Study of AR-DNA association in Spinal Bulbar Muscular Atrophy.

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Spinal and bulbar muscular atrophy (SBMA) is a progressive neuromuscular disorder characterized by the death of lower motor neurons. SBMA is caused by a CAG repeat expansion in the androgen receptor (AR) gene, located in the X chromosome. The pathogenesis of SBMA is androgen dependent, therefore only males are affected. In addition to the neuromuscular symptoms, such as muscle weakness, muscle atrophy, and fasciculations, patients also experience mild androgen insensitivity. AR is an intracellular receptor that, upon binding of its ligand, dihydrotestosterone (DHT), in the cytoplasm, translocates to the nucleus where it works as a transcription factor. In the nucleus, AR can either activate or repress the activity of specific genes. Despite studies reporting that glutamine expansion in AR can result in transcriptional dysregulation, no study to date has evaluated how the expanded CAG tract can impact the AR binding profile throughout the genome. To evaluate if the CAG tract size can impact AR-DNA biding in specific sites, we transfected HEK 293 cells with either the control (22 CAG repeats) or the affected (48 CAG repeats) AR expressing plasmids, tagged with GFP. After the addition of DHT to the media, AR-GFP translocation was observed from the cytoplasm to the nuclei of transfected cells. We next sought to evaluate whether the CAG expansion influence the binding of AR to the SORT1 and ELK3 loci by chromatin immunoprecipitation (ChIP) qPCR. Both genes have well known AR binding sites. SORT1 is associated with protein trafficking whereas ELK3 is involved in transcriptional regulation. These pathways have been previously associated with SBMA pathogenesis. The ChIP qPCR revealed a higher enrichment of AR48Q peaks in comparison to the normal length AR, over the input DNA. These preliminary results suggest that expansion of the CAG tract in the AR gene can result in altered AR-DNA binding. To expand this study, we will investigate genome wide AR-DNA association by ChIP sequencing and define the contributions of AR mutation to SBMA pathogenesis in iPSC-derived skeletal muscles and motor neurons.