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Review on the doctoral dissertation of Ms Róża Szlendak entitled: "Developmental and epileptic encephalopathies as synaptopathies – assessment of the role of NMDA receptors in the etiopathogenesis of disease".

I read with great interest the thesis dissertation written by Ms Róża Szlendak on the work she has carried out under the joint supervision of Professor Dorota Hoffman-Zacharska (Institute of Mother and Child, Warsaw, Poland) and Dr Julie Perroy (Institute of Functional Genomics, Montpellier, France).

The objective of her thesis work was to characterise the molecular basis of a severe form of epilepsy, the Developmental and Epileptic Encephalopathies (DEE), by analysing a large cohort of affected individuals, focusing on a panel of implicated genes. Among these, she selected one specific variant in the synaptic gene *GRIN2*, encoding a subunit of the NMDA receptor, for detailed functional analysis. Her overall goal was to determine the contribution of this receptor to the etiopathogenesis of DEE.

The manuscript is divided into four main parts: Introduction, Materials and methods, Results and Discussion. In addition, there are appended tables that provide a detailed description of the 49 genes included in the EIEE gene panel and the variants identified therein.
Róża started with a comprehensive and well-written introduction of the subject, which deals with the study of the etiology of DEE. These constitute a heterogeneous group of diseases in terms of clinical symptoms and genetic background, and therefore proper syndromic classification is important for prognosis and adapted therapeutic choices. Initially, epilepsies were considered as channelopathies, but thanks to next generation sequencing (NGS), novel epilepsy-associated genes have been identified, among which variants in synaptic genes, including the ionotropic Glutamate receptor NMDAR. This class of receptors and their synaptic function is very well presented in the Introduction, as well as their implication in these so-called synaptopathies.
The Materials and Methods section is very exhaustive and detailed. This reflects the enormous amount of different and cutting-edge technologies Róża has used and developed during her thesis studies. It should be pointed out that she successfully derived cortical neurons from human iPSC obtained from patients, which is a very challenging process.

In the very clear and well-structured Results section, Róża presents the data she obtained during her thesis work, both at the Institute of Mother and Child in Warsaw and at the Institute of Functional Genomics in Montpellier. She analysed a cohort of 694 individuals clinically diagnosed with DEE or drug-resistant epilepsy, by targeted NGS of 49 panel genes, among which were 17 synaptic genes. Using a pipeline of bioinformatic analyses (NGS data Filtering and prioritizing of genetic variants), she could reinterpret the cases and increase the diagnosis yield, enabling 18,3% of patients to have a

confirmed molecular diagnosis. She then decided to focus her studies on variants in the *GRIN* genes, encoding NMDA receptor subunits proteins, and more specifically on the *GRIN2B* subunit, carrying variants in the less well characterized C-terminal domain. Róża then selected the GRIN2B pGlu839Ter nonsense variant for further detailed analysis.

She confirmed the pathogenicity of this variant and characterized the properties of the mutated protein *in vitro* and in iPSC-derived neurons. In HEK cells, she found a reduced cytotoxicity and cell surface expression of the mutated receptor, while the interaction with the other receptor subunits was not affected. Using electrophysiology, she showed that the mutated NMDA receptor displayed reduced NMDA current amplitude with higher sensitivity to magnesium blockade, indicative of a strong dysfunction of the receptor. Finally, in iPSC-derived neurons, she could show a delayed response following NMDA addition, while the global NMDA-induced Calcium influx was unaffected.

In her Discussion, Róża puts into perspective all the results she obtained in her study. She analyses with a lot of maturity the accuracy of her approaches and makes a critical assessment of her results in view of the literature. In addition, she proposes new experiments and analyses of the databases in order to improve the yield of genetic identification of genes implicated in DEE, as part of the scientific effort to establish the background of these severe epilepsies.

In summary, Róża Szlendak carried out a very comprehensive work, combining human molecular and clinical genetics with molecular and cellular biology, biochemistry and electrophysiology, both in heterologous cell systems and in human iPSC-derived neurons derived from patient fibroblasts. In doing so, she has demonstrated her mastery and her capacity to conduct a successful research project. The results of Róża's thesis are very interesting and solid, and **there is no doubt that her work will be published in a good journal in the near future**. Róża will be the first author of a paper that will include the results presented in the thesis: from the identification of a GRIN mutation in a patient to the characterisation of the molecular dysfunctions of the receptor and the consequences on the cell signalling of neurons derived from the patient's iPSCs.

In conclusion, Ms Róża Szlendak has carried out a very rigorous, substantial and excellent scientific work that opens up very important perspectives in the field of understanding the etiopathology of the most severe drug-resistant form of epilepsy, the developmental and epileptic encephalopathies. Therefore, I give a **very positive opinion on the public defense of her thesis**.

Furthermore, I consider **the PhD dissertation to be distinguished**, given the high quality of the manuscript and the scientific work.

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