

PPP2R2B DNA STRUCTURE AND GENE EXPRESSION IN SCA12 PATIENTS FROM INDIA

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The spinocerebellar ataxias (SCAs) are neurodegenerative disorders that share a complex neurological presentation of ataxia of adult-onset and an autosomal dominant inheritance. To date, at least 41 genetic loci have been assigned to particular SCAs. In SCA12, the disease is the result of an expanded (CAG)_n in the 5' of the PPP2R2B gene. The SCA12 (CAG)_n expansion is the most common ataxia encountered in the clinics in northern and eastern India. In order to understand why the disease seems largely restricted to a particular ethnic group, we carried out some genetic studies. To tease out the underlying genetic structure of the sequences around the gene and the CAG stretch we genotyped the patient samples using the Global Screening array V3 and constructed LD maps of this region in SCA12 patients who had tested positive and others who had tested negative. We also utilized sequence data from the 1000 genomes project to construct similar maps for other world populations.

On Genotyping 208 individuals with a neurological diagnosis, we identified 29 patients with an expanded allele at the SCA12 locus (>43 CAG repeats) at our hospital. The SCA12 patients had a normal allele, ranging from 9-38 (9 being most common) and a larger allele which was pathogenic with CAG repeats ranging from 44-65. Individuals who tested negative for SCA12 had CAG repeats in the range of 1-32 with 9 repeats being the most frequent and 9.94 being the average. The LD structure for the 466kb region seemed to be broadly conserved across populations. Interestingly this LD structure seemed to be slightly disrupted in individuals who were SCA12 positive.

The *PPP2R2B* gene is transcribed into several neuron-specific splice variants which differ in the 5' UTR region, these include - i) CAG repeat containing coding transcripts V3 and V10, ii) CAG repeat containing non-coding transcripts V11 and V12 iii) Transcripts without CAG repeat V2, V4, V5, V6, V8 and V9. We studied the expression pattern of four PPP2R2B splice variants (V3, V10, V11 and V12) in two brain regions- the cerebellum and temporal pole- using a patient's post-mortem brain sample diagnosed with SCA12 (84Y/Male; CAG Repeat Count- 13/60) and a matched control. Tissues were obtained from the NIMHANS brain bank. RNA was isolated and, cDNA synthesis was carried out. qPCR was performed in triplicate on QuantStudio 6 Flex using SYBR Green Chemistry and relative gene expression was calculated. We observed a lower expression of all four variants in the case compared to the control sample. In addition, the expression of all variants was lower in the cerebellum when compared with the temporal pole. It is important to understand the molecular genetics of this locus which is the causative gene for SCA12 which is one of the most prevalent causes of ataxia in many parts of India.