with 1 positive association, oxidized lipid species, and 2 negative associations (sphingosine and sphingosine-1-phosphate; Figure 4 and Supplementary Table S8).

3.3 | Lipid species correlation with platelet count and PF4

Spearman correlation analysis was performed to assess the association of lipids with platelet count and PF4 levels. While several lipid species demonstrated positive or negative correlations with these endpoints (Supplementary Tables S9 and S10), none of the associations remained statistically significant after controlling for multiple comparisons.

4 | DISCUSSION

In this study, we demonstrate a distinct lipid profile in VITT, which differs from individuals who received ChAdOx1-S vaccine but did not develop VITT and from standard unprovoked VTE. While the role of

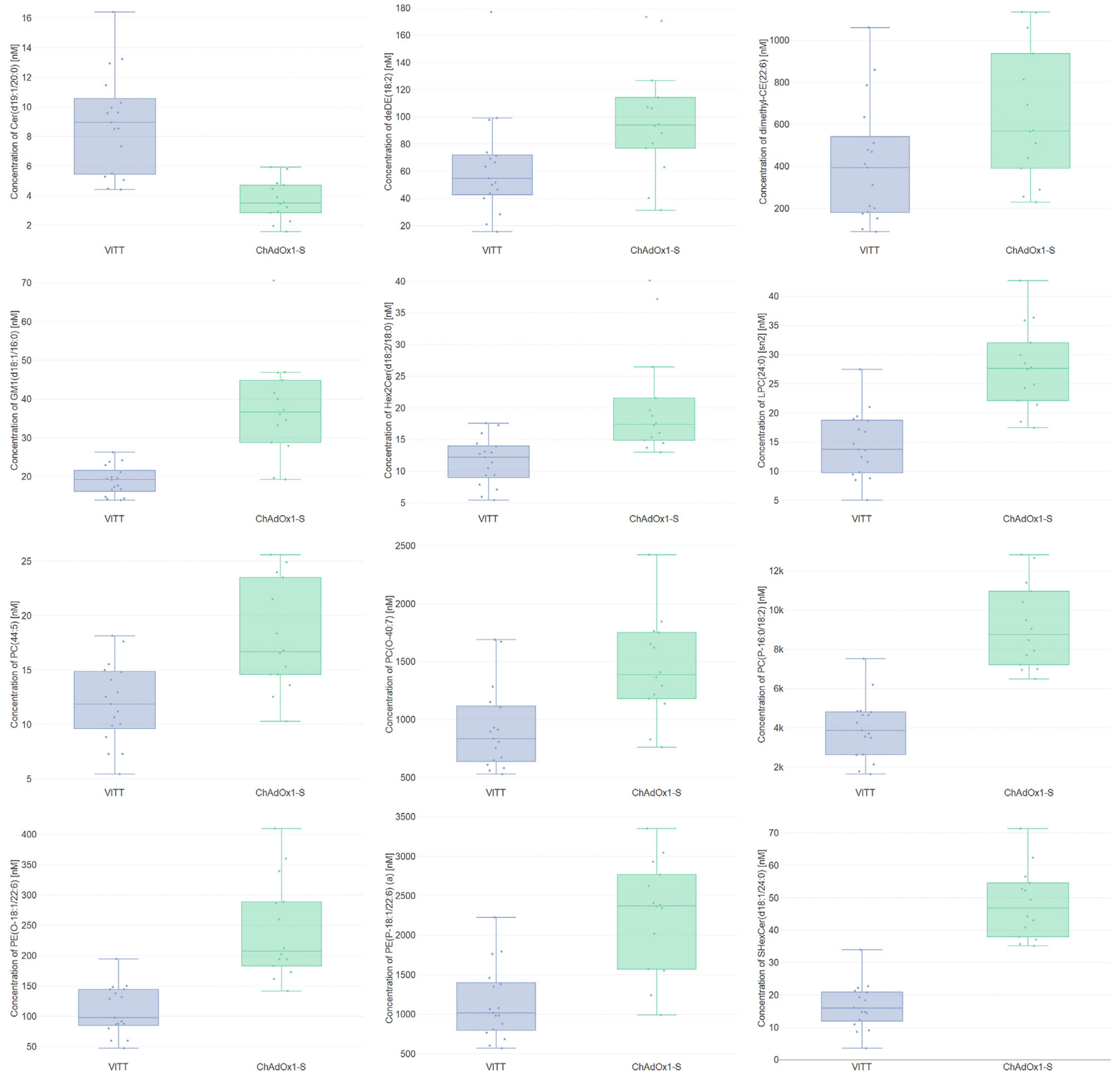

FIGURE 1 Representative figures from 12 of the 163 most significant lipid species from unique lipid classes in VITT vs ChAdOx1-S analysis. There were 163 significant lipid species from 29 classes following Benjamini–Hochberg correction. Cer, ceramide; deDE, dehydrodesmosteryl; dimethyl-CE, dimethyl-cholesteryl ester; GM1, GM1 ganglioside; Hex2Cer, dihexosylceramide; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; PC(O), alkylphosphatidylcholine; PC(P), alkenylphosphatidylcholine; PE(O), alkylphosphatidylethanolamine; PE(P), alkenylphosphatidylethanolamine; SHexCer, sulfatide.

clinical lipids such as cholesterol and triglycerides in thrombosis is well established [12,13], our findings reveal the importance of minor lipid species, such as PS, Cers, PC(P) plasmalogens, and AC species. The alterations in lipid species seen in this study highlight the importance of lipid-mediated interactions in thrombotic disorders while also improving our understanding of the pathophysiology of VITT and differentiating VITT from more common thrombotic disorders such as unprovoked VTE.

Our study identified 163 lipid species that were dysregulated in VITT patients compared with ChAdOx1-S–vaccinated controls, with a notable elevation in the prothrombotic phospholipid, PS. PS acts as a potent prothrombinase cofactor, promoting a procoagulant platelet state and subsequent thrombin generation when exposed to platelet membranes [19]. Additionally, platelets release extracellular vesicles containing high levels of procoagulant PS and other prothrombotic lipids, such as Cers [20]. Consistent with the known central role of

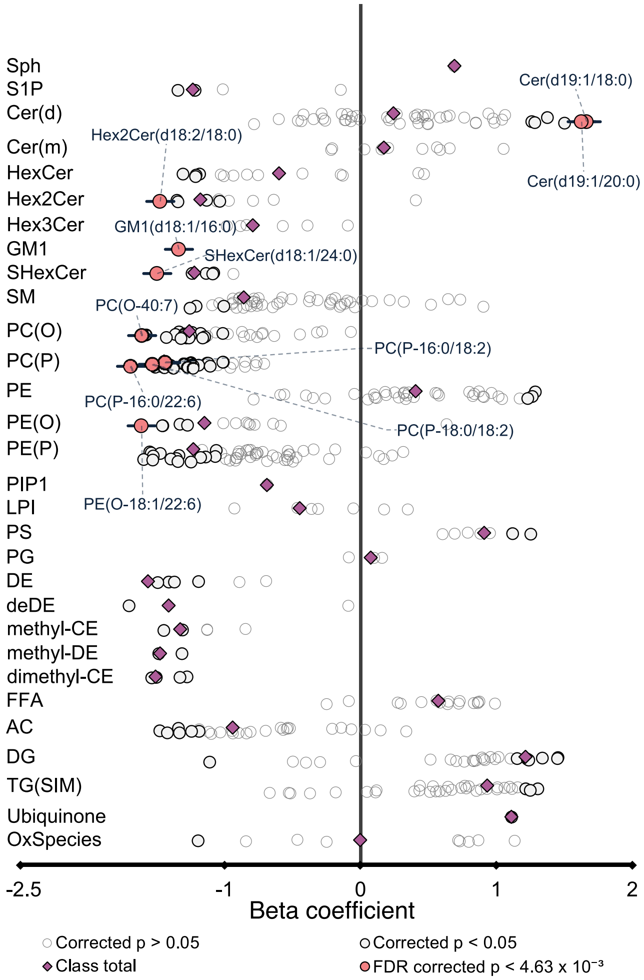

FIGURE 2 Linear regression of the lipid species and classes in VITT vs ChAdOx1-S control cohort. AC, acylcarnitine; Cer(d), ceramide; Cer(m), deoxyceramide; DE, dehydrocholesteryl ester; deDE, dehydrodesmosteryl ester; dimethyl-CE, dimethyl-cholesteryl ester; DG, diacylglycerol; FDR, false discovery rate; FFA, free fatty acids; GM1, GM1 ganglioside; HexCer, monohexosylceramide; Hex2Cer, dihexosylceramide; Hex3Cer, trihexosylceramide; LPI, lysophosphatidylinositol; methyl-CE, methyl-cholesteryl ester; methyl-DE, methyl-dehydrocholesteryl ester; OxSpecies, oxidized lipids; PC(O), alkylphosphatidylcholine; PC(P), alkenylphosphatidylcholine; PE, phosphatidylethanolamine; PE(O), alkylphosphatidylethanolamine; PE(P), alkenylphosphatidylethanolamine; PG, phosphatidylglycerol; PIP1, phosphatidylinositol phosphate; PS, phosphatidylserine; S1P, sphingosine-1-phosphate; SHexCer, sulfatide; SM, sphingomyelin; Sph, sphingosine; TG(SIM), triacylglycerol (SIM, for sum class information).

procoagulant platelets in VITT pathogenesis [8,21] and the finding of platelet PS as the central mediator of thrombosis in heparin-induced thrombocytopenia [22], our study highlights 2 specific PS species, PS(38:3) and PS(40:5), that are significantly elevated in VITT. These findings reinforce the hypothesis that platelets, through increased PS exposure and thrombin generation, play a pivotal role in the pathogenesis of VITT and associated thrombus formation.

Interestingly and somewhat unexpectedly, PS levels were observed to be higher in VTE compared with VITT. While VITT is primarily associated with antibody-mediated procoagulant platelet activation [21], this observation suggests that distinct or additional mechanisms may be responsible for driving PS exposure in VTE. Further investigations are warranted to understand the pathways influencing PS levels in VTE and their contributions to thrombus formation.

Emerging evidence positions Cers, a subclass of sphingolipids, as drivers of procoagulant and proinflammatory responses. Lipidomic analysis of plasma has demonstrated that Cers correlate with a range of major cardiovascular events and can serve as indicators of CVD risk in patients with established CVD and asymptomatic individuals, independent of conventional risk factors [23,24]. While the precise role of Cers in thrombotic diseases continues to be explored, notable associations with inflammatory markers, such as interleukin-6, tumor necrosis factor-α, and nuclear factor κB pathways, have been established [25]. Cers also contribute to the generation of reactive oxygen species and endothelial dysfunction [25,26], which may have subsequent downstream effects on platelets and leukocytes. The current study has found 6 species of Cers to be positively associated with VITT compared with ChAdOx1-S–vaccinated controls, indicating their potential significance in this disease and pointing to the possibility that Cer species could be markers in early detection of VITT.

In the recently published COVIRS, Ryan et al. [17] showed that 9 lipid species are altered following ChAdOx1-S vaccination when compared with prevaccination samples. Using the same lipidomic panel as in the current study, the altered lipid species included several plasmalogen species, as well as reduced levels of PS(36:2) and AC(26:0), which were not observed to be altered following vaccination with BNT162b2 (Pfizer-BioNTech) vaccination [17]. Plasmalogens, including PC(P), are a type of glycerophospholipid that act as natural antioxidants, with lower levels linked to oxidative stress [27]. These lipids have been implicated in immunothrombosis, as evidenced by reduced levels in patients with coronary artery disease and COVID-19 infection [24,28,29].

Consistent with the COVIRS [17], our study identified an inverse correlation with several plasmalogen species, including PC(P-16:0/18:2), PC(P-16:0/22:6), and PC(P-18:0/18:2). The observation of reduced plasmalogens after ChAdOx1-S vaccination (but not BNT162b2) and in patients with VITT suggests a potential link between vaccine-induced changes in plasmalogens and increased oxidative stress. The lower levels of plasmalogens may contribute to a prothrombotic state and potentially predispose individuals to developing VITT, but further studies are needed to confirm these associations.

Our data indicate a reduction in the plasma levels of 7 AC species in VITT compared with the ChAdOx1-S postvaccination controls. Long-chain ACs are known to exhibit anticoagulant properties through inhibition of factor Xa, and lower levels of ACs have previously been associated with VTE [30]. Notably, COVIRS also observed reduced levels of AC(26:0) after ChAdOx1-S vaccination compared

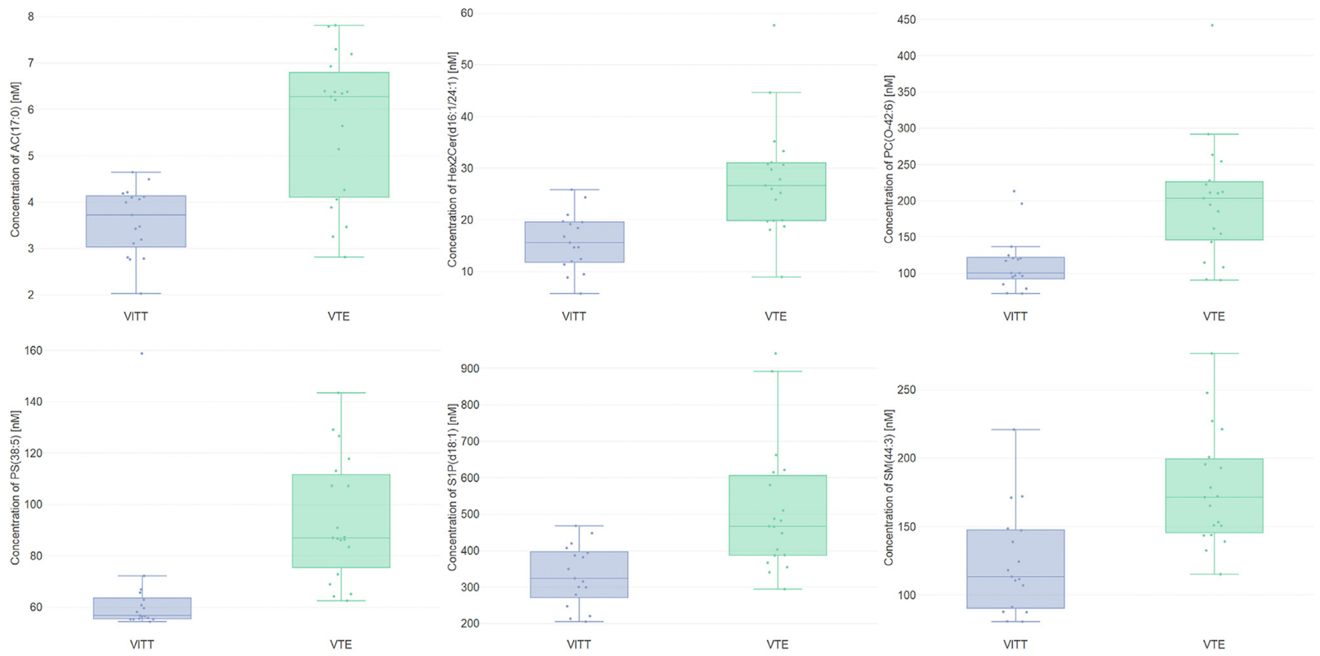

FIGURE 3 Representative figures from each class of significant lipid species in VITT vs unprovoked venous thromboembolism (VTE). There were 12 significant lipid species from 6 classes following Benjamini–Hochberg correction. All species belonging to the same class showed similar β coefficients, and so a representative species from each of the 6 classes is shown here. AC, acylcarnitine; Hex2Cer, dihexosylceramide; PC(O), alkylphosphatidylcholine; PS, phosphatidylserine; S1P, sphingosine-1-phosphate; SM, sphingomyelin.

with prevaccination controls, suggesting that ChAdOx1-S vaccination may disrupt the regulatory anticoagulant function of AC species. In our study, the reduced levels of AC species may contribute directly to the prothrombotic environment observed in VITT and highlight a mechanistic link between altered AC metabolism and thrombosis risk in these patients.

Interestingly, it should be noted that many features of the VITT lipidomic profile align with previous lipid profiles related to insulin resistance, such as elevated DG, triacylglycerol, phosphatidylethanolamine, and Cer species with decreased PC(P) and PC(O) [31]. This profile may therefore represent a metabolic phenotype that is more susceptible to VITT. Further work with larger numbers and clinical phenotyping will be required to resolve this issue.

In our lipidomic analysis comparing VITT with standard VTE, we observed very few significant associations, indicating a similarity in the lipidomic signatures between the 2 conditions. Nonetheless, several associations were demonstrated, particularly an inverse correlation with 5 AC species (AC[17:0], AC[18:2], AC[18:3], AC[20:4], and AC [22:5]). Given the known anticoagulant functions of ACs, these reduced levels are notable and suggest a possible mechanistic pathway by which the thrombotic potential of VITT is elevated above that of standard VTE.

There are important strengths and some limitations that must be noted in our study. VITT is a rare complication, with reported rates between 1 in 25,000 and 1 in 50,000 individuals [32], such that large study numbers are not feasible. Here, our prospective study has included all available samples with VITT confirmed through rigorous clinical adjudication consistent with definitions [16]. Additionally, including the 2 control cohorts (ChAdOx1-S–vaccinated controls and unprovoked VTE) provides a nuanced view of how the pathophysiology of VITT may differ from healthy vaccinated individuals as well as other thrombotic conditions. Limitations include the relatively small sample size and the lack of ethnic diversity, which may limit the applicability of the findings to all populations. Additionally, the 3 cohorts were not matched for age and sex. While these variables were adjusted for in the final analysis, age and sex may influence metabolite profiles, potentially leading to confounding. Furthermore, clinical information such as CVD and diabetes was not collected in the study. Finally, it is well established that VITT is primarily a syndrome of activated and prothrombotic platelets, demonstrated by increased levels of platelet P-selectin, glycoprotein IIb/IIIa, and externalization of PS [8,21]. Evaluating markers of platelet activation, such as glycoprotein VI or glycocalicin, or analyzing the platelet lipidome—particularly focusing on lipids such as 12-lipoxygenase, which mediate prothrombotic platelet responses through FcγRIIa [33,34] – could further enhance our understanding of this rare syndrome. However, these analyses were not feasible in the study due to the small volume of plasma available and the plasma-based nature of the investigation.

5 | CONCLUSION

Dysregulated lipid metabolism underpins many thrombotic conditions, with lipid species involved in both procoagulant and anticoagulant interactions. Here, we demonstrate that VITT has a similar metabolic phenotype to unprovoked VTE but is associated with distinct

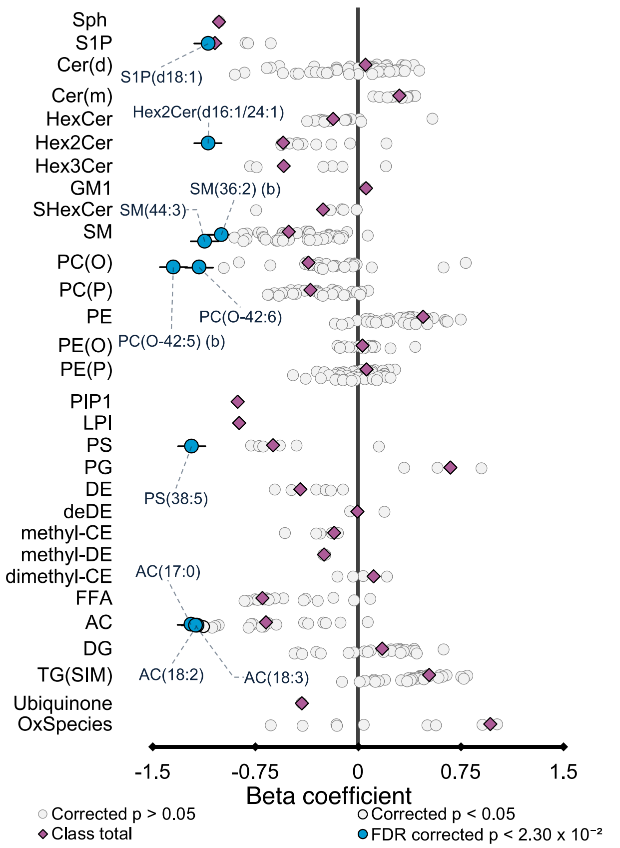

FIGURE 4 Linear regression of the lipid species and classes in VITT vs unprovoked venous thromboembolism. AC, acylcarnitine; Cer(d), ceramide; Cer(m), deoxyceramide; DE, dehydrocholesteryl ester; deDE, dehydrodesmosteryl ester; dimethyl-CE, dimethyl-cholesteryl ester; DG, diacylglycerol; FDR, false discovery rate; FFA, free fatty acids; GM1, GM1 ganglioside; HexCer, monohexosylceramide; Hex2Cer, dihexosylceramide; Hex3Cer, trihexosylceramide; LPI, lysophosphatidylinositol; methyl-CE, methyl-cholesteryl ester; methyl-DE, methyl-dehydrocholesteryl ester; OxSpecies, oxidized lipids; PC(O), alkylphosphatidylcholine; PC(P), alkenylphosphatidylcholine; PE, phosphatidylethanolamine; PE(O), alkylphosphatidylethanolamine; PE(P), alkenylphosphatidylethanolamine; PG, phosphatidylglycerol; PIP1, phosphatidylinositol phosphate; PS, phosphatidylserine; S1P, sphingosine-1-phosphate; SHexCer, sulfatide; SM, sphingomyelin; Sph, sphingosine; TG(SIM), triacylglycerol (SIM, for sum class information).

alterations in lipid metabolism and diverges significantly from the lipid signature seen in ChAdOx1-S–vaccinated controls. In particular, the increase in PS suggests a potential role of procoagulant platelet extracellular vesicles, and reduced levels of the PC(P) plasmalogens and anticoagulant AC species suggest further mechanistic pathways for the prothrombotic nature of VITT. The identification of specific lipid species, including PS, plasmalogens, AC, and Cers, that are differentially expressed in VITT furthers our understanding of the pathophysiology of disease and highlights the complex interplay between lipid metabolism and coagulation pathways. With VITT or VITT-like syndromes now being reported in individuals following adenovirus or other viral infections [35,36], there is a need for further research to understand the pathophysiology and risk factors associated with VITT within diverse populations.

ACKNOWLEDGMENTS

We would like to thank Joanne Haywood at Monash Health for her involvement in the collection and storage of VITT samples. We would also like to thank all of those involved in participant recruitment and sample processing for COVIRS, including staff at SA Pathology, Simone Barry, Stephen Blake, Natalie Stevens, and Yee Tee.

FUNDING

This study received funding from the National Health and Medical Research Council – Medical Research Future Fund (#2015305). K.P. is supported by a National Health and Medical Research Council (NHMRC) Level 3 Investigator Fellowship. J.D.M. is supported by a Heart Foundation Future Leader Fellowship.

AUTHOR CONTRIBUTIONS

H.S.: Study design, sample collection (VTE), sample preparation (VTE/VITT), data collection (VTE/VITT/COVIRS), data analysis and interpretation, drafting of article, revision of article, final approval of article. J.D.M.: Study design, sample collection (VTE), data interpretation, drafting of article, revision of article, final approval of article. N.A.M.: Sample preparation (VTE/VITT), data collection (VTE/VITT), data analysis and interpretation (VTE/VITT/COVIRS), revision of article, final approval of article. D.J.L.: Sample collection (COVIRS), sample preparation (COVIRS), data collection (COVIRS), revision of article, final approval of article. T.D.: Sample preparation (VTE/VITT), data collection (VTE/VITT), data analysis and interpretation (VTE/VITT/COVIRS), final approval of article. C.G.: Data analysis and interpretation (VTE/VITT/COVIRS), final approval of article. J.J.: Sample collection (COVIRS), sample preparation (COVIRS), data collection (COVIRS), final approval of article. R.B.: Sample collection (COVIRS), sample preparation (COVIRS), data collection (COVIRS), final approval of article. G.E.: Sample collection (COVIRS), sample preparation (COVIRS), data collection (COVIRS), final approval of article. M.L.: Sample collection (COVIRS), sample preparation (COVIRS), data collection (COVIRS), final approval of article. P.M.: Sample collection (VITT), final approval of article. P.J.M.: Data analysis and interpretation, revision of article, final approval of article. S.C.: Sample collection (VITT), data collection (VITT), final approval of article. K.P.: Sample collection (VTE), data interpretation, revision of article, final approval of article. H.T.: Study design oversight, sample collection (VITT), data collection (VITT), data analysis and interpretation, drafting of article, revision of article, final approval of article.

RELATIONSHIP DISCLOSURE

There are no competing interests to disclose.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this work, the authors used ChatGPT to assist in refining and improving the structure of already drafted content. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

ORCID

Hannah Stevens https://orcid.org/0000-0003-3394-9768
Huyen Tran https://orcid.org/0000-0003-1989-959X

X

Hannah Stevens @hannahpstevens

REFERENCES

[1] Msemburi W, Karlinsky A, Knutson V, Aleshin-Guendel S, Chatterji S, Wakefield J. The WHO estimates of excess mortality associated with the COVID-19 pandemic. *Nature*. 2023;613:130–7.

[2] Haldane V, De Foo C, Abdalla SM, Jung AS, Tan M, Wu S, et al. Health systems resilience in managing the COVID-19 pandemic: lessons from 28 countries. *Nat Med*. 2021;27:964–80.

[3] Greinacher A, Thiele T, Warkentin TE, Weisser K, Kyrle PA, Eichinger S. Thrombotic thrombocytopenia after ChAdOx1 nCov-19 vaccination. *N Engl J Med*. 2021;384:2092–101.

[4] Schultz NH, Sørvoll IH, Michelsen AE, Munthe LA, Lund-Johansen F, Ahlen MT, et al. Thrombosis and thrombocytopenia after ChAdOx1 nCoV-19 vaccination. *N Engl J Med*. 2021;384:2124–30.

[5] Scully M, Singh D, Lown R, Poles A, Solomon T, Levi M, et al. Pathologic antibodies to platelet factor 4 after ChAdOx1 nCoV-19 vaccination. *N Engl J Med*. 2021;384:2202–11.

[6] See I, Su JR, Lale A, Woo EJ, Guh AY, Shimabukuro TT, et al. US case reports of cerebral venous sinus thrombosis with thrombocytopenia after Ad26.COV2.S vaccination, March 2 to April 21, 2021. *JAMA*. 2021;325:2448–56.

[7] McFadyen JD, Peter K. The known knowns and known unknowns of vaccine-induced thrombotic thrombocytopaenia. *Cardiovasc Res*. 2021;117:e147–50.

[8] McFadyen JD, Sharma P, Moon MJ, Noonan J, Goodall E, Tran HA, et al. Activation of circulating platelets in vaccine-induced thrombotic thrombocytopenia and its reversal by intravenous immunoglobulin. *Br J Haematol*. 2022;196:234–7.

[9] Leung HHL, Perdomo J, Ahmadi Z, Zheng SS, Rashid FN, Enjeti A, et al. NETosis and thrombosis in vaccine-induced immune thrombotic thrombocytopenia. *Nat Commun*. 2022;13:5206. https://doi.org/10.1038/s41467-022-32946-1

[10] Emerging Risk Factors Collaboration, Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK, Thompson A, et al. Major lipids, apolipoproteins, and risk of vascular disease. *JAMA*. 2009;302:1993–2000.

[11] Palasubramaniam J, Wang X, Peter K. Myocardial infarction-from atherosclerosis to thrombosis. *Arterioscler Thromb Vasc Biol*. 2019;39:e176–85.

[12] Varbo A, Benn M, Tybjærg-Hansen A, Nordestgaard BG. Elevated remnant cholesterol causes both low-grade inflammation and ischemic heart disease, whereas elevated low-density lipoprotein cholesterol causes ischemic heart disease without inflammation. *Circulation*. 2013;128:1298–309.

[13] Do R, Willer CJ, Schmidt EM, Sengupta S, Gao C, Peloso GM, et al. Common variants associated with plasma triglycerides and risk for coronary artery disease. *Nat Genet*. 2013;45:1345–52.

[14] Deguchi H, Elias DJ, Griffin JH. Minor plasma lipids modulate clotting factor activities and may affect thrombosis risk. *Res Pract Thromb Haemost*. 2017;1:93–102.

[15] Thrombosis and Haemostasis Society of Australia and New Zealand. THANZ Multidisciplinary VITT Guideline for Doctors. https://rheumatology.org.au/Portals/2/Documents/Public/Professionals/VITT%20guideline%20for%20doctors.pdf?ver=2021-07-04-131821-603; 2021 [accessed April 3, 2024].

[16] Schönborn L, Pavord S, Chen VMY, Pai M, Gwarzo DH, Buttery J, et al. Thrombosis with thrombocytopenia syndrome (TTS) and vaccine-induced immune thrombocytopenia and thrombosis (VITT): Brighton Collaboration case definitions and guidelines for data collection, analysis, and presentation of immunisation safety data. *Vaccine*. 2024;42:1799–811.

[17] Ryan FJ, Norton TS, McCafferty C, Blake SJ, Stevens NE, James J, et al. A systems immunology study comparing innate and adaptive immune responses in adults to COVID-19 mRNA and adenovirus vectored vaccines. *Cell Rep Med*. 2023;4:100971. https://doi.org/10.1016/j.xcrm.2023.100971

[18] Huynh K, Barlow CK, Jayawardana KS, Weir JM, Mellett NA, Cinel M, et al. High-throughput plasma lipidomics: detailed mapping of the associations with cardiometabolic risk factors. *Cell Chem Biol*. 2019;26:71–84.e4.

[19] Bevers EM, Comfurius P, Zwaal RF. The nature of the binding for prothrombinase at the platelet surface as revealed by lipolytic enzymes. *Eur J Biochem*. 1982;122:81–5.

[20] Braig D, Nero TL, Koch HG, Kaiser B, Wang X, Thiele JR, et al. Transitional changes in the CRP structure lead to the exposure of proinflammatory binding sites. *Nat Commun*. 2017;8:14188. https://doi.org/10.1038/ncomms14188

[21] Althaus K, Möller P, Uzun G, Singh A, Beck A, Bettag M, et al. Antibody-mediated procoagulant platelets in SARS-CoV-2-vaccination associated immune thrombotic thrombocytopenia. *Haematologica*. 2021;106:2170–9.

[22] Zlamal J, Singh A, Weich K, Jaffal H, Uzun G, Pelzl L, et al. Platelet phosphatidylserine is the critical mediator of thrombosis in heparin-induced thrombocytopenia. *Haematologica*. 2023;108:2690–702.

[23] Havulinna AS, Sysi-Aho M, Hilvo M, Kauhanen D, Hurme R, Ekroos K, et al. Circulating ceramides predict cardiovascular outcomes in the population-based FINRISK 2002 cohort. *Arterioscler Thromb Vasc Biol*. 2016;36:2424–30.

[24] Meikle PJ, Wong G, Tsorotes D, Barlow CK, Weir JM, Christopher MJ, et al. Plasma lipidomic analysis of stable and unstable coronary artery disease. *Arterioscler Thromb Vasc Biol*. 2011;31:2723–32.

[25] Shalaby YM, Al Aidaros A, Valappil A, Ali BR, Akawi N. Role of ceramides in the molecular pathogenesis and potential therapeutic strategies of cardiometabolic diseases: what we know so far. *Front Cell Dev Biol*. 2021;9:816301. https://doi.org/10.3389/fcell.2021.816301

[26] Akawi N, Checa A, Antonopoulos AS, Akoumianakis I, Daskalaki E, Kotanidis CP, et al. Fat-secreted ceramides regulate vascular redox state and influence outcomes in patients with cardiovascular disease. *J Am Coll Cardiol*. 2021;77:2494–513.

[27] Bozelli Jr JC, Azher S, Epand RM. Plasmalogens and chronic inflammatory diseases. *Front Physiol*. 2021;12:730829. https://doi.org/10.3389/fphys.2021.730829

[28] Schuurman AR, Léopold V, Pereverzeva L, Chouchane O, Reijnders TDY, Brabander J, et al. The platelet lipidome is altered in patients with COVID-19 and correlates with platelet reactivity. *Thromb Haemost*. 2022;122:1683–92.

[29] McFadyen JD, Stevens H, Peter K. The emerging threat of (micro) thrombosis in COVID-19 and its therapeutic implications. *Circ Res*. 2020;127:571–87.

[30] Deguchi H, Banerjee Y, Trauger S, Siuzdak G, Kalisiak E, Fernández JA, et al. Acylcarnitines are anticoagulants that inhibit

factor Xa and are reduced in venous thrombosis, based on metabolomics data. *Blood*. 2015;126:1595–600.

[31] Beyene HB, Hamley S, Giles C, Huynh K, Smith A, Cinel M, et al. Mapping the associations of the plasma lipidome with insulin resistance and response to an oral glucose tolerance test. *J Clin Endocrinol Metab*. 2020;105:dgaa054. https://doi.org/10.1210/clinem/dgaa054

[32] Dix C, McFadyen J, Huang A, Chunilal S, Chen V, Tran H. Understanding vaccine-induced thrombotic thrombocytopenia (VITT). *Intern Med J*. 2022;52:717–23.

[33] Cebo M, Dittrich K, Fu X, Manke MC, Emschermann F, Rheinlaender J, et al. Platelet ACKR3/CXCR7 favors antiplatelet lipids over an atherothrombotic lipidome and regulates thromboinflammation. *Blood*. 2022;139:1722–42.

[34] Yeung J, Tourdot BE, Fernandez-Perez P, Vesci J, Ren J, Smyrniotis CJ, et al. Platelet 12-LOX is essential for FcγRIIa-mediated platelet activation. *Blood*. 2014;124:2271–9.

[35] Schönborn L, Esteban O, Wesche J, Dobosz P, Broto M, Puig SR, et al. Anti-PF4 immunothrombosis without proximate heparin or adenovirus vector vaccine exposure. *Blood*. 2023;142:2305–14.

[36] Warkentin TE, Baskin-Miller J, Raybould AL, Sheppard JI, Daka M, Nazy I, et al. Adenovirus-associated thrombocytopenia, thrombosis, and VITT-like antibodies. *N Engl J Med*. 2023;389:574–7.

SUPPLEMENTARY MATERIAL

The online version contains supplementary material available at https://doi.org/10.1016/j.rpth.2025.102677