The CAD risk locus 9p21 increases the risk of vascular calcification in an iPSC-derived VSMC model

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Research

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Abstract

Background: Coronary artery disease (CAD) is the leading cause of death worldwide. Chromosome locus 9p21 was the first to be associated with increased risk of CAD and coronary artery calcification (CAC). Vascular calcification increases the risk for CAD. Vascular smooth muscle cells (VSMCs) are one of the major cell types involved in the development of vascular calcification.

Methods: So far, mainly animal models or primary SMCs have been used to model human vascular calcification. In this study, a human in vitro assay using iPSC-derived VSMCs was developed to examine vascular calcification. Human iPSCs were derived from a healthy non-risk (NR) and risk (R) donor carrying SNPs in the 9p21 locus. Additionally, 9p21 locus knockouts of each donor iPSC line (NR and R) were used. Following differentiation, the iPSC-derived VSMCs were characterized based on cell type, proliferation and migration rate, along with calcium phosphate (CaP) deposits. CaP deposits were confirmed using Calcein and Alizarin Red S staining and then quantified.

Results: The data demonstrated significantly more proliferation, migration, and CaP deposition in VSMCs derived from the R and both KO iPSC lines than in those derived from the NR line. Molecular analyses confirmed upregulation of calcification markers. These results are consistent with recent data demonstrating increased calcification when the 9p21 murine ortholog is knocked-out.

Conclusion: Therefore, in conclusion, genetic variation or deletion of the CAD risk locus leads to an increased risk of vascular calcification. This in vitro human iPSC model of calcification could be used to develop new drug screening strategies to combat CAC.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

Figures
Characterization of WT iPSCs and derived VSMCs. A) NR (Top) and R (Bottom) WT iPSCs show similar iPSC morphology, as seen in the phase contrast (PhC) images. Scale bars represent 100 um. Staining with the pluripotency marker NANOG (green) confirmed nuclear expression, as seen in the overlaid image (Merge). Nuclei were counterstained with DAPI (blue). Scale bars represent 50 um. B) NR (Top) and R (bottom) WT iPSC-derived VSMCs show colocalization of CNN1 (red) and TAGLN (green) in the contractile apparatus of the cells. No differences were observed between R and NR WT VSMCs. Nuclei were counterstained with DAPI. Scale bars represent 50 um.
Figure 2

RNA expression analyses of pluripotency (A,C) and SMC-associated (B,D) genes. A) Expression of pluripotency-associated markers NANOG, OCT4, and SOX2, was lower in VSMCs derived from NR (green) and R WT (red) iPSCs than in undifferentiated iPSCs. B) SMC-associated markers TAGLN, CNN1, and CALD1 were upregulated in VSMCs derived from NR (green) and R WT (red) iPSCs compared with undifferentiated iPSCs. C) NRKO (orange) and R KO (blue) lines showed significant downregulation of pluripotency genes NANOG, OCT4, and SOX2. D) SMC-associated markers TAGLN, CNN1, and CALD1 were upregulated in VSMCs derived from NR (green) and R WT (red) iPSCs compared with undifferentiated iPSCs.
Figure 3

The 9p21 locus influences proliferation and migration of iPSC-derived VSMCs. A) R WT (red) derived VSMCs showed significantly higher proliferation rates than those derived from NR WT (orange). B) RKO (orange) VSMCs showed higher proliferation rates than those derived from NR KO (blue). C) RWT (red) VSMCs showed significantly higher migration rates than those derived from NR WT (orange). D) There were no differences in migration rate between R KO (orange) and NR KO (blue) VSMCs.
Figure 4

ARS and Calcein staining of calcifying iPSC-derived VSMCs. NR WT cells were negative for ARS (left) and Calcein (right) expression. R WT-, R KO-, and NR KO- derived VSMCs were positive for ARS (red, left) and Calcein (green, right), suggesting the presence of CaP deposits.
Calcification-associated markers are increased in R WT, but decreased in NR WT calcifying 512 VSMCs. A–E) R WT-derived calcifying VSMCs show increased expression of calcification markers ALPL (A), CSF1 (B), and CTSK (C) (clone 1 (orange)) and decreased or unchanged expression in the other two clones (blue and green). RUNX2 (D) was upregulated significantly in two out of three R WT clones. OPN (E) expression did not change significantly. F–L) In NR WT-derived calcifying VSMCs, the markers ALPL (F), CSF1 (G), and CTSK (H), and RUNX2 (K) were downregulated in one or both clones. OPN (L) expression was unchanged in NR WT cells.
Anja Trillhaase, Figure 6

Figure 6

Upregulated expression of most calcification-associated markers in 9p21 KO calcifying VSMCs. A–E) R KO-derived calcifying VSMCs showed upregulation expression of markers ALPL (A), CSF1 (B), and RUNX2 (D) in both clones. Expression of CTSK (C) and OPN (E) was either unchanged or downregulated in one clone of the R KO cells. F–L) In the NR KO-derived calcifying VSMCs, the markers CSF1 (G) and RUNX2 (K) were upregulated in both clones. ALPL (F), CTSK (H) and OPN (L) were unchanged or downregulated in one clone of the NR 526 KO cells.

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