Genetics and Development (including Gene Regulation)

GENETICS AND DEVELOPMENT AND NON-STEROID HORMONE SIGNALING II

CRISPR / Cas9 Genomic Edition of the CDH2 Gene in Zebrafish Leads to Eye and Cardiac Malformation

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Introduction: The *CDH2* gene encodes a transmembrane protein responsible for calcium-dependent cell-cell adhesion that participates in the process of embrionic development. Neural development is achieved by neuronal neurulation, migration and differentiation, as well as axon growth and synapse formation. During pituitary development, *CDH2* was associated to the epithelium-to-mesenchymal transition, with cell migration from the marginal zone to the anterior pituitary gland, followed by terminal differentiation. Thus, a dysfunction in n-cadherins disrupts the architecture of the neural tube, cortical architecture of the embryonic brain and pituitary development. In a previous study in our laboratory, a patient was diagnosed with a homozygous variant located in the N-terminal region of CDH2 (p.Val289IIe) that culminated in congenital hypopituitarism and pituitary hypoplasia. Together, these observations indicate that cadherins, especially N-cadherin, play an indispensable role in the organization of neuroepithelial layers. Zebrafish has been widely used as a model for studies of gene functionality, as it has 70% genetic homology to humans, besides being a small animal with rapid development. Our goal was to generate a zebrafish knockout for the *CDH2* gene, using CRISPR Cas9 genomic edition to study its importance during development and analyze this gene in patients with characteristics similar to those observed in zebrafish. **Material and methods**: Three guides were drawn for the CDH2 gene using crispor program. sgRNA, produced by in vitro transcription, and Cas9 protein were injected into one cell stage. All developmental parameters were observed under a microscope up to 96 hours post fertilization (hpf). Mortality rate were calculated at 24, 48, 72 and 96 hpf. The embryos were genotyped to confirm the deleterious allelic variant. *CDH2* coding region was evaluated in 3 female siblings, born from consanguineous parents, presenting micro/anophtalmia and short stature. Exons 2 to 16 were sequenced by the Sanger method. **Results:** 352 eggs were injected and several deformities such as absence of somites, cardiac edema, spinal curvature, cranial malformation and microphthalmia or total absence of eyes were observed. The mortality rates were 26%, 31%, 40% and 62% at 24, 48, 72 and 96 hpf, respectively. The Sanger sequencing from DNA extracted from the whole animal presented deleterious effect classified as insertions, deletions and missense changes. No deleterious allelic variant was observed in the 15 analyzed exons in the 3 patients. **Conclusion**: The CDH2 gene is important for neurodevelopment and eyes formation in the zebrafish although pathogenic allelic variants in this gene was not found in the studied patients with short stature and eye abnormalities.

Bone and Mineral Metabolism

BONE DISEASE FROM BENCH TO BEDSIDE

Growth Hormone Replacement in Adults During 8 Years Leads to Sustained Increase in Bone Mineral Density

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Introduction: Adult growth hormone deficiency (AGHD) is associated with lower bone mass and likely with increased risk of fragility fractures. GH replacement leads to increase in bone mineral density (BMD). However, only few studies longer than 2 years exist. **Aim:** To assess long-term effect of recombinant GH replacement on BMD and bone turnover markers duringperiod of 8 years. **Patients & Methods:** Prospective follow-up of all (N=63) AGHD patients at one single center. All patients with adult GHD followed at single center. All participants were replaced with daily injection of recombinant human (rh) GH in IGF-1 normalizing regimen according to Endocrine Society Guidelines. Every 2 years, lumbar spine (L-spine) and total hip (TH) BMD using dual X-ray absorptiometry on Hologic Discovery device, was assessed. All patients were assessed for bone turnover markers; carboxy-terminal collagen crosslinks (CTx) and osteocalcin (OC), and 25(OH)D levels. Deficiencies of other pituitary axes were treated if necessary. All patients were supplemented with 800 IU /day of cholecalciferol and 1000-1200mg/day of calcium as recommended by International Osteoporosis Foundation. **Results:** Study group consisted of 38 males and 25 females (35 with adult onset (AO) /28 with childhood onset (CO); mean age at diagnosis 25,1 yrs) AGHD patients. All patients ended 8 years follow-up period without any treatment discontinuation during this period. Treatment was well tolerated, without any serious adverse event. IGF-1 has reached the normal ranges during first 6 months and remains normal during whole study period documenting good adherence to treatment (average dose of rhGH=0,4 mg/day). Both, L-spine and TH BMD increased significantly after 8 years of GH replacement (+8 % for L-spine BMD, +7,7% for TH BMD, both $p<0,01$). The highest peak of BMD was observed after 6 years of treatment. CTx increased by 35% ($p<0,05$) and remain stable, and no significant change in OC was observed during study period. Levels of 25(OH)D increased by 32% ($p<0,05$) from baseline. No clinical fractures were observed. **Conclusion:** Long-term GH replacement in adult GHD together with sufficient levels of vitamin D levels led to increase in BMD and CTx. This study supported fact that GH has sustained effect on bone mass and bone turnover and is safe and well -tolerated for the long time period.