BRIEF REPORT

Overlap of vitamin A and vitamin D target genes with CAKUT-related processes [version 1; peer review: 1 approved with reservations]

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Abstract
Congenital Anomalies of the Kidney and Urinary Tract (CAKUT) are a group of abnormalities affecting the kidneys and their outflow tracts, which include the ureters, the bladder, and the urethra. CAKUT patients display a large clinical variability as well as a complex aetiology, as only 5% to 20% of the cases have a monogenic origin. It is thereby suspected that interactions of both genetic and environmental factors contribute to the disease. Vitamins are among the environmental factors that are considered for CAKUT aetiology. In this study, we collected vitamin A and vitamin D target genes and computed their overlap with CAKUT-related gene sets. We observed significant overlaps between vitamin A target genes and CAKUT causal genes, or with genes involved in renal system development, which indicates that an excess or deficiency of vitamin A might be relevant to a broad range of urogenital abnormalities.

Keywords
CAKUT, vitamin A, vitamin D, nutrients, environmental factor, renal development
Introduction

Congenital Anomalies of the Kidney and Urinary Tract (CAKUT) are a group of abnormalities affecting the kidneys and their outflow tracts, including the ureters, the bladder, and the urethra. In the European Union, the overall prevalence of CAKUT (in live plus stillbirths) between 2011 and 2018 was approximately 35:10,000. The main anomalies observed in CAKUT are hydronephrosis (13.02:10,000) and multicystic renal dysplasia (4.33:10,000); these are followed by posterior urethral valves (1.26:10,000), bilateral renal agenesis (1.27:10,000) and bladder extrophy/epispadias (0.62:10,000); many other anomalies with low prevalence can also be observed. This clinical variability observed in patients is accompanied by a variable severity of the phenotypes.

Approximately 40 different genes are known to be associated with monogenic causes of CAKUT in humans, but they explain only 5% to 20% of the cases. The clinical variability and the complex aetiology of CAKUT cases suggest a multifactorial origin with complex interactions of both genetic and environmental factors contributing to the disease.

The role of different environmental factors in CAKUT pathogenesis has been studied previously. It has been shown, for instance, that the drugs inhibiting the angiotensin-converting enzymes cause a specific form of CAKUT, namely tubular dysgenesis. Many prenatal and maternal factors were also assessed for their involvement in CAKUT and related anomalies. For example, studies have observed CAKUT associations with pregestational maternal diabetes mellitus, gestational maternal diabetes mellitus, abnormal volume of amniotic fluid, urogenital infections before the pregnancy, any infection during the pregnancy, maternal overweight and obesity, and lower infant birth weight. These maternal and prenatal factors are modulated by environmental risk factors. In particular, maternal diabetes and overweight/obesity as well as low birth weight are related to the nutrition of the maternal-fetal dyad. In this regard, trace nutrients, like vitamins, are likely to play important roles that still await full characterization.

Vitamins are known to regulate renal development. The association of maternal vitamin A deficiency with nephron reduction was studied on a rat model. The inactivation of the retinoic acid nuclear receptors also resulted in renal malformations in mice. An additional study on rats showed that vitamin A deficiency downregulates RET expression which is essential for epithelial-mesenchymal interaction during renal development. Goodyear et al. compared a group of pregnant women in Bangalore with a high (55%) prevalence of vitamin A deficiency with a group of pregnant women in Montreal with negligible vitamin A deficiency. The authors found that renal volume in newborns, adjusted for body surface area, was significantly lower in the vitamin A-deficient population. While the two populations are different for other genetic and environmental reasons, these results suggest that vitamin A deficiency may have a role in human CAKUT. El-Khashab et al. reported a similar finding in their study on a cohort of Egyptian mother-child pairs; children of vitamin A deficient mothers had significantly lower kidney sizes.

The research on the effects of vitamin D deficiency on kidney development has generated interesting results. Rogers et al. observed that incubation of metanephroi with vitamin D3 prior to implantation into adult rats increased the number of glomeruli. Conversely, Maka et al. and Nascimento et al. observed that maternal vitamin D deficiency stimulated nephrogenesis in rats. Following these studies, Boyce et al. examined the long term effects of maternal vitamin D deficiency and observed that adult male rat offspring of vitamin D deficient dams had reduced creatinine clearance, indicating reduced renal functional capacity. They also observed a significant upregulation of renal renin mRNA expression in fetuses and adult male offspring, and reported smaller kidneys in the offspring. Miliku et al. examined the association of 25-hydroxyvitamin D levels during mid-pregnancy with childhood kidney outcomes among 4212 mother-child pairs. They observed that children of mothers who were vitamin D deficient during pregnancy had a larger combined kidney volume compared to children of mothers who had optimal vitamin D levels.

In this study, we assessed whether vitamin A and vitamin D target genes are related to gene sets involved in CAKUT and renal system development. We first created a list of vitamin A and vitamin D target genes, extracting information from the Comparative Toxicogenomics Database (CTD) as well as from two publications. We then constructed different gene sets relevant to CAKUT, including genes mutated in monogenic forms of CAKUT, genes involved in renal system development, and genes involved in different pathways of interest. Finally, we performed overlap analyses to identify the genes involved in these different gene sets as well as the significant overlaps.

Methods

Vitamin A target genes
We first queried vitamin A and downloaded all gene interactions of vitamin A and its descendants from the Comparative Toxicogenomics Database (CTD). We selected only the interactions supported by at least two references. This gave a list of 1086 target genes.
Balmer and Blomhoff reviewed published data from 1191 articles to identify genes regulated by retinoic acid (vitamin A acid) in humans and other species. The authors provide a list of retinoic acid target genes split into four categories ranging from strong evidence to indirect regulation through a transcriptional intermediary. We selected the genes from all of these categories, converted gene symbols to human orthologs, and updated gene symbols where necessary using the HUGO Gene Nomenclature Committee (HGNC), Rat Genome Database (RGD), Mouse Genome Database (MGD), BioMart and SynGo. The final list of genes obtained from Balmer and Blomhoff contains 521 target genes, of which 229 are common with genes from CTD.

**Vitamin D target genes**

We queried vitamin D and downloaded all gene interactions of vitamin D and its descendants from the CTD. We selected only the interactions supported by at least two references, and obtained a list of 263 target genes.

In Ramagopalan et al., 230 genes with significant expression changes in response to vitamin D were identified on lymphoblastoid cell lines using microarrays. We checked the gene names using HGNC and obtained a list of 210 target genes.

There are only 15 common genes between the list from CTD and the list from Ramagopalan et al. The combined list of vitamin D target genes (458 genes) has 134 genes in common with the combined list of vitamin A target genes (1378 genes).

**CAKUT-related gene sets**

We collected complementary gene sets relevant to CAKUT and renal system development from several sources, outlined below.

**CAKUT causal genes set**

First, we created a set of genes known to be mutated in the monogenic form of CAKUT. To do so, we combined two lists of genes provided in the studies of van der Ven et al. Only six genes are different between these two lists, and their union leads to 42 causal genes (see Extended data).

**GO kidney development gene sets**

We extracted the genes annotated with the three terms below from Gene Ontology (GO). Please note that these terms have parent-child relationships.

- GO:0072001 Renal system development (315 genes)
- GO:0001822 Kidney development (306 genes)
- GO:0060993 Kidney morphogenesis (96 genes)

**Reactome pathways**

We selected different pathways of interest for CAKUT. The renin-angiotensin system is essential for kidney development and mutations in the genes of this system result in CAKUT. In addition, Davis et al. discussed the role of RET signaling in kidney development and CAKUT. In multiple studies the roles of WNT, NOTCH, and Hedgehog signaling in kidney development and kidney diseases are discussed. Based on these studies we included the following Reactome pathways:

- R-HSA-2022377 Metabolism of angiotensinogen to angiotensins (18 genes)
- R-HSA-8853659 RET signaling (38 genes)
- R-HSA-195721 Signaling by WNT (328 genes)
- R-HSA-157118 Signaling by NOTCH (233 genes)
- R-HSA-5358351 Signaling by Hedgehog (149 genes)
WikiPathways

From WikiPathways Rare Disease Portal\textsuperscript{42} we obtained the following four CAKUT-related pathways:

- WP5053 Development of ureteric collection system (47 genes)
- WP4823 Genes controlling nephrogenesis (43 genes)
- WP5052 Nephrogenesis (17 genes)
- WP4830 GDNF/RET signalling axis (23 genes)

Overlap analyses

We computed the overlaps between vitamin target gene sets and the gene sets of interest defined previously. After obtaining the overlap results, we calculated the significance of the overlaps (hypergeometric test, p-values adjusted by Benjamini-Hochberg (BH) method) (see Underlying data and Extended data\textsuperscript{55}).

Results and discussion

The results of the overlap analyses between vitamin A and D target genes and CAKUT-related gene sets are presented in Tables 1 and 2, and the corresponding data is available in Underlying data\textsuperscript{55}.

Vitamin A target genes and CAKUT-related gene sets show significant overlaps

Vitamin A target genes obtained from both CTD and Balmer and Blomhoff are significantly enriched in the set of CAKUT causal genes, with an overlap of ten and six genes, respectively (Table 1).

A significant overlap is also observed focusing on GO terms related to renal system development, kidney development, and kidney morphogenesis. These results are expected due to the recognized role of vitamin A in differentiation.\textsuperscript{43,44} It should be noted that all overlapping CAKUT causal genes except NRIP1 are also part of the kidney development GO term.

Concerning the overlap with Reactome pathways, the vitamin A target genes obtained from CTD are significantly enriched in signaling by NOTCH. The NOTCH signalling pathway is mainly involved in cell-to-cell communication,\textsuperscript{45} and it plays a role in the development of most organs and tissues.\textsuperscript{46} Other Reactome terms also have overlapping genes, even if not significant. This includes the WNT signalling pathway (which is mainly involved in developmental processes such as patterning, differentiation/proliferation shift, and migration) and the metabolism of angiotensinogen to angiotensins pathway, from which CTSD, CTSG and MME are vitamin A targets and play roles in the generation of different angiotensin peptides.\textsuperscript{47,48,49} The impaired conversion of angiotensin I to angiotensin II is the pharmacodynamic adverse mechanism underlying the fetopathy caused by ACE-inhibiting drugs.\textsuperscript{50} One main feature of this teratogen-induced fetopathy, namely renal tubular dysgenesis, belongs to the CAKUT spectrum. In addition, an overproduction of angiotensin II might have a role in CAKUT anomalies involving renal tubules, as observed in cases of the autosomal recessive polycystic kidney disease.\textsuperscript{51}

Finally, vitamin A target genes extracted from both CTD and Balmer and Blomhoff are significantly enriched in the development of ureteric collection system, genes controlling nephrogenesis, and GDNF/RET signalling axis pathways, as defined in WikiPathways. The target genes extracted from CTD are also significantly enriched in nephrogenesis. Signaling by GDNF through the RET receptor is required for normal growth of the ureteric bud.\textsuperscript{52} Localized expression of GDNF by the metanephric mesenchyme is important to elicit and correctly position the initial budding event from the Wolffian duct and to promote the continued branching of the ureteric bud. Cells that lack RET are unable to contribute to the tip of the ureteric bud.\textsuperscript{52}

Overall, the significant overlaps observed between vitamin A and the CAKUT gene sets indicate that vitamin A might be relevant to a broad range of urogenital abnormalities.

Vitamin D target genes overlap with renal system development

In the analyses of vitamin D target gene sets, we observed very few significant overlaps with CAKUT-related gene sets overall. Vitamin D target genes obtained from CTD are only significantly enriched in renal system development, kidney development, and kidney morphogenesis GO terms. It should be noted that in each case 80% of the enriched genes are also vitamin A target genes (according to CTD or Balmer and Blomhoff).

<table>
<thead>
<tr>
<th>Source</th>
<th>Term name</th>
<th>p-value (BH adjusted) and overlap with genes from Comparative Toxigenomics Database</th>
<th>p-value (BH adjusted) and overlap with genes from Balmer and Blomhoff</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pub</strong></td>
<td>CAKUT causal genes</td>
<td>(2.02e-04) BMP4, EYA1, GATA3, GREB1L, HNF1B, NRIP1, PBX1, RET, SLIT2, WNT4</td>
<td>(2.18e-03) AGTR1, BMP4, HNF1B, ITGA8, NRIP1, RET</td>
</tr>
<tr>
<td><strong>GO</strong></td>
<td>Renal system development</td>
<td>(3.36e-26) ACTA2, ALDH1A2, ASS1, BAX, BCL2, BMP2, BMP4, BMP7, CASP9, CAT, CDKN1C, CFLAR, COL4A1, CTNNB1, CYP26A1, CYP26B1, Dcn, Efnb2, Egfr, Eya1, Fgf10, Fgf2, Fgf8, Foxc1, Gata3, Greb1L, Has2, Hes1, Hnf1B, Hoxb7, Hpgd, Id2, Id3, Irx3, Itga3, Jag1, Lama5, Lhx1, Mmp9, Myc, Nfia, Notch1, Notch2, Odc1, Pbx1, Pdgfa, Pdgfra, Pdgfrb, Plce1, Rara, Rab8, Rab9, Rbp4, Rdhl0, Ret, Rida, Sdc1, Serpinf1, Shh, Slit2, Smad2, Smad3, Sox9, Stat1, Stra6, Sulf2, Tactst2, Tafap2a, Tgfb1, Tgfb2, Tgfb3, Tiparp, Vegfa, Wnt4, Wnt5a, Wnt1, Zbtb16</td>
<td>(5.98e-17) ACTA2, Albg1, Bcl2, BMP2, BMP4, BMP7, Ca2, Calbl1, Col4a1, Cttnnb1, Egr1, Fgf1, Fgf2, Fgf82, Hnf1b, Hoxd11, Il6r, Itga8, Lhx1, Mme, Mmp9, Myc, Notch1, Odc1, Pdgfa, Pdgfra, Pdgfrb, Pecam1, Rara, Rab8, Rbp4, Ret, Shh, Sox9, Stat1, Stra6, Tafap2a, Tgfb1, Tgfb2, Tgfb3, Tiparp, Vegfa, Wnt1, Wnt1</td>
</tr>
<tr>
<td><strong>GO</strong></td>
<td>Kidney development</td>
<td>(3.91e-25) ACTA2, ALDH1A2, ASS1, BAX, BCL2, BMP2, BMP4, BMP7, CASP9, CAT, CDKN1C, CFLAR, CTNNB1, CYP26A1, CYP26B1, Dcn, Efnb2, Egfr, Eya1, Fgf10, Fgf2, Fgf8, Foxc1, Gata3, Greb1L, Has2, Hes1, Hnf1B, Hoxb7, Hpgd, Id2, Id3, Irx3, Itga3, Jag1, Lama5, Lhx1, Mmp9, Myc, Nfia, Notch1, Notch2, Odc1, Pbx1, Pdgfa, Pdgfra, Pdgfrb, Plce1, Rara, Rab8, Rab9, Rbp4, Rdhl0, Ret, Rida, Sdc1, Serpinf1, Shh, Slit2, Smad2, Smad3, Sox9, Stat1, Stra6, Sulf2, Tactst2, Tafap2a, Tgfb1, Tgfb2, Tgfb3, Tiparp, Vegfa, Wnt4, Wnt5a, Wnt1, Zbtb16</td>
<td>(3.62e-16) ACTA2, Albg1, Bcl2, BMP2, BMP4, BMP7, Ca2, Calbl1, Col4a1, Cttnnb1, Egr1, Fgf1, Fgf2, Fgf82, Hnf1b, Hoxd11, Il6r, Itga8, Lhx1, Mme, Mmp9, Myc, Notch1, Odc1, Pdgfa, Pdgfra, Pdgfrb, Pecam1, Rara, Rab8, Rbp4, Ret, Shh, Sox9, Stat1, Stra6, Tafap2a, Tgfb1, Tgfb2, Tgfb3, Tiparp, Vegfa, Wnt1, Wnt1</td>
</tr>
<tr>
<td><strong>GO</strong></td>
<td>Kidney morphogenesis</td>
<td>(2.01e-12) Bcl2, BMP2, BMP4, BMP7, Cttnnb1, Eya1, Fgf10, Fgf2, Fgf8, Gata3, Greb1L, Hes1, Hnf1B, Hoxb7, Irx3, Lama5, Lhx1, Myc, Pbxi, Pdgfrb, Shh, Sox9, Stat1, Tactst2, Tgfb1, Tgfb2, Tgfb3, Tiparp, Vegfa, Wnt4, Wnt5a, Wnt1, Zbtb16</td>
<td>(6.95e-12) Bcl2, BMP2, BMP4, BMP7, Ca2, Calbl1, Cttnnb1, Egr1, Fgf1, Fgf2, Fgf82, Hnf1b, Hoxd11, Il6r, Itga8, Lhx1, Mme, Mmp9, Myc, Notch1, Odc1, Pdgfa, Pdgfra, Pdgfrb, Pecam1, Rara, Rab8, Ret, Shh, Sox9, Stat1, Stra6, Tafap2a, Tgfb1, Tgfb2, Tgfb3, Tiparp, Vegfa, Wnt1, Wnt1</td>
</tr>
<tr>
<td>Reac</td>
<td>Metabolism of Angiotensinogen to Angiotensins</td>
<td>(4.33e-01) Ctsd, Ctsg</td>
<td>(5.95e-02) Ctsd, Ctsg, Mme</td>
</tr>
<tr>
<td>Reac</td>
<td>RET signaling</td>
<td>(1.84e-01) Grb10, Irs2, Pik3r1, Prka, Ret</td>
<td>(3.02e-01) Gfra1, Prka, Ret</td>
</tr>
<tr>
<td>Reac</td>
<td>Signaling by WNT</td>
<td>(1.49e-01) Akt1, Cltb, Ctb2, Cttnbn1, Dact1, Dkk1, Fzd2, Gnb4, Gng4, Gsk3b, H2ac6, H2bc12, H4-16, Itpr3, Myc, Ppp3ca, Prickle1, Prka, Prkcb, Psmb8, Psmb9, Psm2e, Rac1, Sox3, Sox7, Sox9, tert, Wnt3, Wnt4, Wnt5a, Xiap</td>
<td>(5.48e-01) Camk2a, Clta, Cttnbn1, Lef1, Myc, Ppp3ca, Ppp3cb, Prka, Runx3, Sox9, Tert, Wnt1, Wnt3a, Wnt8a</td>
</tr>
<tr>
<td>Reac</td>
<td>Signaling by NOTCH</td>
<td>(1.12e-03) Acta2, Adam10, Akt1, Ccnd1, Creb1, Dlk1, Egfr, Elf3, Fabp7, H2ac6, H2bc12, H4-16, Hes1, Hey1, Jag1, Jun, Lefng, Mdk, Myc, Notch1, Notch2, Pbx1, Psmb8, Psmb9, Psm2e, Runx1, Smad3, Stg1al4, Stg1al6, Stat1, Tfdp2, Tp53</td>
<td>(5.48e-01) Acta2, Cttnbn1, Egfr, Fcer2, Jun, Mdk, Myc, Notch1, Stat1, Tp53</td>
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### Table 1. Continued

<table>
<thead>
<tr>
<th>Source</th>
<th>Term name</th>
<th>p-value (BH adjusted) and overlap with genes from Comparative Toxicogenomics Database</th>
<th>p-value (BH adjusted) and overlap with genes from Balmer and Blomhoff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reac</td>
<td>Signaling by Hedgehog</td>
<td>(8.84e-01) GSK3B, PSMB8, PSMB9, PSME2, SHH, TUBA4A, TUBB2B, TUBB3</td>
<td>(9.87e-01) IHH, SHH</td>
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<tr>
<td>WP</td>
<td>Development of ureteric collection system</td>
<td><strong>(1.84e-05)</strong> BMP4, CCND1, CTNNB1, FOXC1, GATA3, GREB1B, LHX1, MYCN, RARA, RARB, RARG, RET, SHH, SLIT2, TUBA4, TUBB2, WT1</td>
<td><strong>(6.69e-08)</strong> BMP4, CTNNB1, FGFR2, GFRA1, HOXD11, ITGA8, LHX1, MYCN, RARA, RARB, RARG, RET, SHH, TUBA4, TUBB2, WT1</td>
</tr>
<tr>
<td>WP</td>
<td>Genes controlling nephrogenesis</td>
<td><strong>(2.05e-05)</strong> CTNNB1, EYA1, FGFR2, FOXC1, ITGA3, KDR, LHX1, NOTCH2, PDGFRB, RET, SHH, SLIT2, VEGFA, WNT4, WT1</td>
<td><strong>(2.71e-04)</strong> CTNNB1, FGFR2, HOXD11, ITGA8, LHX1, PDGFRB, RET, SHH, VEGFA, WT1</td>
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<td>WP</td>
<td>Nephrogenesis</td>
<td><strong>(2.02e-04)</strong> ALDH1A2, BMP7, FGFR2, GREM1, ITGA3, PDGFRB, RET, SHH, SMAD7, TACSTD2, VEGFA, WNT4</td>
<td><strong>(3.23e-01)</strong> BMP7, MEIS1</td>
</tr>
<tr>
<td>WP</td>
<td>GDNF/RET signalling axis</td>
<td><strong>(1.62e-03)</strong> BMP4, CTNNB1, EYA1, FOXC1, GATA3, LHX1, RET, SLIT2</td>
<td><strong>(1.50e-02)</strong> BMP4, CTNNB1, GFRA1, LHX1, RET</td>
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</table>

### Table 2. Overlap analysis results for vitamin D.

<table>
<thead>
<tr>
<th>Source</th>
<th>Term name</th>
<th>p-value (BH adjusted) and overlap with genes from Comparative Toxicogenomics Database</th>
<th>p-value (BH adjusted) and overlap with genes from Ramagopalan et al.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pub</td>
<td>CAKUT causal genes</td>
<td>(1)</td>
<td>(1)</td>
</tr>
<tr>
<td>GO</td>
<td>Renal system development</td>
<td><strong>(1.35e-05)</strong> ANGPT2, BAX, BCL2, CA2, CALB1, CD24, CYP26B1, DCN, EFN2, GREM1, ID2, ITGA3, MYC, OSR2, SMAD7, TACSTD2, TACSTD2</td>
<td>(1) CFLAR, SULF2</td>
</tr>
<tr>
<td>GO</td>
<td>Kidney development</td>
<td><strong>(1.35e-05)</strong> ANGPT2, BAX, BCL2, CA2, CALB1, CD24, CYP26B1, DCN, EFN2, GREM1, ID2, ITGA3, MYC, OSR2, SMAD7, TACSTD2, TACSTD2</td>
<td>(1) CFLAR, SULF2</td>
</tr>
<tr>
<td>GO</td>
<td>Kidney morphogenesis</td>
<td><strong>(4.71e-02)</strong> BCL2, CALB1, GREM1, MYC, TACSTD2</td>
<td>(1)</td>
</tr>
<tr>
<td>Reac</td>
<td>Metabolism of Angiotensinogen to Angiotensins</td>
<td>(1)</td>
<td>(9.46e-01) CTSZ</td>
</tr>
<tr>
<td>Reac</td>
<td>RET signaling</td>
<td>(1)</td>
<td>(9.46e-01) FRS2, PIK3CA</td>
</tr>
<tr>
<td>Reac</td>
<td>Signaling by WNT</td>
<td>(1) ITPR1, MYC, XPO1</td>
<td>(1) DAAM1, PIP5K1B, RAC2</td>
</tr>
<tr>
<td>Reac</td>
<td>Signaling by NOTCH</td>
<td><strong>(8.24e-01)</strong> CCND1, ELF3, HIF1A, LFG, MYC, MYC, NCO2</td>
<td>(1) HIF1A, KAT2B, MAML1</td>
</tr>
<tr>
<td>Reac</td>
<td>Signaling by Hedgehog</td>
<td>(1)</td>
<td>(1)</td>
</tr>
<tr>
<td>WP</td>
<td>Development of ureteric collection system</td>
<td><strong>(8.24e-01)</strong> CCND1, TACSTD2</td>
<td>(1)</td>
</tr>
<tr>
<td>WP</td>
<td>Genes controlling nephrogenesis</td>
<td>(1) ITGA3</td>
<td>(9.46e-01) CD2AP, CXCR4</td>
</tr>
<tr>
<td>WP</td>
<td>Nephrogenesis</td>
<td>(1)</td>
<td>(1)</td>
</tr>
<tr>
<td>WP</td>
<td>GDNF/RET signalling axis</td>
<td>(9.37e-01) GREM1</td>
<td>(1)</td>
</tr>
</tbody>
</table>
CTSZ, which cleaves angiotensin I to yield angiotensin II, is the only vitamin D target in the metabolism of angiotensinogen to angiotensins pathway. Thus, within CAKUT, vitamin D might also have a role in renal tubular defects related to anomalies of the renin-angiotensin system.

WNT and NOTCH signaling pathways have genes that are targets of vitamin D but there is a small, non-significant overlap, and the overlapping genes are not the WNT and NOTCH receptors nor ligands.

**Conclusions**

In this study we examined the overlaps of vitamin A and vitamin D target gene sets extracted from different databases and publications with CAKUT-related genes and biological processes. While we only observed significant enrichment of vitamin D target genes in GO kidney development terms, there is significant enrichment of vitamin A target genes in most of the gene sets we tested. Indeed, we observed that vitamin A target genes are enriched in CAKUT causal genes set, kidney development pathways, the signaling by NOTCH pathway, and the GDNF/RET signalling axis pathway. There is a strong overlap with the signaling by the WNT pathway, although it is not statistically significant. Overall, the significant overlaps observed between vitamin A and CAKUT gene sets indicate that vitamin A might be relevant to a broad range of urogenital abnormalities. Several environmental chemicals may impinge on retinoid-regulated pathways, and the influence of environment and nutrition cannot be ruled out. Our study is dedicated to the generation of hypotheses. Further study is needed, using up-to-date datasets obtained with new omics techniques, to investigate the fine-grained effects of vitamin excess and deficiency on CAKUT, and to decipher the pathophysiological mechanisms.

**Data availability**

**Underlying data**


This project contains the following underlying data in the ‘Data’ folder:

- VitA-Balmer2002-Genes.txt (list of vitamin A target genes from Balmer and Blomhoff used for overlap analyses).
- VitA-CTD-Genes.txt (list of vitamin A target genes from CTD used for overlap analyses).
- VitD-Ramagopalan2010.txt (list of vitamin D target genes from Ramagopalan et al. used for overlap analyses).
- VitD-CTD-Genes.txt (list of vitamin D target genes from CTD used for overlap analyses).
- PathwaysOfInterest.gmt (CAKUT-related gene sets used for overlap analyses).
- PathwaysOfInterestBackground.txt (list indicating which background GMT file to be used for the analysis of each CAKUT-related gene set).
- hsapiens.GO:BP.name.gmt (all gene sets from GO:BP used as background information for overlap analyses).
- hsapiens.REAC.name.gmt (all gene sets from Reactome used as background information for overlap analyses).
- hsapiens.WP.name.gmt (all gene sets from WikiPathways used as background information for overlap analyses).

This project contains the following underlying data in the ‘Result’ folder:

- VitA-CTD-Genes.csv (overlap analysis results for vitamin A targets from CTD).
- VitA-Balmer2002-Genes.csv (overlap analysis results for vitamin A targets from Balmer and Blomhoff).

- VitD-CTD-Genes.csv (overlap analysis results for vitamin D targets from CTD).

- VitD-Ramagopalan2010.csv (overlap analysis results for vitamin D targets from Ramagopalan et al.).


- VitD-MergedTexVersion.txt (merged table in LaTeX format for the results presented in VitD-CTD-Genes.csv and VitD-Ramagopalan2010.csv).

Extended data


This project contains the following extended data:

- overlapAnalysis.py (Python script used for all the analyses).

This project contains the following extended data in the ‘Supp’ folder:

- CAKUT-CausalGenesPMCM5748921-6115658Comb.txt (list of genes known to be mutated in the monogenic form of CAKUT).

- targetsFromDifferentSourcesOverlap.odt (contains the information on vitamin A and D targets from different sources (CTD, publications), presents source specific genes and common genes).

- VitA-Balmer2002-GeneMapping.csv (detailed table for the gene list from Balmer and Blomhoff).

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

Author contributions

Ozisik O: Conceptualization, Data Curation, Formal Analysis, Project Administration, Writing – Original Draft Preparation; Ehrhart F: Conceptualization, Writing – Review & Editing; Evelo C: Conceptualization, Supervision, Writing – Review & Editing; Mantovani A: Conceptualization, Supervision, Writing – Original Draft Preparation; Baudot A: Conceptualization, Data Curation, Project Administration, Supervision, Writing – Original Draft Preparation.

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References

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The manuscript "Overlap if vitamin A and vitamin D target genes with CAKUT-related processes" is aimed to indirectly evaluate the possible relationship between Vitamin A and D intake imbalance and congenital anomalies of the kidney and urinary tract (CAKUT). The work considers genes reported as dysregulated in CAKUT and key genes involved in different urinary morphogenetic pathways. Both were overlapped with target genes of Vitamin A and Vitamin D (extracted from CTD and from literature: Balmer and Blomhoff, 2002 for Vitamin A, Ramagopalan et al., 2010 for Vitamin D). As expected considering the biological activity of the Vitamin A morphogenetic derivative (retinoid acid), significant overlaps are reported in the manuscript between Vitamin A target genes and genes involved in several urinary morphogenetic processes and signalling. Interestingly, significant overlaps were observed between Vitamin A target genes and CAKUT-related genes. As far as Vitamin D is concerned, overlappings were limited to some kidney morphogenetic genes but no overlapping was evidenced with CAKUT-related genes.

In general, this work can be considered of interest in order to postulate a possible relationship between Vitamin imbalance (mainly Vitamin A) and CAKUT and to suggest that a nutritional evaluation in pregnancy is essential to ensure a physiological development and maternal/fetal health.

Some suggestions in order to improve the impact of the manuscript:

Abstract:
The abstract should be rewritten in order to better clarify aim and results of the work. In particular, Vitamin D results are lacking.

Introduction:
The aim should be better explained. A brief description for the reason of the choice of literature (Balmer and Blomhoff, 2002 for Vitamin A, Ramagopalan et al., 2010 for Vitamin D) could enhance the comprehension.
Methods:
Indicate retinoic acid as "the active metabolite of vitamin A" instead of "vitamin A acid". Better explain the statistical analysis.

Results:
Tables are not fully self explicative. I suggest:
- a separate column for p-value;
- write in bold the genes both overlapping with CAKUT and urinary pathways/signalling.

Moreover, (1) in table 2 is not explained.

Looking in detail at Vitamin A table, four CAKUT-causal genes (BMP4, HNF1B, NRIP1 and RET, overlapping both with CTD and Balmer and Blomhoff) appear as a leitmotiv in different vitamin-A related morphogenetic pathways. In my personal opinion, this result should be stressed and a brief description of functions of BMP4, HNF1B, NRIP1 and RET in kidney and urinary tract development introduced in Results and/or Conclusions.

Conclusions:
Can be rewritten in order to better focus the relevance of the nutritional management during pregnancy in order to ensure both maternal and fetal health. A general consideration should be introduced, considering also the pleiotropic functions of the involved genes.

References:
Please check references, i.e. reference 54 seems not pertinent.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Developmental toxicology

I confirm that I have read this submission and believe that I have an appropriate level of
expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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