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Upstream open reading frames regulate nicotinic acetylcholine receptor subunits associated with smoking and smoking-related disorders

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Introduction

Nicotine addiction poses a major health problem worldwide and is known to considerably increase the risk for diseases such as cancer and cardiovasculopathies. Nicotine both modulates nicotinic acetylcholine receptor (nAChR) subunit expression in various, mostly still unknown ways and acts as a receptor ligand. The genes coding for nAChRs are therefore suspected to play a key role concerning smoking behaviour and related disorders. Especially post-transcriptionally regulatory mechanisms are considered to be involved in the modulation of nAChR subunit expression by nicotine such as internal ribosomal entry sites, microRNA-binding sites and upstream open reading frames (uORFs) located within the untranslated regions (UTR).

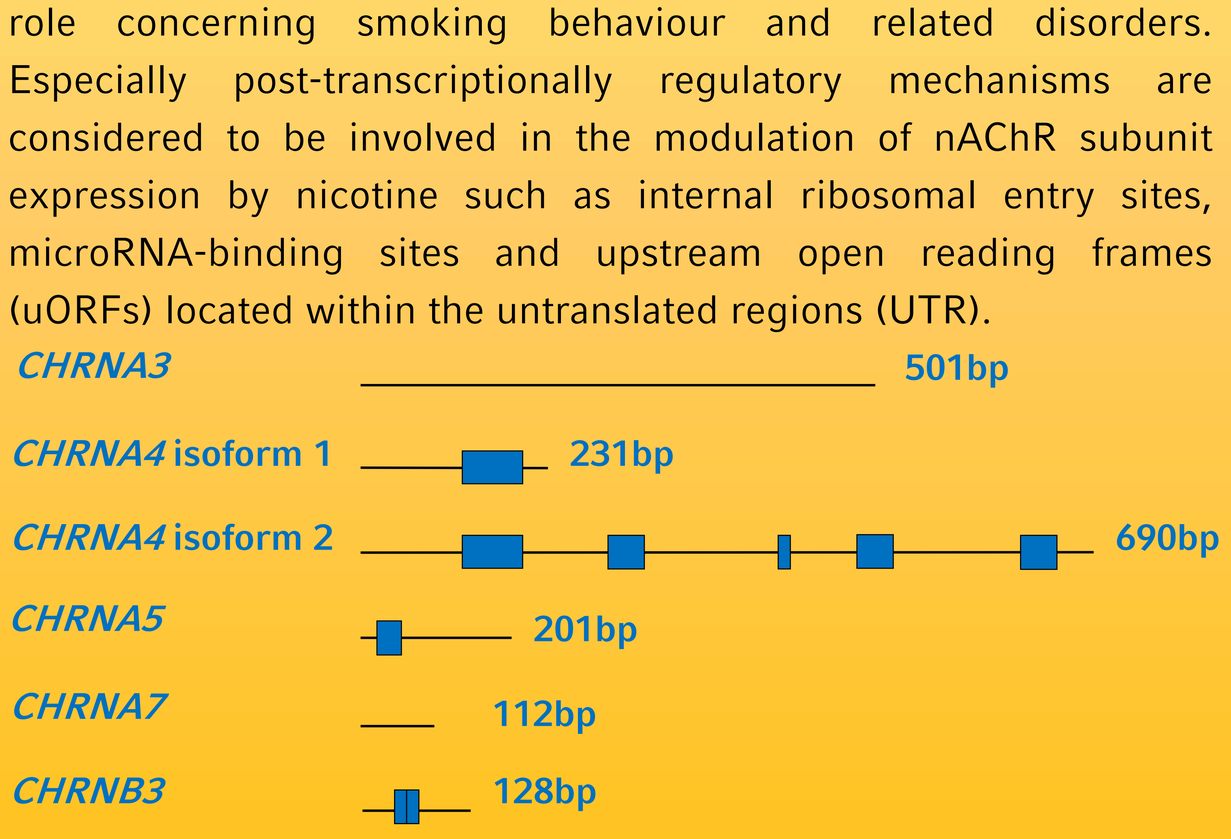

Fig. 1: 5'UTRs of nicotine dependence-associated nAChR subunit genes with putative uORFs (blue squares).

Material and Methods

We performed a systematic search for functionally relevant uORFs in the 5'UTR of the nAChR genes *CHRNA3*, *CHRNA4* isoform 1 and 2, *CHRNA5*, *CHRNA7* and *CHRNB3* (Fig.1). The 5'UTR of the nAchR subunits α4 isoform 1 and 2, α5 and ß3 were cloned into the vector pGL4.10+TK. Constructs with intact start codons were compared to constructs lacking the start codon (ATG mutated to TTG). HEK293 cells were co-transfected with the above-mentioned pGL4.10+TK constructs and the control plasmid pGL4.74. We measured the firefly and renilla luciferase activities by Dual-Glow luciferase assay. The mRNA quantity of firefly and renilla luciferases were assessed by qPCR. The ratio of firefly luciferase and renilla luciferase activity was calculated and normalized to pGL4.10+TK. The various 5'UTR constructs are expressed as fold change to pGL4.10+TK (Fig. 2 and 3).

Results

Reporter gene assays revealed that the uORFs of *CHRNA4* isoform 1 and *CHRNA5* are able to significantly downregulate the luciferase protein expression and are thus functional (Fig. 2 A and Fig. 3 A).

For *CHRNA4* isoform 2 and *CHRNB3* uORFs no significant results were obtained. As to *CHRNA4* isoform 1 and *CHRNA5,* qPCR did not show a significant difference in mRNA quantity when comparing intact versus switched-off uORFs (Fig. 2 B and Fig. 3 B). Therefore, we could rule out for both genes that the luciferase assay results were due to a transcriptional effect.

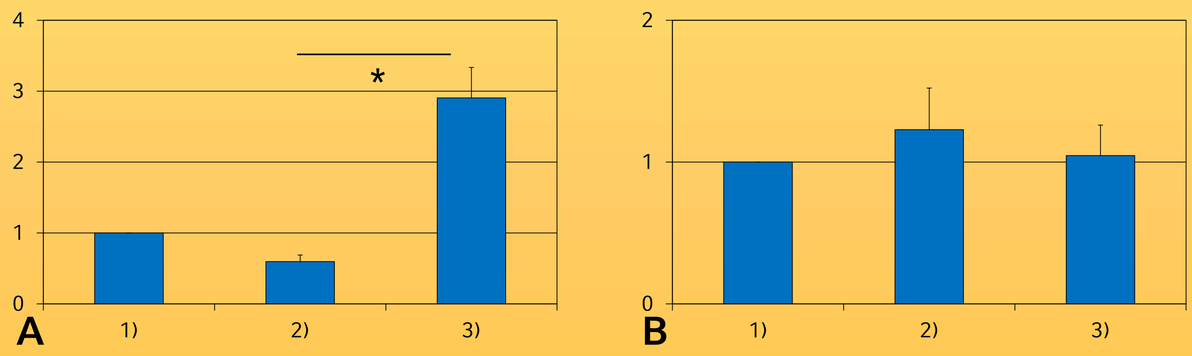

Fig. 2 A: *CHRNA4* isoform 1 contains a post-transcriptionally functional uORF. **B:** Relative qPCR for *CHRNA4* isoform 1 5'UTR showed no significant differences of mRNA amount when comparing intact with switched-off uORF. 1) pGl4.10+TK; 2) *CHRNA4* isoform 1 with intact uORF; 3) *CHRNA4* isoform 1 with switched-off uORF.

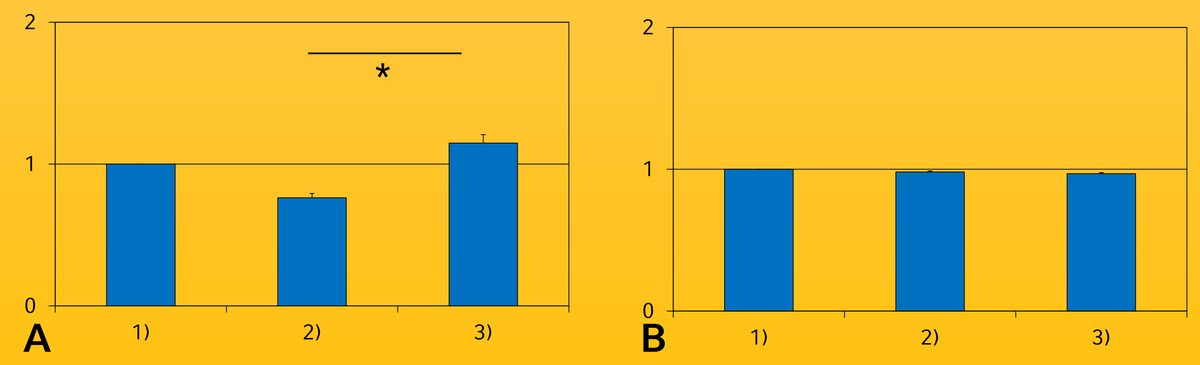

Fig. 3 A: Luciferase assay of *CHRNA5* 5'UTR showed a significant increase in protein expression after switching off the uORF. **B:** Relative qPCR for *CHRNA5* 5'UTR demonstrated no significant differences of mRNA amount when comparing intact with switched-off uORF. 1) pGl4.10+TK; 2) *CHRNA5* with intact uORF; 3) *CHRNA5* with switched-off uORF.

Discussion

The data presented here strongly suggest that uORFs within the 5'UTR of *CHRNA4* isoform 1 and *CHRNA5* are important regulators of protein translation. Interestingly the uORF found to be functional in *CHRNA4* isoform 1 is also present in *CHRNA4* isoform 2 but does not seem to reduce gene expression in the latter sequence context. A possible reason could be the presence of additional, so far unknown regulatory motifs that only exist in the long 5'UTR of isoform 2. To our knowledge this presents the first example of an uORF with isoform-specific functional relevance.

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