Medical Research Archives

ESMED
European Society of Medicine

OPEN ACCESS

Published: June 30, 2023

Citation: Fliri AF and Kajiji S, 2023. Vitamin D Deficiency-Associated Comorbidities: A Protein Network Dynamics Perspective, Medical Research Archives, [online] 11(6). https://doi.org/10.18103/mra.v11i6.3996

DOI
https://doi.org/10.18103/mra.v11i6.3996

ISSN: 2375-1924

RESEARCH ARTICLE

Vitamin D Deficiency-Associated Comorbidities: A Protein Network Dynamics Perspective

Anton F. Fliri,* Founder/President/CSO; SystaMedic Inc., 24 W. Main Street, Suite 216, Clinton, Connecticut 06413
Shama Kajiji, CEO; SystaMedic Inc., 24 W. Main Street, Suite 216, Clinton, Connecticut 06413

Correspondence* anton.fliri@systamedic.com

Author contribution. Dr. Fliri developed the methodology for analyzing cause-effect relationships examined in this manuscript. Dr. Fliri researched literature and is the primary author.
Dr Kajiji reviewed findings, also researched the literature, and contributed equally to writing the paper.

ABSTRACT
Vitamin D deficiency has been linked to numerous comorbid diseases. Despite this recognition, the effectiveness of vitamin D supplementation in improving clinical outcomes remains uncertain. To shed light on the underlying cause-effect relationships and molecular mechanisms involved, we analyzed effects of over 4000 diseases on information transfers between biological processes in human tissues. Spectral clustering of the data generated identified relationships between disease phenotypes and perturbations of protein network-network interactions mediating information transfers in tissues. Examination of these relationships discovered an integrated regulatory scheme involving interactions between 188 proteins responsive to vitamin D (vitamin D interactome). Functional analysis established a central role of fifteen proteins (core vitamin D interactome) in vitamin D pharmacology and their involvement in multiple reciprocal feedback loops. Identification of functions affected by this core regulatory framework provides new insights into relationships between vitamin D deficiency-associated comorbidities, epigenetic regulation of vitamin D pharmacology, and impact of mutations on disease. Recognition of these functional relationships suggests that vitamin D associated comorbidities may not be fully treatable by vitamin D supplementation alone. Further examination of consequences of perturbations of protein interactions within the core vitamin D interactome identified 590 morbidities exhibiting physical changes co-expressed in vitamin D deficiency. Further analysis revealed that these comorbidities can be differentiated based on cause-effect relationships defined by characteristic patterns of physical abnormalities and effects on protein network interactions. These findings demonstrate the utility of systems biology-based cause-effect analyses to unravel complex relationships involving multiple diseases and multiple biological processes; thereby providing a more comprehensive understanding of comorbidities and the impact of vitamin D deficiency on health.

Introduction

Comorbidity and multimorbidity play crucial roles in disease susceptibility, progression, treatment response, and mortality, presenting a significant and escalating public health challenge. [1, 2] Vitamin D deficiency is a worldwide condition and several studies have identified the association of low vitamin D levels with various chronic and acute comorbidities. Several high-risk patient groups, including those using specific medications, are prone to vitamin D deficiency.[3] However, the role of vitamin D supplementation in treatment of these conditions is currently a subject of debate because large interventional studies have been unable to show a clear benefit. One of the reasons for this ambiguity is the predominant focus on single disease domains, despite the recognition of vitamin D's involvement in multiple diseases across the human body. Further, there is tremendous variability of vitamin D levels, dose, form, and route of administration needed. There is a need for a tailored approach to vitamin D based on the specific mechanisms underlying vitamin D deficiency in the different diseases, and it is apparent that desirable levels of vitamin D and the amount of vitamin D required to reach such levels will vary depending upon the disease and the organ systems involved.[4] Additionally, the impact of multimorbidity/comorbidity on overall disease severity lacks comprehensive characterization.

The perplexing clinical observations surrounding vitamin D strongly suggest that, despite advances in systems biology and integrative medicine, our understanding of how variations in vitamin D levels contribute to different medical conditions and serve as markers of poor health remains limited.[5, 6, 7] . This lack of understanding arises partly from the structural and dynamic complexity of the systems involved and partly from the traditional approach of Western medicine, which tends to view health through the lens of isolated structural and functional elements rather than fully integrated dynamic systems. [8, 9, 10, 11, 12]

To analyze effects of perturbations in these complex systems, we and others have developed network- and Information Theory-based methods to characterize underlying cause-effect relationships.[13, 14, 15, 16, 17, 18] These approaches provide valuable insights into comorbidities and their impact on health. Identification of molecular origins of disease[19] and analysis of molecular mechanisms generating response to stressors such as, mutations of genes or drug treatments has traditionally focused on delineating functions of target proteins embedded in protein networks.[20, 21, 22] Protein networks consist of nodes, which can be individual proteins, groups of proteins, or other networks. [23, 24] While some of these networks have well defined structures, others exhibit inducible connections and variable topologies.[25, 26] Traditional cause-effect analysis uses fixed network topologies and examines topological properties of core proteins, such as degree and modularity in network topology comparisons. These methodologies have been used for identifying comorbidities associated with specific diseases. [27, 28, 29] However, it is important to note that diseases and drug responses are often co-expressed, which limits the reliability of this methodology in identifying causal relationships between comorbidity/multimorbidity and disease outcomes or deficiencies.

With the recognition that protein networks have inducible edges came the realization that even a single protein can engage in multiple functions by dynamically changing its connectivity with other proteins.[30, 31] Thus, several studies demonstrated that interactions between proteins are dynamically regulated and perturbation of connectivity of even single proteins can be linked to multiple disorders.[32, 33] Connecting network nodes transfers information and leads to generation of emergent properties, [34, 35, 36] and the rules governing connections between network nodes vary across organ systems, physiological states, and different environments.[37, 38, 39, 40, 41] Understanding how diseases impact protein network dynamics and the generation of emergent properties is crucial for comprehending disease susceptibility, the origins of comorbidity, and determining appropriate treatment strategies.[42, 43, 44]

Insights into how protein networks interact and generate emergent properties can be inferred from their modular design. [45, 46, 47] Nature's utilization of modular network designs suggests that information transfer between cells, tissues and organ systems relies on properties of certain groups of proteins which exhibit swarm-like behavior by dynamically establishing and breaking connections with other proteins.[48, 49, 50, 51, 52]

For considering protein network dynamics in cause-effect analysis, we have developed methodology that uses information associated with small groups of proteins (protein swarms) mediating information transfer between biological processes in human tissues and spectral clustering of cause-effect associated information for ascertaining the organizations of proteins swarms resulting from perturbations.[53, 54, 55] This methodology has been successfully applied for identifying novel combinations for treatment of chronic pain,[56] repurposing of drugs for intercepting acute kidney

injuries induced by last resort antibiotics,[57] and repurposed drug combinations for treating infections caused by pathogens with diverse origins.[58] Herein we employ this methodology for evaluating effects of over 4000 diseases on information transfers between biological processes in human tissues and gaining insight into molecular mechanisms precipitating comorbidity in the context of vitamin D deficiency.

Materials and Methods

We have developed a systems pharmacology-based methodology to analyze effects of disease-induced perturbations on protein network dynamics. This approach, using Information flow-based cause-effect analysis, discovers links between protein networks and minimizes connectivity bias in protein network construction.[59, 60, 61, 62, 63, 64, 65, 66] The method uses spectral clustering of measurements that assess the potential of diseases to impact information transfers between biological processes in human tissues. Protein swarms employed consist of no more than 5 proteins per swarm. These swarms are used for identifying co-citation frequencies between diseases and protein swarm members in the Medline database. These measurements provide estimates on the potential of a disease to impact information transfers mediated by a protein swarm. Protein swarms are derived using the tissue-specific expression of protein-encoding genes and gene enrichment analysis to determine overlaps between tissue and biological process networks.

To conduct system-wide cause-effect relationship analysis, we extract information from various sources, including Medline,[67] the Human Protein Atlas,[68] and the STRING platform.[69] The STRING platform is also used for gene enrichment analysis and protein network construction. Through these data sources and tools, we identified over 7900 groups of proteins, each comprising less than five members capable of transferring information between hundreds of different biological processes in human tissues.

For evaluating how over 4000 diseases impact information transfer capacity of these 7900 protein swarms, we employ a data-mining algorithm developed by SystaMedic Inc. in collaboration with the University of Connecticut. This algorithm determines the sum of co-citation frequencies of a disease with members of a protein swarm in the Medline database. These measurement collections (similarity matrices) are analyzed using UPGMA clustering with cosine correlation as similarity measure.[70] Likewise, for determining disease phenotypes, we determined the co-citation frequencies of the name of a disease with a standardized name of a pathophysiology referred to as the Monarch term, physical abnormality in the Medline database and followed by UPGMA clustering of the collected measurements using cosine correlation as similarity measure.[71, 72] TIBCO Spotfire Data Visualization and Analytics Software are used for hierarchical clustering and data visualization.[73, 74]

Results

Identification of clusters of protein swarms involved in vitamin D signaling:

Vitamin D is a fat-soluble vitamin that plays a crucial role in maintaining the calcium level in the body. Vitamin D interacts with the nuclear vitamin D receptor (VDR) to form a complex, which then binds to the promoter region and modulates the expression of target genes.[75] Additionally, vitamin D can also exert its effects through non-genomic actions, where it binds to VDR and activates various signaling pathways, indirectly influencing the transcription of numerous genes.[76]

Evaluation of impacts of diseases on the information transfer capacity of protein swarms involved spectral clustering of information density measurements of 4257 diseases with 7906 protein swarms. The first step in this cause-effect analysis generated a heat map (Figure 1) which identified six protein swarms (VDR 1-6) containing the vitamin D receptor VDR within a confidence in cluster similarity threshold of 0.9. The second step involved identifying proteins within each of the VDR clusters 1-6 and using the collected proteins for constructing protein networks. Functional analysis of these protein networks was performed on a cluster-to-cluster basis and in the form of a single interactome.[77] These steps allowed for a comprehensive evaluation of how diseases impact the information transfer capacity of protein swarms involved in VDR mediated signaling.

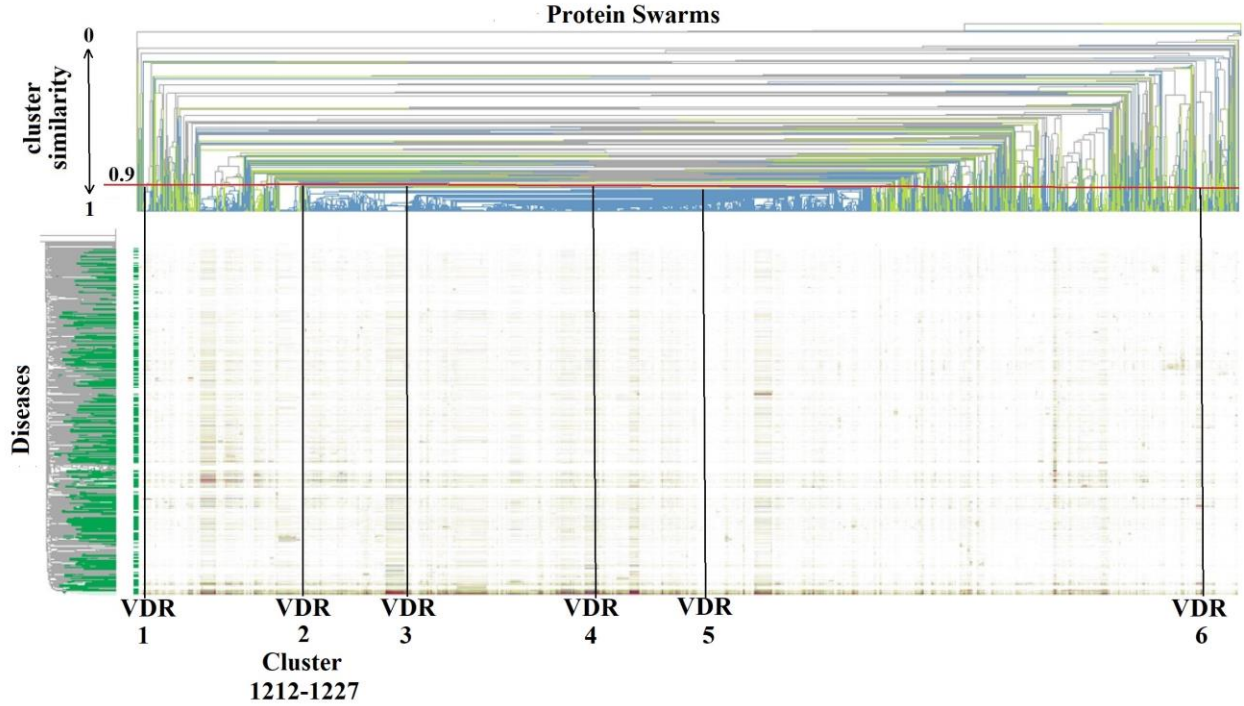

Figure 1. Global assessment of effects of diseases on biological processes in all tissues. The vertical dendrogram axis identifies associations between diseases while the horizontal dendrogram identifies associations between protein swarms. Positions of six protein swarms containing the vitamin D receptor (VDR) are identified by vertical lines. Diseases affecting information transfers in swarm clusters 1-6 are highlighted in green.

Construction of the disease-associated VDR interactome:

The connectivity between 188 proteins captured in VDR clusters **1-6** (Figure 1) defining a cause-effect relationship derived VDR interactome, was determined using the STRING platform and known physical interactions between proteins.[78, 79] This step produced the protein interaction network shown in Figure 2. Functional characterization using the STRING platform revealed that these 188 proteins participate in >2000 biological processes. Moreover, statistically significant enrichment of disease-associated mutations (70/188 nodes; false discovery rate of 10^{-10}) indicated that this protein network plays a crucial role in regulating physiologic functions in health and disease.[80]

To examine the core functions of the VDR interactome, particular attention was given to proteins highlighted in green at the center of the network in Figure 2.[81] These proteins are expressed in all tissues and belong to the swarm clusters 1212-1227, representing VDR cluster 2. Examining proteins in swarm clusters 1212-1227 identified 15 proteins.[82, 83]

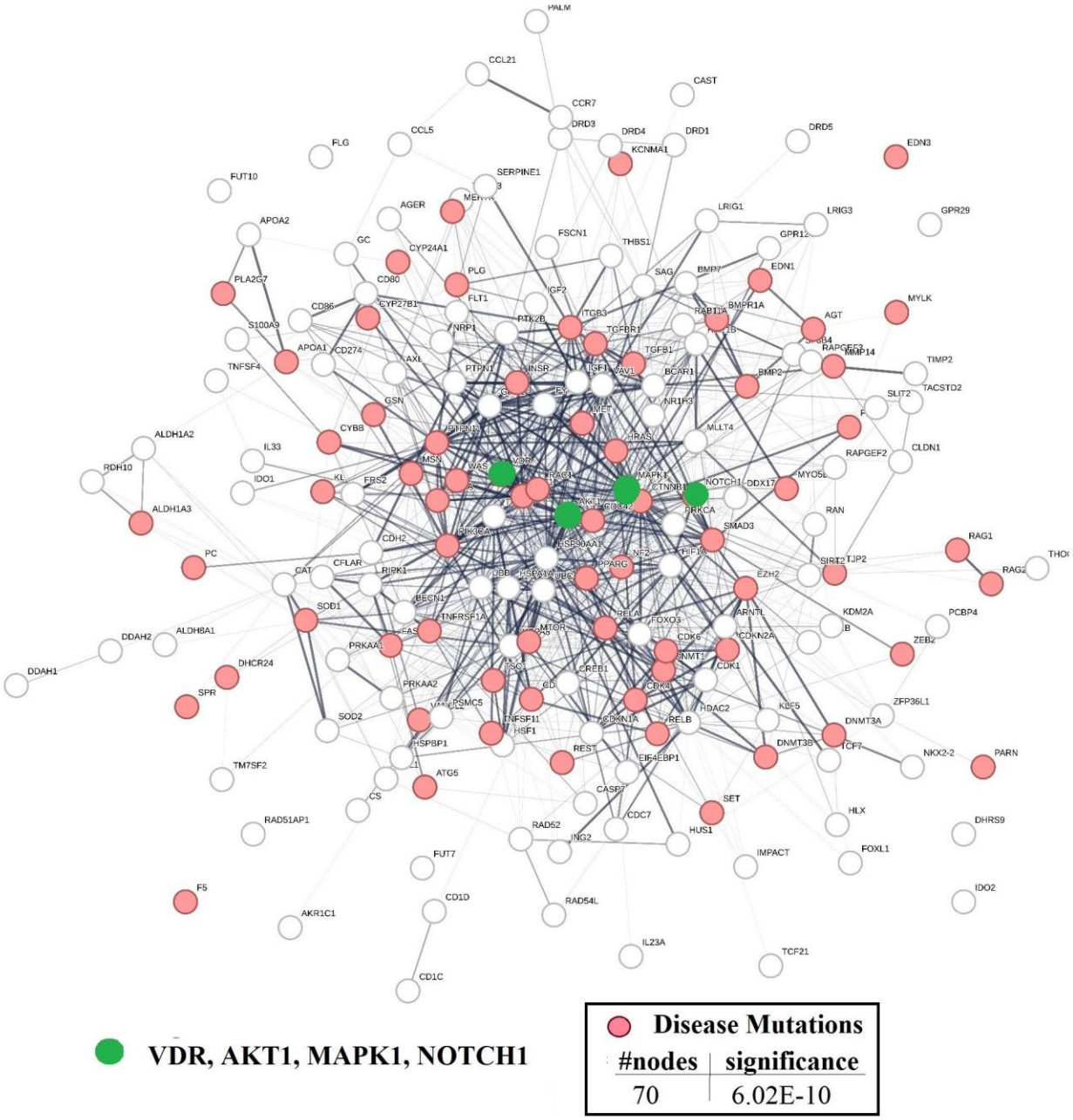

Figure 2. VDR Interactome: Protein interaction network identifying the physical interactions between 188 proteins constituting protein swarms in VDR clusters **1-6**. Proteins at the center belonging to VDR cluster 2 are circled in green (VDR, AKT1, MAPK1 and NOTCH1). These proteins are members of swarm clusters 1212-1227 identified in Figure1.

Functional analysis of swarm clusters 1212-1227:
The connectivity between fifteen proteins in swarm clusters 1212-1227 shown in Figure 3 was determined using the STRING platform. A significant finding provided by protein network construction is that mutations in any one of these fifteen proteins result in disease: VDR,[84] AKT1,[85] CFLAR,[86] CYP24A1,[87] CYP27B1,[88] DDAH1,[89] DDAH2,[90] DNMT3B,[91] MAPK1,[92] NOTCH1,[93] PROC,[94] RAN,[95] REST,[96] TNFRSF1A,[97] ZEB2,[98]. Therefore, defects in vitamin D signaling can be expected to contribute to or worsen impacts of perturbations in signaling pathways involving these fifteen proteins, and vice versa. [99] Evaluating functions affected by mutations in these proteins shows that these proteins play crucial roles in regulating key system processes such as apoptosis, redox signaling, stem cell differentiation, cell proliferation, developmental processes, responses to vitamins, hormones, growth factors, nitric oxide

(NO) production, lipid metabolism, oxidative stress responses, transport, coagulation, wound healing and activities of minerals.[100,101, 102,103,104,105,106,107,108,109,110,111]

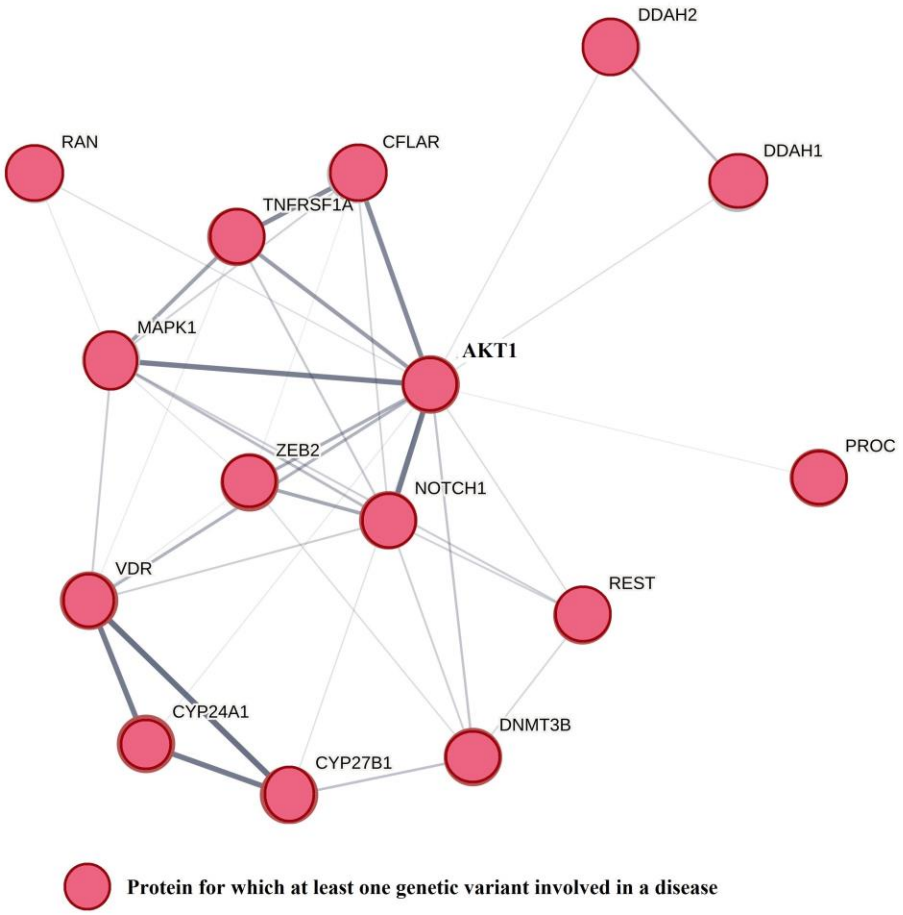

Figure 3. Core regulatory scheme within the VDR Interactome showing known connectivity of proteins in swarm clusters 1212 -1227. Proteins for which at least one genetic variant is involved in a disease are identified by red circles. The serine-threonine protein kinase AKT1 at the center of Figure 3 interacts with all proteins suggesting that AKT1 plays a key role in vitamin D actions and regulating functions of the core vitamin D interactome.

A better understanding of how vitamin D signaling impacts functions of an integrated regulatory schemes can be gained by examining protein interactions shown in Figure 3. Among the core VDR interactome proteins, the serine-threonine protein kinase, AKT1 emerges as a central player interacting with all the other proteins. AKT1 is involved in several processes, such as insulin signaling, regulation of endothelial function, metabolism, [112] and activation of nitric oxide (NO) production by phosphorylating endothelial NO synthase (eNOS) at serine 1177.[113] The binding of vitamin D to VDR activates the mitogen-activated phosphokinase (MAPK)- and AKT1-mediated pathways, leading to increases in NO production.[114, 115, 116] Elevation of NO levels generates a positive feedback loop, as the resulting activation of PI3K and MAPK signaling pathways further increases NO production.[117] The observation that inhibition of AKT1 decreases NO production but increases the expression of the calcitriol-producing enzyme CYP27B implicates that this feedback loop plays a role in regulating NO and vitamin D signaling.[118]

The key role of the VDR interactome in regulation of NO levels is further supported by the findings that NOTCH1, besides having a role in activating canonical transcriptional pathways and affecting endothelial functions, also regulates NO production through redox signaling-mediated feedback loops.[119] Thus, inhibition of NOTCH1 signaling increases the expression of inducible NO synthase (iNOS), enhances the production of superoxide and peroxynitrite, and reduces phosphorylation of eNOS and AKT1. Conversely, oxidative stress-induced activation of NOTCH1 reduces peroxynitrite production and increases NO production.[120, 121]

Core proteins within the VDR interactome also play a crucial role in regulating apoptosis which in-turn is tightly linked to oxidative stress.[122] This regulation involves the fine-tuning of the activity of CASP8 and FADD-like apoptosis regulator, CFLAR which is expressed in multiple organs and regulates inflammation. The functions of CFLAR are also regulated by NO and vitamin D levels. Thus, inhibition of NO synthesis, for example by inhibiting AKT1 activity, increases the sensitivity of stem cells to apoptosis.[123, 124] Likewise, vitamin D deficiency increases the sensitivity to apoptosis. Counteracting these effects, vitamin D-stimulated NO production decreases the sensitivity to apoptosis.[125]

A key role of the core VDR interactome in regulating oxidative stress responses is further supported by the observation that calcitriol downregulates transcriptional activation of DNA methyltransferases 1 3B promoter (DNMT3B). This promoter together with the Silencing Transcription Factor, REST[126] and the G protein, RAN[127] plays a crucial role in regulating oxidative stress responses in stem cells.[128, 129]

Yet another multifunctional protein within the VDR interactome is NOTCH1. The activation of Src-by NOTCH1 triggers the phosphorylation of the eNOS inhibitor Caveolin 1 (CAV1) at tyrosine 14 which causes (1) the activation of eNOS and (2) an increased NO and calcitriol production by a feedback loop involving caveolae-mediated endocytosis.[130, 131] Activation of caveolae-mediated endocytosis promotes the hydrolysis of the eNOS inhibitor dimethylarginine by dimethylaminohydrolase 1 (DDAH1), which is another protein interacting with AKT1. [132,133] DDAH1, impacts NO production by two different mechanisms.[134] First, by degrading the eNOS inhibitor, asymmetric dimethylarginine and second, by directly activating AKT1.[135] Evidence supporting the impact of vitamin D on this feedback loop comes from the observations that patients with vitamin D deficiency have significantly higher levels of asymmetric dimethylarginine.[136]

Furthermore, interactions of tumor necrosis factor-α (TNF) with its receptor TNFRSF1A shown in Figure 3 also affects functions of the aforementioned feedback loop. Thus, the interaction of TNF with its receptor not only activates eNOS and AKT1 by inducing the phosphorylation of AKT1 at Ser 473 and of eNOS at Ser 1179,[137] respectively, which increases NO production but also induces the formation of additional caveolae.[138] Closing the loop, this increased NO production increases the production of TNF-α transcripts[139] and the formation of additional caveolae. These interlinked feedback loops regulate vitamin D activation, NO production and caveolae-mediated endocytosis.

Activation of caveolae functions impacts additional feedback loops. One of these loops is a result of AKT1 and NOTCH1 activation which triggers the expression of the transcription factor ZEB2.[140] This transcription factor plays a key role in transforming growth factor β (TGFβ)-signaling which affects the immune system, the activation of Akt-signaling, [141, 142, 143] and via this mechanism NO production. Degradation of TGFβ by caveolae-mediated endocytosis results in a negative feedback loop as it reduces TGFβ-mediated AKT1 activation.[144] Hence, ZEB2 like NO is a core regulator that plays a pivotal role in the physiological regulation of adult cell proliferation, metabolism, survival, gene expression, mesoderm and vascular differentiation based on its ability to regulate NO, vitamin D activation, and caveolae-mediated endocytosis through TGFβ-mediated feedback loops. [145]

Additionally, protein C (PROC) functions as an anti-thrombogenic component of the coagulation system. The effects of vitamin D deficiency on this system are well documented as activated protein C activates AKT1. [146, 147] This observation highlights yet another role of the core VDR interactome and connects vitamin D and NO levels with functions of the coagulation cascade.[148]

In summary, functional analysis of central portion of the VDR interactome (Figure 2) reveals a regulatory scheme comprising of multiple feedback loops that control NO production, vitamin D activation, oxidative stress responses, TGF b-mediated signaling, apoptosis, caveolae-mediated functions, coagulation cascade, epigenetic regulation, and immune responses. These findings provide substantial evidence that the core VDR interactome plays a fundamental role in maintaining the health of cellular and organ systems. These observations align with the vast amounts of available observational data and physical associations linking vitamin D to energy homeostasis, and regulation of the immune and endocrine systems. [149]

Identification of diseases affecting protein interactions within the core VDR interactome:

Perturbations of identified feedback loops affected by vitamin D signaling are expected to impact functions of the protein interaction network that regulates over 2000 biological processes

(Figure 2). Inspecting information densities in pertinent dendrogram relationships in Figure 1 and highlighted in Figure 4 showed that 590 diseases and vitamin D deficiency modify the information transfer capacities of swarm clusters 1212 – 1227. This observation is suggestive of cause-effect relationships between perturbations of the core VDR interactome and development of disease.

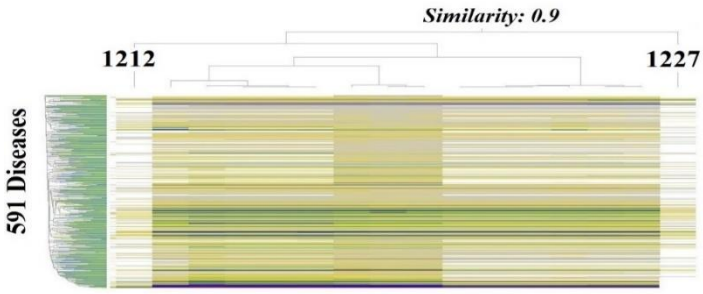

Figure 4. Relationships between protein swarms 1212-1227 grouped within a confidence similarity value of 0.9. The vertical dendrogram identifies 591 diseases impacting on the information transfer capacity of proteins in swarms 1212-1227.

Identification of vitamin D deficiency-associated physical abnormalities:

The term "physical abnormality" refers to standardized ontological terms that are used to describe and computationally analyze phenotypic changes found in human diseases.[150] To identify the physical consequences / pathophysiological changes resulting from perturbations of protein interactions in the VDR cluster 1212-1227, hierarchical clustering was performed using co-citation frequencies between the names of 591 diseases and the names of disease associated phenotypic abnormalities.[151, 152]

As shown in Figure 5A and detailed in TABLE 1, these 591 diseases exhibit physical abnormalities commonly observed in cases of vitamin D deficiency. The heterogeneous effect patterns (pathophysical abnormalities) associated with disease clusters (highlighted in Figure 5B) can be attributed to at least three factors (1) variations in vitamin D levels; (2) differential contributions of feedback loops regulated by protein interactions within the VDR interactome, and (3) additional mechanism of actions. Figure 5B provides a closer examination of the heat map shown in Figure 5A and illustrates the occurrence with respect to a subset of clusters and suggests the presence of distinct mechanisms underlying disease phenotypes (as described in TABLE 2). Moreover, these 590 diseases are grouped into discrete phenotypes which exhibit characteristic patterns of physical abnormalities (highlighted in Figure 5B).

These findings provide an interpretation of cause-effect relationships centered around VDR. The differential expressions of these physical abnormalities among different phenotypes could be attributed to variations in vitamin D levels between phenotypes and/or contributions of mechanisms of action beyond swarms 1212-1227 in VDR cluster 2. Other potential contributors, such as VDR clusters 1, 3-6 and other protein swarm clusters associated with disease phenotype, have not yet been analyzed but can be explored through further analysis of disease phenotypic protein swarm associations identified by the dendrograms depicted in the global analysis shown in Figure 1 linking these 591 comorbidities and beyond just the contributions of vitamin D deficiency.

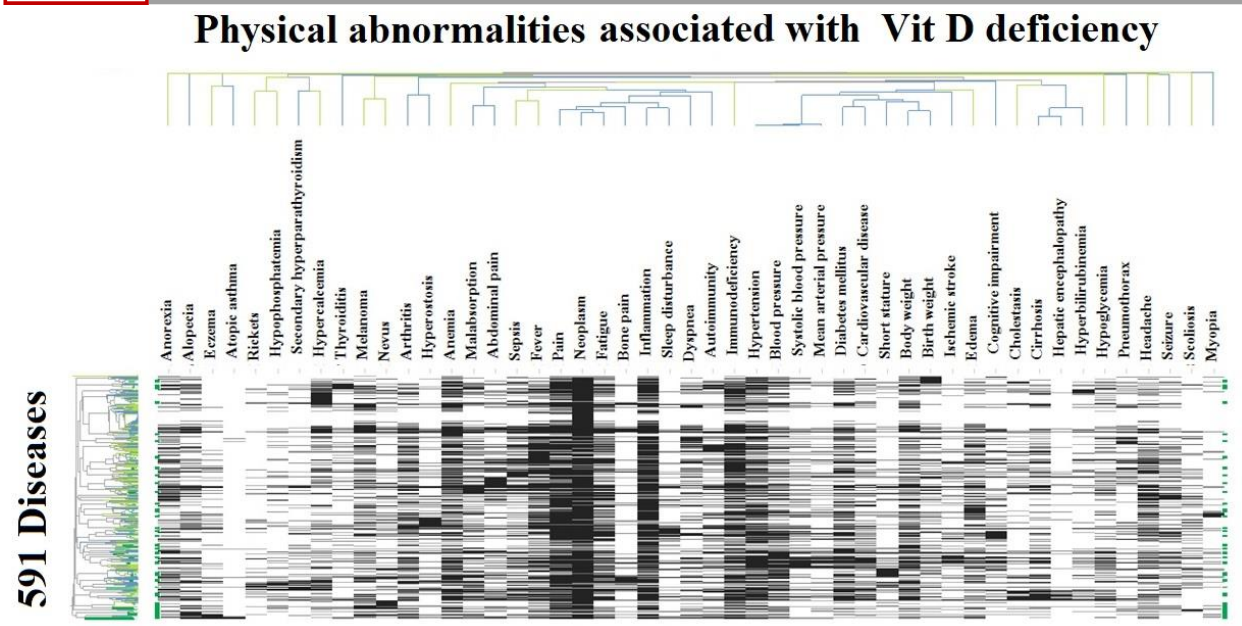

Figure 5A. The heatmap shows frequencies of 50 vitamin D-associated physical abnormalities (TABLE 1) for 591 diseases that affect the Core VDR interactome.

TABLE 1: List of 50 Physical Abnormalities of 591 Diseases Associated With Vitamin D Deficiency

Abdominal pain	Cholestasis	Hyperostosis	Pain
Age	Cirrhosis	Hypertension	Pneumothorax
Alopecia	Cognitive impairment	Hypoglycemia	Rickets
Anemia	Diabetes mellitus	Hypophosphatemia	Scoliosis
Anorexia	Dyspnea	Immuno-deficiency	Secondary hyperparathyroidism
Arthritis	Eczema	Inflammation	Seizure
Atopic asthma	Edema	Ischemic stroke	Sepsis
Autoimmunity	Fatigue	Malabsorption	Short stature
Birth weight	Fever	Mean arterial pressure	Sleep disturbance
Blood pressure	Headache	Melanoma	Systolic blood pressure
Body weight	Hepatic Encephalopathy	Myopia	Thyroiditis
Bone pain	Hyper-bilirubinemia	Neoplasm	
Cardiovascular disease	Hypercalcemia	Nevus	

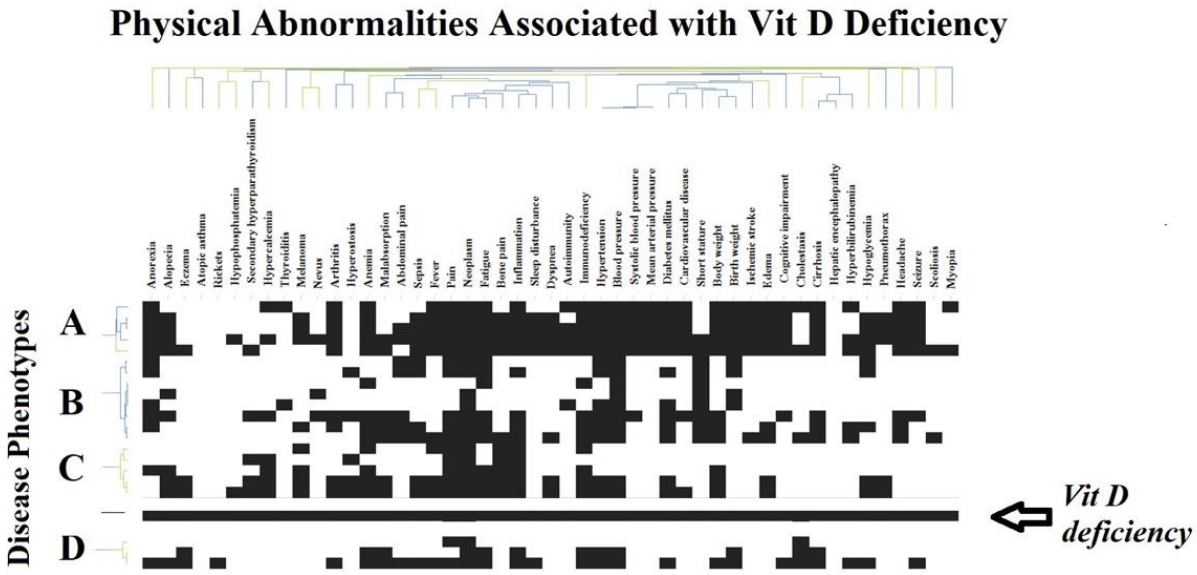

Figure 5B. The heat map compares vitamin D-associated physical abnormalities in 4 different disease phenotypes A-D (see TABLE 2) with that of vitamin D deficiency.

TABLE 2: Diseases in Disease Phenotypes A-D

A	B	C	D
artery disease	adrenal hyperplasia	multiple myeloma	bile duct carcinoma
coronary artery disease	beta thalassemia	myeloma	cholestasis
heart disease	brachydactyly	Paget's disease of bone	intrahepatic cholestasis
hypercholesterolemia	congenital adrenal hyperplasia	plasmacytoma	
myocardial infarction	hypogonadism	prostate carcinoma	
	iron overload		
	thalassemia		
	Turner syndrome		

Discussion

These results expand our previous work on utilizing protein-protein interaction networks to unravel complex cause-effect relationships.[153] We now demonstrate that integration of dynamic properties of protein interaction networks with cause-effect analysis provides an *in-silico* approach to guide improved prevention and management of comorbidity and multimorbidity, a key factor responsible for increased morbidity and a major challenge to health systems worldwide.

Vitamin D deficiency was selected as the model system; it has emerged as a modern epidemic and there is a well-established association between suboptimal vitamin D levels and various comorbidities. Most studies on the relationship between vitamin D and morbidity have focused solely on effects within single disease domains, despite the association of vitamin D biology with several diseases throughout the human body. The objective of this investigation was to decipher functionality of the vitamin D interactome based on analysis of effects of diseases on protein network dynamics and thereby, generate an improved understanding of the molecular origin(s) and variability[154] of treatment outcomes in vitamin D deficiency- associated morbidities.

Since diseases serve as indicators of perturbations of dynamic properties of protein networks and affect information exchange in human tissues, we examined information densities at the intersections between > 4000 disease phenotypes and ~8000 protein swarm clusters (Figure 1) This vantage point allowed us to evaluate how stressors such, as vitamin D deficiency impact network-network interactions mediating information transfers in

human tissues on a system wide scale, and identified six protein swarm associations, containing in-aggregate 188 proteins. The capacity of these 188 proteins to interact and to transmit vitamin D-induced signaling was determined using known physical interactions to identify edges between network nodes for network construction (interactome). These cause-effect and functional analyses led to identification of the VDR interactome shown in Figure 2. The significance of this network in regulating biological functions is evidenced by the high number of disease-associated mutations (70/188 nodes; false discovery rate of 10^{-10}). The importance of this regulatory scheme in regulating protein network dynamics and vitamin D pharmacology is further supported by the finding that mutations of the 15 proteins (Figure 3) at the center (hub) of VDR interactome and residing in protein swarm associations 1212-1227 (Figure 1) cause disease. Since this regulatory scheme integrates functions of multiple feedback loops that respond to vitamin D- and NO-signaling, morbidities associated with vitamin D deficiency may not be fully treatable by vitamin D supplementation alone.

From a systems perspective, hubs are of central functional importance as they have a significant impact on a wide range of molecular processes. Disease-associated mutations in these hubs not only affect vitamin D signaling but also multiple functional categories interconnected through intricate regulatory feedback loops. The central role of AKT1,[155] the neurogenic locus, NOTCH1 [156] and tumor necrosis factor receptor TNFRSF1A[157] (Figure 3) in vitamin D pharmacology has been recognized in prior studies. However, the significance of dimethylarginine dimethylaminohydrolase DDAH2, DNA methyltransferases 3B and homeodomain factors ZEB2 in enabling epigenetic regulation of vitamin D and NO responses and functions of these various feedback loops have not previously been recognized.[158, 159, 160, 161, 162, 163, 164]

It is well-established that vitamin D has widespread involvement in numerous molecular processes and is believed to have ancient origins, with vitamin D usage and VDR being conserved across diverse species of plants and animals.[165, 166] However, the VDR interactome identified in previous studies using theoretical arguments such as, centrality, node degree and confidence in protein associations has very little overlap with the regulatory scheme identified in our system-wide cause-effect analysis.[167] Such discrepancies between interactomes are well recognized and stem from inconsistencies between proteomics datasets and use of network connectivity criteria that are biased towards large, well-characterized proteins and underestimate connectivity contributed by smaller, less investigated proteins.[168, 169]

Our cause-effect analysis methodology addresses the limitations arising from the use of confidence in connectivity criteria and topological arguments for constructing and interpreting protein interaction networks relevant to vitamin D pharmacology. We have validated the relevance of the core VDR interactome by (1) using functional evidence to demonstrate that the proteins shown in Figure 3 are key drivers of vitamin D pharmacology, (2) identifying that perturbations of the regulatory scheme precipitate 590 vitamin D deficiency-associated comorbidities (Figure 4), [170] and (3) that diseases identified using the core VDR interactome as the mechanism of action rationale express the characteristic physical abnormalities associated with vitamin D deficiency (Figures 5A and 5B). These observations illustrate that information flow-based, cause-effect analysis using protein swarms to integrate dynamic properties of proteins increases confidence in interactome construction for using proteomics data to influence decision-making.

Investigation of the pathophysiology of changes (also referred to as physical abnormalities) manifested by these 590 diseases, as understanding the pathophysiology is a crucial determinant for medical practice: it can assist in the treatment of acute and chronic illnesses, managing medications, assisting with diagnostic tests, and managing general health care and disease prevention for patients and their families. More importantly, it can help understand with speed and accuracy why any abnormal health changes have occurred in a patient, why they happened, and what can be done to resolve the situation. Thus, the methodology presented here offers a roadmap for investigating whether comorbidities have additional positive and negative effects beyond those directly caused by vitamin D deficiency. Moreover, it can be extended to assess the collective impact of comorbidities on the manifestation of pathophysiologies and further applied to study the effects of multimorbidity.

Conclusion

Vitamin D is widely recognized as an important, inexpensive, and safe adjuvant therapy for many diseases and all stages of life. However, a clear benefit of vitamin D supplementation for remedying vitamin D deficiency-associated comorbidities has not yet been established.

To unravel this paradox requires a deeper grasp of how dysfunctions in protein network interactions, induced by vitamin D deficiency, impact cellular and organ systems. We demonstrate that spectral clustering of protein swarm-associated, cause-effect information combined with standard protein network analytics represents a compelling technology to examine relationships between diseases from a protein network dynamics perspective. Our findings implicate involvement of the core VDR interactome, comprising of 15 proteins that control multiple feedback loops, as an important vitamin D-responsive regulator of health. Perturbations of protein interactions within this core regulatory scheme precipitates 590 morbidities that can be grouped into phenotypes manifesting discrete sets of physical abnormalities typically associated with vitamin D deficiency. Accuracy in defining endophenotypes of "typical diseases" across different but interrelated datasets will enable better definition of variants and their stratification in therapeutic trials.

In summary, interactome networks capture the complexity of molecular machinery that regulates organismal behavior. Built into the structures of these complex machines is resilience to failure in case of breakdown of communications between system components; thus, preventing cell death or disease. The VDR interactome is a prototypical example of how these safeguarding mechanisms work. Study of cause-effect relationships to understand how disease-induced dysfunctions in protein network interactions affect cellular and organ systems represents a significant contribution to a technically challenging frontier in medical and pharmaceutical research.

Key takeaways:

a) Treatment of vitamin D deficiency-associated morbidities will require interventions, beyond mere vitamin D supplementation, to rebalance functions of multiple biological processes.
b) Comorbidities associated with inadequate levels of vitamin D can be potentially managed or prevented through holistic approaches and nutraceuticals like Cardio Miracle which have been shown to support the complex feedback loops affected by vitamin D deficiency. [171, 172]

Declarations

Conflict of interest: There are no conflicts of interest.

Submission: This is original research that has not been published previously nor is it being considered elsewhere.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

[1] Hurst JR, Dickhaus J, Maulik PK, Miranda JJ, Pastakia SD, Soriano JB, et al. Global Alliance for Chronic Disease researchers' statement on multimorbidity. *Lancet Glob Health*. 2018;6:e1270–1.

[2] Gupta P, Prabhakaran D, Mohan S. Multimorbidity or multiple long-term conditions: need for bridging the evidence & care gaps to address an emerging priority public health issue in India. *Indian J Med Res*. 2022 Sep;156(3):381-383. doi: 10.4103/ijmr.ijmr_1822_21. PMID: 36751739; PMCID: PMC10101353.

[3] Amrein K, Scherkl M, Hoffmann M, Neuwersch-Sommeregger S, Köstenberger M, Tmava Berisha A, Martucci G, Pilz S, Malle O. vitamin D deficiency 2.0: an update on the current status worldwide. *Eur J Clin Nutr*. 2020 Nov;74(11):1498-1513. doi: 10.1038/s41430-020-0558-y. Epub 2020 Jan 20. PMID: 31959942; PMCID: PMC7091696.

[4] Bilezikian JP, Formenti AM, Adler RA, Binkley N, Bouillon R, Lazaretti-Castro M, Marcocci C, Napoli N, Rizzoli R, Giustina A. Vitamin D: Dosing, levels, form, and route of administration: Does one approach fit all? *Rev Endocr Metab Disord*. 2021 Dec;22(4):1201-1218. doi: 10.1007/s11154-021-09693-7. Epub 2021 Dec 23. PMID: 34940947; PMCID: PMC8696970.

[5] Bouillon R, Manousaki D, Rosen C, Trajanoska K, Rivadeneira F, Richards JB. The health effects of vitamin D supplementation: evidence from human studies. *Nat Rev Endocrinol*. 2022;18(2):96-110. doi:10.1038/s41574-021-00593-z

[6] Autier P, Boniol M, Pizot C, Mullie P. vitamin D status and ill health: a systematic review. *Lancet Diabetes Endocrinol*. 2014;2(1):76-89. doi:10.1016/S2213-8587(13)70165-7

[7] Ivanov PC. The New Field of Network Physiology: Building the Human Physiolome. *Front Netw Physiol*. 2021 Jun 30;1:711778. doi: 10.3389/fnetp.2021.711778. PMID: 36925582; PMCID: PMC10013018.

[8] Wang K. et al. Classification of common human diseases derived from shared genetic and environmental determinants. *Nat. Genet*. 2017; 49, 1319–1325.

[9] Clauset A, Moore C, Newman ME. Hierarchical structure and the prediction of missing links in networks. *Nature*. 2008 May 1;453(7191):98-101.

[10] Liu CC, Tseng YT, Li W, et al. DiseaseConnect: a comprehensive web server for mechanism-based disease-disease connections. *Nucleic Acids Res*. 2014;42(Web Server issue):W137-W146. doi:10.1093/nar/gku412

[11] Zhang XY, Birtwistle MR, Gallo JM. A General Network Pharmacodynamic Model-Based Design Pipeline for Customized Cancer Therapy Applied to the VEGFR Pathway. *CPT Pharmacometrics Syst Pharmacol*. 2014;3(1):e92. Published 2014 Jan 15. doi:10.1038/psp.2013.65

[12] Guo P, Chen S, Wang H, Wang Y, Wang J. A Systematic Analysis on the Genes and Their Interaction Underlying the Comorbidity of Alzheimer's Disease and Major Depressive Disorder. *Front Aging Neurosci*. 2022;13:789698. Published 2022 Jan 20. doi:10.3389/fnagi.2021.789698

[13] Fliri Anton Franz Joseph, Method and descriptors for comparing object-induced information flows in a plurality of interaction networks. *US11120346B2*·2021.

[14] Fliri AF, Loging WT, Thadeio PF, Volkmann RA. Analysis of drug-induced effect patterns to link structure and side effects of medicines. Nat Chem Biol. 2005;1(7):389-397. doi:10.1038/nchembio747

[15] Fliri AF, Loging WT, Volkmann RA. Analysis of information flows in interaction networks: implication for drug discovery and pharmacological research. *Discov Med*. 2011;11(57):133-143.

[16] Ying KC, Lin SW. Maximizing cohesion and separation for detecting protein functional modules in protein-protein interaction networks. PLoS One. 2020 Oct 13;15(10):e0240628. doi: 10.1371/journal.pone.0240628. PMID: 33048996; PMCID: PMC7553341.

[17] L. Gao, p. sun, j. song., "Clustering algorithms for detecting functional modules in protein interaction networks.": *Journal of Bioinformatics and Computational Biology*, 2009, Volume: 7, Issue: 1, pp. 217-242

[18] Freitas, C.G.S., Aquino, A.L.L., Ramos, H.S. et al. A detailed characterization of complex networks using Information Theory. *Sci Rep.;* 2019; 9, 16689. https://doi.org/10.1038/s41598-019-53167-5

[19] Oerton E, Roberts I, Lewis PSH, Guilliams T, Bender A. Understanding and predicting disease relationships through similarity fusion. *Bioinformatics*. 2019;35(7):1213-1220. doi:10.1093/bioinformatics/bty754

[20] Goh KI, Cusick ME, Valle D, Childs B, Vidal M, Barabási AL. The human disease network. *Proc Natl Acad Sci U S A*. 2007;104(21):8685-8690. doi:10.1073/pnas.0701361104

[21] Vasam Manjveekar Prabantu, Nagarajan Naveenkumar, Narayanaswamy Srinivasan. Influence of Disease-Causing Mutations on Protein Structural Networks. *Front Mol Biosci*. 2020; 7: 620554.

[22] Sommariva, S., Caviglia, G., Ravera, S. et al. Computational quantification of global effects induced by mutations and drugs in signaling networks of colorectal cancer cells. *Sci Rep*.2021; 11, 19602. https://doi.org/10.1038/s41598-021-99073-7

[23] Takahashi, K., Abe, K., Kubota, S.I. et al. An analysis modality for vascular structures combining tissue-clearing technology and topological data analysis. *Nat Commun* 2022;13: 5239.

[24] Yadav KK, Singh AK. Topology-based protein–protein interaction analysis of oral cancer proteins. *CURRENT SCIENCE*. 2022 Nov 25;123(10):1216.

[25] Panditrao, G., Bhowmick, R., Meena, C. et al. Emerging landscape of molecular interaction networks: Opportunities, challenges and prospects. *J Biosci*. 2022;47, 24. https://doi.org/10.1007/s12038-022-00253-y

[26] D. Hitchcock, C.A. Glasbey, K. Ritz. Image analysis of space-filling by networks: application to a fungal mycelium *Biotechnol. Tech*. 1996; 10, pp. 205-210

[27] de Lichtenberg U, Jensen LJ, Brunak S, Bork P. Dynamic complex formation during the yeast cell cycle. *Science*. 2005;307(5710):724-727. doi:10.1126/science.1105103

[28] Taylor IW, Linding R, Warde-Farley D, et al. Dynamic modularity in protein interaction networks predicts breast cancer outcome. *Nat Biotechnol*. 2009;27(2):199-204. doi:10.1038/nbt.1522

[29] Langhauser F, et al. A diseasome cluster-based drug repurposing of soluble guanylate cyclase activators from smooth muscle relaxation to direct neuroprotection. *NPJ Syst. Biol. Appl*. 2018;4:1–13. doi: 10.1038/s41540-017-0039-7

[30] Yue R, Dutta A. Computational systems biology in disease modeling and control, review and perspectives. *NPJ Syst Biol Appl*. 2022 Oct 3;8(1):37. doi: 10.1038/s41540-022-00247-4. PMID: 36192551; PMCID: PMC9528884.

[31] Hidalgo CA, Blumm N, Barabási AL, Christakis NA. A dynamic network approach for the study of human phenotypes. *PLoS Comput Biol*. 2009;5(4):e1000353. doi:10.1371/journal.pcbi.1000353

[32] Zhong Q, Simonis N, Li QR, et al. Edgetic perturbation models of human inherited disorders. *Mol Syst Biol*. 2009;5:321. doi:10.1038/msb.2009.80

[33] Ma X, Gao L, Tan K. Modeling disease progression using dynamics of pathway connectivity. *Bioinformatics*. 2014;30(16):2343-2350. doi:10.1093/bioinformatics/btu298

[34] Bhalla US, Iyengar R. Emergent properties of networks of biological signaling pathways. *Science*. 1999;283(5400):381-387. doi:10.1126/science.283.5400.381

[35] Bhalla, U. S., & Iyengar, R.. Emergent properties of networks of biological signaling pathways. *Science,* 1999; 283(5400), 381-387.

[36] Fesce R. Subjectivity as an Emergent Property of Information Processing by Neuronal Networks. *Front. Neurosci*. 2020; 14:548071. doi: 10.3389/fnins.2020.548071

[37] Bashan A, Bartsch RP, Kantelhardt JW, Havlin S, Ivanov PCh. Network physiology reveals relations between network topology and physiological function. *Nat Commun*. 2012;3:702. Published 2012 Feb 28. doi:10.1038/ncomms1705

[38] Shah, N.A., Sarkar, C.A. Variable cellular decision-making behavior in a constant synthetic network topology. *BMC Bioinformatics*;2,019; 20, 237 https://doi.org/10.1186/s12859-019-2866-6
[39] R. Schmidt et al.: Inferring Topology of Networks With Hidden Dynamic Variables. *IEEE Access*;2022;10;76682-76692
[40] Dumont, Grégory, and Boris Gutkin. "Macroscopic phase resetting-curves determine oscillatory coherence and signal transfer in inter-coupled neural circuits." *PLoS computational biology*; 2019; 15.5): e1007019.
[41] Wodak SJ, Vlasblom J, Turinsky AL, Pu S. Protein-protein interaction networks: the puzzling riches. *Curr Opin Struct Biol*. 2013;23(6):941-953. doi:10.1016/j.sbi.2013.08.002
[42] Patrick Finze. How we become ill. *EMBO Reports* (2017)18:515-518
[43] Galas, D.. "systems biology." *Encyclopedia Britannica,* 2018; December 7. https://www.britannica.com/science/systems-biology.
[44] Khan A, Uddin S, Srinivasan U. Comorbidity network for chronic disease: A novel approach to understand type 2 diabetes progression. *Int J Med Inform*. 2018;115:1-9. doi:10.1016/j.ijmedinf.2018.04.001
[45] Pedro A. M. Mediano, Fernando E. Rosas et al. Greater than the parts: a review of the information decomposition approach to causal emergence. *Philos Trans A Math Phys Eng Sci*. July 11, 2022; 380(2227): 20210246.
[46] Lage K, et al. Dissecting spatio-temporal protein networks driving human heart development and related disorders. *Mol Syst Biol*. 2010 Jun 22;6:381. doi: 10.1038/msb.2010.36.
[47] Clauset, A., Moore, C., & Newman, M. E.. Hierarchical structure and the prediction of missing links in networks. *Nature*, 2008; 453(7191), 98-101.
[48] McLennan-Smith TA, Roberts DO, Sidhu HS. Emergent behavior in an adversarial synchronization and swarming model. *Phys Rev E*. 2020;102(3-1):032607. doi:10.1103/PhysRevE.102.032607
[49] Lei X, Yang X, Wu FX. Artificial Fish Swarm Optimization Based Method to Identify Essential Proteins. *IEEE/ACM Trans Comput Biol Bioinform*. 2020;17(2):495-505. doi:10.1109/TCBB.2018.2865567
[50] Dresp-Langley, B. Seven properties of self-organization in the human brain. *Big Data and Cognitive Computing*, 2020; 4(2), 10.
[51] Cubillos-Ruiz, A., Guo, T., Sokolovska, A. et al. Engineering living therapeutics with synthetic biology. *Nat Rev Drug Discov* .2021; 20, 941–960). https://doi.org/10.1038/s41573-021-00285-3
[52] Olaf Witkowski and Takashi Ikegami. Emergence of Swarming Behavior: Foraging Agents Evolve Collective Motion Based on Signaling. *PLoS One*. 2016; 11(4): e0152756.
[53] See Reference 10
[54] Fliri AF, Kajiji S. Functional characterization of nutraceuticals using spectral clustering: Centrality of caveolae-mediated endocytosis for management of nitric oxide and vitamin D deficiencies and atherosclerosis. *Front Nutr*. 2022;9:885364. Published 2022 Aug 15. doi:10.3389/fnut.2022.885364
[55] Berahmand, Kamal, Elahe Nasiri, and Yuefeng Li. "Spectral clustering on protein-protein interaction networks via constructing affinity matrix using attributed graph embedding." *Computers in Biology and Medicine* 138 (2021): 104933.
[56] Manivasakam P, Ravi A, Ramesh J, et al. Autophagy: An Emerging Target for Developing Effective Analgesics. *ACS Omega*. 2023;8(10):9445-9453. Published 2023 Mar 3. doi:10.1021/acsomega.2c06949
[57] Fliri Anton; Manivasakam Palaniyandi; Sutcliffe Joyce. Autophagy Activators and Inhibitors Of Ferroptosis For Preventing Acute Renal Failure And Neurotoxicity Induced By Certain Antibiotics. *US2019056855W*·2019-10-18
[58] Fliri Anton Franz Joseph; Method For Identifying Treatment Of Infections Caused By Pathogens Of Diverse Origin.*US2022249467A1*·2022-08-11
[59] Schaefer MH, Serrano L, Andrade-Navarro MA. Correcting for the study bias associated with protein-protein interaction measurements reveals differences between protein degree distributions from different cancer types. *Front Genet*. 2015 Aug 4;6:260. doi: 10.3389/fgene.2015.00260. PMID: 26300911; PMCID: PMC4523822.
[60] Fierro-Monti I, Wright JC, Choudhary JS, Vizcaíno JA. Identifying individuals using proteomics: are we there yet? *Front Mol Biosci*. 2022 Nov 29;9:1062031. doi: 10.3389/fmolb.2022.1062031.
[61] Zhu X, Shen X, Qu J, Straubinger RM, Jusko WJ. Multi-Scale Network Model Supported by Proteomics for Analysis of Combined Gemcitabine and Birinapant Effects in Pancreatic Cancer Cells. *CPT Pharmacometrics Syst Pharmacol*. 2018 Sep;7(9):549-561. doi: 10.1002/psp4.12320.
[62] Kustatscher G, Collins T, Gingras AC, et al. Understudied proteins: opportunities and challenges for functional proteomics. *Nat Methods*. 2022;19(7):774-779.
[63] Ivanov PC, Bartsch RP. Network physiology: mapping interactions between networks of physiologic networks. In: D'Angostino G, Scala A, editors. Networks of networks: the last frontier of complexity. *Cham: Springer*; 2014. p. 203–22.
[64] Broido, A.D., Clauset, A. Scale-free networks are rare. *Nat Commun*.2019; 10, 1017. https://doi.org/10.1038/s41467-019-08746-5
[65] Woessmann J, Kotol D, Hober A, Uhlén M, Edfors F. Addressing the Protease Bias in Quantitative Proteomics. *J Proteome Res*. 2022 Oct 7;21(10):2526-2534. doi: 10.1021/acs.jproteome.2c00491.
[66] Bantscheff M, Schirle M, Sweetman G, Rick J, Kuster B. Quantitative mass spectrometry in proteomics: a critical review. *Anal Bioanal Chem*. 2007;389(4):1017-1031. doi:10.1007/s00216-007-1486-6

[67] US National Library of Medicine. *MEDLINE: Overview* 2021.
[68] Uhlén M, Fagerberg L, Hallström BM, et al. Proteomics. Tissue-based map of the human proteome. *Science*. 2015;347(6220):1260419. doi:10.1126/science.1260419
[69] Damian Szklarczyk, Annika L Gable, Katerina C Nastou, David Lyon, Rebecca Kirsch, Sampo Pyysalo, Nadezhda T Doncheva, Marc Legeay, Tao Fang, Peer Bork, Lars J Jensen, Christian von Mering, The STRING database in 2021: customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets, *Nucleic Acids Research*,2021; Volume 49, Issue D1, Pages D605–D612, https://doi.org/10.1093/nar/gkaa1074
[70] Fliri AF, Loging WT, Thadeio PF, Volkmann RA. Biospectra analysis: model proteome characterizations for linking molecular structure and biological response. *J Med Chem*. 2005;48(22):6918-6925. doi:10.1021/jm050494g
[71] Dunn K, Marshall JG, Wells AL, Backus JEB. Examining the role of MEDLINE as a patient care information resource: an analysis of data from the Value of Libraries study. *J Med Libr Assoc*. 2017 Oct;105(4):336-346. doi: 10.5195/jmla.2017.87.
[72] Finlayson SG, LePendu P, Shah NH. Building the graph of medicine from millions of clinical narratives. *Sci Data*. 2014 Sep 16;1:140032. doi: 10.1038/sdata.2014.32.
[73] Szklarczyk D, Franceschini A, Kuhn M, et al. The STRING database in 2011: functional interaction networks of proteins, globally integrated and scored. *Nucleic Acids Res*. 2011;39(Database issue):D561-D568. doi:10.1093/nar/gkq973
[74] Kaushal D, Naeve CW. An overview of Spotfire for gene-expression studies. *Curr Protoc Hum Genet*. 2005;Chapter 11:. doi:10.1002/0471142905.hg1109s45
[75] Pike JW, Meyer MB. The vitamin D receptor: new paradigms for the regulation of gene expression by 1,25-dihydroxyvitamin D(3). *Endocrinol Metab Clin North Am*. 2010 Jun;39(2):255-69, table of contents. doi: 10.1016/j.ecl.2010.02.007. PMID: 20511050; PMCID: PMC2879406.
[76] Hii CS, Ferrante A. The Non-Genomic Actions of vitamin D. *Nutrients*. 2016 Mar 2;8(3):135. doi: 10.3390/nu8030135. PMID: 26950144; PMCID: PMC4808864.
[77] Missiuro PV, Liu K, Zou L, et al. Information flow analysis of interactome networks. *PLoS Comput Biol*. 2009;5(4):e1000350. doi:10.1371/journal.pcbi.1000350
[78] Soong TT, Wrzeszczynski KO, Rost B. Physical protein-protein interactions predicted from microarrays. *Bioinformatics*. 2008;24(22):2608-2614. doi:10.1093/bioinformatics/btn498
[79] Kotlyar M, Pastrello C, Malik Z, Jurisica I. IID 2018 update: context-specific physical protein-protein interactions in human, model organisms and domesticated species. *Nucleic Acids Res*. 2019;47(D1):D581-D589. doi:10.1093/nar/gky1037
[80] Chen JC, Alvarez MJ, Talos F, Dhruv H, Rieckhof GE, Iyer A, Diefes KL, Aldape K, Berens M, Shen MM, Califano A. Identification of causal genetic drivers of human disease through systems-level analysis of regulatory networks. *Cell*. 2014 Oct 9;159(2):402-14.
[81] Csermely P, Korcsmáros T, Kiss HJ, London G, Nussinov R. Structure and dynamics of molecular networks: a novel paradigm of drug discovery: a comprehensive review. *Pharmacol Ther*. 2013;138(3):333-408. doi:10.1016/j.pharmthera.2013.01.016
[82] Valente TW, Coronges K, Lakon C, Costenbader E. How Correlated Are Network Centrality Measures? *Connect (Tor)*. 2008 Jan 1;28(1):16-26. PMID: 20505784; PMCID: PMC2875682
[83] Jeong H, Mason SP, Barabási AL, Oltvai ZN. Lethality and centrality in protein networks. Nature. 2001;411(6833):41-42. doi:10.1038/35075138
[84] Malloy PJ, Feldman D. Genetic disorders and defects in vitamin D action. *Endocrinol Metab Clin N Am* 2010;39:333–346
[85] Shrestha Bhattarai, T., Shamu, T., Gorelick, A.N. et al. AKT mutant allele-specific activation dictates pharmacologic sensitivities. *Nat Commun*. 2022; 13, 2111. https://doi.org/10.1038/s41467-022-29638-1.
[86] Luke Humphreys, Margarita Espona-Fiedler, Daniel B. Longley. FLIP as a therapeutic target in cancer. *The FEBS Journal*. 2018; Volume 285, Issue 22 p. 4104-4123.https://doi.org/10.1111/febs.14523
[87] Schlingmann KP, Kaufmann M, Weber S, et al. Mutations in CYP24A1 and idiopathic infantile hypercalcemia. *N Engl J Med*. 2011;365(5):410-421. doi:10.1056/NEJMoa1103864
[88] Demir K, Kattan WE, Zou M, et al. Novel CYP27B1 Gene Mutations in Patients with vitamin D-Dependent Rickets Type 1A. *PLoS One*. 2015;10(7):e0131376. Published 2015 Jul 1. doi:10.1371/journal.pone.0131376
[89] Ding H, Wu B, Wang H, Lu Z, Yan J, Wang X, Shaffer JR, Hui R, Wang DW. A novel loss-of-function DDAH1 promoter polymorphism is associated with increased susceptibility to thrombosis stroke and coronary heart disease. *Circ Res*.2010; 106:1145–1152
[90] Hallmark B, Karafet TM, Hsieh P, Osipova LP, Watkins JC, Hammer MF. Genomic Evidence of Local Adaptation to Climate and Diet in Indigenous Siberians. *Mol Biol Evol*. 2019;36(2):315-327. doi:10.1093/molbev/msy211
[91] Okano M, Bell DW, Haber DA, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell*. 1999;99(3):247-257. doi:10.1016/s0092-8674(00)81656-6
[92] Zou Y, Deng W, Wang F, et al. A novel somatic MAPK1 mutation in primary ovarian mixed germ cell tumors. Oncol Rep. 2016;35(2):725-730. doi:10.3892/or.2015.4402

[93] Petrackova A, Turcsanyi P, Papajik T, Kriegova E. Revisiting Richter transformation in the era of novel CLL agents. *Blood Rev*. 2021;49:100824. doi:10.1016/j.blre.2021.100824
[94] Yue Y, Liu S, Han X, et al. Pathogenic variants of PROC gene caused type I activity deficiency in a familial Chinese venous thrombosis. *J Cell Mol Med*. 2019;23(10):7099-7104. doi:10.1111/jcmm.14563
[95] Cleary JD, Pattamatta A, Ranum LPW. Repeat-associated non-ATG (RAN) translation. *J Biol Chem*. 2018;293(42):16127-16141. doi:10.1074/jbc.R118.003237
[96] Mahamdallie SS, Hanks S, Karlin KL, et al. Corrigendum: Mutations in the transcriptional repressor REST predispose to Wilms tumor. *Nat Genet*. 2016;48(4):473. doi:10.1038/ng0329-473d
[97] Blaschek A, V Kries R, Lohse P, et al. TNFRSF1A and MEFV mutations in childhood onset multiple sclerosis. *Eur J Paediatr Neurol*. 2018;22(1):72-81. doi:10.1016/j.ejpn.2017.08.00
[98] Cordelli DM, Di Pisa V, Fetta A, et al. Neurological Phenotype of Mowat-Wilson Syndrome. *Genes* (Basel). 2021;12(7):982. Published 2021 Jun 27. doi:10.3390/genes12070982
[99] Wang H, Chen W, Li D, Yin X, Zhang X, Olsen N, Zheng SG. vitamin D and Chronic Diseases. *Aging Dis*. 2017 May 2;8(3):346-353. doi: 10.14336/AD.2016.1021. PMID: 28580189; PMCID: PMC5440113.
[100] J. Wesley Pike et al. The vitamin D receptor: contemporary genomic approaches reveal new basic and translational insights. *J Clin Invest*. 2017;127(4):1146-1154. https://doi.org/10.1172/JCI88887.
[101] Nitulescu GM, Van De Venter M, Nitulescu G, Ungurianu A, Juzenas P, Peng Q, Olaru OT, Grădinaru D, Tsatsakis A, Tsoukalas D, Spandidos DA, Margina D. The Akt pathway in oncology therapy and beyond (Review). *Int J Oncol*. 2018 Dec;53(6):2319-2331. doi: 10.3892/ijo.2018.4597.
[102] Kopan R. Notch signaling. *Cold Spring Harb Perspect Biol*. 2012 Oct 1;4(10):a011213. doi: 10.1101/cshperspect.a011213.
[103] Braicu C, Buse M, Busuioc C, Drula R, Gulei D, Raduly L, Rusu A, Irimie A, Atanasov AG, Slaby O, Ionescu C, Berindan-Neagoe I. A Comprehensive Review on MAPK: A Promising Therapeutic Target in Cancer. *Cancers* (Basel). 2019 Oct 22;11(10):1618. doi: 10.3390/cancers11101618.
[104] Tsuchiya Y, Nakabayashi O, Nakano H. FLIP the Switch: Regulation of Apoptosis and Necroptosis by cFLIP. *Int J Mol Sci*. 2015 Dec 18;16(12):30321-41. doi: 10.3390/ijms161226232.
[105] Kümpfel, T., Hohlfeld, R. TNFRSF1A, TRAPS and multiple sclerosis. *Nat Rev Neurol 5*, 528–529 (2009). https://doi.org/10.1038/nrneurol.2009.154
[106] Birkhoff JC, Huylebroeck D, Conidi A. ZEB2, the Mowat-Wilson Syndrome Transcription Factor: Confirmations, Novel Functions, and Continuing Surprises. *Genes* (Basel). 2021 Jul 3;12(7):1037. doi: 10.3390/genes12071037.
[107] Oh D, Yu CH, Needleman DJ. Spatial organization of the Ran pathway by microtubules in mitosis. *Proc Natl Acad Sci U S A*. 2016;113(31):8729-8734. doi:10.1073/pnas.1607498113
[108] Sedaghat Y, Bui HH, Mazur C, Monia BP. Identification of REST-regulated genes and pathways using a REST-targeted antisense approach. *Nucleic Acid Ther*. 2013;23(6):389-400. doi:10.1089/nat.2013.0445
[109] Esmon CT. The protein C pathway. *Chest*. 2003;124(3 Suppl):26S-32S. doi:10.1378/chest.124.3_suppl.26s
[110] Okano M. Bell D.W. Haber D.A. Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell*. 1999; 99: 247-257
[111] Liu X, Hou L, Xu D, et al. Effect of asymmetric dimethylarginine (ADMA) on heart failure development. *Nitric Oxide*. 2016;54:73-81. doi:10.1016/j.niox.2016.02.006
[112] Alwhaibi A, Verma A, Adil MS, Somanath PR. The unconventional role of Akt1 in the advanced cancers and in diabetes-promoted carcinogenesis. *Pharmacol Res*. 2019;145:104270. doi:10.1016/j.phrs.2019.104270
[113] Chen J, Somanath PR, Razorenova O, Chen WS, Hay N, Bornstein P, Byzova TV. Akt1 regulates pathological angiogenesis, vascular maturation and permeability in vivo. *Nat Med*. 2005;11:1188–96.
[114] Takayanagi H. Osteoimmunology: shared mechanisms and crosstalk between the immune and bone systems. *Nat Rev Immunol*. 2007; 7(4): 292– 304
[115] Tamayo M, Martin-Nunes L, Val-Blasco A, Piedras MJ, Larriba MJ, Gómez-Hurtado N, Fernández-Velasco M, Delgado C. Calcitriol, the Bioactive Metabolite of vitamin D, Increases Ventricular K+ Currents in Isolated Mouse Cardiomyocytes. *Front Physiol*. 2018 Aug 24;9:1186. doi: 10.3389/fphys.2018.01186.
[116] Mahmoud AM, Szczurek M, Hassan C, Masrur M, Gangemi A, Phillips SA. vitamin D Improves Nitric Oxide-Dependent Vasodilation in Adipose Tissue Arterioles from Bariatric Surgery Patients. *Nutrients*. 2019 Oct 18;11(10):2521. doi: 10.3390/nu11102521. PMID: 31635396; PMCID: PMC6835261.
[117] Doronzo G, Viretto M, Russo I, et al. Nitric oxide activates PI3-K and MAPK signalling pathways in human and rat vascular smooth muscle cells: influence of insulin resistance and oxidative stress. *Atherosclerosis*. 2011;216(1):44-53. doi:10.1016/j.atherosclerosis.2011.01.01
[118] Pike JW, Meyer MB. The unsettled science of nonrenal calcitriol production and its clinical relevance. *J Clin Invest*. 2020 Sep 1;130(9):4519-4521. doi: 10.1172/JCI141334. PMID: 32716362; PMCID: PMC7456237.
[119] Miloudi K, Oubaha M, Ménard C, et al. NOTCH1 signaling induces pathological vascular permeability in diabetic retinopathy. *Proc Natl Acad Sci U S A*. 2019;116(10):4538-4547. doi:10.1073/pnas.1814711116
[120] Pei, H., Yu, Q., Xue, Q. et al. Notch1 cardioprotection in myocardial ischemia/reperfusion involves reduction of oxidative/nitrative stress. *Basic Res Cardiol*.2013; 108, 373. https://doi.org/10.1007/s00395-013-0373

[121] Boopathy AV, Pendergrass KD, Che PL, Yoon YS, Davis ME. Oxidative stress-induced Notch1 signaling promotes cardiogenic gene expression in mesenchymal stem cells. *Stem Cell Res Ther*. 2013 Apr 18;4(2):43. doi: 10.1186/scrt190. PMID: 23597145; PMCID: PMC3706823.
[122] Kannan K, Jain SK. Oxidative stress and apoptosis. *Pathophysiology*. 2000;7:153–163. doi: 10.1016/S0928-4680(00)00053-5.
[123] Dash PR, McCormick J, Thomson MJ, Johnstone AP, Cartwright JE, Whitley GS. Fas ligand-induced apoptosis is regulated by nitric oxide through the inhibition of fas receptor clustering and the nitrosylation of protein kinase Cepsilon. *Exp Cell Res*. 2007;313(16):3421-3431. doi:10.1016/j.yexcr.2007.05.030
[124] Uriarte SM, Joshi-Barve S, Song Z, et al. Akt inhibition upregulates FasL, downregulates c-FLIPs and induces caspase-8-dependent cell death in Jurkat T lymphocytes. *Cell Death Differ*. 2005;12(3):233-242. doi:10.1038/sj.cdd.4401549
[125] Tabasi N, Rastin M, Mahmoudi M, Ghoryani M, Mirfeizi Z, Rabe SZ, Reihani H. Influence of vitamin D on cell cycle, apoptosis, and some apoptosis related molecules in systemic lupus erythematosus. *Iran J Basic Med Sci*. 2015 Nov;18(11):1107-11. PMID: 26949498; PMCID: PMC4764112.
[126] Ho SY, Ling TY, Lin HY, et al. SDF-1/CXCR4 Signaling Maintains Stemness Signature in Mouse Neural Stem/Progenitor Cells. *Stem Cells Int*. 2017;2017:2493752. doi:10.1155/2017/2493752
[127] Datta S, Snow CJ, Paschal BM. A pathway linking oxidative stress and the Ran GTPase system in progeria. *Mol Biol Cell*. 2014 Apr;25(8):1202-15. doi: 10.1091/mbc.E13-07-0430. Epub 2014 Feb 12. PMID: 24523287; PMCID: PMC3982987.
[128] Lai GR, Lee YF, Yan SJ, Ting HJ. Active vitamin D induces gene-specific hypomethylation in prostate cancer cells developing vitamin D resistance. *Am J Physiol Cell Physiol*. 2020;318(5):C836-C847. doi:10.1152/ajpcell.00522.2019
[129] Mirza I, Mohamed A, Deen H, et al. Obesity-Associated vitamin D Deficiency Correlates with Adipose Tissue DNA Hypomethylation, Inflammation, and Vascular Dysfunction. *Int J Mol Sci*. 2022;23(22):14377. Published 2022 Nov 19. doi:10.3390/ijms232214377
[130] Zimnicka AM, Husain YS, Shajahan AN, Sverdlov M, Chaga O, Chen Z, Toth PT, Klomp J, Karginov AV, Tiruppathi C, Malik AB, Minshall RD. Src-dependent phosphorylation of caveolin-1 Tyr-14 promotes swelling and release of caveolae. *Mol Biol Cell*. 2016 Jul 1;27(13):2090-106. doi: 10.1091/mbc.E15-11-0756. Epub 2016 May 11. PMID: 27170175; PMCID: PMC4927282.
[131] Fliri AF, Kajiji S. Functional characterization of nutraceuticals using spectral clustering: Centrality of caveolae-mediated endocytosis for management of nitric oxide and vitamin D deficiencies and atherosclerosis. *Front Nutr*. 2022;9:885364. Published 2022 Aug 15. doi:10.3389/fnut.2022.885364
[132] Schaefer RJ, Bonor JC, Joglekar MS, van Golen KL, Nohe AG. 1,25 Dihydroxyvitamin D3 uptake is localized at caveolae and requires caveolar function. *J Biomed Nanotechnol*. 2013;9(10):1707-1715. doi:10.1166/jbn.2013.1662
[133] Xie Z, Hou L, Shen S, Wu Y, Wang J, Jie Z, Zhao X, Li X, Zhang X, Chen J, Xu W, Ning L, Ma Q, Wang S, Wang H, Yuan P, Fang X, Qin A, Fan S. Mechanical force promotes dimethylarginine dimethylaminohydrolase 1-mediated hydrolysis of the metabolite asymmetric dimethylarginine to enhance bone formation. *Nat Commun*. 2022 Jan 10;13(1):50. doi: 10.1038/s41467-021-27629-2. PMID: 35013196; PMCID: PMC8748781.
[134] Chen Y, Li Y, Zhang P, Traverse JH, Hou M, Xu X, Kimoto M, Bache RJ. Dimethylarginine dimethylaminohydrolase and endothelial dysfunction in failing hearts. *Am J Physiol Heart Circ Physiol*. 2005;289:H2212–9.
[135] Zhang P, Hu X, Xu X, Chen Y, Bache RJ. Dimethylarginine dimethylaminohydrolase 1 modulates endothelial cell growth through nitric oxide and Akt. *Arterioscler Thromb Vasc Biol*. 2011 Apr;31(4):890-7. doi: 10.1161/ATVBAHA.110.215640.
[136] Choi HR, Lee SW, Yeom H, Jeon DH, Kim HC, Youm Y. Association between vitamin D status and asymmetric dimethylarginine (ADMA) concentration in the Korean elderly population. *Maturitas*. 2017;102:13-17. doi:10.1016/j.maturitas.2017.05.002
[137] Barsacchi R, Perrotta C, Bulotta S, Moncada S, Borgese N, Clementi E. Activation of endothelial nitric-oxide synthase by tumor necrosis factor-alpha: a novel pathway involving sequential activation of neutral sphingomyelinase, phosphatidylinositol-3' kinase, and Akt. *Mol Pharmacol*. 2003;63(4):886-895. doi:10.1124/mol.63.4.886
[138] Dargelos E, Renaud V, Decossas M, Bure C, Lambert O, Poussard S. Caveolae-mediated effects of TNF-α on human skeletal muscle cells. Exp Cell Res. 2018;370(2):623-631. doi:10.1016/j.yexcr.2018.07.027
[139] Magrinat G, Mason SN, Shami PJ, Weinberg JB. Nitric oxide modulation of human leukemia cell differentiation and gene expression. *Blood*. 1992;80(8):1880-1884.
[140] Jeong MH, Kim HR, Park YJ, Chung KH. Akt and Notch pathways mediate polyhexamethylene guanidine phosphate-induced epithelial-mesenchymal transition via ZEB2. *Toxicol Appl Pharmacol*. 2019;380:114691. doi:10.1016/j.taap.2019.
[141] Rosati J, Spallotta F, Nanni S, et al. Smad-interacting protein-1 and microRNA 200 family define a nitric oxide-dependent molecular circuitry involved in embryonic stem cell mesendoderm differentiation. *Arterioscler Thromb Vasc Biol*. 2011;31(4):898-907. doi:10.1161/ATVBAHA.110.214478

[142] Bassez G, Camand OJ, Cacheux V, Kobetz A, Dastot-Le Moal F, Marchant D, Catala M, Abitbol M, Goossens M (March 2004). "Pleiotropic and diverse expression of ZFHX1B gene transcripts during mouse and human development supports the various clinical manifestations of the "Mowat-Wilson" syndrome". *Neurobiology of Disease*. 15 (2): 240–50. doi:10.1016/j.nbd.2003.10.004. PMID 15006694. S2CID 25770329.

[143] Theodossiou SK, Murray JB, Hold LA, Courtright JM, Carper AM, Schiele NR. Akt signaling is activated by TGFβ2 and impacts tenogenic induction of mesenchymal stem cells. *Stem Cell Res Ther*. 2021;12(1):88. Published 2021 Jan 26. doi:10.1186/s13287-021-02167-2

[144] Shi C, Sun B, Wu H, et al. Dysfunction of Caveolae-Mediated Endocytic TβRI Degradation Results in Hypersensitivity of TGF-β/Smad Signaling in Osteogenesis Imperfecta. *J Bone Miner Res*. 2023;38(1):103-118. doi:10.1002/jbmr.4734

[145] Pallotta F, Rosati J, Straino S, et al. Nitric oxide determines mesodermic differentiation of mouse embryonic stem cells by activating class IIa histone deacetylases: potential therapeutic implications in a mouse model of hindlimb ischemia [published correction appears in Stem Cells. 2014 Jun;32(6):1688-9]. *Stem Cells*. 2010;28(3):431-442. doi:10.1002/stem.300

[146] Mohammad S, Mishra A, Ashraf MZ. Emerging Role of vitamin D and its Associated Molecules in Pathways Related to Pathogenesis of Thrombosis. *Biomolecules*. 2019 Oct 24;9(11):649. doi: 10.3390/biom9110649. PMID: 31653092; PMCID: PMC6920963.

[147] Maehata Y, Miyagawa S, Sawa Y. Activated protein C has a protective effect against myocardial I/R injury by improvement of endothelial function and activation of AKT1. *PLoS One*. 2012;7(8):e38738. doi: 10.1371/journal.pone.0038738. Epub 2012 Aug 20. PMID: 22916090; PMCID: PMC3423409.

[148] Mohammad S, Mishra A, Ashraf MZ. Emerging Role of Vitamin D and its Associated Molecules in Pathways Related to Pathogenesis of Thrombosis. *Biomolecules*. 2019 Oct 24;9(11):649. doi: 10.3390/biom9110649. PMID: 31653092; PMCID: PMC6920963.

[149] Bouillon R, Carmeliet G, Lieben L, Watanabe M, Perino A, Auwerx J, et al. Vitamin D and energy homeostasis: of mice and men. *Nat Rev Endocrinol*. 2014;10:79–87. doi: 10.1038/nrendo.2013.226.

[150] Luo L, Yan S, Lai PT, et al. PhenoTagger: A Hybrid Method for Phenotype Concept Recognition using Human Phenotype Ontology [published online ahead of print, 2021 Jan 20]. *Bioinformatics*. 2021;btab019. doi:10.1093/bioinformatics/btab019

[151] Köhler S, Gargano M, Matentzoglu N, et al. The Human Phenotype Ontology in 2021. *Nucleic Acids Res*. 2021;49(D1):D1207-D1217. doi:10.1093/nar/gkaa1043

[152] Choi KW, Batchelder AW, Ehlinger PP, Safren SA, O'Cleirigh C. Applying network analysis to psychological comorbidity and health behavior: Depression, PTSD, and sexual risk in sexual minority men with trauma histories. *J Consult Clin Psychol*. 2017 Dec;85(12):1158-1170. doi: 10.1037/ccp0000241.

[153] Fliri AF, Loging WT, Volkmann RA. Cause-effect relationships in medicine: a protein network perspective. *Trends Pharmacol Sci*. 2010;31(11):547-555. doi:10.1016/j.tips.2010.07.005.

[154] See reference 4

[155] Zhang Y, Zhang J, Studzinski GP. AKT pathway is activated by 1, 25-dihydroxyvitamin D3 and participates in its anti-apoptotic effect and cell cycle control in differentiating HL60 cells. *Cell Cycle*. 2006;5(4):447-451. doi:10.4161/cc.5.4.2467

[156] Domingues-Faria C, Chanet A, Salles J, Berry A, Giraudet C, Patrac V, Denis P, Bouton K, Goncalves-Mendes N, Vasson MP, Boirie Y, Walrand S. Vitamin D deficiency down-regulates Notch pathway contributing to skeletal muscle atrophy in old wistar rats. *Nutr Metab* (Lond). 2014 Sep 30;11(1):47. doi: 10.1186/1743-7075-11-47. PMID: 25317198; PMCID: PMC4195890.

[157] Bhat IA, Mir IR, Malik GH, et al. Comparative study of TNF-α and vitamin D reveals a significant role of TNF-α in NSCLC in an ethnically conserved vitamin D deficient population. *Cytokine*. 2022;160:156039. doi:10.1016/j.cyto.2022.156039

[158] Nguyen, TP., Scotti, M., Morine, M.J. et al. Model-based clustering reveals vitamin D dependent multi-centrality hubs in a network of vitamin-related proteins. *BMC Syst Biol 5*, 195 (2011). https://doi.org/10.1186/1752-0509-5-195

[159] Lai GR, Lee YF, Yan SJ, Ting HJ. Active vitamin D induces gene-specific hypomethylation in prostate cancer cells developing vitamin D resistance. *Am J Physiol Cell Physiol*. 2020;318(5):C836-C847. doi:10.1152/ajpcell.00522.2019

[160] Larriba MJ, García de Herreros A, Muñoz A. Vitamin D and the Epithelial to Mesenchymal Transition. *Stem Cells Int*. 2016;2016:6213872. doi: 10.1155/2016/6213872. Epub 2016 Jan 6. PMID: 26880977; PMCID: PMC4736588.

[161] Fetahu IS, Höbaus J, Kállay E. Vitamin D and the epigenome. *Front Physiol*. 2014 Apr 29;5:164. doi: 10.3389/fphys.2014.00164. PMID: 24808866; PMCID: PMC4010791.

[162] Rajendran G, Shanmuganandam K, Bendre A, Muzumdar D, Goel A, Shiras A. Epigenetic regulation of DNA methyltransferases: DNMT1 and DNMT3B in gliomas [published correction appears in J Neurooncol. 2011 Sep;104(2):495. Mujumdar, Dattatreya [corrected to Muzumdar, Dattatraya]]. *J Neurooncol*. 2011;104(2):483-494. doi:10.1007/s11060-010-0520-2

[163] Skrypek N, Bruneel K, Vandewalle C, et al. ZEB2 stably represses RAB25 expression through epigenetic regulation by SIRT1 and DNMTs during epithelial-to-mesenchymal transition. *Epigenetics Chromatin*. 2018;11(1):70. Published 2018 Nov 16. doi:10.1186/s13072-018-0239-4

[164] Li J, Sun L, Li Y. Regulation of dimethylarginine dimethylaminohydrolase 2 expression by NF-κB acetylation. *Exp Ther Med*. 2021 Feb;21(2):114. doi: 10.3892/etm.2020.9546. Epub 2020 Dec 3. PMID: 33335577; PMCID: PMC7739820.

[165] Bouillon R, Carmeliet G, Verlinden L, et al. Vitamin D and human health: lessons from vitamin D receptor null mice. *Endocr Rev*. 2008;29(6):726-776. doi:10.1210/er.2008-0004

[166] Bikle DD. Vitamin D: an ancient hormone. *Exp Dermatol*. 2011;20(1):7-13. doi:10.1111/j.1600-0625.2010.01202.x

[167] Nguyen, TP., Scotti, M., Morine, M.J. et al. Model-based clustering reveals vitamin D dependent multi-centrality hubs in a network of vitamin-related proteins. *BMC Syst Biol 5*, 195 (2011). https://doi.org/10.1186/1752-0509-5-195

[168] Wodak SJ, Vlasblom J, Turinsky AL, Pu S. Protein-protein interaction networks: the puzzling riches. *Curr Opin Struct Biol*. 2013;23(6):941-953. doi:10.1016/j.sbi.2013.08.002

[169] Gillis J, Ballouz S, Pavlidis P. Bias tradeoffs in the creation and analysis of protein-protein interaction networks. *J Proteomics*. 2014 Apr 4;100:44-54. doi: 10.1016/j.jprot.2014.01.020. Epub 2014 Jan 27. PMID: 24480284; PMCID: PMC3972268.

[170] Alberca GGF, Alberca RW. Role of vitamin D deficiency and comorbidities in COVID-19. World *J Virol*. 2022 Jan 25;11(1):85-89. doi: 10.5501/wjv.v11.i1.85. PMID: 35117974; PMCID: PMC8788214.

[171] Khan A, Dawoud H, Malinski T. Nanomedical studies of the restoration of nitric oxide/peroxynitrite balance in dysfunctional endothelium by 1,25-dihydroxy vitamin D3 - clinical implications for cardiovascular diseases. Int J *Nanomedicine*. 2018 Jan 19;13:455-466. doi: 10.2147/IJN.S152822. PMID: 29416330; PMCID: PMC5788997.

[172] See reference 49.