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**CONSTRUCTION OF HUMAN SCN5A GENE
MUTANT L1001Q AND ITS EXPRESSION IN VITRO**

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Objective To construct human SCN5A gene fusion protein vectors EGFP-L1001Q-hNav1.5 and eukaryotic expression vectors, to study the function of mutant L1001Q with methods of

Abstracts

molecular biology, and to further discuss the pathogenesis of atrioventricular block.

Methods The missense mutation pEGFP-L1001Q-hNav1.5 was generated by site-directed mutagenesis using pEGFP-WT-hNav1.5 as the template. The desired mutations in mutant plasmid were confirmed by direct sequencing. Wild type EGFP-hNav1.5 and mutant EGFP-L1001Q-hNav1.5 were linearised by restriction enzymes HindIII/KpnI and were cloned into pCDNA3.0 vector. All insert sequences as well as mutated sites were confirmed by dideoxyribo-nucleotide sequencing. The recombinant vectors were transfected into HEK293 cells with lipofectamine 2000 for immunochemical staining and visualised by confocal microscopy, and the channels protein were examined by western blot analysis.

Results The mutant plasmid were correctly constructed. pEGFP-WT-hNav1.5 and pEGFP-L1001Q-hNav1.5 were expressed in the cell membrane, and the mutant L1001Q channels protein analysis were similar to wild-type channels. These results collectively suggest that the L1001Q mutation of SCN5A channels does not impair normal protein expression in HEK 293 cells.

Conclusion The construction of the eukaryotic expression vector of missense mutation of human SCN5A gene mutant L1001Q and provide a solid foundation for further electrophysiology experimental studies on the mechanism of atrioventricular block induced by missense mutation.

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