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Evaluation of the effect of c.2946+1G>T mutation on splicing in the *SCN1A* gene

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Mutations in the SCN1A gene have commonly been associated with a wide range of mild to severe epileptic syndromes. They generate a wide spectrum of phenotypes ranging from the relatively mild generalized epilepsy with febrile seizures plus (GEFS+) to other severe epileptic encephalopathies, including myoclonic epilepsy in infancy (SMEI), Cryptogenic Focal Epilepsy (CFE), Cryptogenic Generalized Epilepsy (CGE) and a distinctive subgroup termed as Severe Infantile Multifocal Epilepsy (SIMFE). The present study was undertaken to investigate the potential effects of a transition in the first nucleotide at the donor splice site of intron 15 of the SCN1A gene leading to CGES. Functional analyses using site-directed mutagenesis by PCR and subsequent *ex-vivo* splicing assays, revealed that the c.2946+1G>T mutation lead to a total skipping of exon 15. The exclusion of this exon did not alter the reading frame but induced the deletion of the amino acids (853 Leu - 971 Val) which are a major part in the fourth, fifth and sixth transmembrane segments of the SCN1A protein. The theoretical implications of the splice site mutations predicted with the bioinformatic tool Human Splice Finder were investigated and compared with the results obtained by the cellular assay.

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Cloning, expression and concentration of antiviral protein from the hemolymph of *Lonomia obliqua* in bacterial system

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Insect hemolymph studies demonstrated the presence of active principles such as antiviral. The aim of this work was cloning, expression and concentration of the recAVLOEc protein with antiviral activity from *L. obliqua* hemolymph. For that, a PCR with specific primers for the antiviral protein, based on the sequence previously cloned in bac-to-bac system, was made. Restriction sites were inserted in the primer for connection to the plasmid pET28a. First reaction was performed with the restriction enzyme BamHI, followed by digestion with the enzyme HindIII. After binding insert in the vector, the construction was selected in *E. coli* BL21 (DE3) pLysS and the cloning was confirmed by sequencing. The protein expression was induced with IPTG (1mM) at 0.7 DO at 37°C for 4h. In order to concentrate the target-protein, precipitation by salting-out method was used. Initially, ultrasound bacterial lysis was performed. After centrifugation (8,000 rpm x 10 min), the saturated salt solution (sodium or ammonium sulfate) was added to the supernatant phase of cellular lysate. This solution was maintained for 12h at 4°C until the formation of the precipitate phase. The solubility curve of recAVLOEc was determined by measuring the protein composition of the supernatant phase. The effectiveness of salts as precipitant agents was verified. In addition, antiviral tests were performed and a titration was made with Measles virus infecting VERO cells, in which 24 µg recAVLOEc/mL resulted in the viral title 256-fold lower than control.

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