***Figure 1. Unsupervised multivariate analysis of patient and control CSF metabolite concentrations. A- C. PCA score plots showing the separation of patients from controls on the first two principal components.*** *A) All samples**B) MeCP2 and CDKL5. C) hypo-glutamatergic.* ***D and E. Ward hierarchical clustering showing log-transformed metabolite concentrations.*** *Both MeCp2 /CDKL5 (C) and hypo-glutamatergic (D) patients form homogeneous groups regardless of genotype. There is some overlap with controls, but mostly patients cluster together and have a markedly decreased expression of most metabolites*

***Figure 2. Identification of significantly altered metabolites and their impact on group classification A-B)*** *OPLS-DA score plots showing separations of controls compared to* ***A)******RTT*** *and* ***B)******hypo-glutamatergic patients.*** *Although control samples showed high intergroup variability, both OPLS-DA models showed good separation between the groups (R2Y(cum) > 0.9 ,Q2Y(cum) > 0.8, RMSEE < 0.2).* ***C-D) VIP scores.*** *Both diseases show similar metabolic alterations.* ***C) RTT*** *had 18 metabolites with VIP score > 1* ***D)******hypo-glutamatergic*** *patients ha 17. The highest VIP scores in both cases belonged to tryptophan metabolites.* ***E-F) Integrated results of UVA and MVA analysis. E) RTT*** *had a subset of metabolites that were only identified by UVA* ***F) hypo-glutamatergic patients.*** *For both groups there was a small number of metabolites identified as altered by both univariate and multivariate analyses. Both diseases showed similar metabolic alterations, though RTT (****C****) had a higher number of significantly altered metabolites. Interestingly, RTT patients showed some metabolites that were significantly altered but did not contribute to the classification of the OPLS-DA model.* ***G-H)******Hierarchical clustering using only the selected metabolites*** *showed perfect separation between patients and controls for both groups.* ***G) RTT patients*** *showed a few more metabolites that had increased concentrations when compared to controls than did* ***H) hypo-glutamatergic patients****, but overall the altered metabolites were decreased in both groups of patients.*

***Figure 4. Pathways affected by selected metabolites.*** *Altered metabolites for both RTT and hypo-glutamatergic patients had the highest impact in galactose metabolism, amino sugar and nucleotide sugar metabolism, and glycerolipid metabolism. The pathways that were most altered were ABC transporters, galactose metabolism, and tryptophan metabolism.*

***Figure 5.*** ***Alterations in amino acid metabolism. A)*** *All patients showed decreased levels of amino acids transported by LAT1. Leucine, phenylalanine, and the tyrosine metabolite 4-hydroxyphenyllactic acid were significantly decreased in RTT patients, while hypo-glutamatergic patients showed a similar tendency but were only significantly altered in phenylalanine and had slightly increased 4-hydroxyphenyllactic acid. Both groups had non-significantly decreased levels of valine.* ***B)******Alterations in tryptophan metabolism.*** *Of the metabolites involved in tryptophan metabolism that were analyzed, only 5-Hydroxyindole-3-acetic acid was significantly decreased in both disease groups, while kynurenine was significantly decreased in RTT patients and non-significantly decreased in hypo-glutamatergic patients. Tryptophan, N-Acetyl-5-hydroxytryptamine, anthranilic acid, and kynurenic acid showed a slight decrease that was not statistically significant in both RTT and hypo-glutamatergic patients. 3-Hydroxyanthranilic acid did not vary between either group and the controls.*

***Supplementary Figure 1.***

***Supplementary Table 1.****.*