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RESEARCH LETTER

Galectin-3-binding protein and future venous thromboembolism

1 | INTRODUCTION

Venous thromboembolism (VTE), a term encompassing deep vein thrombosis and pulmonary embolism, affects annually more than 10 million people worldwide [1]. Knowledge of VTE risk factors is key for effective prevention and subsequent reduction of the disease burden in society. Nevertheless, up to 50% of incident VTEs occur in the absence of any recognized predisposing factor [2], indicating that there are still fundamental open questions regarding disease pathogenesis. Galectin-3-binding protein (gal3bp), a protein originally described as a tumor-associated antigen, interacts with various proteins, including gal3 [3]. The interaction between Gal3bp and gal3 promotes cell-to-cell adhesion and has been involved in regulation of inflammation and extracellular matrix remodeling [3,4]. A murine stasis model of venous thrombosis revealed that blocking gal3bp with antibodies or knocking out gal3 reduced thrombus weight [4], suggesting that these proteins might serve as therapeutic targets for VTE. Further, in the Atherosclerosis Risk in Communities study, gal3 levels were associated with the risk of future VTE [5]. However, to our knowledge, no study has evaluated the prospective association between gal3bp and VTE in the general population. Therefore, we aimed to investigate the association between plasma gal3bp levels and risk of future incident VTE in a population-based nested case-control study.

2 | METHODS

A population-based nested case-control study, including 415 patients with incident VTE and 847 randomly sampled age- and sex-matched controls, was derived from the fourth survey of the Tromsø Study cohort (Tromsø 4, 1994-2007). The details of the nested case-control design have been described elsewhere [6]. In this design, the temporal sequence between exposure and outcome is preserved since gal3bp was measured in plasma samples collected at inclusion in the parent cohort in 1994-1995. The regional committee for medical and health research ethics approved the study, and all participants provided written informed consent.

A VTE was classified as provoked or unprovoked depending on the presence of provoking factors closely preceding the VTE diagnosis [6]. At inclusion in Tromsø 4 (1994-1995), baseline assessment comprised body mass index (BMI), information on history of cancer and arterial cardiovascular disease (CVD), as well as collection of nonfasting blood samples in EDTA [6]. High-sensitivity C-reactive protein (CRP) and gal3bp were measured in platelet-free plasma in duplicates by enzyme-immunoassay using commercially available reagents (R&D Systems) [6]. The intra- and interassay coefficients of variation for CRP were 2.6% and 9.1%, respectively [6], whereas the intra and interassay coefficients of variation for gal3bp were 3% and 15.8%, respectively.

Statistical analyses were performed using Stata version 16 (StataCorp LLC) and R version 4.0.5 (The R Foundation for Statistical Computing). Gal3bp levels were categorized according to quartile cutoffs determined in controls. Unconditional logistic regression was used to estimate odds ratios (ORs) for VTE with 95% CIs according to gal3bp quartiles. The association between gal3bp levels and VTE was adjusted for age and sex and further for BMI and high-sensitivity CRP (inflammatory marker), given their potential to act as confounders. Based on previous findings of higher gal3bp levels in women than in men [7], we also performed analyses where gal3bp was categorized according to sex-specific quartile cutoffs. Results based only on baseline measurement of gal3bp could be affected by regression dilution bias due to the long follow-up time in the parent cohort [8]. To address this, we performed an analysis considering the time elapsed between blood sampling at baseline and the occurrence of VTE events while keeping all controls in the analyses. The methodological details of this analysis were previously described [6].

3 | RESULTS AND DISCUSSION

The distribution of baseline characteristics across quartiles of gal3bp plasma levels is shown in the Table. The mean age and BMI, median CRP levels, and the proportion of women and subjects with self-reported history of arterial CVD increased with higher gal3bp quartiles. The mean age at the time of the VTE event was 68 ± 14 years; 48% were men, 62% of the VTE events were deep vein thrombosis, and 58% were provoked VTEs.

The ORs for VTE according to quartiles of plasma gal3bp levels are shown in Figure 1A. In the age- and sex-adjusted model, the ORs

TABLE Distribution of baseline characteristics across quartiles of galectin-3-binding protein (gal3bp) plasma levels.

	Plasma Gal3bp levels (μg/mL)			
Characteristics	Quartile 1	Quartile 2	Quartile 3	Quartile 4
	<3.16	3.16-4.27	4.27-5.87	≥5.87
n	318	300	308	336
Age, y	57.7 ± 13.9	60.0 ± 13.5	60.3 ± 13.7	62.9 ± 13.6
Sex, women	43.7 (139)	49.3 (148)	54.9 (169)	63.4 (213)
BMI, kg/m^2	25.4 ± 4.0	25.7 ± 3.7	26.6 ± 4.0	27.8 ± 4.8
hsCRP, mg/L	1.00 (0.57-2.20)	1.18 (0.65-2.35)	1.36 (0.74-2.82)	1.94 (1.01-3.42)
Cancer[a]	3.8 (12)	3.7 (11)	3.6 (11)	7.14 (24)
CVD[a]	9.8 (31)	15 (45)	16.6 (51)	21.7 (73)

Continuous variables are shown as mean (± SD) or median (25th-75th percentile). Categorical variables are shown as percentages with numbers in brackets.

BMI, body mass index; CVD, cardiovascular disease; hsCRP, high-sensitivity C-reactive protein.

[a]Self-reported history of cancer or arterial cardiovascular disease (myocardial infarction, angina, or stroke) at baseline.

for VTE showed no association across gal3bp quartiles. Participants with plasma gal3bp levels in the highest quartile (≥5.87 μg/mL) had an OR for VTE of 1.19 (95% CI, 0.86-1.65) compared with those with gal3bp in the lowest quartile (<3.16 μg/mL). Risk estimates for VTE were attenuated to unity after adding BMI and CRP to the regression models. Results similar to the main analysis were obtained when sex-specific quartile cutoffs were applied (Figure 1A) and with the exclusion of participants with a self-reported history of arterial CVD or cancer at baseline (data not shown). Even when considering extreme levels of gal3bp (ie, ≥90th and ≥95th percentiles of the control population), no association between this protein and VTE risk was found (data not shown).

To evaluate the possibility of underestimating the true association because of regression dilution bias, we estimated ORs as a function of time between blood sampling and VTE events (Figure 1B). Although the ORs for VTE by high vs low levels of gal3bp were higher with shortened time between blood sampling and VTE events, risk estimates were not significant. Of note, this analysis should be interpreted with caution given its limited statistical power due to few events within the first years after blood sampling.

To our knowledge, this is the first study with a prospective design that evaluated the association between gal3bp and incident VTE in the general population. In a murine stasis model of venous thrombosis, DeRoo et al. [4] showed that mice given anti-gal3bp antibody prior to venous thrombosis induction had a significant decrease in thrombus weight compared with control mice. The potential lack of association between gal3bp and VTE in our study seems to be inconsistent with the murine model, implying that the experimental findings might not be clinically relevant for the development of VTE. Still, gal3bp could be relevant in selected conditions associated with inflammation. Interferon has been shown to upregulate gal3bp expression [9]. In line with this, gal3bp has previously been found to be elevated in diseases associated with low-grade inflammation and increased interferon activity, such as systemic lupus erythematosus [9]. As low-grade inflammation is reported to be associated with VTE [1], gal3bp could serve as a link between conditions associated with increased inflammatory response and VTE. This hypothesis would explain the apparent association between gal3bp and VTE in patients with systemic lupus erythematosus [10].

Some limitations of the study warrant attention. Blood samples were stored for more than 20 years and subjected to 1 additional freeze-thaw cycle before measuring gal3bp. However, because blood samples were stored in the same way, for the same duration, and subjected to the same number of freeze-thaw cycles in cases and controls, any potential misclassification would be non-differential with regards to VTE status, thereby introducing a possibility for underestimating the true associations. Gal3 is involved in biological pathways potentially relevant to VTE pathogenesis, including the regulation of inflammatory responses [4]. Thus, it would have been interesting to also investigate the impact of gal3 on VTE risk, but this protein was not measured in the nested case-control study. The majority of study participants were White, and caution is needed to generalize our findings to other ethnicities.

In conclusion, we found that plasma levels of gal3bp were not associated with the risk of future incident VTE, suggesting that this protein may not play a relevant role in VTE pathogenesis in the general population.

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AUTHOR CONTRIBUTIONS

E-S.H. analyzed data, interpreted the results, and drafted the manuscript. M.S.E. interpreted the results and revised the manuscript. P.A. and T.U. performed the laboratory analysis, interpreted the results, and revised the manuscript. J-B.H. and S.K.B. designed the study, organized data collection, interpreted the results, and revised the manuscript. V.M.M. designed the study, interpreted the results, contributed to the manuscript draft, and revised the manuscript. All authors reviewed and approved the final version of the manuscript.

RELATIONSHIP DISCLOSURE

There are no competing interests to disclose.

A

	Controls	Cases	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
Gal3bp quartiles (μg/mL)					
<3.16	211	107	Ref.	Ref.	Ref.
3.16-4.27	211	89	0.84 (0.59-1.18)	0.81 (0.58-1.15)	0.79 (0.56-1.12)
4.27-5.87	214	94	0.87 (0.62-1.23)	0.82 (0.59-1.16)	0.78 (0.56-1.11)
≥5.87	211	125	1.19 (0.86-1.65)	1.06 (0.75-1.48)	0.97 (0.69-1.36)
P for trend			0.3	0.7	0.9
Gal3bp sex-specific quartiles					
Quartile 1	209	106	Ref.	Ref.	Ref.
Quartile 2	215	107	0.98 (0.70-1.36)	0.95 (0.68-1.33)	0.92 (0.66-1.29)
Quartile 3	212	78	0.72 (0.51-1.03)	0.68 (0.48-0.97)	0.65 (0.45-0.93)
Quartile 4	211	124	1.16 (0.84-1.60)	1.03 (0.74-1.44)	0.94 (0.67-1.33)
P for trend			0.7	0.8	0.4

B

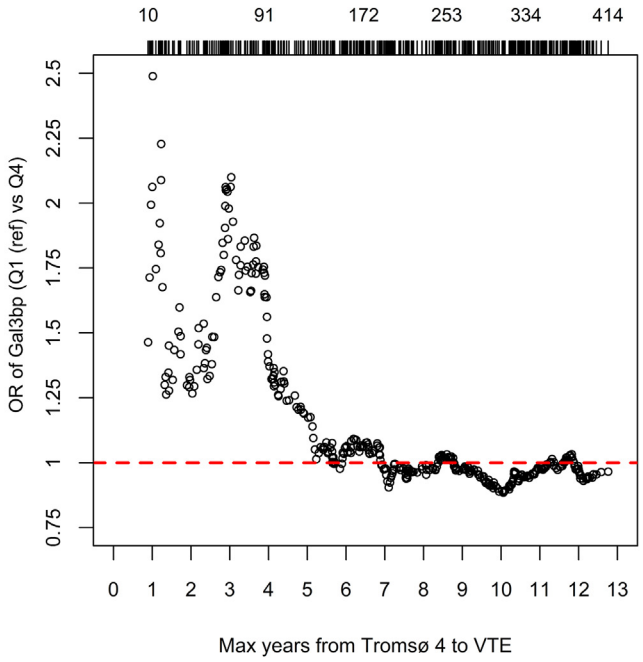

FIGURE 1 Plasma levels of galectin-3-binding protein (gal3bp) and venous thromboembolism (VTE). (A) Odds ratios (OR) with 95% CI for venous thromboembolism (VTE) according to quartiles of gal3bp plasma levels. Model 1, adjusted for age and sex; model 2, adjusted for age, sex, and body mass index (BMI); model 3, adjusted for age, sex, BMI, and high-sensitivity C-reactive protein. Women, quartile 1: <3.42 μg/mL; quartile 2: 3.42-4.82 μg/mL; quartile 3: 4.82-6.71 μg/mL; and quartile 4: ≥6.71 μg/mL. Men, quartile 1: <2.92 μg/mL; quartile 2: 2.92-3.96 μg/mL; quartile 3: 3.96-5.20 μg/mL; and quartile 4: ≥5.20 μg/mL. (B) Plots of estimated ORs for overall VTE as a function of time from blood sampling in Tromsø 4 (1994-1995) to VTE events. Participants with plasma levels of gal3bp in the highest quartile (Q4) were compared with those with gal3bp in the lowest quartile (Q1, reference category). Analyses were adjusted for age, sex, BMI, and high-sensitivity C-reactive protein. Risk estimates were not statistically significant ($P < .05$). The number of VTE events is depicted above the plot. Note that because of a missing value in BMI, there were 414 VTE events when BMI was used as an adjustment variable.

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