Institute of Physiology CAS

Prof. Ladislav Vyklický M.D., DSc.
Department of Cellular Neurophysiology
Institute of Physiology CAS
Vídeňská 1083, 142 20 Prague 4

Telephone: (+420) 24106 2450
E-mail: Ladislav.Vyklicky@fgu.cas.cz
www.biomed.cas.cz/odd031/

Dissertation thesis report

Thesis title: Mouse models for Angelman syndrome: generation and characterization
Author of the thesis: Linn Amanda Sydinge

The dissertation thesis, with 70 pages in total (56 pages Abstract, Introduction, Aims, Methods, Results, and Discussion), is based on one original publication, one submitted paper, and two review papers. The project aimed to generate two mouse models connected to Angelman syndrome. One was developed by a large deletion of the entire *Ube3a* gene, and the other by targeting the *Gabra5* gene. The first model was subsequently used to characterize Angelman syndrome's genetic landscape and study the phenotypic presentation. The other was used to understand the consequences of the deletion of one GABA$_A$ receptor for excitability and to study the stress response and anxiety-like behavior.

The introductory part of the thesis (19 pages) presents information on the clinical manifestation of Angelman syndrome, locus arrangement, disease genetics, and different mouse models. This section is easy to read and gives detailed information about the disease, mainly concerning genetic causes. Given the absence of behavioral tests in the Results section, I find the inclusion of a chapter on "Phenotyping of AS models" describing various behavioral tests and their importance not entirely warranted. Conversely, due to the inclusion of the chapter "Echocardiography reveals differences between WT and novel AS strain" in the Results section, I missed a section in the Introduction that would have dealt with somatic problems in Angelman syndrome, particularly in the cardiovascular system.

The section on Methods and materials (5 pages) is condensed; in some cases, only the reference to manuscripts is given for the methods used. I see this as a problem since manuscript 2 was submitted, is not publicly available, and is not included as part of the Ph.D. dissertation. It may be only my problem, but I do not understand some abbreviations used in figure 2.1. such as ERG, ECG, and Echn. These are not explained in the figure legend nor listed in section 8. (List of abbreviations).

I found the chapter "2.4 Neuronal differentiation of P19 cells" very interesting and I can say from my own experience that the differentiation of pluripotent cells into neurons is not always successfully achieved. Therefore, I am a bit disappointed that the author did not describe this part in more detail and did not include a photomicrograph of the differentiated neurons to judge the morphology of neurons.

The Results section (7 pages) responds briefly but accurately to the stated aims. I want to emphasize that the creation of animal models of human diseases conditioned by genetic changes is the focus of the best laboratories in the world. The methodological steps involved in their preparation are time-consuming, laborious, and in most cases, the goal can only be achieved with enormous effort. The result is the seemingly simple statement: "We have created a genetic model". Dr. Sydinge has succeeded. She has (i.) evaluated the expression of AS/PWS locus genes upon deletion of Ube3a and (ii.) generated and characterized a model spanning the entire *Ube3a* gene and a model targeting the *Gabra5* gene. The gained results are a step forward in understanding molecular and genetic events underlying the symptoms of Angelman syndrome and, perhaps in the future, may lead to an efficient cure for this and related neurodevelopmental diseases.

The Discussion section (5 pages) recapitulates and discusses the results.

Conclusion: The present dissertation contains results obtained mainly by challenging molecular biology methods, some of which have significantly advanced the scientific understanding of the genetic background of Angelman syndrome. The results of Dr. Sydinge also offer new animal models that can be used in designing new strategies for the treatment of neurodevelopmental diseases. The submitted dissertation contains original findings that significantly increase our knowledge of genetically determined nervous system diseases. Since, in the submitted dissertation, the author has demonstrated that she can address experimental questions of basic research at a professional level using modern experimental methods, I recommend that Dr. Linn Amanda Sydinge be awarded the degree of Ph.D.

In Prague 15.12.2022   Prof. Ladislav Vyklický

Questions:
1. One of the typical features of AS is microcephaly. Is this phenomenon preserved also in the murine models of AS? Is neurogenesis altered in Angelman syndrome? Ataxia is frequently associated with defects in the cerebellum. Were there some anatomical defects in the cerebellum in the genetically modified mouse model of the disease?
2. P19 cells in their pluripotent state were transfected with AAV-EF1a-Ube3a to rescue the decreased expression of paternally expressed genes. The transfection revealed that the expression of Ubiquitin-protein ligase E3A (UBE3A) was approximately 20-fold upregulated in the Ube3a-KO transfected cells. But it did not rescue the expression of any PEGs to WT levels. What percentage of cells was transfected?
3. The methods section describes the differentiation of pluripotent P19 teratocarcinoma cells into neuronal-like cells. It is stated that they were kept in a DMEM:F12 media supplemented by nutrients for two days for synapse maturation. DMEM:F12 contains 50 μM glutamate and the same concentration of aspartate. Both these amino acids are highly toxic to neurons (the effect is well characterized and known as excitotoxicity and thought to underlie neurodegeneration in man). What is the reason for including glutamate and aspartate in the media? Since you have used electrophysiological techniques to characterize GABA$_A$ receptors, did you use this technique to characterize neuronal-like cells regarding synaptic transmission and responses to glutamate and GABA?