# The effect of vitamin D receptor silencing on voltage sensitive calcium channel alpha 1 D in cortical neurons

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Amyloid plaques that have amyloid beta peptide as a core component are one of the major pathological hallmarks of Alzheimer's disease [1]. Our previous studies showed certain polymorphism of vitamin D receptor (VDR) gene increases the risk of developing AD indicating the probable role of vitamin D ($1,25(OH)_2D_3$) in AD [2]. Some clues have pointed out that vitamin D can exert protective effects on nervous system by modulating the synthesis of neurotrophins, calcium channels and calcium binding proteins [2, 3, 4, 5]. Vitamin D regulates the expression of calcium channels in several cell types. Our previous study has showed that amyloid β (Aβ) treatment eliminated VDR protein in cortical neurons up regulated L type voltage sensitive calcium channels alpha1 C (LVSCC A1C) [4]. These results might indicate the potential role of vitamin D and vitamin D mediated mechanisms in neurodegeneration [5]. However there was no data about the regulation of L type voltage sensitive calcium channels alpha1 D (LVSCC A1D) under the condition of VDR repression. The aim of this study was to investigate the expression levels of LVSCC A1D in VDR silenced primary cortical neurons.

Cerebral cortex dissected from brains of Sprague Dawley rat embryos on the embryonic day 16 and cultured (Figure 1). qRT-PCR and Western blotting methods were performed for determining the expressions of LVSCC A1D. Localization of LVSCC A1D protein was shown by immunofluorescent labelling. Cytotoxicity levels were determined by ELISA. Apoptotic cell death was investigated with TUNEL method.

Our findings showed that VDR silencing in cortical neurons did not affect LVSCC-A1D mRNA or protein expression (Figure 2). Immunoreactivity for LVSCCA1D was localized in cytoplasm of cortical neurons (Figure 3). No significant difference was observed in the apoptotic index and LDH release between siRNA-treated groups and control groups.

Our results indicated that the VDR silencing does not effect LVSCC A1D production. Although expression of this protein was altered by supplementation with vitamin D, this regulation might not be from VDR.

*References*

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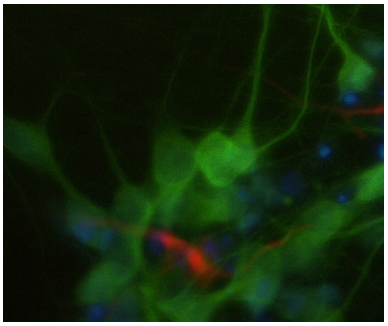

***Figure 1.*** *7 days old primary cortical neuron culture. Glia ratio of the culture found as 5%. Neurons, green (FITC tagged Pan Neuronal Marker antibody); glia red (TR tagged GFAP antibody). x40*

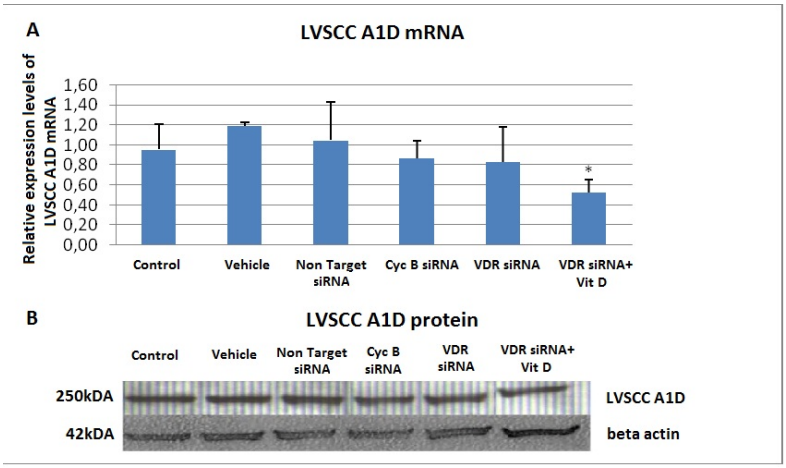


***Figure 2.*** *siRNA-mediated knockdown of VDR does not effect expression of LVSCC A1D mRNA and protein but vitamin D does. A) Comparison of LVSCC A1D mRNA levels. * LVSCC A1D mRNA levels from VDRsiRNA and vitamin D treated neurons were statistically lower than in other groups (p <0.05). B)Detection of LVSCC A1D protein by western blot.*

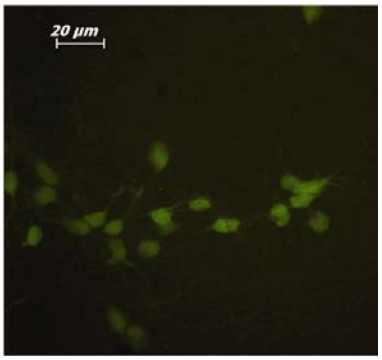


***Figure 3.*** *7 day old primary cortical neurons. Immunoreactivity for LVSCC A1D is localized mostly in cytoplasm. FITC tagged anti-LVSCC A1D antibody. X40*