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Unraveling the Clotting Cascade in Vitamin K Deficiency Associated Comorbidities

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Abstract

The coagulation mechanism can be affected by vitamin K (VK) deficiency, a severe nutritional imbalance that is associated with a number of comorbidities. In this study, we incorporate data from the Gene Expression Omnibus (GEO) archive to provide a study into the unraveling of the clotting cascade in the context of VK deficiency. Maintaining hemostasis is crucial, and VK is an essential cofactor for the synthesis of coagulation factors. A lack of VK has been associated with a higher risk of bleeding problems and other comorbidities. In the present investigation, we utilized publicly accessible GEO datasets to examine the transcriptional alterations in genes linked with the clotting cascade when deficiency of VK and its associated conditions are involved. The results of our study suggested that there were significant variations in the expression of genes associated with fibrinolysis, vascular homeostasis, and coagulation in response to VK deficiency. These alterations were significantly connected to the risk of thrombotic and hemorrhagic episodes, as well as various diseases that have been associated with VK deficiency-associated disease phenotypes, including diabetes, placenta, prostate, hepatic, renal, and cardiovascular disorders.

Keywords: Vitamin K, Transcriptome, Coagulation, Comorbidities, Pathways

Introduction:

Vitamin K (VK) plays a crucial role in blood coagulation which is termed as Koagulations-Vitamin in both German and Scandinavian languages (1). When blood vessels rupture, a complicated physiological process known as blood coagulation, or clotting, prevents excessive bleeding. Because it is essential for the synthesis of the various proteins involved in the formation of blood clots, VK plays a key role for this process. Prothrombin and a group of proteins called the VK-dependent clotting factors (Factors II, VII, IX, and X) (Table 1) are the two primary proteins that are dependent on VK (2). VK is required for the synthesis of inactive precursor proteins that play a vital role in coagulation, which are then converted into their active forms through a process called carboxylation (3). When a blood vessel is injured,

a series of events are triggered to form a blood clot, and this process, known as the coagulation cascade, involves a sequence of enzymatic reactions which ultimately leads to the formation of a stable blood clot. While VK-dependent clotting factors are integral to this cascade, without VK, these factors may not be activated (3). Once the clotting factors are activated, they participate in the formation of fibrin, a protein mesh that stabilizes the blood clot wherein fibrin binds blood cells together and forms a barrier at the site of injury to prevent further blood loss (4). VK deficiency can lead to impaired blood clotting, which can result in excessive bleeding or hemorrhage. Individuals with a deficiency in VK, such as those with certain medical conditions or those taking medications that interfere with VK metabolism, are at risk of bleeding disorders. In contrast, VK antagonists, such as warfarin, are used in medical settings to reduce blood clotting and prevent thrombosis by inhibiting the function of VK-dependent clotting factors (2).

Table 1. Genes related to VK-dependent blood coagulation. Mutations or deficiencies in the above listed genes can lead to bleeding disorders or clotting disorders, depending on whether the gene product is deficient, overactive or nonfunctional. Understanding the genetics of these coagulation factors is crucial for diagnosing and managing such disorders.

Genes	Encodes	Crucial roles
F2 (Factor II)	Factor II, or prothrombin, is produced from the F2 gene	Prothrombin is a precursor to thrombin, which is a key enzyme in the coagulation process
F7 (Factor VII)	Coagulation factor VII, which is also known as proconvertin	Involves in the initiation of the coagulation cascade
F9 (Factor IX)	Coagulation factor IX, also called Christmas factor	Deficiency in factor IX leads to hemophilia B
F10 (Factor X)	Factor X is encoded by the F10 gene	Role in the common pathway of the coagulation cascade
Protein C (PROC) and Protein S (PROS1)	Protein C is an anticoagulant protein, and it is activated by the thrombin-thrombomodulin complex	The PROC gene encodes protein C. Protein S, which acts as a cofactor for protein C, is encoded by the PROS1 gene
Protein Z (PROZ)	Protein Z is another protein involved in the regulation of blood coagulation	It is encoded by the PROZ gene

We have earlier worked on systems genetics integration of variants associated with VK and associated disease phenotypes and identified DEGs and variants associated with them (5). In this work, we aimed to identify the clotting cascades associated with VK deficiency by employing gene expression omnibus (GEO) database and discerning the role of DEGs associated in these datasets. The work coalesces taking the GEO and GEO RNA-seq Experiments Interactive Navigator (GREIN) for downstream analyses.

Material and Methods:

The GEO is a publicly accessible repository of transcriptome data from various biological experiments. We retrieved the data of four datasets associated with VK disease phenotypes from the GEO database pertaining to blood coagulation in humans, *viz.* ranging from development and disease to diabetes, Alzheimer and cardiovascular diseases, placenta, and hepatic functions in VK2 (Table 1). We used an interactive web platform GREIN that analyzes

Table 2. Research data from GEO datasets.

Study	Title	Number of GEO samples	Reference
GSE109265	Bioinformatics analysis of transcriptome related to blood stasis syndrome in diabetes mellitus patients	6	(7)
GSE111366	Apolipoprotein E4 Expression Causes Gain of Toxic Function in Isogenic Human Induced Pluripotent Stem Cell-Derived Endothelial Cells	12	(8)
GSE171223	Intersection of Regulatory Pathways Controlling Hemostasis and Hemochorial Placentation [human]	8	(9)
GSE76098	Connexin 32-mediated cell-cell communication is essential for hepatic differentiation from human embryonic stem cells	16	(10)

GEO RNA-seq data (6) using the shiny framework. The results of this study can be taken to further investigations on VK's role in coagulation and make important contributions to improve deficiency.

Results and Discussion:

There are significant DEGs common between the datasets as we compared the 42 datasets. The GSE109265 harbours specific genes associated with diabetes to blood coagulation and VK wherein the coagulation cascade, helps in synthesizing a variety of proteins, and aids in development of clotting factors. However, there are some indirect associations between diabetes, blood coagulation, and VK. A protein called matrix Gla, which is dependent on VK, protects and maintains soft tissues and blood arteries from acquiring calcification. While undercarboxylated MGP may arise from insufficient VK intake, this may increase vascular calcification. Diabetic patients often develop vascular calcification, which is associated with cardiovascular problems. Another protein that is dependent on VK is osteocalcin. Despite being mostly linked to bone health, some research indicates that osteocalcin might also be involved in glucose metabolism. It has been suggested to affect the sensitivity and secretion of insulin. Changes in VK status may have an indirect effect on the function of osteocalcin and may have an effect on diabetes. The development and progress of diabetes is significantly impacted by oxidative stress and inflammation. Due to its anti-inflammatory properties, VK may be linked to lower levels of inflammation, according to specific study results. Insulin resistance and diabetes can both be increased by inflammation. The GSE111366 study (8) is about apolipoprotein which plays a major role in Alzheimer disease, cardiovascular disorders that impacts the cerebral and vascular system. The third study GSE171223 is about hemostasis on maternal-fetal interface (9). Prothrombotic challenges, particularly those induced on by placentation, are countered by TFPT. TFPI was found in trophoblast cells lining uterine spiral arterioles and other places in the placenta that functioned as blood delivery channels at the placentation site. TFPI is positioned in such a manner to prevent blood coagulation cascades from being triggered, which might interfere with blood flow into the placenta (9). The last study GSE76098 is about how VK2 regulates the maturation of human pluripotent stem cells to hepatocytes (10). The results indicated that up-regulation of Cx32 may be highly beneficial for the maturation of hepatic functions, as VK2 demonstrated improved maturation for hepatic functions.

The set of three genes PHACTR1, lncRNAs and novel protein are found common in the datasets of diabetes, ApoE, placenta, Pca and renal. The protein ENSG00000112137 (Phosphatase and Actin Regulator 1) encoded by PHACTR1 gene is a member of the phosphatase and actin regulator family of proteins which can bind actin and regulate the reorganization of the actin cytoskeleton (11). It plays a role in tubule formation and in endothelial cell survival. PHACTR1 SNPs are associated with susceptibility to myocardial infarction, coronary artery disease and cervical artery dissection (12). The two lncRNAs are ENSG00000241135 (LINC00881); ENSG00000253819 - LINC01151 and novel protein (ENSG00000273259) whose function is unknown. The group of hepatic, prostate and renal datasets has three common DE genes. The biological function of lncRNA genes ENSG00000235160 LINC02248 (lncRNA) and ENSG00000260372 AQP4-AS1 (AQP4 Antisense RNA 1) are unknown; and the gene ENSG00000283683 MYOCOS (Myocilin Opposite Strand) involves in neuronal development and diseases (13). ENSG00000274461 (LINC02930) and ENSG00000211893 (IGHG2), a Immunoglobulin Heavy Constant Gamma 2 (G2m Marker) was found common between the hepatic, myocardial and prostate datasets, that mainly involves heparin-induced Thrombocytopenia Pathway (14). ENSG00000086289 and ENSG00000133110 were found to be common between diabetes, apolipoprotein, placenta, hepatic, myocardial and prostate datasets. The gene ENSG00000086289 EPDR1 (Ependymin Related 1) may play a role in calcium-dependent cell adhesion that associates various types of cancer includes (15–18) and the gene ENSG00000133110 Periostin (POSTN) encodes a secreted extracellular matrix protein that functions in tissue development and regeneration, including wound healing, and ventricular remodeling following myocardial infarction. Further it binds to integrins to support adhesion and migration of epithelial cells that plays a role in cancer stem cell maintenance and metastasis (19–21). The gene ENSG00000225255 PSLNR (Prostate Enriched LncRNA) is found common between hepatic and pca datasets (Supplementary Table 1).

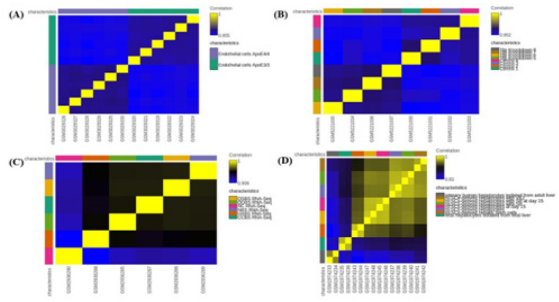

Figure. 1: Visualized relationships between the genes across different datasets in correlation plots representing (A) apolipoprotein (B) placenta (C) diabetes mellitus (D) hepatic functions.

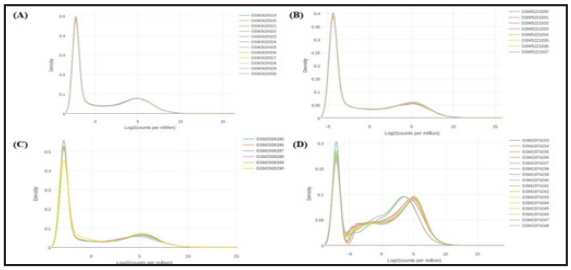

Figure. 2: Visualized the potential subpopulation genes in density plots representing (A) apolipoprotein (B) placenta (C) diabetes mellitus (D) hepatic functions

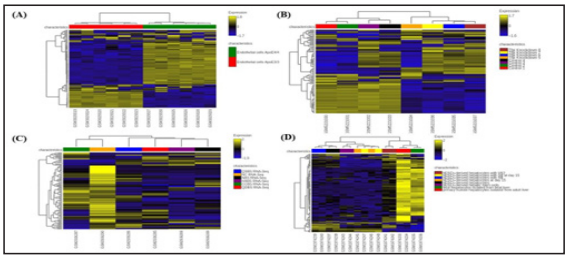

Figure. 3: Graphical representation of gene expression patterns across datasets in Interactive heatmaps (A) apolipoprotein (B) placenta (C) diabetes mellitus (D) hepatic functions. The colors in the heatmap represent the expression levels of genes, yellow represents up-regulated genes and blue represents the down-regulated genes. Intermediate expression levels may be represented by colors like black.

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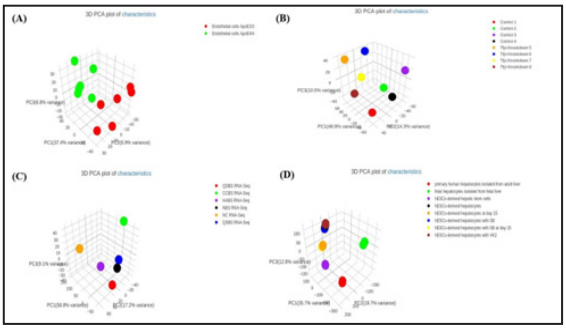

Figure. 4: Visualized the three-dimensional space created in PCA plots representing, (A) apolipoprotein (B) placenta (C) diabetes mellitus (D) hepatic functions.

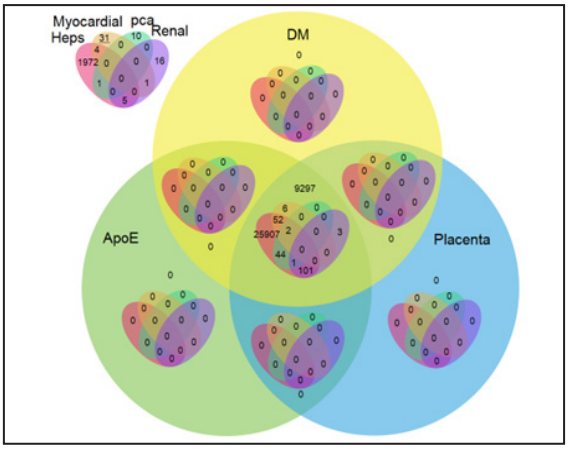

Figure 5. A multi-venn representing common significant DEG among seven datasets.

Overall, VK genes play various roles in human health and metabolism (22) such as coagulation (23), bone health (24), skeletal development and disease (25), cardiovascular and renal aspects (26) and so on. In this study, we have also mentioned the role of VK key genes responsible for four different comorbidities compared with our previous study transcriptome datasets (5). In comparison with VK genes, the gene ENSG00000123843 complement component 4 binding protein (C4BPB) has been identified as common between GSE109265 and GSE111366. C4BPB has a regulatory role in the coagulation system and, mediated through the beta-chain binding of protein S, a VK-dependent protein that serves as a cofactor of activated protein C (27). ENSG00000110799, a Von Willebrand Factor (VWF) has been identified as common between the datasets GSE111366 and GSE76098. VWF is protein coding gene that codes for a glycoprotein which plays crucial role in normal hemostasis and a research study (28) identified apolipoprotein as novel high-affinity VWF binding partner that regulates proteolysis and functions of VWF under arterial shear and also involved in pathogenesis of cardiovascular diseases (29). Taken together, we did not find any common DEGs between the seven datasets, however, our work could be construed as a prototype in screening candidate DEGs (Supplementary Table 2).

VK is a vital nutrient that supports the blood coagulation process by facilitating the activation of key clotting factors and enabling the formation of a stable blood clot when needed to prevent excessive bleeding. Although there are correlations between VK, blood coagulation, and comorbidities, it is crucial to study these linkages which are complex, and more studies are required to completely comprehend the mechanisms at function While the associations are not well established, VK metabolism or the activity of VK-dependent proteins may be indirectly impacted by genetic variables associated with these comorbidities. As VK is essential for overall health, it is essential that individuals with diabetes maintain a balanced diet that includes adequate amounts of the VK.

Conclusion

We provide an overview of the role that VK plays in preserving hemostasis and emphasize the significant consequences of VK insufficiency when considering related comorbidities. Our results add to the accumulating literature of information about the complex functions of VK and contribute to the developing of personalized approaches for addressing VK deficiency in a clinical context, which may enhance the general health of those who are deficient.

Authors' contributions: PS and PBK conceived the work. SR performed the analyses and wrote the first draft. JN added a compendium of figures. PS and PBK proofread the manuscript before all authors agreed to it.

Conflict of Interest

The authors declare no conflict of interest.

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