# Introduction

Neurodevelopmental disorders

Neurodevelopmental disorders (NDDs) are a group of neuropsychological pathologies characterized by their manifestation between infancy and adolescence. NDDs include attention deficit/hyperactivity disorder (ADHD), autism spectrum disorder (ASD), motor and mood disorders, and intellectual and learning disabilities. Despite the multiple genetic mutations that have been identified as likely causal factors of NDDs, diagnosis remains challenging because of the diseases’ low genotype-phenotype correlation and the common presence of comorbidities (Moretto et al. 2018).

Interestingly, many of the genes altered in NDDs are involved in pathways related to protein synthesis, transcriptional regulation, and synaptic signaling (Reviewed in Mullins et al. 2016). Because of this, there has been great interest in the biomolecules involved in these pathways as potential biomarkers or therapeutic targets for different NDDs. For example, several studies have found the excitatory neurotransmitter glutamate to be increased in the blood and brain tissue of ASD patients. This has led to the theory that ASD is caused by an increase in the excitation/inhibition (E/I) ratio due to either a lack of GABA-ergic neurons or a deficiency in their activity (Rubenstein and Merzenich, 2003; Nelson and Valakh, 2015; Robertson et al., 2016; Uzunova et al., 2016; Lopatina et al. 2019). Similarly, Rett syndrome (RTT) patients and animal models have shown higher levels of glutamate and a reduced expression of metabotropic glutamate receptors 5 and 7 (mGlu5, mGlu7)  (Gogliotti et al. 2016; Gogliotti et al. 2017).

Tryptophan is an essential amino acid that is the precursor of several neuroactive compounds. The majority of tryptophan is catabolized through the kynurenine pathway into the coenzyme nicotinamide adenine dinucleotide (NAD+), which is a vital component of energy metabolism. Kynurenic (KYNA) is an intermediate metabolite of the kynurenine pathway that acts as an antagonist to the N-methyl-D-aspartate receptors (NMDARs). Studies have found that KYNA to be significantly increased in the cerebrospinal fluid (CSF) and brain tissue of patients with schizophrenia (Sathyasaikumar et al. 2011; Holtze et al. 2012)

Recent research has shed light on omics technologies as useful tools in the search for biomarkers and therapeutic targets for complex diseases. Metabolomics is a relatively new area of research that can capture patient-specific variation such as response to treatment, exposure to environmental conditions, or disease progression, which makes it a promising avenue for personalized medicine. One of the main advantages of metabolomics is that it offers a more accurate picture of the phenotype. It allows researchers to study not only the information encoded in the genome of a patient, but also the effect that genomic, proteomic regulation have on its expression. For diseases like NDDs, a metabolomic approach could provide the missing link between their molecular profiles and their clinical presentation. For that reason, this study focused on the comparison of the metabolic profiles of patients affected with different NDDs and healthy controls. Special focus was given to tryptophan and energy metabolism because of

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Rett syndrome (RTT) is a severe X-linked neurodevelopmental disorder with an incidence of 1 in 10,000 to 20,000 live births, primarily affecting girls. Patients may appear to develop normally until around 6 to 18 months of age, when their language and motor skills plateau and then begin a rapid regression. The severity of symptoms is highly varied, but children tend to deteriorate around age 10 and are frequently wheelchair-bound by adulthood. RTT Patients have a higher incidence of sudden and unexpected death (26%) than is seen in their age peers that is mostly due to cardiac and respiratory complications. The main criteria for RTT diagnosis is loss of spoken language, loss of purposeful hand skills, gait and motor abnormalities, and stereotypic hand movements. Other important symptoms include autistic behaviors, seizures, autonomic dysfunction, and profound intellectual disability. RTT is classified as typical when a patient presents with all four main criteria, and as atypical when a patient shows at least two of the main criteria and five out of eleven supporting criteria. The disease is monogenic, with 97-98% of typical cases and 85% of atypical cases caused by loss-of-function mutations in the gene methyl-CpG-binding protein 2 (*MECP2)*. Atypical RTT can also be caused by mutations in *MECP2* or more rarely by mutations in other genes such as forkhead box G1 gene (*FOXG1*), which causes a preserved speech variant, or the cyclin-dependent kinase-like 5 (*CDKL5*), which causes a variant with early-onset seizures. *CDKL5* can also cause

Mutations in syntaxin binding protein 1 *(STXBP1*) appear in a variety of early infantile encephalopathies, with one common type being Ohtahara syndrome/early infantile epileptic encephalopathy 4 (EIEE4). Patients present a variety of severities of intellectual disability (~80%), developmental delay (95%), motor disorders (~55%), epileptic seizures (~90%), and behavioral abnormalities (~35%) (Reviewed in Cali et al. 2022). Regression, autism, and stereotypic movements are sometimes also present, which has led to patients being diagnosed with atypical Rett syndrome (Olson et al 2015). *STXBP1* is extremely important for normal brain development, as it is necessary for the formation of the SNARE complex that controls neurotransmitter secretion. Loss-of-function mutations in this gene are strongly negatively selected, with *STXBP1* null neurons showing no synaptic activity at all. The main pathogenic mechanism appears to be haploinsufficiency in patients with heterozygous mutations that reduce synaptic activity in both excitatory and inhibitory neurons. GABAergic neurons have been reported to be more greatly impacted by *STXBP1* deficiency, which potentially causes a net hyperexcitatory state that results in the observed epileptic symptoms.

GRIN-related encephalopathies cause a similar phenotype to the previously mentioned disorders, with intellectual disability, autism, movement disorders, and epilepsy commonly seen in patients. These disorders are caused by mutations in *GRIN* genes, which encode the subunits that form the N-methyl-D-aspartate (NMDA) ionotropic glutamate receptors. The most common mutations occur in genes *GRIN1*, *GRIN2A*, and *GRIN2B*. The severity of symptoms varies depending on the gene that is mutated, with *GRIN1* mutations generally resulting in more severe cases (Santos-Gómez et al. 2021). In contrast, both loss- and gain-of-function mutations have been reported to result in similar phenotypes (Swanger et al. 2016) (XiangWei et al. 2017)