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Original Research

Analysis of potential interactions between vitamins D and C using gene expression profiles from mouse models

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Abstract: Vitamin D (VD) and vitamin C (VC) have been recognized as essential elements for human health. Animal models, especially mouse models, have been widely used in the study of VD and VC. This study is to investigate how VD and VC interact at molecular level using whole genome expression profiles from spleens of 111 mouse strains and livers from 40 mouse strains. We first identified the genes that are closely correlated to vitamin D receptor (Vdr) and gulonolactone oxidase (Lgo). We next analyzed the potential molecular pathways of Vdrand Lgo correlated genes and examined how these two sets of pathways are connected in liver and spleen. Our results indicated that Vdrand Lgo associate to distinct groups of genes. While most of genes are not the same, a few of them are associated to both Vdrand Lgo. Our gene network construction suggests that there are interactions between Vdrand Lgopathways. Our work laid down the foundation for future study of the interactions between VD and VC and revealed the potential bias when using animals for the study of VD or VC, as humans do not produce VC.

Key words: Gene Expression; Mouse; Network; Vitamin C; Vitamin D.

Introduction

Vitamin D (VD) is an essential element that mainly regulates the absorption of calcium and phosphorus as well as direct cell communication in humans. VD could be either synthesized by sunlight or obtained from a diet of fish liver oils and egg yolks (1). In liver, VD is converted into the prohormone calcidiol and then converted into calcitriol in kidney. The active VD metabolite calcitriol mediates its biological effects by binding to the vitamin D receptor (*Vdr*), which is expressed by cells in most organs (2-4). Concomitantly, spleen as a center of activity of the mononuclear phagocyte system, may participate in the metabolism of VD.

Vitamin C (VC) is a water-soluble vitamin found mostly in vegetables and fruits. It serves to protect cells from damage from free radicals, which are formed during food synthesis, as well as creating collagen, a protein used to heal wounds (5). In mammals, ascorbate is produced by glucose in liver, which is extracted from glycogen; ascorbate synthesis is a glycogenolysis-dependent process. VC is found in high concentrations in immune cells, and is consumed quickly during infections. Although how VC interacts with the immune system is still not certain, it has been hypothesized that VC modulates the activities of phagocytes, the production of cytokines and lymphocytes, and the number of cell adhesion molecules in monocytes (6). Although plants or animals could survive without VC, humans cannot synthesize it. We would have to consume VC because human cells cannot perform the final step of VC biosynthesis, which converts I-gulono-g-lactone into ascorbic acid, catalyzed by gulonolactone oxidase (*Lgo* or *Gulo*). VC is essential for the synthesis of collagen and also plays a role in maintaining a balanced collagen network (7,8).

Animal models have been widely used in the study of VD and VC. One study suggests that VD may bind and activate VD receptor to aid in the treatment of established breast cancer (9, 10). Another study used a mouse model to investigate VD as a possible treatment for multiple sclerosis (MS) (11, 12). Another study claimed that VC, in tandem with N-acetyl cysteine, acts to diminish the tumor genesis by decreasing DNA damage and mutation (13).

Vdr gene and *Lgo* gene as the essential relevant genes are selected as the pivotal searched terms in this gene network. Since humans do not have a functional LGO gene, humans must obtain VC from food It is known that VC includes both ascorbic acid and dehydroascorbic acid which acts as a water-soluble antioxidant and also have antiscorbutic effects. *Lgo* gene, which encodes *Lgo* enzyme, is considered to be the critical gene in the biosynthesis of VC (14-16). Meanwhile, the genomics and genetics of mouse have been extensively studied (17-19). The *Lgo*-/- mouse has become the model of choice in studying the role of VC in complex diseases (20). *Vdr* is a ligand-dependent transcription factor and its primary function is to maintain calcium homeostasisthrough regulating target gene expression

in response to VD (21). Therefore, mice lacking *Vdr* are an appropriate model to elucidate the overall functions of VD in selected tissues (22).

VD and VC are associated with several diseases and play important roles in human health. Base on the above evidence, we hypothesize that liver and spleen are related to the metabolism of VD and VC. In present study, by implicating gene network analysis, we respectively investigate genes that are associated with both Vdr and Lgo in liver and spleen. Our objective is to explore the interactions between VD and VC.

Materials and Methods

Whole genome expression profiles of BXD RI strains

Data for this study come from gene expression profiles form two types of tissues, the spleen and the liver (http://www.genenetwork.org/webqtl/main.py). The first set is the data from spleen. In the UTHSC Affy MoGene 1.0 ST Spleen (Dec10) RMA Database, these strains include a total of 111 strains, including 81 BXD strains, both parental strains (C57BL/6J and DBA/2J) and both reciprocal F1 hybrids (B6D2F1 and D2B6F1), and 26 other common inbred strains were quantified.

The second set of data is from the liver. UNC Agilent G4121A Liver LOWESS Stanford (Jan06) Both Sexes Database include profiles of the gene expression in livers of naive mice of both sexes from C57BL/6J, DBA/2J, B6D2F1, and 37 BXD strains were profiled using Agilent oligonucleotide microarrays.

Although these two sets of data from two microarray platforms, the comparison between sexes is based on data within the same platform. The data for female to male comparison in mouse spleen is all from the Affymetric platform. The data for comparison from liver between female and male mice are all from Agilent platform.

Identification of genes that are highly correlated to the expression of Vdr and Lgogenes

For genes correlated in expression levels to *Lgo* and *Vdr*, 10 common genes were found within the top 500 genes of correlated expression (*Tm9sf3, Mdm1, Igk, Chkb, 3321401G04Rik, Mmp14, Rp2h, Ubn2, Ubr2, and Usp15*). In case of multiple probes of a gene, the probe with the highest expression level was chosen to represent the gene.

Identification of genes that have been reported Vdrassociated genes from the highly Lgo correlated genes and the Lgo associated genes from the highly Vdr correlated genes

Identification of bone relevant genes was conducted with a searching tool, PGMapper (http://www.genediscovery.org/pgmapper/index.jsp) (23) with Key word "Vitamin C" or "Vitamin D". For any potential candidates, at least the abstract of one reference was read by two authors to determine a link between the VD and VC. For a gene with more than one reference that indicated its candidacy, at least two references were read and cited in this study. The following articles were found using these methods:

OMIM results:

http://omim.org/entry/612792 (Ptdss1), http://omim.org/entry/138245 (Grik1), http://omim.org/entry/601014 (Dlg1)

PubMed Results:

http://www.ncbi.nlm.nih.gov/pubmed/24584058?dopt=AbstractPlus

http://www.ncbi.nlm.nih.gov/pubmed/20054029?dopt=AbstractPlus

http://www.ncbi.nlm.nih.gov/pubmed/19609519?dopt=AbstractPlus

http://www.ncbi.nlm.nih.gov/pubmed/19592096?dopt=AbstractPlus

http://www.ncbi.nlm.nih.gov/pubmed/16769580?dopt=AbstractPlus

http://www.ncbi.nlm.nih.gov/pubmed/10910926?dopt=AbstractPlus

http://www.ncbi.nlm.nih.gov/pubmed/7601456?dopt=AbstractPlus

http://www.ncbi.nlm.nih.gov/pubmed/8436414?dopt=AbstractPlus

http://www.ncbi.nlm.nih.gov/pubmed/2575643?dopt=AbstractPlus

http://www.ncbi.nlm.nih.gov/pubmed/6158554?dopt=AbstractPlus (Igk),

http://www.ncbi.nlm.nih.gov/pubmed/19423758?dopt=AbstractPlus(Cldn2)

Gene network construction

Gene network construction was conducted using GeneNetwork Graphic function http://www.genenetwork.org/webqtl/main.py

Database selection

To determine the best data set among the alternatives, we used the GN (GeneNetwork)'s advanced search strings to identify all transcripts with an LRS (likelihood ratio statistic) value above 50 and less than 1000 that are located within 10Mb on either side of the gene. GN computed the numbers of transcripts associated with very high LRS or LOD (log of the odds) ratio. The majority of these hits are natural genesgenes that modulate their own expression. Each of liver mRNA alternative data sets was systematically tested. The data of outcomes are shown in Table 1The similarity of platforms was one of the criteria as selection priority of data sets. Based on the information above all, UNC Agilent G4121A Liver LOWESS Stanford (Jan06) Both Sexes Database and UTHSC Affy MoGene 1.0 ST Spleen (Dec10) RMA Database with the maximum number of hits and the similarity platform were selected for this study.

Statistical Analysis

The top 500 associated genes in each dataset on the basis of Pearson correlation were used for plotting Network Graphs in GeneNetwork. An r absolute value > 0.50 was considered to indicate connection line threshold.

Results

Gene network in liver

Normal Probability Plot was employed to evaluate whether the selected data are normally distributed. By applying the Basic Statistics tools provided by Gene-

Table 1. Selected databases UNC Agilent G4121A Liver LOWESS Stanford (Jan06). Both Sexes Database and UTHSC Affy MoGene 1.0 ST Spleen (Dec10) RMA Database data sets are significantly better than all of the other transforms or data sets (n=transcripts associated with LRS values above 50 (a LOD score > 10).

A

	Liver mRNA databases	n
1	GSE16780 UCLA Hybrid MDP Liver Affy HT M430A (Sep11) RMA Database	216
2	SUH BXD Liver CCl4-treated Affy Mouse Gene 1.0 ST (Jun11) RMA Database	129
3	UNC Agilent G4121A Liver LOWESS Stanford (Jan06) Both Sexes Database	339
4	UNC Agilent G4121A Liver LOWESS Stanford (Jan06) Males Database	175
5	UNC Agilent G4121A Liver LOWESS Stanford (Jan06) Females Database	199
6	EPFL/LISP BXD CD+HFD Liver Affy Mouse Gene 1.0 ST (Apr13) RMA Database	248
7	EPFL/LISP BXD HFD Liver Affy Mouse Gene 1.0 ST (Apr13) RMA Database	186
8	EPFL/LISP BXD CD Liver Affy Mouse Gene 1.0 ST (Apr13) RMA Database	118

B

	Spleen mRNA databases	n
1	UTHSC Affy MoGene 1.0 ST Spleen (Dec10) RMA Database	590
2	UTHSC Affy MoGene 1.0 ST Spleen (Dec10) RMA Males Database	225
3	UTHSC Affy MoGene 1.0 ST Spleen (Dec10) RMA Females Database	269
4	UTHSC Affy MoGene 1.0 ST Spleen (Dec10) RMA Exon Level Database	3524
5	UTHSC Affy MoGene 1.0 ST Spleen (Oct10) RMA Database	475
6	UTK Spleen ILM6.1 (Jan10) VST Database	572
7	IoP Affy MOE 430v2 Spleen (May09) RMA Database	122

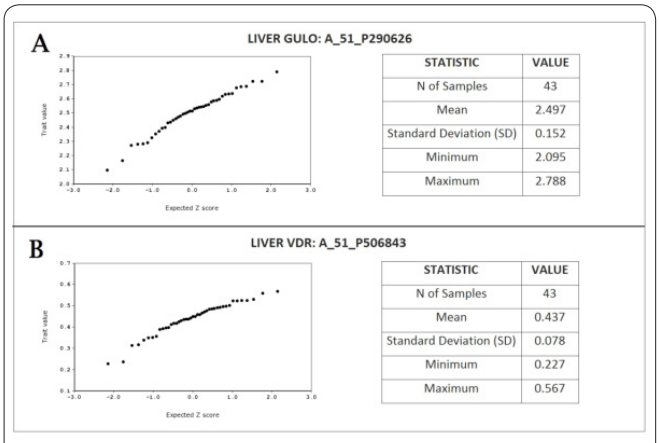

Figure 1. Basic statistics of the databases in liver. Plot A and B respectively evaluates the measured expression value on *Lgo* and *Vdr* mRNA in liver on the y-axis and the expected Z score value (units of deviation from the mean measured in standard deviation units) on the x-axis, indicated that the data are normally distributed.

Network to select data sets, we obtain the plots in Fig.1. The different symbols represent different groups. The y-axis indicates the measured expression value on *Vdr* (A) and *Lgo* (B) mRNA in liver while the x-axis is the expected Z score value (units of deviation from the mean measured in standard deviation units). As described in the plots, the actual values and the predicted values (based on a z score) formed a nearly straight line, which demonstrates that all the data were approximately normally distributed.

Genes that are highly correlated to the expression of Lgo in liver

Type of genes

Values of *Lgo* gene in the UNC Agilent G4121A Liver LOWESS Stanford (Jan06) Both Sexes database were compared to all 20868 records in the same database. The top 500 correlations ranked by the Genetic Correlation (Pearson's r) are obtained by using the commend "Calculate Correlations" for person comparison with "top 500" in "return" (Supplementary Table 1 and 2).

Expression levels of genes

To estimate the correlation between *Lgo* and other candidate genes, Pearson' sample correlation (r) was computed. The Pearson's r values across genes were ranged from 0.694 to -0.665 (regulated *Lgo* gene as 1.0). Among *Lgo* relevant genes, 189 had negative correlation with *Lgo*, indicating that they probably inhibit the expression of the *Lgo* gene. A total of 310 genes were positively correlated to that of *Lgo*, suggesting that they might be co-regulated by a common set of transcription factors (Shown in Fig.2A).

Genes that are highly correlated to the expression of Vdr in liver

Type of genes

Values of *Vdr* gene in the UNC Agilent G4121A Liver LOWESS Stanford (Jan06) Both Sexes database were compared to all 20868 records in the identical database. The top 500 correlations ranked by the Genetic Correlation (Pearson's r) were obtained using the same procedure as that for *Lgo* (Supplementary Table 3 and 4).

Expression levels of genes

Pearson' sample correlation (r) was computed in Gene Network to investigate the correlation of *Vdr* gene and other candidates. The Pearson's r values across genes were ranged from 0.658 to -0.606 (regulated *Vdr* gene as 1.0). Among these genes, 220 were negatively correlated to that of *Vdr* gene, indicating their potential suppress function to *Vdr*. There 279 genes were positively correlated to *Vdr* suggesting that they may have the accelerate effect on *Vdr* gene expression. (Shown in Fig.2B).

Gene network of Vdr and Lgo and their connections

Genes correlated with Vdr and Lgo gene in liver

To identify the genes that are involved in pathways of both VC and VD metabolism, we compared the list of top 500 genes associated to *Vdr* gene and *Lgo* gene. The comparison revealed that the expression levels of

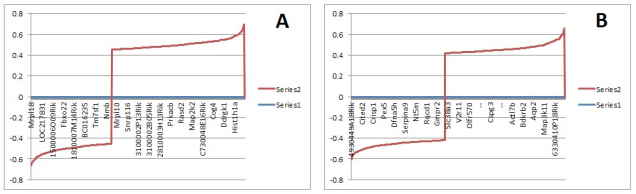

Figure 2. Calculate correlation of genes in liver. A. The Pearson's r values of the top 500 genes are computed, ranged from 0.694 to -0.665 (regulated *Lgo* gene as 1.0). Series 1 represents for the top 500 correlated genes in *Lgo* gene expression, series 2 stand for the Pearson's value of each gene. 189 genes are negative indicate they probably inhibit the expression of the *Lgo* gene. On the contrary, 310 genes are positive suggest they might have promotive effect to *Lgo* gene expression. B. The Pearson's r values of the top 500 genes are calculated valuing from 0.694 to -0.665 (regulated *Vdr* gene as 1.0). Series 1 indicate the top 500 genes in *Vdr* gene expression, while series 2 is described as the Pearson's value of each gene. Despite of *Vdr* gene, 219 negative genes are raised with their feasible suppress function and that 280 positive genes come up with the accelerate effect they may behave in *Vdr* gene expression.

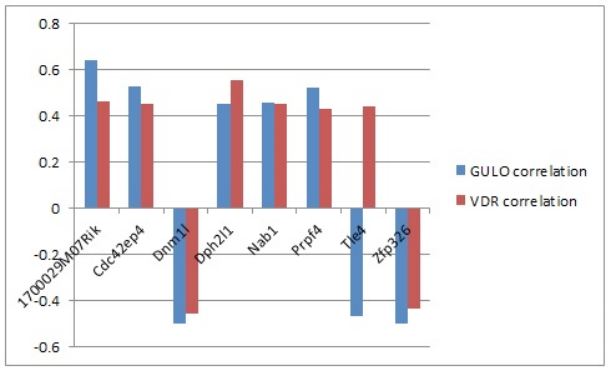

Figure 3. Correlated genes in liver. Pearson's Sample correlation (r) ranged from 0.64 to -0.44, indicate that the correlation among *Lgo* and *Vdr* gene and recommended gene are fairly closed. The gene such as 1700029M07Rik, Cdc42ep4, Dph2l1, Nab1, Prpf4 genes all plays a positive role in both *Lgo* and *Vdr* gene expression (r value =0.43 to 0.64), 1700029M07Rik possess the highest r value. Furthermore, Dnm1l and Zfp326 genes probably have inhibition effect (r value =-0.44 to -0.52) and Tle4 gene may promote *Vdr* expression rather than restrain *Lgo* gene.

8 genes (1700029M07Rik, Cdc42ep4, Dnm1l, Dph2l1, Nab1, Prpf4, Tle4 and Zfp326) were correlated to both the expression of the *Lgo* gene and *Vdr* gene. As shown in Fig.3: all these 8 genes are closely relevant to *Lgo* and *Vdr*, with the Pearson's Sample correlation (r) ranged from 0.64 to -0.44. Moreover, *1700029M07Rik, Cdc42ep4, Dph2l1, Nab1, Prpf4* genes are positively connected to both *Lgo* and *Vdr* gene expression while Dnm1l and Zfp326 genes probably have inhibition effect. In addition, Tle4 may restrain the expression of *Lgo* gene and contrarily promote the *Vdr* expression. 1700029M07Rik exhibited the highest Pearson's Sample correlation value, 0.64 to *Lgo* while a 0.46 R value to that of *Vdr*.

By means of gene network we revealed the correlation between *Lgo* gene and *Vdr* gene expression. Indicated from the comparison, 8 genes are both associated with *Lgo* gene and *Vdr* gene, some of them have synergistic function and others are contrary. Fig.4 shows the relational graphs of the genes, which are radial layout with *Vdr* and *Lgo* as the central nodes. These graphs show the connection between each above gene and *Vdr*, as well as *Lgo*. As indicated from the figure 4, these 8 genes are both associated with *Lgo* gene and *Vdr* gene, some of them have synergistic function and others have opposite effect on expression of *Lgo* and *Vdr*.

Connection of the correlated genes

Based on gene network, we concomitantly explore the interrelation of the correlated genes. As shown in Fig.5, by compared the top 100 genes that were correlated of each of the 8 correlated genes separately, we found that 139 genes appeared to be shared by at least two of the 8 genes. Fig.6 graphed the relationship of all candidate genes. It shows that all 8 genes are associated with both *Vdr* gene and *Lgo* gene. It also shows connec-

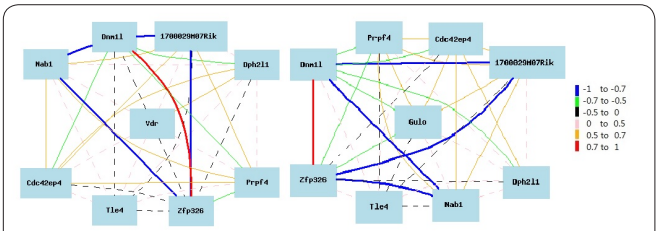

Figure 4. Connections of the correlated genes in liver. The 9 nodes in the each graph show the genes. The 36 edges between the nodes, filtered from the 36 total edges and drawn as curves, show Pearson correlation coefficients greater than 0.0 or less than -0.0. The lines with different color indicate the Pearson's Sample correlation (r).

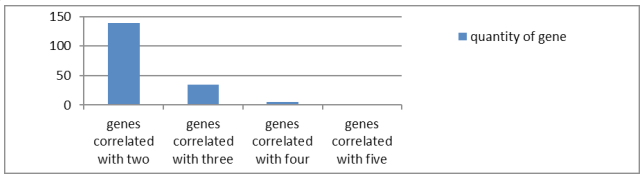

Figure 5. Correlations among the genes. Among the 139 shared genes, 98 genes are correlated with two of the 8 genes, 34 genes are associated with three of them, 5 with four genes, while Dnm1l and Snx1 are shared by five of the correlated 8 genes.

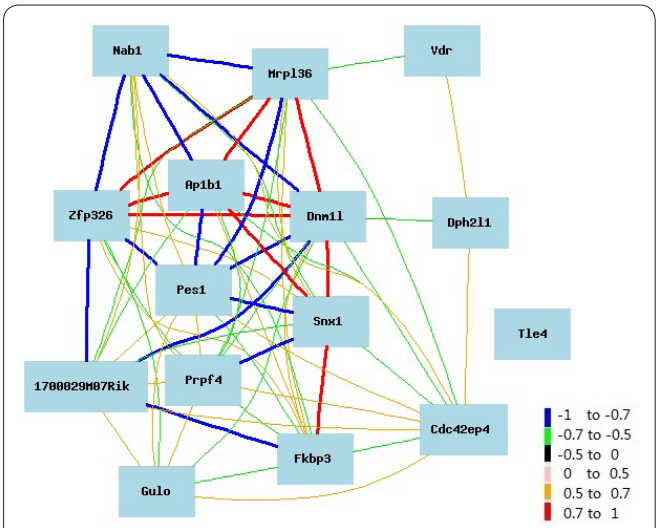

Figure 6. Connections among the associated genes. The nodes exhibit 15 correlated genes, the 65 edges between the nodes, filtered from the 105 total edges and drawn as curves, show Pearson correlation coefficients greater than 0.5 or less than -0.5. Colors of the lines reveal the value of the Pearson's Sample correlation (r).

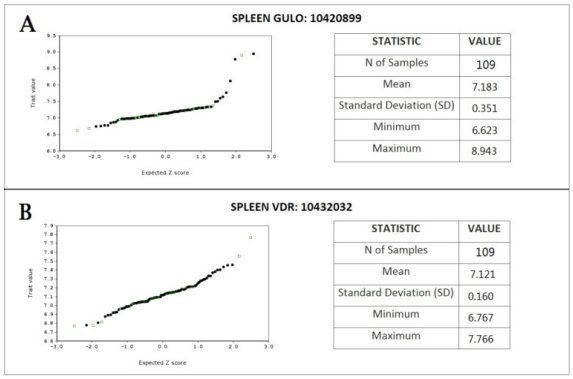

Figure 7. Basic statistics of the databases in spleen. In this probability plot, different symbols represent different groups, the y-axis indicates value on *Vdr* (A) and *Lgo* (B) mRNA expression in spleen while the x-axis stand for Z score value (units of deviation from the mean measured in standard deviation units). As shown, the actual values and the predicted values(z score) form a nearly straight line, which suggests that all the data are approximately normally distributed.

tions of the 7 genes shared by four or five of the 8 common genes. This section of the network indicated that there are connections among the 8 genes which associated with both *Vdr* gene and *Lgo* gene,concomitantly demonstrate the interaction of VD and VC.

Gene network in spleen

Similar to the gene network in liver, the basic statistics of the data is shown in Fig.7, indicated that the selected data are all normal distribution.

Gene that are highly correlated to the expression of Lgo and Vdr in spleen

Type of genes

We intended to respectively calculate the top 500 correlations of *Lgo* and *Vdr* genes, and then search for the related genes shared by them. However, there was no gene that was shared by both *Lgo* and *Vdr* genes. To further explore the potential connection of the two genes, we moved forward to investigate thoroughly the top 1000 genes associated to *Lgo* and *Vdr* genes. Expression levels of *Lgo* gene and *Vdr* gene in the UTHSC Affy MoGene 1.0 ST Spleen (Dec10) RMA database were compared to all 35556 records in the UTHSC Affy MoGene 1.0 ST Spleen (Dec10) RMA database. The top 1000 correlations ranked by the Genetic Correlation (Pearson's r) are obtained for both *Lgo* and *Vdr* genes are obtained by using the commend "Calculate Correlations" for person comparison with "top 1000" in "return" (Supplementary 5-8).

Expression levels of genes

As shown in Fig.8A, the Pearson's r values across genes were ranged from 0.906 to -0.515, to that of *Lgo*. A total of 183 genes may play a negative role in the expression of *Lgo*, while 816 genes could be positive. Simultaneously, in Fig.8B, The 1000 *Vdr* associated genes had the Pearson's r values ranged from 0.522 to -0.527. A total of 233 genes probably inhibit the *Lgo* gene expression and 766 may promote.

Gene network of Vdr and Lgo and their connections

Genes correlated with Vdr and Lgo gene in spleen

After comparison of the top 1000 gene associated to *Lgo* and *Vdr* gene, we found 7 genes that their expression levels were correlated to the expression levels of both *Lgo* and *Vdr* gene. They are *4930429B21Rik, Aldh4a1, Chst7, Cmtm8, Gstm7, Loxl2* and *Rbm39*. As presented in Fig.9, the Pearson's Sample correlations (r) of these genes to that of *Lgo* and *Vdr* are *ranged from 0.43 to -0.42. Among these genes, 4930429B21Rik*, Aldh4a1, Chst7, Cmtm8, *Gstm7, Loxl2* are promotive while Rbm39 are inhibitive for the expression levels of both of *Lgo* and *Vdr* gene. Among these correlated genes, *Rbm39* has the highest correlation value, as -0.42 in *Lgo* and -0.38 in *Vdr*.

This section of gene network revealed that there are connections between *Lgo* and *Vdr* gene expression, however, the connection is not strong.

Connection of the correlated genes

We concomitantly determined interrelation of the common correlated genes with the graph tool in gene network, which is exhibited in Fig.10. The two radial layouts centered with *Vdr* and *Lgo* revealed the correlation of these associated genes directly. Similar to the prior gene network in the liver, we once again compared the top 100 genes of the 7 common associated genes in spleen. We found 83 genes shared by at least 2 of these 7 common genes, including 68 in two and 15 in three of

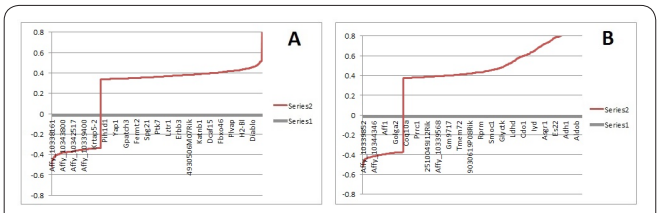

Figure 8. Calculate correlation of genes in spleen. A: Pearson's r values across genes are ranged from 0.906 to -0.515, with the value of *Lgo* gene as 1.0. 183 genes may play a negative role in the expression of *Lgo*, while 816 genes could be positive. In Fig.8B, regulated *Vdr* gene as 1.0, Pearson's r values are from 0.522 to -0.527, there are 233 genes inhibit the *Vdr* gene expression and 766 may promote.

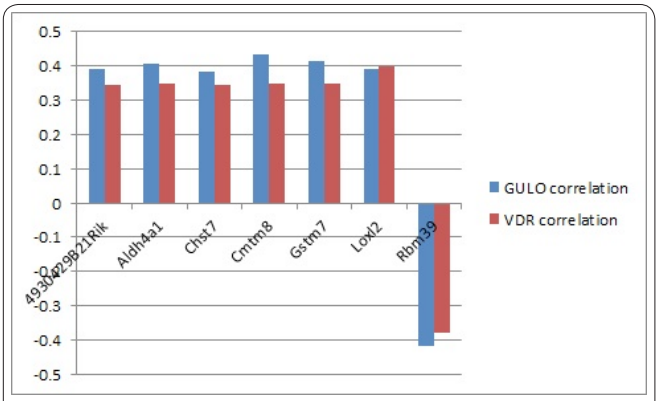

Figure 9. Correlated genes in spleen. Pearson's Sample correlations (r) of the candidate genes are covered from 0.43 to -0.42, indicated that there are significant correlation between *Lgo* and *Vdr* gene expression. 4930429B21Rik, Aldh4a1, Chst7, Cmtm8, Gstm7, Loxl2 and Rbm39 genes all participate in both *Lgo* and *Vdr* gene expression (r value =0.43 to 0.64), as Rbm39 possess the highest r value. In addition, 4930429B21Rik, Aldh4a1, Chst7, Cmtm8, Gstm7 play the positive role (r value 0.40 to 0.43) while Rbm39 is negative.

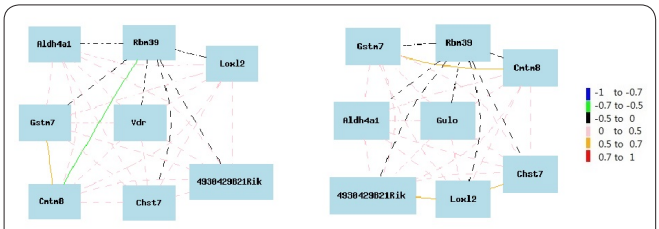

Figure 10. Connections of the correlated genes in spleen. The displayed nodes stands for the correlated genes, in each graph, there are 28 edges and drawn as curves, show Pearson correlation coefficients greater than 0.0 or less than -0.0. Different colors of the lines reveal the value of the Pearson's Sample correlation (r).

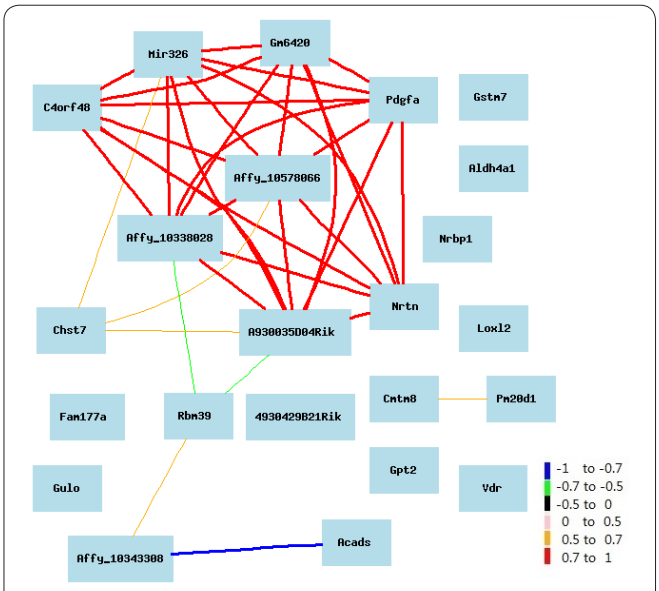

Figure 11. Connections among the associated genes. Nodes exhibit 23 correlated genes, the 36 edges between the nodes, filtered from the 253 total edges and drawn as curves, show Pearson correlation coefficients greater than 0.5 or less than -0.5. Colors of the lines reveal the value of the Pearson's Sample correlation (r).

list of top 100 genes of these common genes. We finally construct the gene network between the 7 commonly shared genes between *Lgo* and *Vdr* and the 15 genes shared by three of these 7 genes. Fig.11 shows the summary of relationship of these 7 genes, the 15 genes, and the *Lgo* and *Vdr*.

Discussion

Using the available gene expression profiles in the gene network, we evaluated the correlation of *Lgo* and *Vdr* in the gene expression pathways. Eight genes in the top 500 associated genes appeared to be shared by both *Vdr* and *Lgo* in liver, while 7 genes in top 1000 in spleen. In the liver, among the 8 genes1700029M07Rik has the highest Pearson's correlation value, which suggests that it might play a critical role in the connection of both pathways of *Lgo* and *Vdr* genes. Additionally, after separately determining the top correlated 100 genes of the 8, we found that the expression levels of Dnm1l was associated to the expression levels of five of the 8 common genes in liver, suggesting that Dnm1l may also connect to the two pathways. Analogously in spleen, *Rbm39* suppressed the expression of both *Lgo* and *Vdr* gene, with high negative correlation R values. The expression of Cmtm8 may also be connected to both the expression levels of *Vdr* and *Lgo* in spleen.

1700029M07Rik gene, which is known as tetratricopeptide repeat domain 9 (*Ttc* 9) genes, is a protein coding gene. Immunostaining and cell fractionation studies revealed that TTC9 is predominantly localized to the endoplasmic reticulum and is a biologically significant protein which is related to hormonal regulation (24). Since VC and VD are all involved in hormonal metabolism, 1700029M07Rik gene is the hypothetical connection in this sense. Dnm1l (dynamin 1-like gene) gene encodes a member of the dynamin family which is localized to the cytoplasm and mitochondrial membrane. The encoded proteins which involved in mitochondrial and peroxisomal division are essential for mitochondrial fission. In mammals, mitochondria are vital organelles known for their essential role in energy metabolism and cell survival (25), which might be the potential function Dnm1l gene playing in Lgoand Vdr pathways. Rbm39 (RNA binding motif protein 39) gene is a transcriptional coactivator. Rbm39 can stimulate transcription mediated by the progesteron and estrogen steroid hormone receptors. It also contributes to alternative splicing by modulation of processing of pre-mRNAs (26-27). It may participate in both vitamin C and vitamin D synthesis. Cmtm8 (CKLF-like MARVEL transmembrane domain containing 8) is one of the chemokine-like factor superfamily members. A study demonstrated that overexpression o*f Cmt*m8 accelerated ligand-induced clearance of epidermal growth factor receptor (*Egfr*) from the cell surface, attenuating *Egfr*-mediated signaling (28). It has also reported that *Cmtm*8 overexpression induced cells undergoing apoptosis via caspase-dependent and -independent pathways (29). Another study revealed that *Egfr* increases Snail1 and downregulates *Vdr* in colonic tumors, whereas *Vdr* suppresses the colonic renin-angiotensin system cascade, limiting *Egfr* signals (30). Together with our data it seems that there should be another cross-talk pathway in CMTM8 gene expression that probably influences both vitamin C and vitamin D metabolism.

This study demonstrated that there are reliable connections between molecular pathways of Lgo and Vdr. We hypothesize that 1700029M07Rik and Dnm1l are genes that are highly correlated with both pathways in liver, while Rbm39 and Cmtm8 genes are considered to be the critical genes in the connection of both pathways in spleen. Additionally, the network in our analysis substantially demonstrated the relationship of *Lgo* and *Vdr* associated genes, brought up a confident prospect of the correlation of VD and VC metabolism. Their network connections also provide evidence of their potential coactions in diseases. To this end, the network connection also suggested a potential treatment strategy. Nevertheless, our current gene network has revealed the correlation of VC and VD metabolism pathway. The mechanisms of these connections are still widely unknown. Further assessments of consequences of these correlations are immanency demanded and it is crucial to fully understand their potential implications. To fully understand the significance of association between Vdr and Lgo molecular pathways, much work is needed to explore this profound knowledge.

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Author contributions

XZ, and YJ designed experiments and analyzed data. MN, AWV, XY, CT, analyzed the microarray data and gene network. Y J, AWV conceived and managed the project. MN, X Z, CT, XY, AWV, YJ contributed to manuscript preparation. All authors contributed to manuscript finalization.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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