

# Reactome Pathway Analysis of Venous Thromboembolism, Peripheral Artery Disease, Stroke, and Coronary Artery Disease

Bengt Zöller<sup>1</sup> Peter J. Svensson<sup>2</sup> Wuqing Huang<sup>1</sup> Ji Jianguang<sup>1</sup>

<sup>1</sup> Department of Clinical Sciences, Center for Primary Health Care Research, Skåne University Hospital, Lund University, Malmö, Sweden

<sup>2</sup> Department of Coagulation Disorders, Skåne University Hospital, Lund University, Lund, Sweden

Address for correspondence Bengt Zöller, MD, PhD, Center for Primary Health Care Research, Jan Waldenströms gata 35, Skåne University Hospital, S-205 02 Malmö, Sweden (e-mail: [bengt.zoller@med.lu.se](mailto:bengt.zoller@med.lu.se)).

Thromb Haemost 2021;121:964–966.

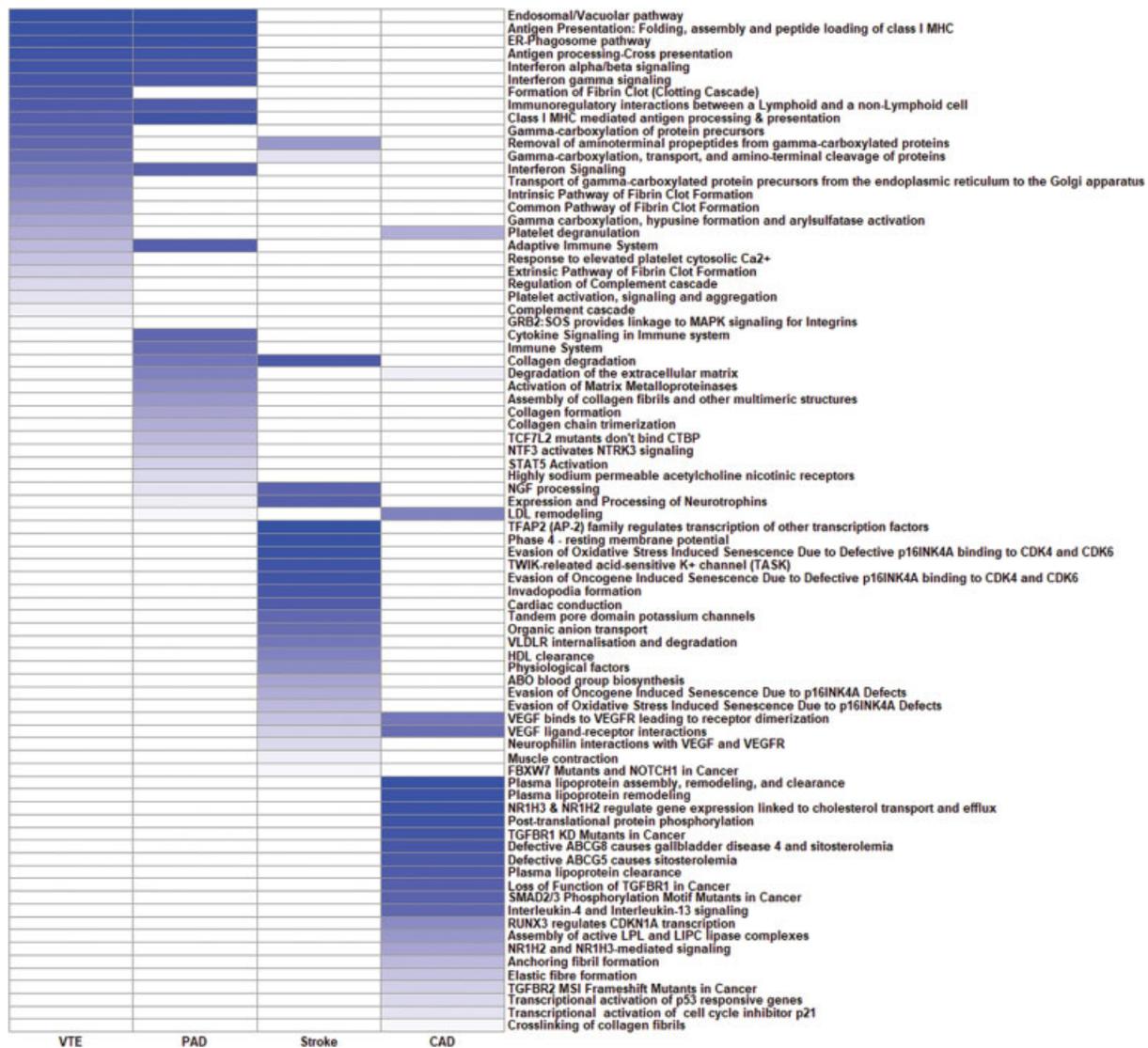
In *Nature Genetics*, Klarin et al reported a genome-wide association study (GWAS) of venous thromboembolism (VTE).<sup>1</sup> The authors identified 33 VTE risk loci, of which 22 were novel. Of special interest is the genetic overlap with arterial vascular disease and especially peripheral artery disease (PAD).<sup>1</sup> The genetic overlap between VTE with stroke and coronary artery disease (CAD) was weaker. Using linkage disequilibrium score regression, a stronger positive correlation between VTE and PAD ( $r=0.47$ ,  $p=2.0 \times 10^{-15}$ ) than for VTE and CAD ( $r=0.27$ ,  $p=1.2 \times 10^{-7}$ ) or VTE and large artery stroke ( $r=0.35$ ,  $p=0.02$ ) was observed throughout the genome.<sup>1</sup> This is in agreement with another GWAS study by Klarin et al that identified factor V Leiden (rs6025) to be a graded risk factor for PAD severity.<sup>2</sup> No association between factor Leiden and CAD or stroke was observed.<sup>2</sup> Interestingly, these findings are also in agreement with previous nationwide epidemiological register-based family studies in Sweden.<sup>3–5</sup> Family history of VTE is a strong risk factor for VTE in Sweden as determined using the world's largest family database—the Swedish Multi-generation Register.<sup>6</sup> However, family history of VTE is not a strong risk factor for CAD or ischemic stroke in Sweden.<sup>3,4</sup> Instead, a weak to moderate association is observed between family history of VTE and PAD in Sweden.<sup>5</sup> Among Swedish families the association between family history of VTE and PAD is especially strong for arterial thrombosis and embolism in the peripheral vasculature in agreement with the GWAS studies by Klarin et al.<sup>1,2,5</sup>

To better understand the genetic overlap reported by Klarin et al<sup>1</sup> for VTE with arterial disease we have performed a Reactome pathway analysis (<https://reactome.org>) for VTE, PAD, stroke, and CAD.<sup>7</sup> Reactome pathway analyses are used to interpret high-throughput experimental results. It could also be used to formulate predictive models of normal and disease pathways.<sup>7</sup> We used risk loci (or nearest loci) as reported in GWAS for VTE,<sup>1</sup> PAD,<sup>2</sup> stroke,<sup>8</sup> and CAD.<sup>9</sup> In

►**Fig. 1** are presented the 25 most relevant pathways identified for VTE, PAD, stroke, and CAD by a Reactome pathway analysis based on reported genes from GWAS of VTE, PAD, stroke, and CAD.<sup>1,2,8,9</sup> VTE shares 10 of the 25 (40%) most relevant pathways with PAD. VTE shares two pathways (8%) with stroke and one (4%) with CAD. PAD shares three (12%) pathways with stroke and two (8%) with CAD. Stroke shares two (8%) pathways with CAD. This is in agreement with a previous Swedish nationwide epidemiological register-based study that indicated that although PAD partially shares familial susceptibility with CAD and stroke, unique inherited site-specific factors are likely to exist.<sup>10</sup>

Of special interest is that all 10 shared pathways for VTE and PAD involve the immune system and inflammation (►**Fig. 1**). Nine of the VTE Reactome pathways are involved in coagulation and three VTE Reactome pathways involve platelets. Two VTE Reactome pathways involve the complement system and one VTE Reactome pathway involves mitogen-activated protein kinase signaling for integrins.

Reactome pathway analysis has not been performed for VTE. Baaten et al performed a Reactome pathway analysis of experimental arterial thrombosis in mouse models.<sup>11</sup> A limitation of the mouse study was the inclusion of a large set of human genes that are linked to inherited bleeding disorders, whereas genes related to the vascular component of thrombosis were limited. Thus, mainly hemostatic pathways were detected. However, as in the present study cancer pathways were identified (►**Fig. 1**).<sup>11</sup> Many studies have linked cardiovascular disorders and cancer.<sup>12,13</sup> Reactome pathway analyses have also been performed on gene expression data from samples of peripheral blood from patients.<sup>14–16</sup> The expression of vascular and liver plasma proteins are not reflected in these analysis.<sup>14–16</sup> Nair et al focused on expression of inflammatory genes obtained by linking PolySearch and CADgene databases.<sup>16</sup> A study by Ghosh et al used GWAS data for Reactome pathway analysis of CAD.<sup>17</sup>



**Fig. 1** The 25 most relevant Reactome pathways (<https://reactome.org>)<sup>7</sup> according to genome-wide association study (GWAS) associated genes for venous thromboembolism (VTE),<sup>1</sup> peripheral artery disease (PAD),<sup>2</sup> stroke,<sup>8</sup> and coronary artery disease (CAD).<sup>9</sup> VTE and PAD share most pathways (10/25 = 40%). Color is graded according to strength of association.

Several CAD pathways identified by Ghosh et al involve mechanism also observed in the present study, that is, metabolism (lipids), signal transduction, extracellular matrix organization, immune system, and transport of small molecules (► **Fig. 1**).<sup>17</sup>

A limitation of the Reactome analysis is that the analysis does not take into account the effect size and the minor allele frequency of respective GWAS variant. Only the genome-wide significant gene loci were entered directly in the Reactome analysis. Moreover, in many cases we do not know which gene locus harbors the functional variant causing the association with VTE, PAD, stroke, and CAD. Still, the Reactome pathway analysis appears to be fairly valid as coagulation Reactome pathways are mainly related to VTE and Lipid Reactome pathways to arterial disease (especially CAD) (► **Fig. 1**). The following coagulation-related pathways are only associated with VTE: formation of fibrin clot; gamma-carboxylation of protein precursors; transport of gamma-carboxylated protein precursors from the endoplasmic reticulum to the Golgi apparatus; intrinsic pathway of

fibrin clot formation; common pathway of fibrin clot formation, gamma carboxylation, hypusine formation, and aryl-sulfatase activation; and extrinsic pathway of fibrin clot formation (► **Fig. 1**). Several lipid pathways are only related to CAD: plasma lipoprotein assembly, remodeling, and clearance; plasma lipoprotein remodeling; NR1H3 and NR1H2 regulate gene expression linked to cholesterol transport and efflux; plasma lipoprotein clearance; low-density lipoprotein remodeling; and plasma lipoprotein clearance (► **Fig. 1**).

In conclusion, two GWAS by Klarin et al genetically link VTE to arterial disease (especially PAD).<sup>1,2</sup> Our Reactome pathway analysis indicates that especially VTE and PAD share many Reactome pathways, while less Reactome pathways are shared for VTE with stroke or CAD. The Reactome pathway analysis not only confirms the involvement of the coagulation system and platelets in VTE but also indicates the importance of the immune system and inflammation in both VTE and PAD. The immune system might be a novel target for the development of new therapeutic agents for VTE and PAD.

**Conflict of Interest**

None declared.

**Acknowledgments**

This work was supported by grants to Bengt Zöller from the Swedish Heart Lung Foundation, Swedish Research Council, and ALF funding by Region Skåne.

**References**

- 1 Klarin D, Busenkell E, Judy R, et al;INVENT Consortium Veterans Affairs' Million Veteran Program. Genome-wide association analysis of venous thromboembolism identifies new risk loci and genetic overlap with arterial vascular disease. *Nat Genet* 2019;51(11):1574–1579
- 2 Klarin D, Lynch J, Aragam K, et al;VA Million Veteran Program. Genome-wide association study of peripheral artery disease in the Million Veteran Program. *Nat Med* 2019;25(08):1274–1279
- 3 Zöller B, Li X, Sundquist J, Sundquist K. Venous thromboembolism does not share strong familial susceptibility with coronary heart disease: a nationwide family study in Sweden. *Eur Heart J* 2011;32(22):2800–2805
- 4 Zöller B, Li X, Ohlsson H, Sundquist J, Sundquist K. Venous thromboembolism does not share strong familial susceptibility with ischemic stroke: a nationwide family study in Sweden. *Circ Cardiovasc Genet* 2011;4(05):484–490
- 5 Zöller B, Li X, Sundquist J, Sundquist K. A nationwide family study of venous thromboembolism and risk of arterial vascular disease. *Heart* 2016;102(16):1315–1321
- 6 Zöller B, Li X, Sundquist J, Sundquist K. Age- and gender-specific familial risks for venous thromboembolism: a nationwide epidemiological study based on hospitalizations in Sweden. *Circulation* 2011;124(09):1012–1020
- 7 Fabregat A, Jupe S, Matthews L, et al. The Reactome Pathway Knowledgebase. *Nucleic Acids Res* 2018;46(D1):D649–D655
- 8 Malik R, Chauhan G, Traylor M, et al;AFGen Consortium Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium International Genomics of Blood Pressure (iGEN-BP) Consortium INVENT Consortium STARNET BioBank Japan Cooperative Hospital Group COMPASS Consortium EPIC-CVD Consortium EPIC-InterAct Consortium International Stroke Genetics Consortium (ISGC) METASTROKE Consortium Neurology Working Group of the CHARGE Consortium NINDS Stroke Genetics Network (SiGN) UK Young Lacunar DNA Study MEGASTROKE Consortium. Publisher Correction: multiethnic genome-wide association study of 520,000 subjects identifies 32 loci associated with stroke and stroke subtypes. *Nat Genet* 2019;51(07):1192–1193
- 9 van der Harst P, Verweij N. Identification of 64 novel genetic loci provides an expanded view on the genetic architecture of coronary artery disease. *Circ Res* 2018;122(03):433–443
- 10 Calling S, Ji J, Sundquist J, Sundquist K, Zöller B. Shared and non-shared familial susceptibility of coronary heart disease, ischemic stroke, peripheral artery disease and aortic disease. *Int J Cardiol* 2013;168(03):2844–2850
- 11 Baaten CCFM, Meacham S, de Witt SM, et al. A synthesis approach of mouse studies to identify genes and proteins in arterial thrombosis and bleeding. *Blood* 2018;132(24):e35–e46
- 12 Koene RJ, Prizment AE, Blaes A, Konety SH. Shared risk factors in cardiovascular disease and cancer. *Circulation* 2016;133(11):1104–1114
- 13 Blaes AH, Shenoy C. Is it time to include cancer in cardiovascular risk prediction tools? *Lancet* 2019;394(10203):986–988
- 14 Masud R, Shameer K, Dhar A, Ding K, Kullo IJ. Gene expression profiling of peripheral blood mononuclear cells in the setting of peripheral arterial disease. *J Clin Bioinforma* 2012;2:6
- 15 Arvind P, Jayashree S, Jambunathan S, Nair J, Kakkar VV. Understanding gene expression in coronary artery disease through global profiling, network analysis and independent validation of key candidate genes. *J Genet* 2015;94(04):601–610
- 16 Nair J, Ghatge M, Kakkar VV, Shanker J. Network analysis of inflammatory genes and their transcriptional regulators in coronary artery disease. *PLoS One* 2014;9(04):e94328
- 17 Ghosh S, Vivar J, Nelson CP, et al. Systems genetics analysis of genome-wide association study reveals novel associations between key biological processes and coronary artery disease. *Arterioscler Thromb Vasc Biol* 2015;35(07):1712–1722