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The role of vitamin D receptor in dental and craniofacial anomalies

Introduction

Vitamin D is an essential endocrine regulator of mineral metabolism. It is crucial for skeletal and tooth formation. Vitamin D signaling can be ligand-dependent or independent, depending on the target gene. The principal targets of the vitamin D receptor (VDR) are regulators of mineral uptake homeostasis, ensuring bone growth in a structurally integrated manner. In rodents, vitamin-D deficient mineralization permanently alters dentin and enamel composition and additionally may modify tooth morphology. *VDR*$^{-/-}$ (VDR-null) mutants have been used to understand how the molecular targets of vitamin D signaling regulate skeletal and tooth mineralization (Berdal et al., 2011; Foster et al., 2014).

Low serum vitamin D levels are associated with a wide range of pathologies, including immune deficiencies, cancer incidence, metabolic disease, and neurological pathologies (Hoel et al., 2016). This emphasizes the significance of vitamin D extra-skeletal activities in relation to the widespread distribution of nuclear vitamin D receptors.

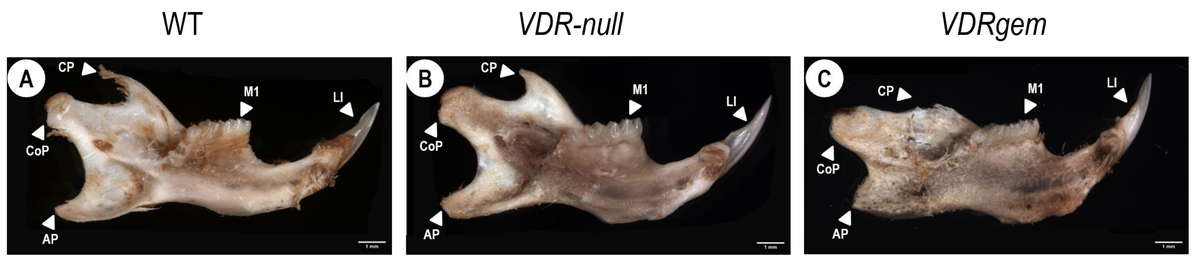

Figure 1. Bone phenotype of adult WT, *VDR-null* and *VDR$_{gem}$* mice.
Gross morphology of adult mandibles in (A) WT, (B) *VDR-null* and (C) *VDR$_{gem}$*. *VDR-null* and *VDR$_{gem}$* bone has a darker appearance, lacking cortical bone in *VDR$_{gem}$*. *VDR$_{gem}$* mandible is smaller with angular and condylar processes less defined. Coronoid process was fractured due to the bone fragility.

Methods and Results

To further investigate VDR signaling and its role during tooth and bone development, we analyzed two models of VDR-deficient signaling, *VDR-null* and *VDR$_{gem}$* mouse lines. *VDR$_{gem}$* mice express a mutated VDR (*VDR$_{gem}$* for gemini) that is unresponsive to endogenous vitamin D [1,25(OH)2D3]. This mutant can therefore be used to selectively distinguish between ligand-dependent VDR defects (such as bone mineralization) and phenotypes due to the absence of non-ligand-dependent VDR, such as alopecia resulting from to hair follicle abnormalities.

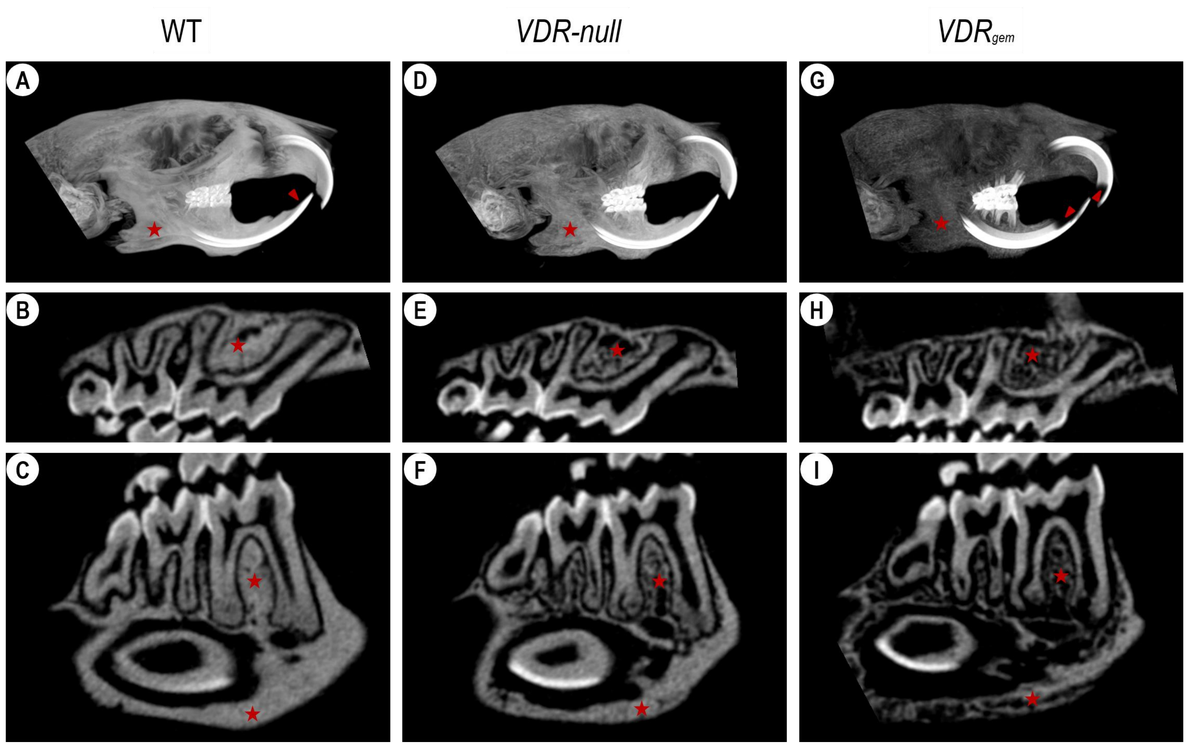

Figure 2. Micro-CT (µCT) imaging of adult WT, *VDR-null* and *VDR$_{gem}$* head and teeth.
(A, B, C) Comparison of WT mice, with normal bone density and dental tissues to (D, E, F) *VDR-null* mutants which have a less dense bone with more trabecular space and a thinner dentin; and to (G, H, I) *VDR$_{gem}$* mice showing a more impaired phenotype with no exposed dentin in lower and upper incisors (red arrowheads) and bone with larger trabecular spaces and no cortical (compare ★ marks).

Overall, dental and skeletal defects are more severe in *VDR$_{gem}$* mice than in *VDR-null* mice. These mice show reduced alveolar bone density, abnormal root and pulp morphology, and severe hypocalcification of alveolar bone and dentin (Figures 1, 2 and 3).

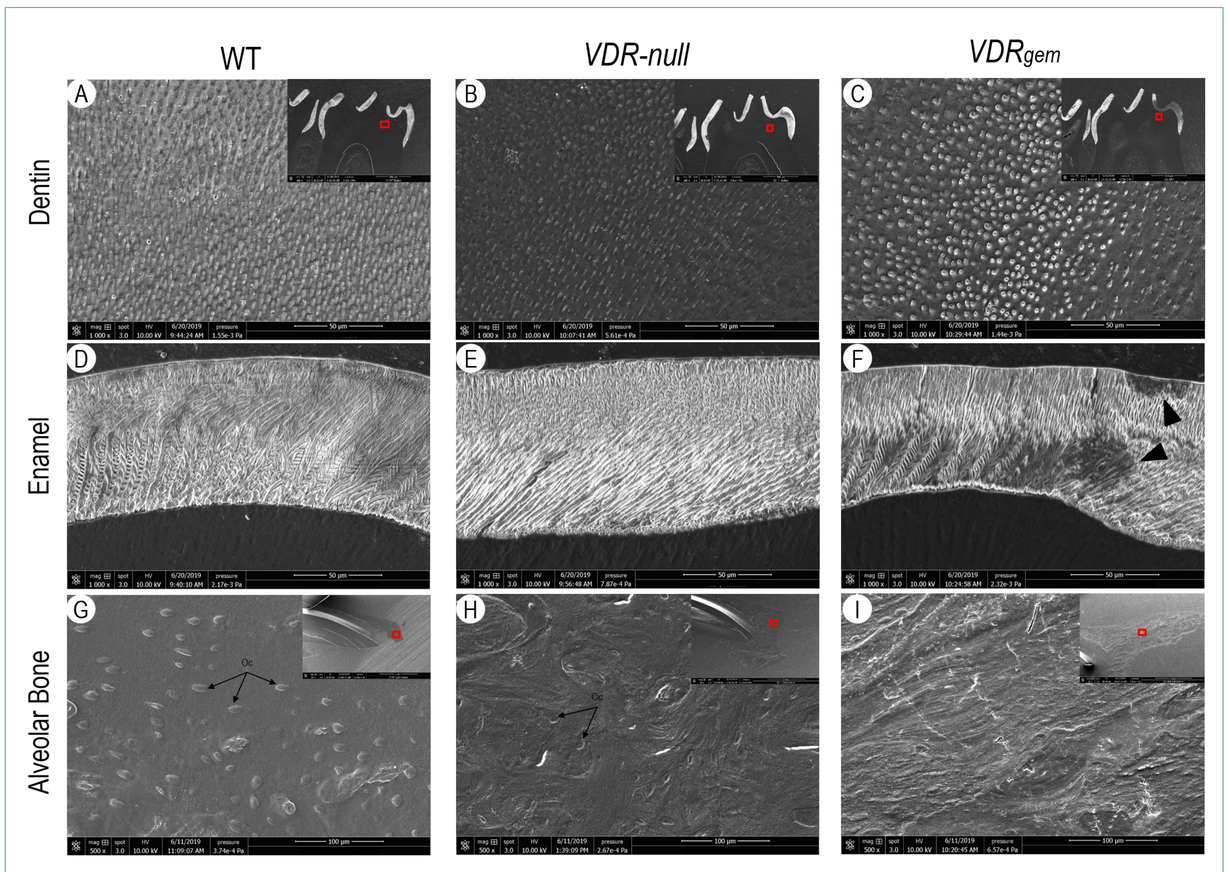

Figure 3. Scanning electron microscopy (SEM) imaging of WT, *VDR-null* and *VDR$_{gem}$* teeth and alveolar bone.
(A-C) SEM of dentin structure showed no significant structural changes whilst an abnormal mineralization pattern in *VDR$_{gem}$* was reported. (D-F) In enamel, *VDR-null* and *VDR$_{gem}$* ultrastructure is altered compared to WT sample with perturbation of enamel structure including prism form and interprismatic space. (F) In *VDR$_{gem}$*, enamel thickness is not constant with a thinner enamel layer in some areas and hypomineralized spots (black arrowheads). (G-I) Alveolar bone analysis in WT, *VDR-null* and *VDR$_{gem}$* shows a highly disorganized bone in both mutants with more impairment in *VDR$_{gem}$* mice. Osteocytes (Oc) are diminished in *VDR-null* and *VDR$_{gem}$* (H, I) compared to WT (G).

Global transcriptomic analysis (RNA sequencing) performed in postnatal day 5 (PN5) lower incisors of *VDR-null* vs. *VDR$_{gem}$* showed different affected pathways in the two models (Figure 4).

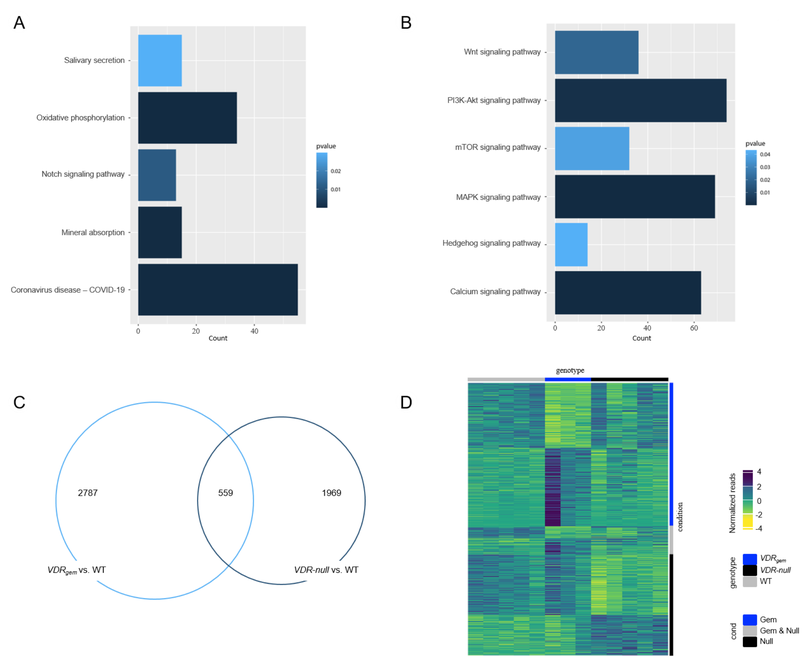

Figure 4. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and genes dysregulated in *VDR-null* and *VDR$_{gem}$*.
KEGG pathway enrichment analysis for up- and down-regulated genes of (A) *VDR-null* and (B) *VDR$_{gem}$* samples. (C) Venn diagram shows deregulated genes comparing WT with *VDR-null* and *VDR$_{gem}$* samples, 341 genes are dysregulated in both *VDR-null* and *VDR$_{gem}$*. (D) Heatmap shows the difference in pattern expression between groups.

Conclusion

Vitamin D, acting through its receptor, enables bone and teeth to accumulate sufficient calcium and phosphorus to ensure their structural integrity. *VDR$_{gem}$* mutants show more severe defects in bone mineral homeostasis compared to *VDR-null* mice, indicating that altered molecular targets may produce greater morphogenic consequences. Mineralization and bone homeostasis targets showed greater impairment in *VDR$_{gem}$* vs. *VDR-null* mutant incisors, indicating that *VDR$_{gem}$* cannot respond to endogenous vitamin D, resulting in a more severe phenotype marking complete vitamin D deficiency.

All experimental protocols were approved by the IGBMC's Animal Care and Use Committee and the French ministry of Higher Education and Research (APAFIS #15589-201806191874970v4)

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