Developing novel transgenic mouse model of atherogenesis with conditional oxidative stress by introduction of epithelium-specific inducible mitochondrial PolG with mutagenic activity

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Background and Aims:

Murine strain with corrupted PolG gene coding mitochondrial polymerase γ, which fidelity and proofreading activity are dramatically decreased in a way it catalyzes DNA synthesis with mismatch nucleotides in synthesized DNA strand already exists. Constitutive appearance of the mutations in mitochondrial genome trigger chronic oxidative stress, which is one of the major atherogenic factors. Since normal development of the animal is affected by chronic inflammation provoked by oxidative stress in mitochondria, this model lacks tissue-specificity and conditionality of the pathologic processes being studied in atherosclerosis research with existing mitochondrial DNA damage model of vascular endothelium inflammation and further degeneration.

Methods:

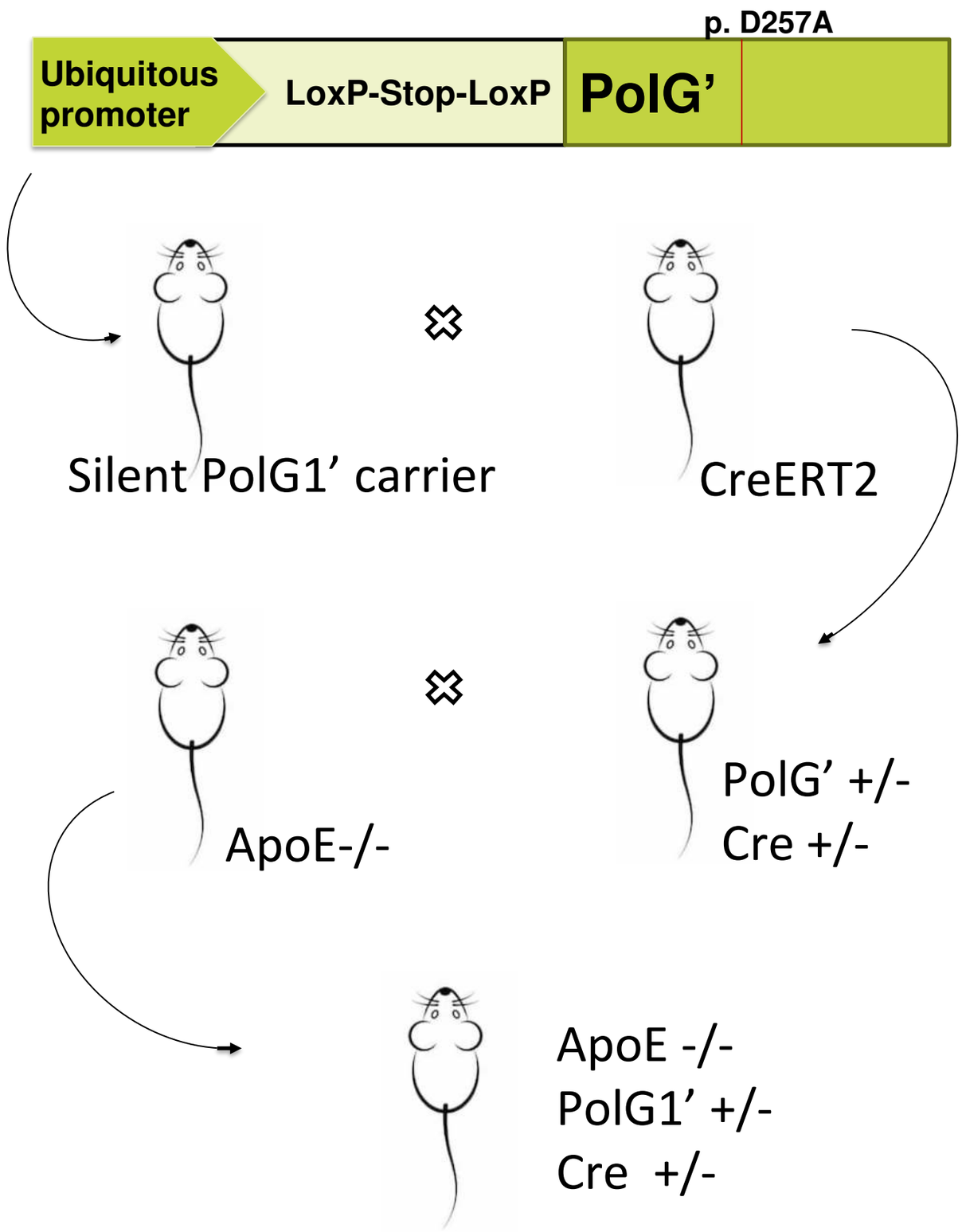
Crucial improvement of mitochondrial oxidative stress model of atherosclerosis we propose is inducibility of the engineered corrupted PolG1 gene and its epithelial specific localization. Introducing floxed stop cassette in proximal 3' region of the promoter of the PolG by CRISPR-Cas9 genome editing system is the first step for the novel strain development. Further crossbreeding of obtained transgenic animals with constitutive or inducible expressants of Cre recombinase in endothelium (Vascular Endothelial CreERT2) is a way to obtain mice strain with normal development and ability to start oxidative stress by tamoxifen addition into the mice chow.

Results

Developed construction for transgenesis according to the plan, in vitro validation is undergoing before transfection.

Conclusions:

This improvement of existing atherosclerosis model could be more accurate representation of the atherogenic processes in mice. Tissue specificity of the inducible by tamoxifen expression of the impaired PolG would reproduce pathologic processes of the naturally occurring atherosclerosis in Human patients, we suppose.



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