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MEETING ABSTRACT

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An *in vitro* model to study the human-specific nicotinic acetylcholine receptor gene *CHRFAM7A*

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Background: Human-specific polymorphisms in nicotinic acetylcholine receptor (nAChR) genes have been associated with several disorders including cognitive deficits, epilepsy, language impairment, acute stress disorder, attention deficit hyperactivity disorder, schizophrenia, hypotonia and many others. The *CHRNA7* gene, which encodes for the α7 nAChR subunit, is located on the proximal part of chromosome 15, one of the least stable regions in the genome with frequent microdeletions and duplications. In this unstable region, about one million years ago, the *CHRNA7* gene duplicated partially and fused with the gene *FAM7A* forming a novel human-specific gene called *CHRFAM7A*. This gene encodes for the dupα7 nAChR subunit, which is slightly truncated at the extreme N-terminus, and therefore lacks the agonist binding site, but otherwise shares all structural elements with α7. Since *CHRNA7* and *CHRFAM7A* are still in unstable gene regions both genes can be duplicated or deleted, resulting in humans with varying copy numbers of either of the two genes. In the current project, we want to study the phenotypical effect of neurons which express constant levels of α7 but increased or reduced levels of dupα7.

Methods: There is a lack of animal models for human-specific genes and human neurons as a cell source are even more limited. Therefore, we differentiated human induced pluripotent stem cells (hiPSCs) with different dupα7 expression levels into cortical neurons in order to investigate the functional properties of α7- and dupα7-containing nAChRs using Fura-2 Ca^{2+} imaging and/or patch-clamp electrophysiology.

Results: We started to differentiate a hiPSC line expressing average levels of α7- and dupα7-nicotinic receptors. First, neural rosettes including neural progenitor cells (NPCs) were obtained from hiPSCs. Next, terminally differentiated cortical neurons were generated from NPCs. Concordantly, hiPSC-derived neurons were stained by using fluorescently labelled neuronal markers for molecular characterization. Additionally, we used Fura-2 Ca^{2+} imaging and patch-clamp electrophysiology to assess the functionality of neurons. We found that hiPSC derived neurons expressed the neuronal markers MAP2 and vGluT1. Moreover, we observed many neurons firing healthy trains of action potentials. They evoked voltage-dependent responses resulting in sodium currents and rapidly inactivating potassium currents. For Fura-2 Ca^{2+} imaging, hiPSC-derived neurons responded to the α7-specific agonists choline or PNU-282987 with or without the positive allosteric modulator PNU-120596.

Discussion: We were able to differentiate hiPSC into functional cortical neurons and started to characterize them using patch-clamp electrophysiology and Fura-2 Ca^{2+} imaging. In future experiments, we plan to repeat those experiments using hiPSCs with higher expression levels of dupα7 (after lentiviral infection) or with reduced expression levels (through CRISPR/Cas9 technology). In conclusion, analyzing the molecular consequences of human-specific *CHRFAM7A* copy number variations will be critical for understanding the possibly disease-causing phenotypes of this nAChR polymorphism.

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Keywords: human induced pluripotent stem cell (hiPSC) – nicotinic acetylcholine receptor (nAChR)

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