Abstract

Angelman syndrome (AS) is a neurodevelopmental disease found in 1 to 10,000 to 40,000 births, exhibiting an equal gender ratio. Key characteristics of the disease include an ataxic gait with tremor, severe mental retardation, profound speech impairment and seizures. Behavioral deficits such as increased anxiety and autism spectrum disorder features is found in affected individuals as well. The disease stems from the imprinted region 15q11.2-13q where genes are either maternally or paternally expressed as a result of parent-of-origin specific expression of the alleles. There are four main genetic etiologies causing AS namely, i) a large deletion ranging from 4-6 Mb on the maternally inherited allele including imprinted and bi-allelically expressed genes, ii) maternal deletion of the Ubitiquin ligase E3 (*UBE3A*) gene, iii) paternal uniparental disomy and iv) imprinting defect leading to inappropriate methylation of the locus. So far, there is no cure for AS rather the symptoms are ameliorated using a multidisciplinary approach. The goal of the doctoral study was to further decipher the role of *Ube3a* and *Gabra5* using two mouse models to gain more knowledge about the involvement of these two genes for future therapeutic interventions in for Angelman syndrome.

One model generated was a full gene deletion of *Ube3a* from 5'UTR to 3'UTR encompassing approximately 76 Kb with all coding and non-coding elements abolished. Although there are many mouse models available targeting the *Ube3a* gene in different manners there is no model with the entire gene missing, which offers a larger genetic similarity to the situation in patients as the majority harbor a large deletion. Also, a large variability of phenotypes in other AS models have been reported depending on experimenter, strain, and age of the animals, clearly presenting a barrier for development of therapeutics. To assess the suitability of the model in AS research we subjected it to a battery of tests particularly aimed towards the AS pathology, including motor skill evaluation and behavioral paradigms. We found that the novel AS model recapitulated motor skill deficits seen in DigiGait and rotarod tests. Furthermore, behavioral aberrancies were confirmed seen as underperformance in nest building and tail suspension test. The model did however not exhibit any underperformance in memory-dependent tests such as Barnes maze and novel object recognition. However, when subjected to the more difficult place reversal task in the IntelliCage setup, they did indeed underperform. We also observed differences in circadian rhythm activity and hypoactivity. The results obtained match well with phenotypes reported in other AS models

and has by the tests performed by us not provided any clear advantage in terms of studying AS. However, more tests addressing phenotypes such as autism spectrum disorder features, electrophysiology and EEG should be conducted and can lay basis for future publications.

Additionally, we targeted the Gamma-aminobutyric acid receptor alpha 5 (*Gabra5*) gene. This gene is frequently deleted on the maternal allele in patients with a large deletion. The cluster of Gamma-aminobutyric acid (GABA) subreceptor genes beta 3, gamma 3 and of course, alpha 5, is believed to be an important contributor to electrophysiological phenotypes but have also been linked to panic disorder and anxiety, phenotypes consistent in AS patients. We decided to study the anxiety-like behavior in *Gabra5* deficient mice by both behavioral tests but also assessing corticosterone levels, as dysregulation of the hypothalamic-pituary-adrenal- (HPA) axis has been linked to anxiety disorders. Furthermore, the HPA-axis is under GABAergic regulation however, the subreceptor expression and their contribution to the HPA-axis regulation, is still not clear, presenting a knowledge gap. We found that Gabra5$^{-/-}$ mice had lower corticosterone levels, which was rather surprising as disinhibition of GABAergic signaling has been reported to result in increased excitability. Additionally, the Gabra5$^{-/-}$ mice did not appear more anxious in open field and elevated plus maze tests. Furthermore, rearing behavior was decreased, suggesting a lower level of experienced anxiousness. Lastly, we did a functional analysis of hippocampal slices, a brain region known to contribute to anxiety regulation. The pact-clamp experiments revealed that Gabra5$^{-/-}$ derived neurons were hyperpolarized in several parameters tested. Based on this we believe that there must be a functional compensation of probably calcium or chloride channels to explain our observations. Although we were not able to attribute phentoypes to the alpha 5 channel we could clearly show the importance of examining functional compensation in constitutive models.

Finally, we conducted several gene expression analyses detecting expression of genes belonging to the AS locus using RT-qPCR. We generated two knock-out cell lines using CRISPR/Cas9 targeting resulting in a *Ube3a* deficient line and another line knocking out a putative enhancer residing within the intron 4-5 of said gene.

We evaluated expression of the genes in teratocarcinoma P19 cell line in both its pluripotent state and differentiated into neurons. We found a significant decrease in all paternally expressed genes. In rescue experiments with UBE3A overexpression, a restoration of gene expression was absent. We proceeded to do the same evaluation but with the putative enhancer knocked-out and found

that it recapitulated the observations made in *Ube3a* knock out cells. The results of the RT-qPCR generated experimental data pointing towards a possible function of the enhancer however additional tests are needed and eventually in the mouse organism.