## Abstract: 61O

**Epileptic Encephalopathies associated with gain-of-function $GABA_A$ receptor variants are more severe than loss.**

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γ-aminobutyric acid type A ($GABA_A$) receptors mediate both synaptic and extrasynaptic neuronal inhibition. Recently, a wealth of variants in $GABA_A$ receptor subunit-encoding genes have been identified in patients with developmental and epileptic encephalopathies. Patients present with a wide phenotypic spectrum including an age of onset from birth to several years old, mild to severe intellectual disability, behavioural deficits, and unpredictable drug responses. The prevailing paradigm is that $GABA_A$ receptor variants exclusively cause epileptic disorders via a loss of cell surface expression or function, however this dogma cannot explain the wide phenotypic spectrum.

To resolve the molecular mechanism underlying the phenotypic spectrum, we performed a large-scale genotype/phenotype correlation on 54 variants in the *GABRB3* gene. We found that patients segregated into two distinct functional groups of gain-of-function (increased GABA sensitivity) and loss-of-function (impaired GABA sensitivity). Surprisingly, gain-of-function variants were associated with a more severe phenotype, with a significantly younger age of onset, higher prevalence of severe intellectual disability, hypotonia, microcephaly and poorer response to treatment. Analysis of electroencephalogram and seizure types similarly demonstrated distinct phenotypic differences between the two groups. We further analysed the desensitizing properties of *GABRB3* gain-of-function variants and found that several variants with severe manifestations of the disorder reduced the steady-state equilibrium, further increasing GABAergic activity to exacerbate the clinical phenotype. We further functionally analysed a pathogenic *GABRA4* variant and identified a gain-of-function variant with increased maximum open probability. Heterozygous knock-in gain-of-function *GABRB3* mouse models displayed high lethality around the age of birth and prior to weaning.

We conclude that the current paradigm that loss-of-function $GABA_A$ receptor variants are the exclusive cause of seizures is incorrect. Instead, somewhat counter-intuitively, increased GABAergic activity from gain-of-function $GABA_A$ receptor variants are a greatly underappreciated cause of severe epileptic encephalopathies, and precision medicine approaches are required to be developed that target the cause of epilepsy in these patients.