Pilot Study to Implement Molecular Detection of Triplet Expansion Repeats in A Cohort of Patients Suspected to Have Friedreich's Ataxia

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Background: Friedreich ataxia is a rare slow progressing autosomal-recessive neurodegenerative disease with a global prevalence of 1 in 40,000. It predominantly affects the nervous system resulting in ataxia of all limbs, cerebellar dysarthria, absent reflexes in the lower limbs, sensory loss and pyramidal signs. Along with non- neurological symptoms such as cardiomyopathy and diabetes. Main objective of this study was to genotype selected variants associated with Friedreich's ataxia in a cohort of clinically suspected Sri Lankan patients. Methodology: We conducted a cross-sectional retrospective study on patients clinically suspected to have Friedreich ataxia. They were tested using the molecular method of GAA repeat expansion. Patients were recruited from the Human Genetics Unit, Faculty of Medicine, University of Colombo following written informed consent. We selected 12 clinically suspected patients. Since this is a rare condition only 12 patients were found on the rare disease database which consists of over 600. DNA sample were extracted from the blood samples using QIAGEN blood DNA extraction kit following manufacturer's protocol. DNA samples were initially analyzed using conventional PCR followed by TP-PCR. Long Range PCR and Sanger sequencing was done to validate the genotyping assay. Results: Patients who were homozygous for Friedreich ataxia didn't give any bands on conventional PCR and gave ladder like bands for TP-PCR. Patients who were homozygous for the wild type of Friedreich ataxia variant had a single band with both conventional PCR and TP-PCR. Three samples were identified as homozygous mutant and nine samples were identified as homozygous wild type for Friedreich ataxia. In LR PCR, two confirmed homozygous mutant cases showed GAA repeats range between 580 to 800. Patient with homozygous wild type showed 5 GAA repeats in their DNA sequencing. Conclusion: This pilot study confirms that molecular detection of GAA triplet expansion repeats in patients suspected to have Friedreich's ataxia as a useful cost- effective confirmatory test that can be implemented in Sri Lanka. We hope to expand the sample population further in a future study.

Keywords: Friedreich's ataxia, GAA triplet expansion, Molecular testing, Sri Lanka, TP - PCR, Rare diseases