Cag/polyglutamine disease diagnostics

Summary

Polyglutamine disorders refer to a group of nine neurodegenerative conditions caused by a single gene mutation, where the expansion of a CAG trinucleotide repeat region in the disease-affected gene leads to the production of a long sequence of glutamine (Q) residues in the associated protein. Considering that almost 60% of the global population resides in Asia, it can be inferred that a significant proportion of HD and SCA patients are either of Asian descent or live in Asia. There is a lack of understanding regarding the prevalence of HD and SCA, genetic subtypes, and disease manifestations in most of Asian countries. There have been no previous studies that have conducted a thorough review of the genetic factors and serum biomarkers related to HD and SCA in the subpopulation of Sri Lanka. The aim of this study is to evaluate the CAG repeat expansion in the genes associated with HD and SCA, analyze the diversity in observable characteristics, and discover new biomarkers linked to proteins and metabolism.

In Chapter 2.1 we have shown that SCA1 was the most common factor, found in a total of 61 (19.0%) individuals of the cohort. This report also documents an extensive SCA1 pedigree in the Sri Lanka population, comprising 38 individuals. While there are limited data globally of large SCA1 pedigrees, our SCA1 pedigree is likely the largest reported in Asia. Even though PCR became the ideal technique for detecting CAG repeat expansion, in our studied cohort 155 patients [SCA; n= 113 (47.8%) and HD; n= 42 (48.3%)] were genetically negative for the tested mutations. Thereby, we suggest that establishing a PAN South Asian poly (Q) disease network to combine resources and conduct NGS analysis through collaboration among research groups and international partners could be of benefit for all partners.

In Chapter 2.2, we have postulated that the absence of comparative data pertaining to potential candidates for gene therapy clinical trials in HD may have resulted in the underrepresentation of South Asian HD patients in multicenter gene therapy clinical trials. Intriguingly, 45 HD patients in Sri Lanka were identified as candidates for available gene therapy (Antisense oligonucleotides- ASOs) using our low-cost molecular diagnostic approach. Further our study suggest a model designing Phase 3 clinical trials for rare diseases, incorporating developing countries through multicenter studies by mitigating issues with patient recruitment, attrition, comorbid conditions and expenses that have been identified as bottlenecks in designing long-duration clinical trials for rare diseases.

The inherited diseases community in Sri Lanka has historically received limited attention and frequently being stigmatized as ”incurable”. The availability of neurogenetic testing in government hospitals in Sri Lanka is extremely limited, while it is only accessible in a handful of private sector centers at exorbitant costs. In Chapter 2.3 we present accumulated knowledge in establishing a pro bono cost-effective, national molecular diagnostic service in Sri Lanka that is provided free of charge. This study offers valuable insights and recommendations that could serve as a blueprint for other developing nations pursuing to implement a similar program. Further, this study demonstrates the diagnostic yield of specific rare neuromuscular and trinucleotide repeat disorders in Sri Lanka, highlighting the challenges faced in conducting these investigations with limited resources and minimal cost. Additionally, it explores the uphill task of establishing a neurobiobank and emphasizes the value of investing in human resources for a sustained existence. Lastly the established neuro-biobank created a national wealth by fostering international collaborations focused on advancing genetic networks within cutting-edge multicenter translational therapeutic initiatives.

The South Asian demographic presents an abundant reservoir of untapped potential for the exploration of human genetic discoveries. To the best of our knowledge, the study discussed in chapter 2.4 is the very first instance documenting c.1982A>G mutation in the DDHD2 gene globally to the best of our knowledge. This newly identified variant is believed to be pathogenic for hereditary spastic paraplegia type 54 (SPG54, OMIM: 615033). This mutation was observed in a 4-year-old male who is clinically presumed to have HSP. Moreover we were able to find out a presence of a missense mutation ZFHX3 c.530C>T, which is responsible for SpinoCerebellar ataxia type 4, which is not a pathogenic variant in a patient clinically diagnosed with SCA.

In Chapter 3.1 we outlined a systemical study to identify and quantify serum proteomic biomarkers in SCA1-3 patients from a limited resources South Asian setting. We targeted a proteomic screen of 1300 proteins using an aptamer-based screening approach in SCA. The data reveal that LCN2 is substantially increased in SCA1-3, while Lactoferrin is significantly increased in SCA2 and SCA3. Further the AUC values from logistic regression analysis, indicating that LCN2 has the potential to be a biomarker in SCA1 patients. This study necessitates additional research aimed at developing techniques to diminish LCN2 levels and transform activated astrocytes into the neuroprotective A2 subtype, which can protect neuronal cells in SCA1. The higher AUC values from logistic regression analysis reinforce the possibility of Lactoferrin as a candidate biomarker for SCA2 and SCA3 patients.

In Chapter 4.1 we documented the serum metabolic signatures of SpinoCerebellar Ataxias (SCA1-3), from a South Asian perspective. In this study, a notable increase in the expression of 1-palmitoyl-2-arachidonoyl-GPI(16:0/20:4) and 2-hydroxypalmitate was observed within the subpathways of lipid metabolism, specifically, phosphatidylinositol(PI) and fatty acid, monohydroxy, respectively, among individuals diagnosed with SCA2. The metabolites,

1-palmitoyl-2-arachidonoyl-GPI(16:0/20:4) and 2-hydroxypalmitate, have not been previously reported to be associated with SCAs or other neurodegenerative disorders. The comparative analysis of metabolites revealed that the altered super pathway in SCA1 (kynurenate), SCA2 (N-acetyl-aspartyl-glutamate, N-acetyl-isoputreanine), SCA3 (8-methoxykynurenate, Valine) were primarily related to amino acid metabolism which could be further validated as metabolic biomarkers in large cohort studies.

Finally in Chapter 4.2 we investigated the composition of essential oils, methanolic and hexane extraction of Bark and leaf extracts of Ceylon cinnamon and its wild species. The findings of the study reveals that the bark of Sri Vijaya and *C. rivulorum*, along with the leaves of Sri Vijaya and *C. sinharajaense*, exhibited remarkable antioxidant properties. The cinnamaldehyde percentage of Sri Gemunu was the highest at 61.63%, while the bark of *C. sinharajaense* contained 59.19%. The leaves of Sri Gemunu had the highest eugenol content, determining at 91.45%. *C. zeylanicum* and its strains have been found to exhibit the highest antioxidant and anti-inflammatory properties. *C. sinharajaense* and *C. rivulorum* are two wild cinnamon species that show potential for potential medicinal uses. This research can open avenues for studying the effect of gut microbiota utilising metabolomic approaches and clinical trials with natural products specific to cinnamon species in disease-modifying, progression, and response to the design of new therapeutic targets.