## Phylogeny

PKDCC/VLK is a member of a novel family of secreted protein kinases (bordoli2014asecretedtyrosine pages 9-10). It is classified within the protein kinase-like (pkinase and pkinase\_Tyr) clan and is phylogenetically related to the FAM69 and SGK196 kinase families (dudkiewicz2013anovelpredicted pages 6-8, sreelatha2015thesecretorypathway pages 2-4). PKDCC/VLK and FAM20C represent distinct secreted kinase families that are more distant from each other than from canonical cytoplasmic kinases, suggesting independent evolutionary origins (unknownauthors2022thesecretedkinase pages 41-45). It is identified as a divergent kinase, SgK493, within the human kinome (hanks2003genomicanalysisof pages 2-3). Sources conflict regarding its classification, with some placing it within the tyrosine kinase family as an atypical kinase (vitorino2015xenopuspkdcc1and pages 1-2) and others noting it is not classifiable into the major kinase groups (bordoli2014asecretedtyrosine pages 1-2). Another source states that secretory pathway kinases like PKDCC were not originally included in the human kinome described by Manning et al. and form a separate evolutionary lineage (sreelatha2015thesecretorypathway pages 1-2).

Orthologs are functionally conserved in vertebrates, including mouse, zebrafish, and *Xenopus laevis*, which has two paralogs, Pkdcc1 and Pkdcc2 (unknownauthors2022thesecretedkinase pages 41-45, vitorino2015xenopuspkdcc1and pages 1-2). There are conflicting reports on its broader evolutionary distribution. Several sources state PKDCC/VLK is a vertebrate-specific innovation with no orthologs in invertebrates (unknownauthors2022thesecretedkinase pages 41-45, unknownauthors2022thesecretedkinase pages 37-41, unknownauthors2022thesecretedkinase pages 37-41). Conversely, other studies report that PKDCC is present across Metazoa, including the sea anemone *Nematostella* and the sea urchin *Strongylocentrotus*, and has remote homologues in plants (dudkiewicz2013anovelpredicted pages 8-8, dudkiewicz2013anovelpredicted pages 6-8, dudkiewicz2013anovelpredicted pages 9-10).

## Reaction Catalyzed

The enzyme catalyzes the ATP-dependent transfer of a phosphate group to a tyrosine residue on a protein substrate (bordoli2014asecretedtyrosine pages 9-10, revollo2020thesecretedtyrosine pages 1-4). Autophosphorylation also occurs on serine residues (unknownauthors2022thesecretedkinase pages 41-45).

ATP + [a protein]-L-tyrosine = ADP + [a protein]-L-tyrosine phosphate.

## Cofactor Requirements

The kinase activity of PKDCC/VLK is dependent on ATP as a phosphate donor, which can be utilized from intracellular stores within the secretory pathway or from extracellular sources, such as that released from platelet dense granules (bordoli2014asecretedtyrosine pages 9-10, revollo2020thesecretedtyrosine pages 10-14). Its kinase activity is enhanced by Mg²⁺ and Ca²⁺ ions (sreelatha2015thesecretorypathway pages 2-4).

## Substrate Specificity

PKDCC/VLK has a strong preference for phosphorylating tyrosine residues on a broad spectrum of secreted and ER/Golgi-resident proteins (bordoli2014asecretedtyrosine pages 1-2, bordoli2014asecretedtyrosine pages 9-10). It does not have a well-defined or clear consensus sequence motif (sreelatha2015thesecretorypathway pages 2-4, revollo2020thesecretedtyrosine pages 1-4). Instead, it often targets tyrosine residues within conserved structural domains, including immunoglobulin (IG), fibronectin type III (FN3), von Willebrand factor A (VWA), and hemopexin domains (bordoli2014asecretedtyrosine pages 9-10, bordoli2014asecretedtyrosine pages 2-3). One specific substrate site identified is Y504 in the fibronectin-type III domain of the EphB2 ectodomain (srikanth2024vlkdrivesextracellular pages 1-4).

## Structure

PKDCC/VLK possesses an N-terminal hydrophobic signal sequence that directs it into the secretory pathway, a proline-glycine rich domain, and a C-terminal catalytic kinase domain (sreelatha2015thesecretorypathway pages 2-4, bordoli2014asecretedtyrosine pages 2-3). It may also contain a cysteine-rich domain and calcium-binding EF-hand motifs (dudkiewicz2013anovelpredicted pages 9-10). The kinase domain is divergent, lacking recognizable homology in subdomains V, IX, X, and XI compared to canonical protein kinases (bordoli2014asecretedtyrosine pages 1-2, unknownauthors2022thesecretedkinase pages 41-45). The canonical ‘DFG’ and ‘HRD’ motifs within the catalytic domain are replaced by alternate, yet functional, residues (sreelatha2015thesecretorypathway pages 2-4).

## Regulation

PKDCC/VLK activity is regulated by post-translational modifications. Autophosphorylation at tyrosine 148 and serine 177 is required for its catalytic activity (unknownauthors2022thesecretedkinase pages 41-45). The protein is also phosphorylated at tyrosine 64 (revollo2020thesecretedtyrosine pages 4-7). It undergoes N-linked glycosylation at five potential sites, a modification that is important for its maturation, stability, and secretion from the cell (bordoli2014asecretedtyrosine pages 2-3, unknownauthors2022thesecretedkinase pages 41-45). The kinase’s own catalytic activity is required for its efficient secretion, as kinase-inactive mutants exhibit defective secretion (bordoli2014asecretedtyrosine pages 9-10, revollo2020thesecretedtyrosine pages 1-4).

## Function

PKDCC/VLK functions as a secreted tyrosine kinase, acting both intracellularly within the ER and Golgi and extracellularly after secretion (bordoli2014asecretedtyrosine pages 9-10). It is highly expressed during embryogenesis in mesenchymal cells involved in bone, lung, and cartilage development (bordoli2014asecretedtyrosine pages 9-10, unknownauthors2022thesecretedkinase pages 41-45). In adults, expression is lower but is high in megakaryocytes and platelets, where it is stored in alpha-granules and secreted upon stimulation (bordoli2014asecretedtyrosine pages 2-3, revollo2020thesecretedtyrosine pages 14-17). It is also secreted from neurons (srikanth2024vlkdrivesextracellular pages 1-4).

PKDCC/VLK phosphorylates a range of substrates, including extracellular matrix (ECM) proteins such as MMP1, MMP13, collagen type I, and osteopontin, as well as ER-resident chaperones like MESD and ERP29 (bordoli2014asecretedtyrosine pages 2-3, unknownauthors2022thesecretedkinase pages 41-45). It is involved in multiple signaling pathways. In Hedgehog (HH) signaling, it genetically interacts with Gli3 to regulate bone development (unknownauthors2022thesecretedkinase pages 41-45). In Wnt signaling, secreted VLK phosphorylates RGMb to regulate LRP5 internalization and also negatively regulates the Wnt/β-catenin pathway (unknownauthors2022thesecretedkinase pages 41-45). It is also involved in the JNK-dependent Wnt/PCP pathway (vitorino2015xenopuspkdcc1and pages 1-2). Additionally, it is essential for normal platelet activation and thrombus formation, and it modulates synaptic plasticity and pain responses by phosphorylating the EphB2 ectodomain to promote its interaction with the NMDAR (revollo2020thesecretedtyrosine pages 1-4, srikanth2024vlkdrivesextracellular pages 1-4).

## Other Comments

Loss of PKDCC/VLK function is associated with severe developmental defects. Global deletion in mice results in perinatal lethality with skeletal abnormalities (limb shortening, reduced bone mineralization), lung hypoplasia, and craniofacial defects including cleft palate (bordoli2014asecretedtyrosine pages 9-10, unknownauthors2022thesecretedkinase pages 41-45). In humans, genome-wide association studies link variants in the *VLK* gene to variations in bone density (bordoli2014asecretedtyrosine pages 9-10). The kinase is also implicated in lung fibrosis and tissue remodeling abnormalities (unknownauthors2022thesecretedkinase pages 41-45). Related kinase families, such as Fam69, are genetically linked to neurological disorders (sreelatha2015thesecretorypathway pages 2-4).

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