De novo germline variants in *Histone 3 Family 3A (H3F3A)* and *Histone 3 Family 3B* (*H3F3B*) associated with a severe neurodegenerative disorder and functional effect unique from their somatic mutations

Histones are nuclear proteins that associate with deoxyribonucleic acid (DNA) and allow DNA to be packaged into condensed chromatin. Histones are dynamically decorated with post-translational modifications (PTMs), which regulate such processes as DNA repair, gene expression, mitosis, and meiosis. The specific Histone 3 Family 3 (H3F3) histones (H3.3), encoded by *H3F3A* and *H3F3B*, mark active genes, maintain epigenetic memory, and maintain heterochromatin and telomeric integrity. Specific somatic mutations in *H3F3A* have been strongly associated with pediatric tumors, but no germline mutations have been described in humans. Here we report 23 patients, ages 4 months to 32 years, with de novo missense germline mutations in *H3F3A* or *H3F3B* who share a core phenotype of progressive neurologic dysfunction and congenital anomalies, but no malignancies yet.

These 16 mutations in 23 patients are all *de novo* and are not found in large population datasets. There are three recurrent mutations in our cohort; the two variants p.R18G and p.A115G were found in two unrelated patients, and one variant, p.T46I, was found in four unrelated patients. We hypothesized that these missense mutations contribute to the shared patient phenotype through the induction of epigenetic dysregulation of histone PTMs. Histones PTMs within the nucleosome affect chromatin state, mitotic initiation, and gene expression.

Therefore, histones from multiple tissues from several *H3F3A* and *H3F3B* patients were analyzed by mass spectroscopy (MS), which revealed that the mutant histone proteins are present at a level similar to that of wild-type H3.3. MS analysis allowed for quantitation of some of the PTMs on mutant histones, many of which showed strikingly aberrant patterns that suggested local, but not global, dysregulation of chromatin structure. These data suggest that the pathogenic mechanism of germline histone mutations is distinct from that of the cancer-associated somatic histone mutations. In addition, RNA-Seq on patient tissues showed a statistically significant upregulation of genes related to mitosis and cell division. Fibroblast lines derived from multiple unrelated patients showed increased proliferative capacity compared to normal human fibroblast control lines. We anticipate that characterization of the pathology behind this novel syndrome will provide insight into new therapeutic targets for the neurologic degeneration in these and non-syndromic patients.