Laboratory Diagnosis Of The Five Common Spinocerebellar Ataxias (Type 1, 2, 3, 6 And 7) In Institute For Medical Research (IMR): Malaysia Experience

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INTRODUCTION

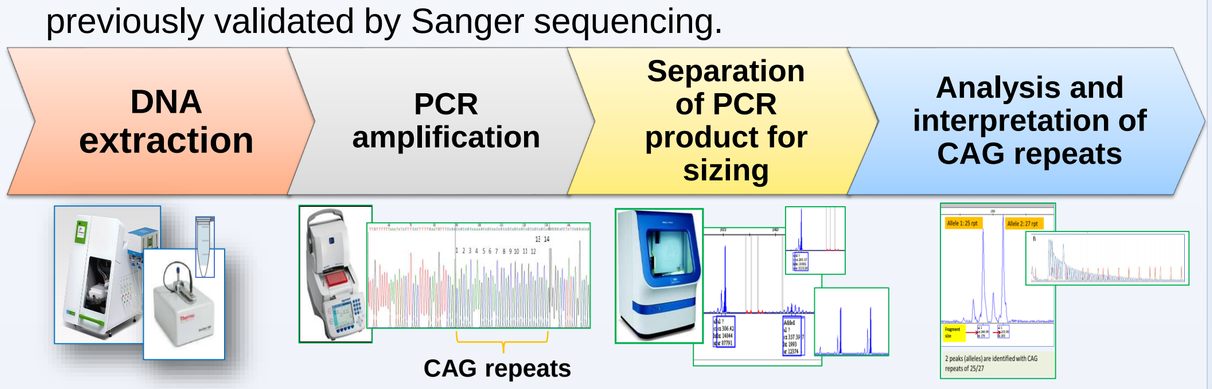
The spinocerebellar ataxias (SCAs) are a group of clinically and genetically heterogeneous neurodegenerative diseases causing progressive cerebellar dysfunction, mostly with adult-onset[1]. There are over 50 distinct subtypes of SCA have been identified to date, and diagnosis is a challenge as it cannot be reliably differentiated on a clinical basis due to variable expressions and overlapping phenotypes. Therefore, an accurate diagnosis of SCAs greatly relies on molecular testing to detect an abnormally expansion mutation of CAG trinucleotide repeats in a specific causative gene. Taken together, SCA1, 2, 3, 6 and 7 are the most prevalent autosomal dominant ataxias worldwide, accounting for approximately 60% of the cases.

OBJECTIVES

1. To describe molecular approaches for confirmation of the five common SCA subtypes, namely SCA1 (*ATXN1* gene), SCA2 (*ATXN2* gene), SCA3 (*ATXN3* gene), SCA6 (*CACNA1A* gene) and SCA7 (*ATXN7* gene).
2. To present a 4-year molecular data (2017-2020) of SCA cases referred to IMR, the referral centre for molecular genetic testing in Malaysia.

MATERIALS & METHODS

- A total of 214 individuals with clinically suspicion of SCAs were referred to IMR for molecular confirmation by clinical geneticists and neurologists throughout Malaysia.
- The genetic testing employed a targeted amplification of the polyglutamine region in *ATXN1* (SCA1), *ATXN2* (SCA2), *ATXN3* (SCA3), *CACNA1A* (SCA6), and *ATXN7* (SCA7) gene by fluorescent-PCR (F-PCR) followed by capillary electrophoresis to generate array of peaks for identification and sizing of the CAG tri-nucleotide repeats number.
- Triplet-primed PCR, (TP-PCR) a second-tier method was carried out to verify the homoallelism status of normal individuals with apparent homozygous and to confirm the presence of large expansion for individuals with expanded allele detected by F-PCR.
- Allele sizes were determined by known-CAG repeat control samples previously validated by Sanger sequencing.



RESULTS & DISCUSSION

- In total, 67 of 214 (31%) referred patients were molecularly confirmed being affected by either SCA1, 2, 3 or 6. Figure 1 illustrated the prevalence of each SCA subtype in Malaysia.
- SCA3 was the commonest, detected in 40 (60%) individuals, followed by SCA2 in 15 (22%), in accordance to the worldwide data[1].
- The third most common SCA subtypes is SCA1, detected in 10 (15%) and the next is SCA6, detected in two (3%) individuals. This is in contrary to the worldwide data in which SCA6 is the third most common SCA genotype followed by SCA1[1].

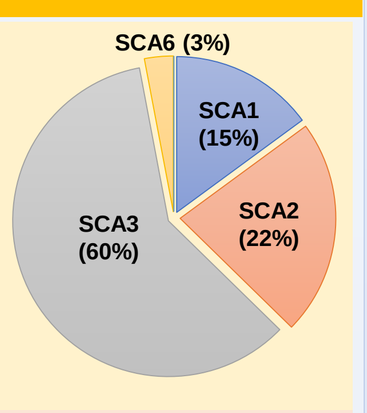


Figure 1 shows the prevalence of SCA subtypes in Malaysia.

- India is among the country with the highest prevalence of SCA1 (22%)[1] and coincidentally, 60% of our SCA1 patients were from Indian ethnicity. Given that Malaysia is a multi-ethnic country, this perhaps explains why our prevalence of SCAs is different from others.
- No positive case for SCA7, making it the least common in this study which also has the lowest prevalence worldwide (0-3%)[1]

RESULTS & DISCUSSION (continued)

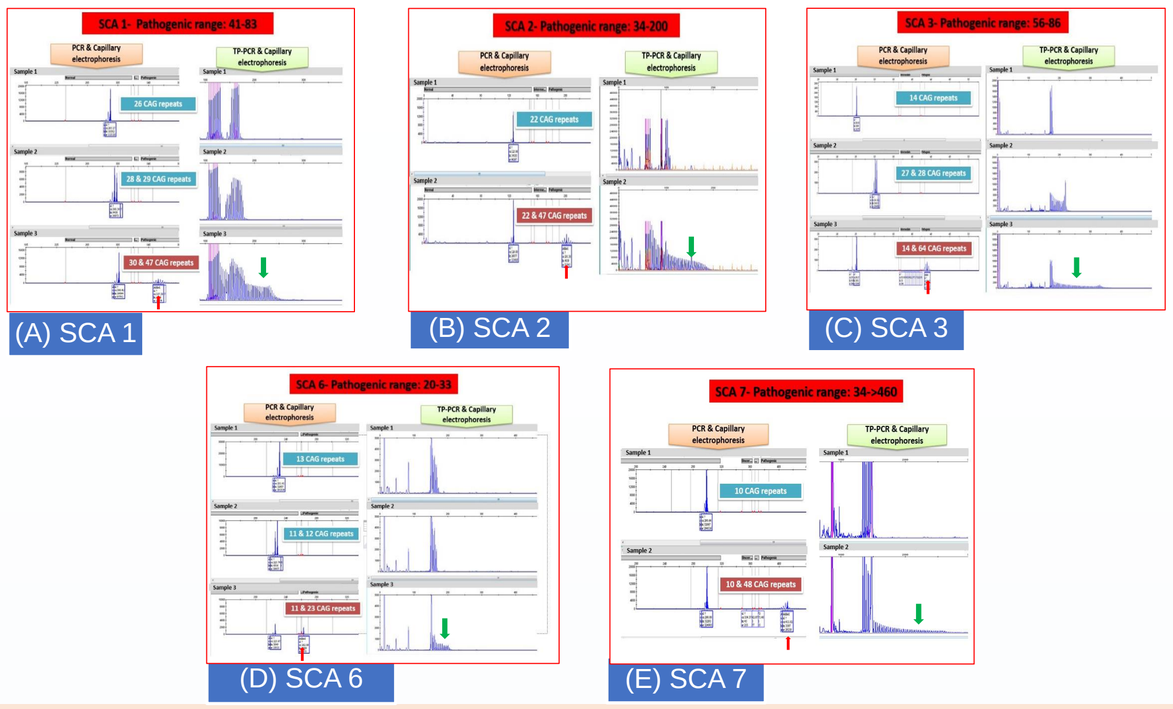


Figure 2 shows electropherograms for each SCA subtype tested. Each panel contains normal (homo and hetero-allelic) and expanded alleles amplified by F-PCR (on the left side) and TP-PCR (on the right side) that was employed to confirm the absence of large expansion when only one normal allele was identified by F-PCR as well as verifying the presence of expansion as illustrated in A, B, C, D and E. The allele size was expressed as number of CAG repeats. Reference range of each SCA subtypes are also presented[2]. Please note that the expanded allele for SCA7 (E) was identified in quality control material but not from the patient sample. For each SCA, the presence of expansion was detected as a bell-shaped array of peaks in F-PCR (red arrow) whereas in TP-PCR showed as a slowly descending array of peaks (green arrow).

- The F-PCR and capillary electrophoresis followed by TP-PCR have been proven as a rapid and cost-effective strategy for diagnosis of SCA.
  - ✓ F-PCR is able to determine an accurate repeat sizing with an acceptable error limit of ± 1 triplet repeat for normal and ±3 triplet repeat for expanded alleles with exception of only ± 1 triplet repeat is acceptable for SCA6 expansions, in accordance with European Molecular Genetics Quality Network (EMQN) guidelines[3]. In addition, it is able to resolve heterozygous alleles differ in 1 CAG-repeat as seen in Fig 2A (28/29), Fig 2C (27/28) and Fig 2D (11/12). We managed to report the genotypes concordant to the consensus result over the past two years' participation in EMQN.
  - ✓ TP-PCR can confirm the presence or absence of a pathogenic allele regardless of limitation of detection range in F-PCR assay, therefore allowing a clear distinction between true homoallelism and expansion. This is particularly important in SCA2 and SCA7 where homoallelism is frequent and very large expansions (>400) are known to be present in juvenile-onset cases[4].
  - ✓ The normal and pathogenic size ranges were also well-characterized. A pathogenic range of 64-80 repeats have been observed from our cohort of SCA3 patients.

CONCLUSION

- We have provided a comprehensive molecular testing for SCAs which enables identification of the five common SCA subtypes followed by a complementary method to address possible inconclusive results.
- Participation in external quality assurance program EMQN since 2019 has thus improved the performance quality and ensuring reliability of SCA genetic testing in our laboratory.

REFERRENCES

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