Abstract

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Title of diploma thesis: Analysis of genomic regions bound and regulated by Ataxin-3

Spinocerebellar ataxia type 3 (SCA3), also known as Machado-Joseph disease, is a dominantly inherited neurodegenerative disease. In SCA3, the disease protein ataxin-3 (ATXN3) contains an abnormally long polyglutamine (polyQ) tract encoded by CAG repeat expansion. ATXN3 binds DNA and interacts with transcriptional regulators pointing toward a direct role of ATXN3 in transcription. It is conceivable that mutant ATXN3 triggers multiple, interconnected pathogenic cascades leading to neurotoxicity, however, the principal molecular pathomechanism remains elusive. Here, PCR analyses of 16 ATXN3-bound genomic regions recently identified by next generation sequencing of immunoprecipitated ATXN3-bound chromatin fragments confirmed enriched binding of ATXN3 to 5 genomic regions next to genes encoding CCAAT/enhancer binding protein delta (CEBPD), period circadian clock-2 (PER2), phosphatase and tensin homolog (PTEN), serine protease inhibitor family F2 (SERPINF2) and thrombospondin-1 (THBS1). To investigate putative regulatory effects of ATXN3, the ATXN3-bound genomic regions were subcloned in, luciferase reporter constructs. Subsequently, wild type (WT) and hemizygous ATXN3-knockout human neuroblastoma cell line (SH-SY5Y) were transfected with the constructs and analyzed for ATXN3-modulated changes in luciferase activity. In ATXN3 knockout cells, a repressive ATXN3-dependent change in luciferase activity was found for CEBPD and THBS1 suggesting that ATXN3 binds to these regions to enhance or maintain transcriptional repression. In cells overexpressing normal or mutant ATXN3, an ATXN3 isoform dependent, regulatory effect was also found for CEBPD and THBS1 suggesting that ATXN3 may be principally involved in the transcriptional regulation of these genes. The identified and analyzed genomic regions may contribute to pathogenetic processes involved in SCA3 disease.