Abstract:

Characterization of *ap4b1-/-* zebrafish as a novel *in vivo* model of SPG47 and its application in small molecule screens

The discovery of treatments for hereditary spastic paraplegia has been limited by the slow disease progression in knockout mouse models, their unsuitability for large-scale *in vivo* screens, and their relatively high costs. Zebrafish is a small vertebrate model that offers key benefits to circumvent these issues. Building on preliminary data in a morpholino oligonucleotide-based model, we engineered *ap4b1* knockout zebrafish lines using CRISPR/Cas9 technology to create the first zebrafish model of *AP4B1*-associated hereditary spastic paraplegia (SPG47). The central hypothesis of this proposal is that *ap4b1-/-* zebrafish exhibit morphological, biochemical and behavioral phenotypes relevant to AP-4 biology and SPG47 in humans and can be employed to test novel small molecule therapies. We anticipate that these phenotypes will (1) provide insight into AP-4-related cell biology *in vivo*; (2) establish a human-to-zebrafish paradigm for drug screens and translational research; (3) inform the *in vivo* modeling of other childhood-onset hereditary spastic paraplegias.

AP-4-associated Hereditary Spastic Paraplegia - A translational approach to an ultra-rare disease

Bi-allelic loss-of-function variants in genes that encode subunits of the adaptor protein complex 4 (AP-4) lead to prototypical yet poorly understood forms of childhood-onset and complex hereditary spastic paraplegia: SPG47 (AP4B1), SPG50 (AP4M1), SPG51 (AP4E1) and SPG52 (AP4S1). Here, we report a detailed cross-sectional analysis of clinical, imaging and molecular data of 156 patients from 101 families. Enrolled patients were of diverse ethnic backgrounds and covered a wide age range (1.0-49.3 years). While the mean age at symptom onset was 0.8 ± 0.6 years [standard deviation (SD), range 0.2-5.0], the mean age at diagnosis was 10.2 ± 8.5 years (SD, range 0.1-46.3). We define a set of core features: early-onset developmental delay with delayed motor milestones and significant speech delay (50% non-verbal); intellectual disability in the moderate to severe range; mild hypotonia in infancy followed by spastic diplegia (mean age: 8.4 ± 5.1 years, SD) and later tetraplegia (mean age: 16.1 ± 9.8 years, SD); postnatal microcephaly (83%); foot deformities (69%); and epilepsy (66%) that is intractable in a subset. At last follow-up, 36% ambulated with assistance (mean age: 8.9 ± 6.4 years, SD) and 54% were wheelchair-dependent (mean age: 13.4 ± 9.8 years, SD). Episodes of stereotypic laughing, possibly consistent with a pseudobulbar affect, were found in 56% of patients. Key features on neuroimaging include a thin corpus callosum (90%), ventriculomegaly (65%) often with colpocephaly, and periventricular white-matter signal abnormalities (68%). Iron deposition and polymicrogyria were found in a subset of patients. AP4B1-associated SPG47 and AP4M1-associated SPG50 accounted for the majority of cases. About two-thirds of patients were born to consanguineous parents, and 82% carried homozygous variants. Over 70 unique variants were present, the majority of which are frameshift or nonsense mutations. To track disease progression across the age spectrum, we defined the relationship between disease severity as measured by several rating scales and disease duration. We found that the presence of epilepsy, which manifested before the age of 3 years in the majority of patients, was associated with worse motor outcomes. Exploring genotype-phenotype correlations, we found that disease severity and major phenotypes were equally distributed among the four subtypes, establishing that SPG47, SPG50, SPG51 and SPG52 share a common phenotype, an 'AP-4 deficiency syndrome'. By delineating the core clinical, imaging, and molecular features of AP-4-associated hereditary spastic paraplegia across the age spectrum our results will facilitate early diagnosis, enable counseling and anticipatory guidance of affected families and help define endpoints for future interventional trials.