

● ● ● CLINICAL TRIALS

Comment on Buil et al, page 4644

## VT study disassociates C4BP from protein S

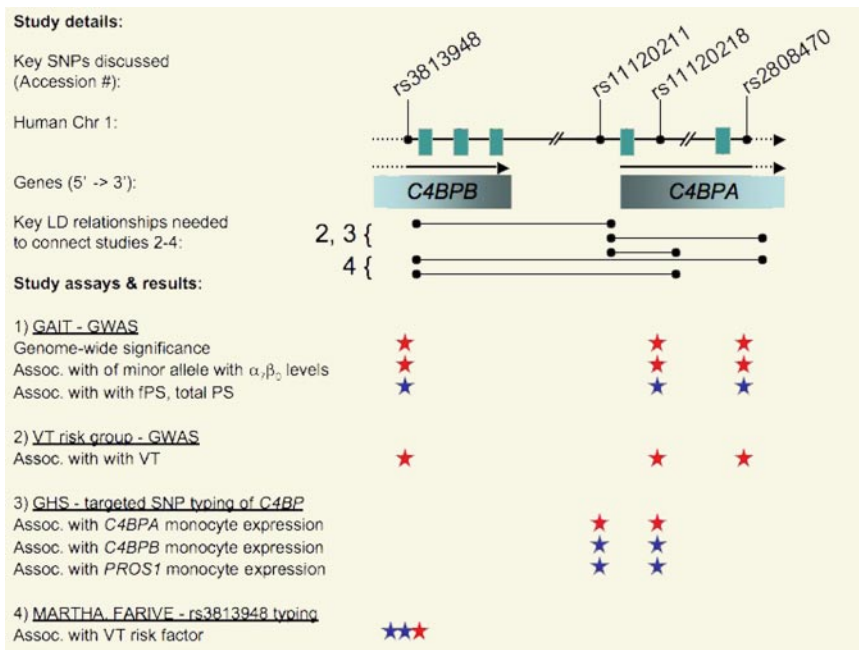
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In this issue of *Blood*, Buil and colleagues combine data from genome-wide associations, a case-control study, and expression and plasma analyses to implicate unbound C4BP, but not protein S, as a risk factor for venous thrombosis (see figure). Their study provides a new basic research direction into the functions of unbound C4BP not involving protein S.

binding protein (C4BP) circulates in the blood in the different isoforms comprising  $\alpha$  and  $\beta$  subunits. The investigators establish that increased *C4BPA* expression in monocytes was associated with SNPs at the locus, and that plasma levels of the unbound  $\alpha_7\beta_0$  isoform were elevated in venous thrombosis patients. Their combination of genetic approaches and validation experiments unexpectedly point to the importance of the  $\alpha_7\beta_0$  isoform, which is the only circulating form of C4BP unable to bind protein S.

Protein S has been an ever-popular candidate locus for venous thrombosis because it confers high risk in human subjects with hereditary protein S deficiency.<sup>3</sup> However, in persons lacking any of the well-characterized mutations, variations in protein S fail to explain much of the more subtle range of quantitative variations in susceptibility to venous thrombosis in the general population.

In contrast, C4BP previously had only been mentioned in the context of venous thrombosis by following the “guilty-by-association” principle.<sup>4</sup> Generally, complement inactivator proteins, which include C4BP, are thought of as involved in immune response and inflammation.<sup>4</sup> Interestingly, whereas the molecular bond between C4BP and protein S has led to the implication of the latter protein in immune response and inflammation, the reverse has not been a popular hypothesis; that is, that the binding of C4BP to protein S implicates C4BP in venous thrombosis. C4BP was mostly noted as a “piggy-back” protein of protein S. However, once the inactivity of C4BP-bound protein S was established, a possible role for C4BP in the context of venous thrombosis emerged.<sup>5</sup> The possibility that C4BP can be independently active in the coagulation pathway was noted in an earlier study as well.<sup>6</sup> Now Buil et al are suggesting that, indeed, in the case of venous thrombosis, C4BP merits to be viewed as an active participant rather than a passive associate of protein S.



The complement binding protein gene cluster containing *C4BPB* and *C4BPA* mapped as new genetic risk factor for venous thrombosis (VT) by means of genome-wide association mapping studies (GWAS) in the GAIT cohort (1), a VT-risk group (2), targeted SNP typing in the GHS study (3), single SNP case-control analyses (rs3813948) of the MARTHA and FARIVE cohorts (4). Key linkage disequilibrium (LD) relationships between key SNPs employed by Buil et al in the context of studies. Significant support and key nonsignificance of assays are shown as red and blue, respectively. Significance was observed only for a combined analysis of both cohorts, but not for each cohort separately. FPS, free plasma protein S; PS, total plasma protein S, *PROS1*, protein S gene.

**B**uil and colleagues, in this issue of *Blood*, fine map a new susceptibility locus for venous thrombosis to 1q32, at the position where the *C4BPA* and *C4BPB* genes are located.<sup>1</sup> Although this feat falls short of the

conclusive identification of causative single nucleotide polymorphisms (SNPs),<sup>2</sup> a strength of the study lies in the efforts to explore the contribution of candidate SNPs to venous thrombosis. C4b-complementary

To be clear, the fact that protein S deficiency is a risk factor for venous thrombosis is not a matter of controversy.<sup>3</sup> Based on my reading of the study, Buil et al are now proposing that the bonding between C4BP and protein S, compelling as it has been due to the function in blood coagulation, may have led to the impression that further investigations into any additional genetic factors with direct ties to this specific pathway were unnecessary.

In the broadest sense, genome-wide association studies liberate basic researchers from preexisting hypotheses so that even if particular loci have dominated discussions in the context of a disease, the genome-wide association approach provides an unbiased method to interrogate the genome for other significant loci. This approach has paid off; their results suggest that C4BP merits its own discussion, apart from protein S binding, as another avenue of investigation into the risk for venous thrombosis.<sup>1</sup>

While the C4BP locus emerged as an independent player on the stage of venous thrombosis from this study, its prominent molecular liaison, protein S, has dropped out of the picture, which feels a bit uncomfortable given the appreciation of the importance of this bond between the 2 proteins.<sup>4</sup> Not only was protein S not present in the genome-wide association studies presented in the paper, but the authors went to some length to confirm that the SNPs were not associated with protein S levels. However, because each of the 4 study components was rather independent, with its own strengths and limitations, the evidence *against* protein S perhaps could have been stronger. Moreover, the authors rely heavily on the interpretation of linkage disequilibrium to connect study components that relied on different sets of SNPs, but in the end, leave the entire CBPB locus standing as a candidate (see figure). Linkage disequilibrium within and between the genes at the CBPB locus likely will render further fine-scale mapping attempts tricky.<sup>2</sup> Finally, attempts to replicate or expand on this study need to consider that linkage disequilibrium between key SNPs might vary between study cohorts drawn from general populations of various genomic backgrounds.<sup>7</sup>

The work by Buil et al should set the stage for basic research into the unknown pathways that contribute to the risk of venous thrombosis in the general population. It is common for the variances in complex

phenotypic trait values that can be explained by candidate SNPs to be underwhelming.<sup>2</sup> Thus, the ability to account for approximately 11% of the variance in expression levels of C4BPA and plasma levels of unbound  $\alpha_7\beta_0$  should be looked upon as great success that is consistent with the authors' view that the study of the unbound C4BP  $\alpha_7\beta_0$  isoform could be a productive field for investigation on its own. However, although compelling, results by others indicate that the study of the interaction between protein S and C4BP continues to yield results of interest with regard to quantitative variation in protein S,<sup>8</sup> and thus, that it may be premature to throw out the baby with the bath water.

In all, the result that the up-regulation of unbound C4b-complementary binding protein (C4BP), but not protein S, emerged from these analyses as a genetic risk factor for venous thrombosis is an unexpected but needed impetus for an expanded view of the factors involved in venous thrombosis. It also merits a shift from protein S-centric research. Despite the potential fallacies of array-based genome-wide association studies,<sup>2</sup> in this and a few other cases a bird's-eye view of the genome has enabled Buil et al to spot a new target well worth pursuing on the ground.<sup>1,9,10</sup>

*Conflict-of-interest disclosure: The author declares no competing financial interests.* ■

## REFERENCES

1. Buil A, Tréguët DA, Souto JC, et al. C4BPB/C4BPA is a new susceptibility locus for venous thrombosis with unknown protein S-independent mechanism: results from genome-wide association and gene expression analyses followed by case-control studies. *Blood*. 2010;115(23):4644-4650.
2. McCarthy MI, Abecasis GR, Cardon LR, et al. Genome-wide association studies for complex traits: consensus, uncertainty and challenges. *Nat Rev Genet*. 2008;9(5):356-369.
3. Seligsohn U, Lubetsky A. Genetic susceptibility to venous thrombosis. *N Engl J Med*. 2001;344(16):1222-1231.
4. Dahlbäck B. The tale of protein S and C4b-binding protein, a story of affection. *Thromb Haemost*. 2007;98(1):90-96.
5. Maurissen LF, Thomassen M, Nicolaes GA, et al. Re-evaluation of the role of the protein S-C4b binding protein complex in activated protein C-catalyzed factor Va-inactivation. *Blood*. 2008;111(6):3034-3041.
6. van de Poel RH, Meijers JCM, Rosing J, Tans G, Bouma BN. C4b-binding protein protects coagulation factor Va from inactivation by activated protein C. *Biochemistry*. 2000;39(47):14543-14548.
7. International HapMap Consortium. A haplotype map of the human genome. *Nature*. 2005;437(7063):1299-1320.
8. Denis CV, Roberts SJ, Hackeng TM, Lenting PJ. In vivo clearance of human protein S in a mouse model: influence of C4b-binding protein and the Heerlen polymorphism. *Arterioscler Thromb Vasc Biol*. 2005;25(10):2209-2215.
9. Tréguët DA, Heath S, Saut N, et al. Common susceptibility alleles are unlikely to contribute as strongly as the FV and ABO loci to VTE risk: results from a GWAS approach. *Blood*. 2009;113(21):5298-5303.
10. Li Y, Bezemer ID, Rowland CM, et al. Genetic variants associated with deep vein thrombosis: the F11 locus. *J Thromb Haemost*. 2009;7(11):1802-1808.

## ● ● ● IMMUNOBIOLOGY

Comment on Kumar et al, page 4725

# Bulging glands? Blame it on B cells

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Gross lymph node morphologic changes often accompany infections. In this issue of *Blood*, Kumar and colleagues show that B cells contribute to lymphoid remodeling via LTs following viral insults.<sup>1</sup>

**A** crucial function of the lymph node (LN) is to facilitate physical interactions among rare immune cells arriving from various tissue compartments. The unique positioning of the LN at the interface between the blood and lymphatic systems allows tissue-derived antigen and antigen-presenting cells (APCs) to congregate in close proximity to blood-derived lymphocytes. Increased lymphocyte recruitment, decreased egress, and

enhanced cytokine/chemokine communication network, together with changes in LN architecture to accommodate the massive cellular influx, all conspire to accomplish one goal: to enhance physical encounters between relevant lymphocytes and APCs that lead to timely and efficient immune activation.<sup>2,3</sup>

In recent years, there has been an increased appreciation of the role of B cells in lymphoid tissue organogenesis and immune response