

Wnt/ β -Catenin Inhibitor Dickkopf 1

A Novel Predictor for Cardiovascular and Cerebrovascular Events

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In this issue of *ATVB*, Ueland et al and Zue et al are reporting the prognostic value for future cardiovascular events of elevated serum levels of DKK1 (Dickkopf 1) at admission in patients with acute myocardial infarction or ischemic stroke, respectively.^{1,2} Raised DKK1 levels were previously reported in smaller patient cohorts with type 2 diabetes mellitus and cardiovascular disease or in acute ischemic stroke patients.^{3,4} Nevertheless, these studies did not excite major attention because of their relatively small cohorts and because they did not consider the prognostic value of elevated DKK1 for future cardiovascular events. The newly published studies in this journal have significantly more patients, which were followed up after their first cardiovascular event for recurrent cardiovascular events, making these cohorts valuable for assessing whether DKK1 is a potential biomarker for unstable atherosclerotic arterial disease.

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The secreted glycoprotein DKK1, a member of the Dickkopf family, is known to antagonize Wnt/ β -signaling by interaction with the LRP5/6 (low-density lipoprotein receptor-related protein 5/6). The Wnt/ β -catenin signaling pathway is an important mediator in cardiovascular disease and influences inflammation, vascular calcification, and proliferation of different cell types in atherosclerotic disease.⁵ DKK1 plays an important role in vertebrate embryogenesis and knocking out of the DKK1 gene leads to impaired limb and head development in mice.⁶ The first step to induce Wnt/ β -signaling is the interaction of Wnt proteins with a receptor complex on the plasma membrane, consisting of a member of the Frizzled protein family and its co-receptor LRP5/6. The intracellular signal transducing protein β -catenin, which normally is degraded in the cell (Figure [A]), consequently accumulates, leading to the transcription of multiple target genes (Figure [B]). The protein structure of LRP5/6 consists of 4 β -propeller domains connected to a transmembrane region.^{7,8} DKK1 binds with high affinity to the first and third β -propeller structure, which also harbors the binding site for Wnt proteins, and consequently abolishes the signaling via the Wnt/ β -catenin

cascade.^{5,9} Furthermore, Kremen receptors act together with DKK1 to induce endocytosis of LRP5/6¹⁰ (Figure [C]). Despite structural similarities between LRP5 and LRP6, both seem to have slightly different roles in the Wnt/ β -catenin signaling pathway. LRP6 is involved in embryonal development, adult bone formation, and was recently reported to also play a role in LDL clearance.^{5,11} Moreover, mutations of LRP6 are strongly associated with cardiovascular disease.⁵ For example, the LRP6_{R611C} mutation strongly affects cholesterol biosynthesis and LDL clearance resulting in high LDL and triglyceride serum levels, which are one of the major risk factors for cardiovascular disease.¹¹ Knockout of LRP6 in ApoE^{-/-} mice led to postnatal lethality.¹² Mutations of LRP5 are associated with an early onset of osteoporosis, an abnormal increase of bone mass, and calcified aortic valves.^{5,13,14} In addition, total knockout of LRP5 in mice leads to abnormal low bone mass.⁵

Many different molecular mechanisms and structural changes are responsible for the formation of unstable atherosclerotic plaques. This includes inflammation, the loss of vascular smooth muscle cells (VSMC), the size of the necrotic core, and microcalcifications in the fibrous cap.^{15,16} Atherosclerotic plaque heterogeneity in terms of cell contribution and morphology further raises the complexity of this field.¹⁷ Oxidized LDL induces the expression of DKK1 in macrophages and in endothelial cells.^{18,19} Furthermore, the presence of monocytes/macrophages and oxidized LDL led to a higher matrix metalloproteinase activity in calcifying vascular cells, causing further instability of the atherosclerotic plaque.²⁰ On the contrary, downregulation of DKK1 impaired monocyte adhesion to endothelial cells and, therefore, attenuated inflammation of the atherosclerotic plaque.²¹ Oscillating shear stress, often present in branches and curves of large arteries, induces DKK1 expression in vitro in a shear stress model with endothelial cells as well as in ApoE^{-/-} mice.²¹ Overexpression of DKK1 in ApoE^{-/-} mice led to enlarged plaque formation and promoted instability of the lesion, because of enhanced apoptosis of endothelial cells. Endothelial cells, macrophages, and platelets are sources of DKK1, whereas VSMC does not secrete DKK1.¹² Interestingly, in co-cultures with endothelial cells, VSMC tend to take up DKK1 and proliferation of VSMC was inhibited by recombinant DKK1.^{19,22} On the contrary, DKK1 seems to correlate negatively with the severity of vascular calcification in patients.²³⁻²⁵ There is plausible evidence that sheet-like calcifications have a stabilizing effect on the atherosclerotic plaque, whereas spotty calcification or microcalcification are often associated with unstable plaques.²⁶ In vitro studies showed that DKK1 was able to attenuate RUNX2 (runt-related transcription factor 2) expression in VSMC, which is an important transcription factor for osteogenic differentiation of VSMC. Additionally, the Frizzled protein/LRP6 agonist Wnt3a, promoted the calcification process.²⁷

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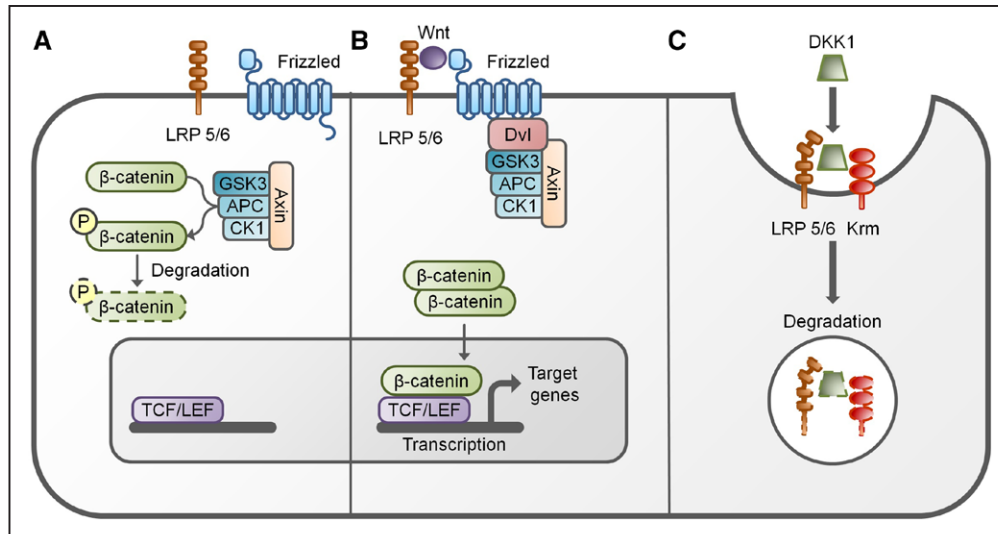


Figure. Schematic representation of the Wnt/ β -catenin signaling pathway and its inhibition by DKK1 (dickkopf-1). **A**, Inactive Wnt/ β -catenin signaling leads to phosphorylation of intracellular β -catenin by the β -catenin destruction complex containing GSK3 β (glycogen synthase kinase 3 β), axin, APC (adenomatous polyposis coli), and CK-1 (casein kinase-1), followed by degradation of the phosphorylated β -catenin via the ubiquitin proteasome system. **B**, Binding of the Wnt protein to a frizzled receptor leads to co-activation of LRP5/6, followed by phosphorylation of the intramembrane part of LRP5/6 and leading to recruitment of DVL (disheveled) protein. This results in inactivation of the β -catenin destruction complex and leads to the accumulation of β -catenin and its translocation to the nucleus where it forms a complex with the TCF/LEF (T-cell factor/lymphoid enhancer factor) and induces transcription of several target genes. **C**, Binding of DKK1 to LRP5/6 leads to a complex formation with the Kremen receptor (Kre), followed by endocytosis and degradation of the LRP5/6 receptor, resulting in the inhibition of Wnt/ β -catenin signaling.

In summary, there is convincing evidence that DKK1 is elevated in unstable compared with stable atherosclerotic plaques. It is tempting to speculate that DKK1 promotes unstable plaque formation by inhibiting the deposition of protective larger calcifications and through influencing the cellular plaque composition. Moreover, DKK1 seems to be a promising biomarker candidate to predict future cardiovascular and cerebrovascular events. Consequently, including the admission DKK1 serum levels in the assessment of patients hospitalized with myocardial infarction or stroke could help to identify those at highest risk of further cardiovascular events. Nevertheless, further research will be needed to clarify the role of DKK1—and Wnt signaling in general—in maintaining plaque stability.

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Disclosures

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References

- Zhu Z, Guo D, Zhong C, et al. Serum Dkk-1 (Dickkopf-1) is a potential biomarker in the prediction of clinical outcomes among patients with acute ischemic stroke. *Arterioscler Thromb Vasc Biol*. 2019;39:285–293. doi: 10.1161/ATVBAHA.118.311960
- Ueland T, Akerblom A, Ghukasyan T, Michelsen AE, Becker RC, Bertilsson M, Himmelmann A, James SK, Siegbahn A, Storey RF, Kontny F, Aukrust P, Wallentin L; for the PLATO Investigators. Admission levels of DKK1 (Dickkopf-1) are associated with future cardiovascular death in patients with acute coronary syndromes: insights from the PLATO trial. *Arterioscler Thromb Vasc Biol*. 2019;39:294–302. doi: 10.1161/ATVBAHA.118.311042
- García-Martín A, Reyes-García R, García-Fontana B, Morales-Santana S, Coto-Montes A, Muñoz-Garach M, Rozas-Moreno P, Muñoz-Torres M. Relationship of Dickkopf1 (DKK1) with cardiovascular disease and bone metabolism in Caucasian type 2 diabetes mellitus. *PLoS One*. 2014;9:e111703. doi: 10.1371/journal.pone.0111703
- Seifert-Held T, Pekar T, Gattlinger T, Simmet NE, Schmagl H, Stojakovic T, Fazekas F, Storch MK. Circulating Dickkopf-1 in acute ischemic stroke and clinically stable cerebrovascular disease. *Atherosclerosis*. 2011;218:233–237. doi: 10.1016/j.atherosclerosis.2011.05.015
- Foulquier S, Daskalopoulos EP, Lluri G, Hermans KCM, Deb A, Blankesteijn WM. WNT signaling in cardiac and vascular disease. *Pharmacol Rev*. 2018;70:68–141. doi: 10.1124/pr.117.013896
- Mukhopadhyay M, Shtrom S, Rodriguez-Esteban C, Chen L, Tsukui T, Gomer L, Dorward DW, Glinka A, Grinberg A, Huang SP, Niehrs C, Izpisua Belmonte JC, Westphal H. Dickkopf1 is required for embryonic head induction and limb morphogenesis in the mouse. *Dev Cell*. 2001;1:423–434. doi: 10.1016/S1534-5807(01)00041-7
- Chen S, Bubeck D, MacDonald BT, Liang WX, Mao JH, Malinauskas T, Llorca O, Aricescu AR, Siebold C, He X, Jones EY. Structural and functional studies of LRP6 ectodomain reveal a platform for Wnt signaling. *Dev Cell*. 2011;21:848–861. doi: 10.1016/j.devcel.2011.09.007
- Cheng Z, Biechele T, Wei Z, Morrone S, Moon RT, Wang L, Xu W. Crystal structures of the extracellular domain of LRP6 and its complex with DKK1. *Nat Struct Mol Biol*. 2011;18:1204–1210. doi: 10.1038/nsmb.2139
- Seménov MV, Tamai K, Brott BK, Kühl M, Sokol S, He X. Head inducer Dickkopf-1 is a ligand for Wnt coreceptor LRP6. *Curr Biol*. 2001;11:951–961. doi: 10.1016/S0960-9822(01)00290-1
- Mao B, Wu W, Davidson G, Marhold J, Li M, Mechler BM, Delius H, Hoppe D, Stannek P, Walter C, Glinka A, Niehrs C. Kremen proteins are Dickkopf receptors that regulate Wnt/ β -catenin signalling. *Nature*. 2002;417:664–667. doi: 10.1038/nature756
- Go GW. Low-density lipoprotein receptor-related protein 6 (LRP6) is a novel nutritional therapeutic target for hyperlipidemia, non-alcoholic fatty liver disease, and atherosclerosis. *Nutrients*. 2015;7:4453–4464. doi: 10.3390/nu7064453
- Ueland T, Otterdal K, Lekva T, Halvorsen B, Gabrielsen A, Sandberg WJ, Paulsson-Berne G, Pedersen TM, Folkersen L, Gullestad L, Oie E, Hansson GK, Aukrust P. Dickkopf-1 enhances inflammatory interaction between platelets and endothelial cells and shows increased expression in atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2009;29:1228–1234. doi: 10.1161/ATVBAHA.109.189761

13. Astiazarán MC, Cervantes-Sodi M, Rebolledo-Enríquez E, Chacón-Camacho O, Villegas V, Zenteno JC. Novel homozygous LRP5 mutations in mexican patients with osteoporosis-pseudoglioma syndrome. *Genet Test Mol Biomarkers*. 2017;21:742–746. doi: 10.1089/gtmb.2017.0118
14. Roetzer KM, Uyanik G, Brehm A, Zwerina J, Zandieh S, Czech T, Roschger P, Misof BM, Klaushofer K. Novel familial mutation of LRP5 causing high bone mass: Genetic analysis, clinical presentation, and characterization of bone matrix mineralization. *Bone*. 2018;107:154–160. doi: 10.1016/j.bone.2017.12.002
15. Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM. Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. *Arterioscler Thromb Vasc Biol*. 2000;20:1262–1275.
16. Hutcheson JD, Maldonado N, Aikawa E. Small entities with large impact: microcalcifications and atherosclerotic plaque vulnerability. *Curr Opin Lipidol*. 2014;25:327–332. doi: 10.1097/MOL.0000000000000105
17. Espitia O, Chatelais M, Steenman M, Charrier C, Maurel B, Georges S, Houlgatte R, Verrecchia F, Ory B, Lamoureux F, Heymann D, Gouëffic Y, Quillard T. Implication of molecular vascular smooth muscle cell heterogeneity among arterial beds in arterial calcification. *PLoS One*. 2018;13:e0191976. doi: 10.1371/journal.pone.0191976
18. Zhang Y, Ge C, Wang L, Liu X, Chen Y, Li M, Zhang M. Induction of DKK1 by ox-LDL negatively regulates intracellular lipid accumulation in macrophages. *FEBS Lett*. 2015;589:52–58. doi: 10.1016/j.febslet.2014.11.023
19. Di M, Wang L, Li M, Zhang Y, Liu X, Zeng R, Wang H, Chen Y, Chen W, Zhang Y, Zhang M. Dickkopf1 destabilizes atherosclerotic plaques and promotes plaque formation by inducing apoptosis of endothelial cells through activation of ER stress. *Cell Death Dis*. 2017;8:e2917. doi: 10.1038/cddis.2017.277
20. Li R, Mittelstein D, Lee J, Fang K, Majumdar R, Tintut Y, Demer LL, Hsiai TK. A dynamic model of calcific nodule destabilization in response to monocyte- and oxidized lipid-induced matrix metalloproteinases. *Am J Physiol Cell Physiol*. 2012;302:C658–C665. doi: 10.1152/ajpcell.00313.2011
21. Li M, Liu X, Zhang Y, Di M, Wang H, Wang L, Chen Y, Liu X, Cao X, Zeng R, Zhang Y, Zhang M. Upregulation of Dickkopf1 by oscillatory shear stress accelerates atherogenesis. *J Mol Med (Berl)*. 2016;94:431–441. doi: 10.1007/s00109-015-1369-9
22. Zhuang Y, Mao JQ, Yu M, Dong LY, Fan YL, Lv ZQ, Xiao MD, Yuan ZX. Hyperlipidemia induces vascular smooth muscle cell proliferation involving Wnt/ β -catenin signaling. *Cell Biol Int*. 2016;40:121–130. doi: 10.1002/cbin.10543
23. Register TC, Hruska KA, Divers J, Bowden DW, Palmer ND, Carr JJ, Wagenknecht LE, Hightower RC, Xu J, Smith SC, Dietzen DJ, Langefeld CD, Freedman BI. Plasma Dickkopf1 (DKK1) concentrations negatively associate with atherosclerotic calcified plaque in African-Americans with type 2 diabetes. *J Clin Endocrinol Metab*. 2013;98:E60–E65. doi: 10.1210/jc.2012-3038
24. Hampson G, Edwards S, Conroy S, Blake GM, Fogelman I, Frost ML. The relationship between inhibitors of the Wnt signalling pathway (Dickkopf-1(DKK1) and sclerostin), bone mineral density, vascular calcification and arterial stiffness in post-menopausal women. *Bone*. 2013;56:42–47. doi: 10.1016/j.bone.2013.05.010
25. Szulc P, Schoppet M, Rachner TD, Chapurlat R, Hofbauer LC. Severe abdominal aortic calcification in older men is negatively associated with DKK1 serum levels: the STRAMBO study. *J Clin Endocrinol Metab*. 2014;99:617–624. doi: 10.1210/jc.2013-3201
26. Mori H, Torii S, Kutyna M, Sakamoto A, Finn AV, Virmani R. Coronary artery calcification and its progression: what does it really mean? *JACC Cardiovasc Imaging*. 2018;11:127–142. doi: 10.1016/j.jcmg.2017.10.012
27. Cai T, Sun D, Duan Y, Wen P, Dai C, Yang J, He W. WNT/ β -catenin signaling promotes VSMCs to osteogenic transdifferentiation and calcification through directly modulating Runx2 gene expression. *Exp Cell Res*. 2016;345:206–217. doi: 10.1016/j.yexcr.2016.06.007

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