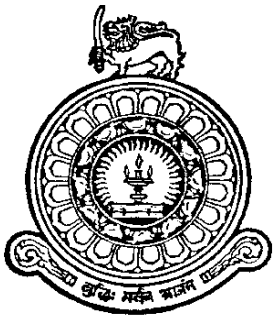
CLINICAL FEATURES AND GENETIC ETIOLOGY OF SPINOCEREBELLAR ATAXIA IN A COHORT OF

SRI LANKAN PATIENTS



BY

WADUWAWATTE LEKAMALAGE DULIKA SANJEEWANI SUMATHIPALA, MBBS

HUMAN GENETICS UNIT

FACULTY OF MEDICINE

UNIVERSITY OF COLOMBO

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Declaration

I declare that the contents of this thesis are my own work, except for that detailed below, for which I would like to thank the following person:

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Abstract

Introduction: Hereditary ataxias are rare neurodegenerative disorders reported from most of the world. Among the dominant ataxias more than 30 are described, with distinct variable genotypes' distribution according to the geographic area. Although there is to this date no cure for the disorders, molecular genetics are a fast expanding field and it is important to determine the disease prevalence and its natural history in all populations. This study is the first attempt at molecular genetic analysis of hereditary ataxia in the Sri Lankan population.

Objectives: The aims of this study were to investigate clinically and molecularly identified Sri Lankan patients with hereditary ataxia and evaluate the spectrum of genotypes-phenotypes correlations.

Methods: The research described in this dissertation included 46 patients; 34 diagnosed with autosomal dominant cerebellar ataxia, 8 with autosomal recessive ataxia and 4 with sporadic ataxia. Clinical history, physical examination findings, psychological and cognitive profiles and investigation findings such as brain CT and MRI were documented. 5 ml of venous blood was drawn from each patient and genomic DNA was extracted. Genetic analysis for SCA 1, 2, 3, 6, 7, 8, 12, 17 and Friedreich's ataxia was performed.

Results: Spinocerebellar ataxia type 1 (SCA1) was identified in 21 patients from 12 families. Of the 21 patients 15 were from a single geographical location in the southern province. A single patient with SCA 2 was also identified. Mean CAG repeat length in the affected allele of SCA 1 patients was 52.0±3.8. SCA 3, 6, 7, 8, 12 and Friedreich's ataxia mutations were not seen. Mean age at onset of patients with SCA 1 was 34.8±110 years, disease duration 7.4±3.1 years, and 76.2 % of the patients were depending on walking aids. Mean SARA and INAS

scores were 18.8±9.7 and 3.6±2.4. Clinically relevant depression was present in 68.4% of the patients.

Conclusion: SCA1 and SCA2 were the only types of SCA identified in Sri Lanka, with SCA 1 as the most prevalent type responsible for 61.7% of autosomal dominant ataxias. There were no major differences between earlier reported SCA 1 phenotypes and genotypes and the present population. Depression comorbidity was high, highlighting the need for supportive care in this progressive neurological disorder. A founder mutation is hypothetized and is an important future area of research.

1.0 Introduction and Background

Ataxia is derived from the Greek words "a" meaning not and "taxis" meaning "order". Hereditary ataxias are a group of neurodegenerative diseases that can be inherited in dominant, recessive or X-linked manner. This thesis describes the first reports about the clinical characteristics and genetic nosology of hereditary ataxia in a selected patient population of Sri Lanka.

1.1 Definitions

'*Ataxia*' refers to the inability to fine tune posture and movement in an orderly manner. It is a non – specific clinical symptom, with many possible underlying causative factors. Types of ataxia may be broadly classified into cerebellar, sensory and vestibular.

Sensory ataxia is caused by the loss of sensory proprioception. The underlying defect is in the sensory fiber peripheral neuropathy, dysfunction of the dorsal column of the spinal cord or cerebrum. This is caused by a variety of disorders: infectious, auto-immune, metabolic, toxic, vascular and hereditary diseases. As this is caused by the lack of sensory input rather than a cerebellar dysfunction, patients are clinically characterized by a discrepancy between near normal movement with open eyes and clearly worsened ataxia with eyes closed. Clinical examination reveals a positive Romberg's sign (Sghirlanzoni *et al.* 2005).

'*Vestibular ataxia'*: Defects in the vestibular system are another cause of ataxia. The acute onset may be accompanied by vertigo, nausea and vomiting. In chronic slowly progressive vestibular ataxia, disequilibrium may be the only symptom. Causative factors may be focal lesions in the vestibular system or vestibular region of the cerebral cortex, exogenous toxic substances such as ethanol and acute labrynthitis.

Cerebellar ataxia refers to disease due to dysfunction in the cerebellum or the afferent and or efferent pathways of the cerebellum. This may be further categorized as:

(1) dysfunction of the vestibulocerebellum (flocculonodular lobe) which impairs the balance and the control of eye movements, (2) dysfunction of the spinocerebellum (vermis and associated areas near the midline) presents itself with a wide-based "drunken sailor" gait (called truncal ataxia), (3) dysfunction of the cerebrocerebellum (lateral hemispheres) presents as disturbances in carrying out voluntary, planned movements by the extremities (Diener *et al.* 1992).

In a genetic perspective ataxia may be inherited or acquired. Inherited ataxias can be further divided into autosomal dominant, autosomal recessive, X – linked and mitochondrial ataxias. Acquired ataxia are divided into primary (congenital) and secondary ataxias (Table 1) (Manto *et al.* 2009). Sporadic ataxia is an interesting group of ataxia that may fall under either acquired or inherited ataxias. Many clinical diagnostic dilemmas arise from sporadic ataxias.

This dissertation will focus mainly on autosomal dominant cerebellar ataxias; however a brief classification of all hereditary ataxia types will be presented.

Table 1 Classification of ataxia (Manto *et al.* 2009)

Hereditary ataxia

Autosomal Dominant Cerebellar Ataxia

- *(a) Spinocerebellar ataxia (1 -36)*
- *(b) Episodic ataxias*

Autosomal Recessive Ataxia

- *(a) Friedreich's ataxia (though primarily a sensory ataxia rather than a cerebellar ataxia)*
- *(b) Ataxia with Occulomotor apraxia type 1 and type 2*
- *(c) Ataxia with isolated Vitamin E deficiency*
- *(d) Ataxia telangiectasia*

X – Linked Cerebellar ataxias

- *Fragile X tremor / ataxia syndrome*

Mitochondrial Disorders

- *Kearns – Sayre syndrome, MEERF (myoclonic epilepsy with ragged – red fibers), MELAS (mitochondrial encephalopathy, lactic acidosis, and stoke like episodes, Leigh syndrome)*
- *POLG (DNA polymerase gamma) ataxia – neuropathy disorder*

Non-hereditary ataxia

Sporadic and Acquired ataxia

(1) Degenerative ataxia

- *(a) multiple system atrophy (MSA)*
- *(b) idiopathic late onset cerebellar ataxia (ILOCA)*

(2) Acquired ataxia

- *(a) Stroke (infarction, hemorrhage)*
- *(b) Toxic induced – ethanol, drugs (antiepileptic agents, lithium salts, antineoplastics, metronidazole), heavy metals, solvents*
- *(c) Immune mediated – multiple sclerosis, gluten ataxia, cerebellar ataxia with anti – glutamic acid decarboxylase (GAD) antibodies, systemic lupus erythematosis, Sjoren syndrome, Cogan syndrome, thyroiditis, Miller – Fisher syndrome, paraneoplastic cerebellar syndrome*
- *(d) Infectious / parainfectious diseases (abscess, cerebellitis)*
- *(e) Traumatic*
- *(f) Neoplastic disorder (cerebellar tumor, metastatic disease)*
- *(g) Endocrine (hypothyroidism)*
- *(h) Structural disease (Chiari malformations, agenesis, hypoplasia, dysplasia)*

1.2 Hereditary Ataxias

Classification of the hereditary ataxias based on phenotype or pathological findings is inadequate, due to the very large phenotype-genotype heterogeneity. Affected subjects with the same genotype have marked phenotype heterogeneity. Conversely, different genotypes produce similar phenotypic features and similar neuropathological features. These circumstances make the classification of hereditary ataxia (HA) difficult.

Autosomal dominant cerebellar ataxia (ADCA) is usually an adult onset disease that is clinically heterogeneous. The main neurological feature is the presence of a progressive cerebellar ataxia. Phenotypical differences between the different ADCA mainly depend on the occurrence of additional non cerebellar symptoms. Clinical-genetic classifications were achieved initially by Anita Harding, who devised a classification (Table 2), which was universally accepted in the pre-molecular era.

Table 2 Harding's Clinical-genetic classification of the hereditary ataxias (Harding 1983)

1. *Autosomal dominant cerebellar ataxia with optic atrophy/ ophthalmoplegia/ dementia/ extrapyramidal features/ amyotrophy (ACDA type 1)*
2. *Autosomal dominant cerebellar ataxia with pigmentary retinal degeneration ± ophthalmoplegia or extrapyramidal features (ADCA type 2)*
3. *'Pure' autosomal dominant cerebellar ataxia of later onset (older than 50 years) (ADCA type 3)*
4. *Autosomal dominant cerebellar ataxia with myoclonus and deafness (ADCA type 4)*

According to the above table Harding distinguished 4 main groups of genetically heterogeneous Autosomal Dominant Cerebellar Ataxia (ADCA I - IV).

Following the identification of genetic mutations leading to autosomal dominant cerebellar ataxia the disease classification underwent a further revolution. The first ataxia gene was identified in 1993 and was called spinocerebeller ataxia type 1(SCA 1) (Kwiatkowski *et al.* 1993). As additional dominant genes were found they were called SCA2, SCA3, etc. Usually, the "type" number of "SCA" refers to the order in which the gene was found. (SCA 1 - 36). At present a purely genetic classification based on the genetic loci of the mutation of spinocerebellar ataxia is accepted.

Episodic ataxia is a hereditary disorder where recurrent episodes of vertigo and ataxia are variably associated with progressive ataxia. There are 7 types of episodic ataxia described in the literature, with type 1 and 2 (EA1, EA2) being the most common. They are also considered as ion- channel disorders and have a high frequency of epilepsy and migraine as associated symptoms (Jen *et al.* 2007).

A genetic classification for Autosomal Recessive Cerebellar Ataxia (ARCA) is still under development, and at present are named haphazardly, according to clinical features, protein dysfunction geographical origin etc. (Friedreich's ataxia, ataxia with vitamin E deficiency, ataxia telangiectasia) (Anheim *et al.* 2012).

Fragile X tremor/ ataxia may be classified among the X-linked hereditary ataxias. Patients exhibit combinations of kinetic tremor, ataxia of gait, Parkinsonism, autonomic dysfunction, polyneuropathy and cognitive deficits (Berry-Kravis *et al.* 2007).

Cerebellar ataxia, particularly gait ataxia, is a frequent finding or often an associated symptom in many mitochondrial disorders, and therefore an etiology to remember in investigation of recessive ataxias (Zeviani *et al.* 2012).

Sporadic ataxia is a term coined when there is an absence of family history as well as any known etiological cause to the ataxia. This is a clinical description of a patient rather than a disease entity in itself.

Genetic as well as clinical features that mask the hereditary nature of a disease are listed below.

Anticipation – A feature associated with triplet repeat disease. With each successive generation the number of CAG repeats increases in the unstable allele. This causes disease phenotypes to manifest earlier with greater severity with each successive generation. Thereby an apparently sporadic ataxia patient may have a parent that manifested a mild ataxic phenotype at an advanced age missed in the information gathering.

De novo mutation – An alteration in a gene that is present for the first time in one family member as a result of a mutation in a germ cell of one of the parents or in the fertilized zygote. In triplet repeat mutations it is the family member that has expansion mutation of the triplet codons in a sufficient number to result in disease manifestation.

Variable expressivity –This is the variation in phenotype with the same underlying genetic mutation. In CAG repeat diseases there is a repeat length dependent, variable phenotype. (e.g.: The largest SCA3 expansions cause disease onset in childhood or teenage years, manifesting with widespread dystonia, spasticity, and ataxia. In contrast, smaller SCA3 expansions lead to late-onset ataxia, commonly with a degree of peripheral neuropathy and motor neuron loss.

The smallest SCA3 expansions, those very close to the lower limit of disease repeats, may result in restless leg syndrome and very little else (Dubourg *et al.* 1995).

X inactivation - A genetic phenomenon is a process by which one of the two copies of the X chromosome present in females is inactivated. With carriers of mutation in the X chromosome this inactivation is skewed with the mutated X chromosome preferably inactivated. In the instances of X – linked disease such as Fragile X ataxia/ tremor the mutation may be derived from the mother but manifest in the son as a sporadic ataxia.

Non penetrance/ reduced penetrance – In triplet repeat mutation carriers; expansions bordering on the normal repeat length may manifest a family history compatible with a sporadic onset of ataxia. This is due to the reduced penetrance seen in those with trinucleotide repeat lengths close to normal levels

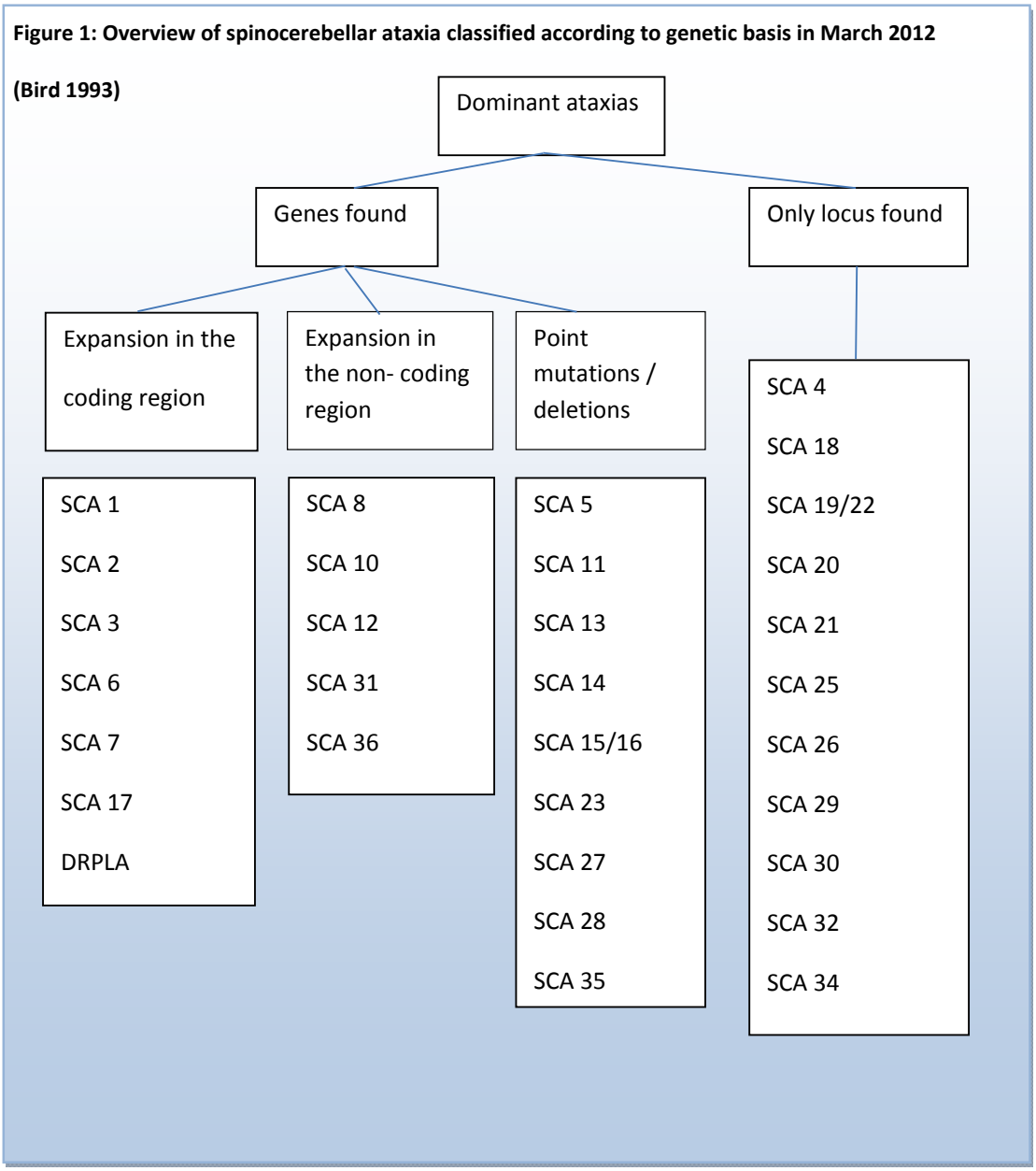
Further complicating factors such as small family size, incorrect family data, adoption with absence of family data, or early death of affected family members may hide the monogenic nature of inheritance within the family.

1.3 *Genetic classification*

Autosomal Dominant Cerebellar Ataxia (ADCA)

The genetic loci of ADCA have been mapped in 34 subtypes of SCA; in 22 the gene has been identified. The genetic mutation in 12 of the 18 is repeat expansion in the corresponding gene (Bird 1993). The rest are conventional mutation resulting in disruption of the gene. Repeat expansion mutations can be further subdivided into CAG trinucleotide mutations in the coding region of genes or polyglutamine expansion mutation and repeat mutation in the noncoding portion of corresponding mutant genes.

Figure 1: Overview of spinocerebellar ataxia classified according to genetic basis in March 2012 (Bird 1993)



Polyglutamine / Coding region Expansion SCA

Polyglutamine expansion SCA are the first subtype of SCA discovered. After identification of SCA1 in 1993, 6 more have been discovered with the same CAG trinucleotide expansion mutation: SCA2, SCA3, SCA6, SCA7, SCA17 and DRPLA between 1994 and 1999. The initial presenting clinical features include gait disturbance in two thirds of those affected along with diplopia, dysphagia, episodic vertigo and change in handwriting. Age of onset is

usually between the third and fourth decade. There is degeneration of cerebellar functions with death resulting from brain stem failure. The MRI findings of patients reveal cerebellar and brainstem atrophy (Durr 2010). Disease phenotypes manifest above a threshold of triplet repeat mutation, which differs with each gene. The average number of CAG repeats that result in disease manifestation is between 37 and 40; however there are exceptions with SCA6 manifesting with CAG repeats above 19 and SCA3 with CAG repeats above 51. The characteristic feature of CAG repeat expansion mutation is anticipation: the increase in disease severity and decrease in age of onset with each subsequent generation. This is due to the unstable trinucleotide expansion that increases in size with gametogenesis, especially in paternal transmission (Chung *et al.* 1993). Apart from the age at onset the disease phenotype also varied according to the CAG repeat length. For example, in patients with SCA3 the frequency of pyramidal signs increased with CAG repeat length and the frequency of altered vibration sense decreased (Dubourg *et al.* 1995).

Non-coding expansion SCAs

SCA 8, 10, 12, 31 and 36 are non-coding expansion SCAs: expansion of repeating segments are present in the intron or untranslated region of the genome. Disease phenotype is mild with a wide range of age of onset of disease. In comparison to coding region mutations these SCA subtypes exhibit less brainstem involvement on MRI (Durr 2010).

SCA8 was the first non-coding expansion SCA to be identified. The underlying mutation of CTG repeat expansion is controversial as there is no correlation between repeat length expansion and penetrance and repeat expansion has been noted in controls and patient with other diseases (Schols *et al.* 2003). In SCA 10 a pentanucleotide expansion, ATTCT has been discovered in intron 9 of *ATXN10* gene. Rather than a gain or loss of function the underlying disease mechanism is thought to be related to the altered chromatin structure caused by the

pentanucleotide expansion (Matsuura *et al.* 2000). A CAG repeat expansion in the *PPP2R2B* has been found to be the causative mutation for SCA12 (Holmes *et al.* 1999). Dysregulation of mitochondrial morphogenesis is thought to be the underlying disease mechanism. SCA 31 is a pentanucleotide repeat expansion TGGAA of the thymidine kinase 2 (*TK2*) gene (Ishikawa *et al.* 2011). The clinical age of onset of ataxia is relatively late (mean of 60 years) and there is an associated hearing impairment. The latest progressive ADCA, SCA36 is caused by heterozygous expansion of an intronic GGCCTG hexanucleotide repeat in the *NOP56* gene on chromosome 20p13 (Kobayashi *et al.* 2011). Unaffected individuals carry 3 to 8 repeats, whereas affected individuals carry 1,500 to 2,000 repeats.

Conventional mutation SCAs

Spinocerebellar ataxias caused by conventional mutations are SCA5, 11, 13, 14, 15/16, 23, 27, 28 and 35. These SCAs are relatively rare and are clinically distinct from polyglutamine expansion SCAs. The age of onset is variable with childhood onset common, but without the rapid progression seen in juvenile onset polyglutamine SCAs. Congenital mental retardation associated with conventional mutations is stable and does not show deterioration with age. Imaging shows global cerebellar atrophy with no brainstem involvement (Durr 2010).

Episodic Ataxia

Episodic ataxias (EA) have seven subtypes and are characterized by episodic bouts of cerebellar symptoms and signs with relatively sparse findings in between. EA 1 and 2 are the most common. The mechanism of disease is mainly through mutations in protein responsible for ion- channels, therefore many of the episodic ataxias are also called chanellopathies (Jen *et al.* 2007).

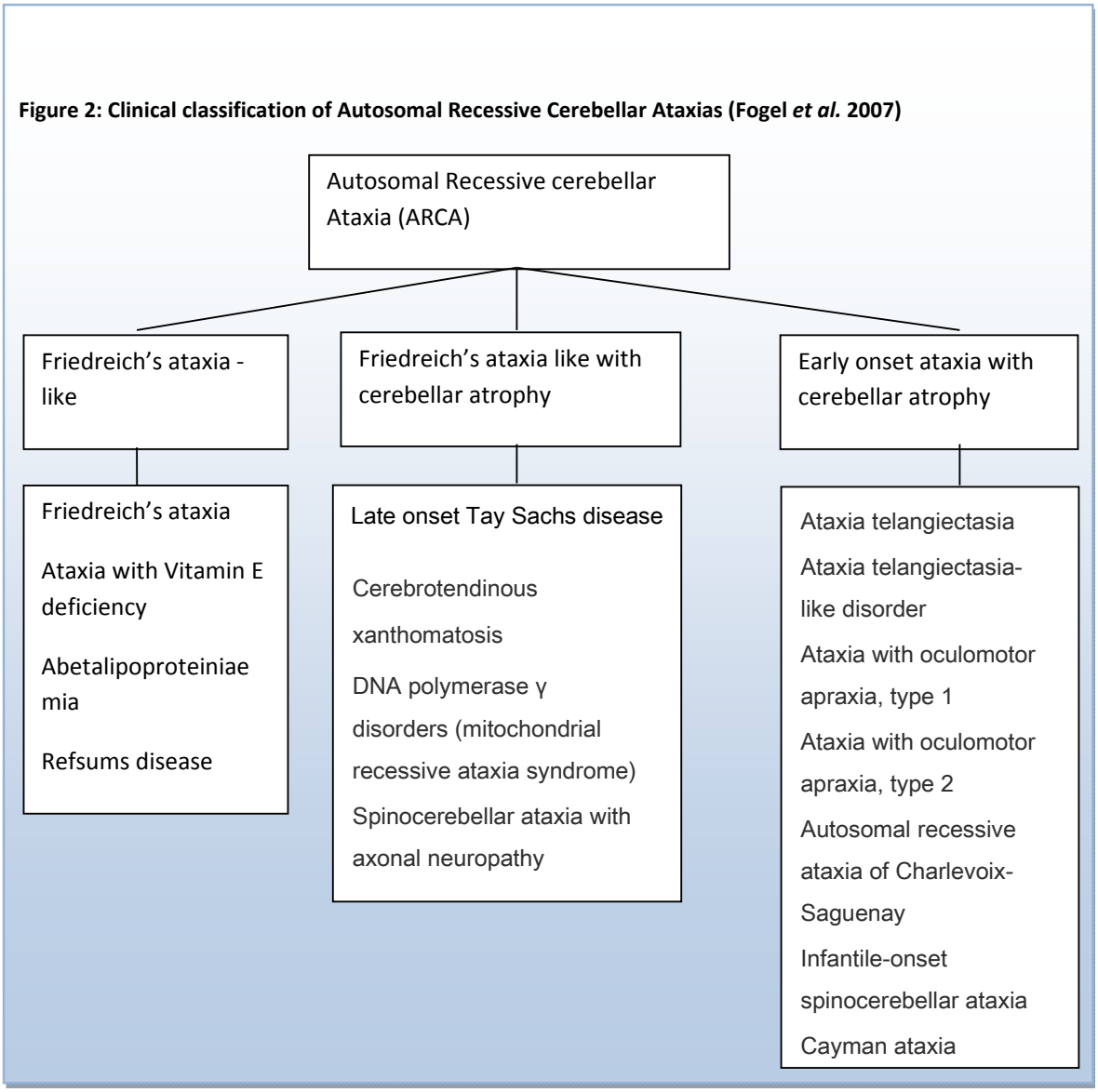
Autosomal Recessive Ataxia

Autosomal recessive cerebellar ataxias are a heterogeneous group of neurodegenerative diseases. There is no consensus for the classifications of recessive ataxias. There are many rare forms described in literature, and the list varies according to the reviewer (Anheim *et al.* 2012).

Autosomal recessive ataxias are heterogeneous with respect to age of onset, severity of disease progression and occurrence of extracerebellar and systemic signs. Age of onset for the majority of ARCA is in childhood (<20 years). However milder variants with adult onset have been described. The most common ARCA is Friedreich's ataxia (FA). Approximately 15% of all patients with Friedreich's ataxia have an age of onset beyond 25 years. Other common ARCA are Ataxia telangiectasia, Ataxia with isolated vitamin E deficiency (AVED) and Ataxia with occulomotor apraxia type 1 and 2 (AOA 1 and 2). AVED and FA are similar phenotypically but are due to different underlying mechanisms (Fogel *et al.* 2007). Another recessive disorder with a phenotype similarities to ataxia telangiectasia is Ataxia with occulomotor apraxia type 1 and 2 (AOA 1 and 2).

Friedreich's ataxia is a prototype of autosomal recessive ataxia and many diagnostic approaches are focused in comparison with this disease. Therefore out of the countless classification methods found in reviews the following was selected as an appropriate classification method for this study as it classified based on comparison with Friedreich's ataxia. (Figure 2)

Figure 2: Clinical classification of Autosomal Recessive Cerebellar Ataxias (Fogel *et al.* 2007)



X-linked Ataxia

Fragile X tremor / ataxia syndrome is an X-linked inherited ataxia syndrome. The main features are action tremor and cerebellar gait ataxia. Other associated features include Parkinsonism, executive function deficit and dementia, neuropathy and dysautonomia. On MRI there is a characteristic increased T2 signal intensity in the middle cerebellar peduncle (MCP sign). The underlying genetic mutation is the moderated expansion (55 - 200) of the CGG trinucleotide repeat in the Fragile X Mental Retardation 1 (FMR1) gene. The pathogenic mechanism is through the toxic over expression of the FMR1 mRNA (Berry-Kravis *et al.* 2007).

1.4 Investigation methods of SCAs

Neuropsychology

There is a high rate of cognitive and psychiatric disorder amongst patient with degenerative cerebellar disease. The cerebellum is believed to play a role in modulation of emotion and cognition (Leroi *et al.* 2002).

Attention and executive functioning are more severely affected in SCA 1 as compared to patients with SCA 2, 3, and 6 (Klinke *et al.* 2010). In addition mild deficits of verbal memory are found in SCA 1, 2, and 3.

In SCAs ataxia severity, gender and SCA subtype are found to be independent predictors of depressive status (Schmitz-Hubsch *et al.* 2011). Depression and memory symptoms may also be the initial presenting symptoms in SCA. The presence of early neuropsychiatric features in SCA emphasizes the need for careful behavioral screening and assessment of these patients (McMurtray *et al.* 2006).

Neuropathology

Autopsy findings for neuropathological features in SCA are rare and rely on autopsies done on patients at the end-stage of disease. Macroscopic appearances of specimens usually show correlation with the clinical features of patients. However significant discrepancies have also been noted: morphologically normal pyramidal tracts and prominent spasticity in SCA1, SCA3 and SCA7; massive neuronal loss in substantia nigra and absent parkinsonism features in SCA2 and SCA3 (Schols *et al.* 2004).

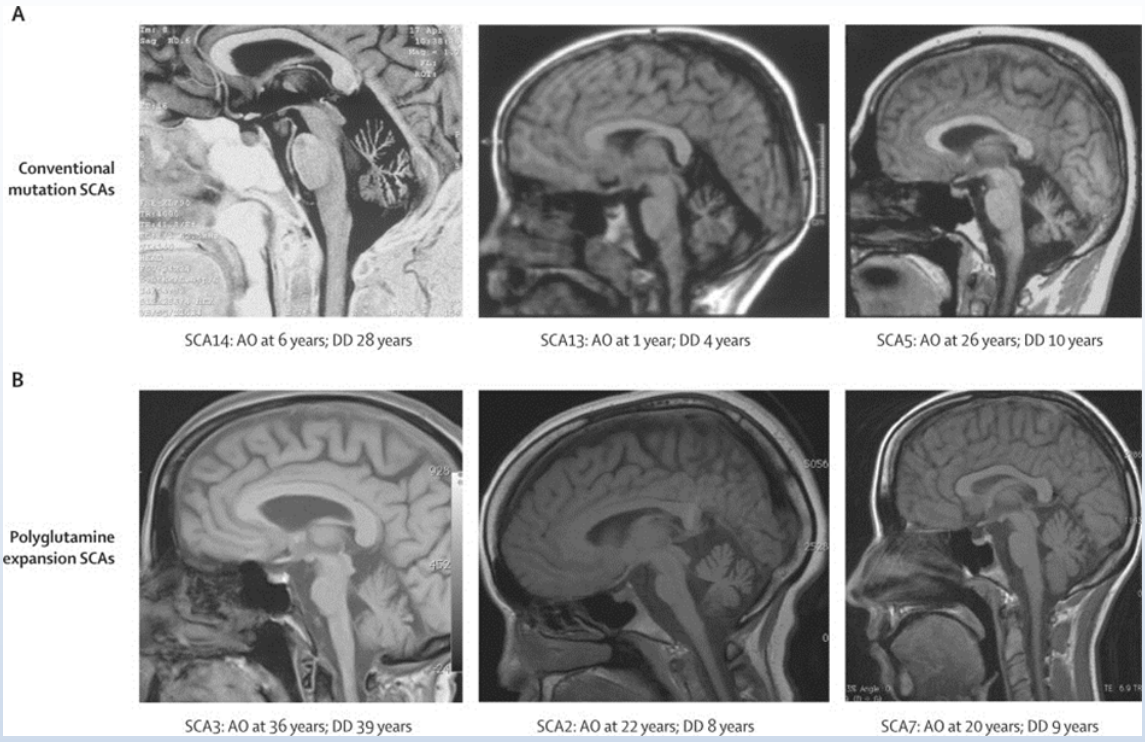
The pathological hallmark of SCA is the abnormal accumulation of polyglutamine in neuronal inclusions in immunohistochemical studies. These intranuclear inclusions are seen mostly in the degenerating brain region of the specific SCA subtype. In SCA1, SCA2, and SCA3 the

pons is affected along with the cerebellum while in SCA6, pathological findings are confined to the cerebellum (Durr 2010).

Neuroimaging

MRI is the imaging standard for SCA and there are fundamental patterns of degeneration seen: spinal atrophy, olivopontocerebellar atrophy, and cortical cerebellar atrophy. Pronounced olivopontocerebellar atrophy is characteristic and occurs early in SCA2 and SCA7. In comparison olivopontocerebellar atrophy is milder in SCA1 and SCA3. In SCA6 atrophy is restricted to the cerebellum. Atrophy of the upper spinal cord is present in SCA1, SCA2, and SCA3 but not in SCA6. It has been shown that CAG repeat length does not affect the severity of atrophy seen in any SCA subtype. In contrast non polyglutamine expansion SCA's (SCA 5, 11, 13, 14, 8, 10, 12) have a more limited atrophy that spares the brainstem (midbrain, pons and medulla) (Durr 2010). (Figure 3)

Figure 3: Differences in cerebellar involvement on MRI between polyglutamine expansion SCAs and conventional mutation SCAs



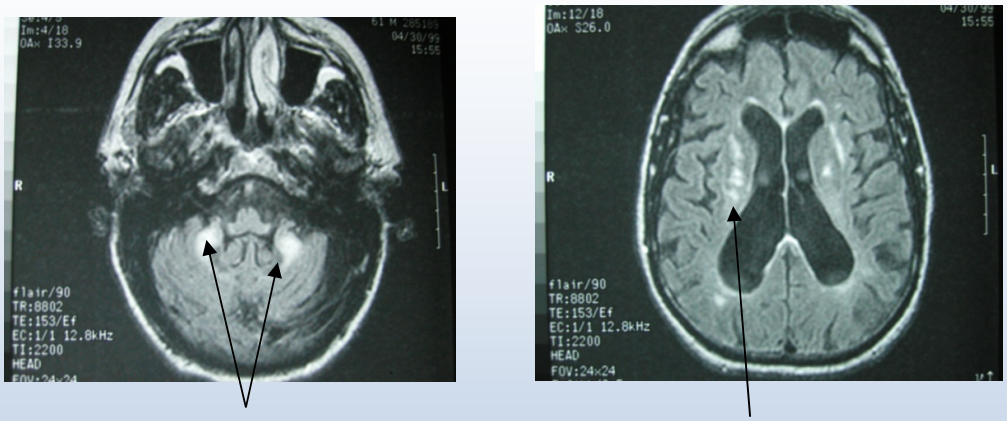
Sagittal MRI scans showing the involvement of the cerebellum only in conventional mutation SCAs (SCA5, SCA13, SCA14; A) and the brainstem with relatively minor involvement of the cerebellum in polyglutamine expansion SCAs (SCA2, SCA3, SCA7; B) in patients with comparable disease durations. SCA=spinocerebellar ataxia. AO=age at onset. DD=disease duration

Durr A. Autosomal dominant cerebellar ataxias: polyglutamine expansions and beyond. Lancet Neurol 2010;9(9):885-894. By permission of author

ARCA with cerebellar ataxia with pure sensory neuropathy such as Friedreich's ataxia and Ataxia with vitamin E deficiency have no cerebellar atrophy on Brain MRI. They however have spinal cord atrophy. In contrast diseases with cerebellar ataxia with sensory motor axonal neuropathy such as ataxia telangiectasia and ataxia with oculomotor apraxia type 1 and 2, have cerebellar atrophy on MRI (Anheim *et al.* 2012).

In Fragile X permutation tremor / ataxia syndrome there is a characteristic hyperintense signal change lateral to the dentate nucleus and extends into the middle cerebellar peduncles. This is often accompanied by signal changes in the supratentorial white matter and generalized brain atrophy (Berry-Kravis *et al.* 2007). (Figure 4)

Figure 4: Fragile X ataxia tremor associated with increase in MCP signal intensity and abnormal white matter signal in the periventricular region with grey and white mater atrophy



Berry-Kravis E, Abrams L, Coffey SM et al. Fragile X-associated tremor/ataxia syndrome: clinical features, genetics, and testing guidelines. Mov Disord 2007;22(14):2018-30 By permission of the author Dr Elizabeth Berry-Kravis

1.5 Pathophysiology

Polyglutamine SCA (SCA 1, 2, 3, 6, 7, 17 and DRPLA) are caused by the expansion of a coding CAG repeat, resulting in polyglutamines (polyQ) in the corresponding proteins. These polyQ tracts lead to instability of the protein, leading to misfolding and nuclear or cytoplasmic intraneuronal inclusions. These polyQ tracts increase the intrinsic toxicity of the proteins resulting in cell death. Recent research has suggested that in the earlier steps of the aggregation process the oligomers formed during misfolding may be underlying the neuronal toxicity in the polyQ diseases (Ross *et al.* 2004).

Apart from the neuronal toxicity these poly Q tracts also sequester various proteins, such as ubiquitin, molecular chaperones and subunits of proteasomes. Through the depletion of these proteins, global impairment of the protein quality system is induced. Some proteins affected by polyQ expansion are involved in protein quality control i.e.; ataxin -3 a ubiquitin – specific cysteine protease (Duenas *et al.* 2006).

Non coding region repeats are seen in SCA 8, 10, 12 31 and 36 (Verbeek *et al.* 2011). The mechanism of neurodegeneration in SCA 8, 10 and 12 is thought to be caused by RNA – induced gain of function. SCA 31 is caused by an insertion in the intronic region between the BEAN and TK genes. The paracentromeric satellite sequence included in this region has been shown to play an essential role in maintaining chromatin conformation and thereby alter gene transcription (Ishikawa *et al.* 2011). In SCA 36 the expanded repeat was shown to lead to an accumulation of RNA foci that sequestered RNA – binding proteins. Whether this is the causative mechanism of disease is to be further investigated (Kobayashi *et al.* 2011).

Conventional mutation SCAs disease mechanism is thought to be correlated with the specific genes or proteins they disrupt. As these disease genes display various biological functions, distinct cellular pathways are thought to be involved. The diverse functions of the defective

genes suggest that a wide range of biological pathways can be disrupted to cause cerebellar degeneration (Paulson 2009).

1.6 Natural History of Disease Progression

EUROSCA natural history study is a multicenter longitudinal cohort study, which was formed to obtain quantitative data on the progression of the most common spinocerebellar ataxias and the factors that influence their progression (Jacobi *et al.* 2011). The Scale for the Assessment and Rating of Ataxia (SARA scale) was used as the primary outcome measure and Inventory of Non- Ataxia Symptoms (INAS) as the secondary outcome measure.

Scale for the Assessment and Rating of Ataxia - The SARA is based on a semi quantitative assessment of cerebellar ataxia and includes eight items (gait, stance, sitting, speech disturbance, finger chase, nose – finger test, fast alternating hand movements, heel – shin slide). It yields a score from 0 (no ataxia) to 40 (very severe ataxia) (Schmitz-Hubsch *et al.* 2006).

Inventory of Non Ataxia Symptoms (INAS scale) - INAS provides structured information on non-ataxia symptoms in ataxia patients. INAS consists of 30 items, each of which is related to one of the following 16 symptoms or syndromes: areflexia, hyperreflexia, extensor plantar response, spasticity, paresis, amyotrophy, fasciculations, myoclonus, rigidity, chorea, dystonia, resting tremor, sensory symptoms, brainstem occulomotor signs (horizontal and vertical ophthalmoparesis, slowing of saccades), urinary dysfunction and cognitive impairment.

In the EUROSCA study results of one and two years follow up data showed disease progression was fastest in SCA 1, followed by SCA2 and 3. The slowest progression was seen in SCA 6. SARA score and INAS score change in a parallel manner, suggesting neurodegenerative change in cerebellar structures run in parallel with noncerebellar structures.

Another finding was that the CAG repeat length determined the severity of ataxia as measured by SARA in SCA1, SCA2 and SCA3 but not in SCA6. Repeat length was also shown to have an influence on the number, type and severity of accompanying non ataxia symptoms. The influence repeat length had on disease progression was less clear. SCA1 and 2 showed some correlation between length of expanded allele on disease progression while SCA3 and 6 did not show such an effect (Jacobi *et al.* 2011).

1.7 Epidemiology

The worldwide incidence of ADCA is estimated at one to three per 100 000, including both polyglutamine expansion and conventional mutation SCAs (Schols *et al.* 2004). However as genetic testing is limited in most epidemiological studies to polyglutamine expansion SCAs, these are the ones more frequently described. Of the polyglutamine expansion subtypes SCA 3 is the most prevalent, followed by in descending order SCA 2, 1 and 8 worldwide. In up to 44% of the cases a genetic diagnosis was not possible with the genetic panels available for SCA.

Founder effects are seen in SCA subtypes. SCA 3 is relatively high in Portugal, Brazil, China, Japan, Netherlands and Germany. SCA 2 subtype is found in Cuba. SCA 1and 2 are more common in the United Kingdom, Italy and India. SCA 10 and DRPLA are found predominantly in Brazil and Japan, respectively (Durr 2010).

Prevalence figures for SCA in Asian countries are relatively limited in comparison to the data available for Western countries. Most published data on Asian populations are derived from Japan, China, India and South Korea. The absence of diagnostic facilities and the limitation of further management options once diagnosed as well as the focus on basic healthcare reduces the encouragement to pursue genetic diagnosis and research in many Asian countries.

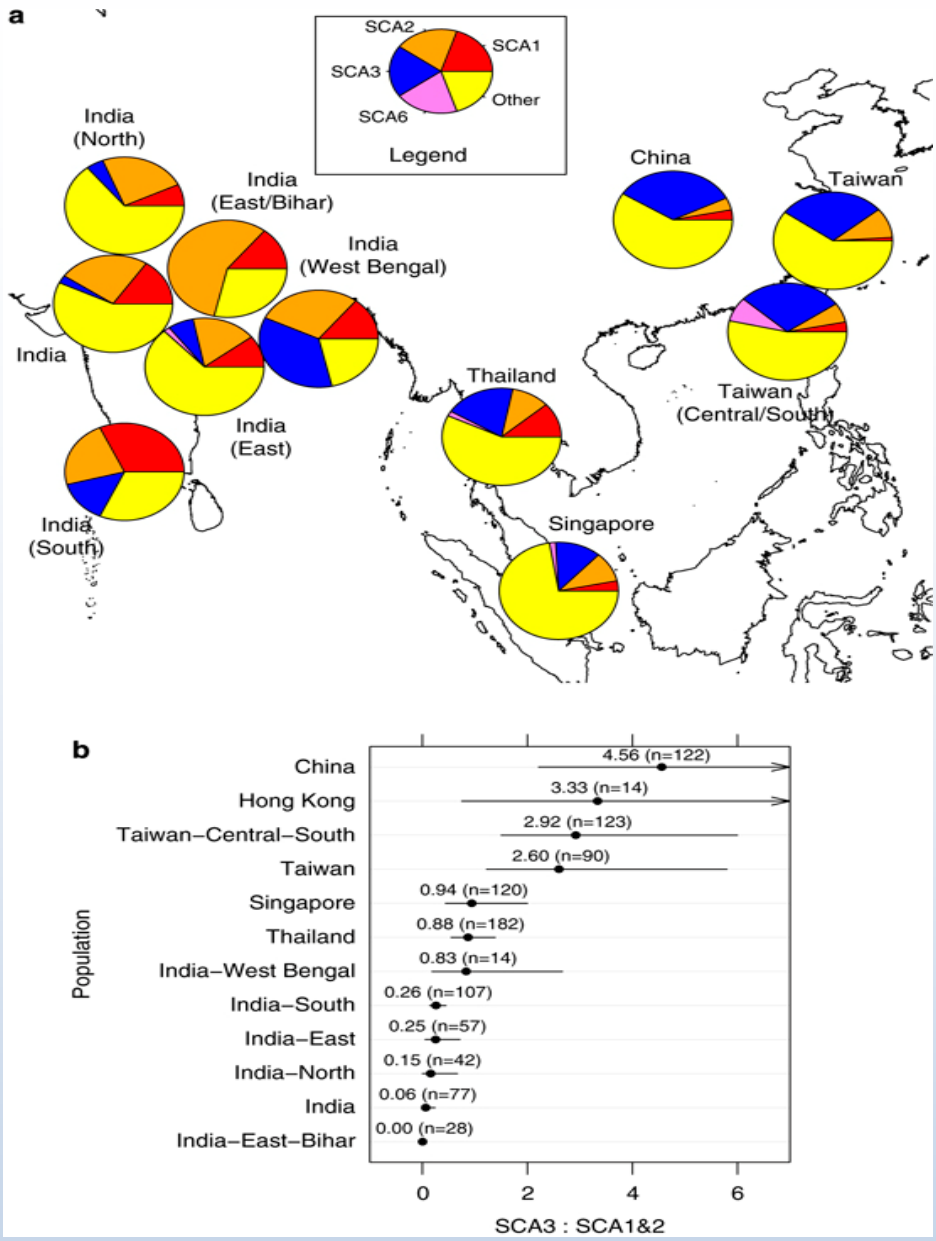
Japan has had several studies in various regions on the prevalence of SCA. Overall SCA 3 and 6 appears the most common SCA subtypes (Sasaki *et al.* 1999). A founder effect for SCA 6 was noted in Western Japan (Mori *et al.* 2001).

Tang B, Liu C, Shen L et al (2000), showed that the prevalence of SCA in those of Chinese kindred to be highest for SCA 3 with 48.23% of the total diagnosed SCA patients (Tang *et al.* 2000).

In the largest published study on South Korean populations SCA 2 was the most frequent hereditary ataxia (12.6%), while SCA 3 and 6 had a prevalence of 4.6 and 6.9% respectively (Jin *et al.* 1999).

The distribution of SCA subtypes in South Asia is depicted below (Figure 5).

Figure 5: Geographical variation in SCA subtypes in south, southeast and east Asia



The odds of having the 'Chinese SCA' (SCA3) rather than 'Indian SCAs' (SCA1 and -2) cases in these populations, with their non-parametric 95% confidence intervals is displayed in (b). Sura T, Eu-Ahsunthornwattana J, Youngcharoen S et al. Frequencies of spinocerebellar ataxia subtypes in Thailand: window to the population history. J Hum Genet 2009; 54(5):284-288. By permission of the author Dr Jakris Eu-ahsunthornwattana (Sura *et al.* 2009)

As our neighboring country, the scenario in India with regard to prevalence of SCA subtypes plays an important role in hypothesizing the possible prevalence figures in Sri Lanka. (Table 3)

Table 3 Prevalence of Spinocerebellar ataxia in the Indian subcontinent according to published studies upto 2012

Region	*Year*	*N*	*SCA subtype*				
			SCA 1	*SCA 2*	*SCA 3*	*SCA 6*	*SCA 7*
India South(Krishna et al. 2007)	*2003 -2006*	*284*	*34 (31.8%)*	*24 (22.4%)*	*15(14%)*	*N/A*	*N/A*
India – South(Rengaraj et al. 2005)	*2005*	*236*	*7.2%*	*N/A*	*N/A*	*N/A*	*N/A*
India East (West Bengal)(Sinha et al. 2004)	*2004*	*28*	*2 (14.3%)*	*4 (28.6%)*	*5 (35.7%)*	*0*	*0*
India Mostly North(Saleem et al. 2000)	*2000*	*42*	*3 (7.1%)*	*10 (23.8%)*	*2 (4.8%)*	*0*	*0*
India East (West Bengal(Basu et al. 2000))	*1997 – 1999*	*57*	*6 (10.5%)*	*10 (17.5%)*	*4(7.0%)*	*1(1.8%)*	*0*

SCA 2 is the predominant subtype in Northern and Eastern India. Q. Saleem et al (2000) found that SCA 2 was predominately present in the families from northern India (Saleem *et al.* 2000). It was concluded that eastern India had the highest prevalence of SCA 2 subtypes

(Sinha *et al.* 2004). A total of 28 families tested and 26 out of 16 families (57%) were found to have the SCA2 mutation.

In contrast SCA 1 subtype had the highest prevalence in Southern India. Krishna et al in 2007 showed that one third of SCA were of 1, 2, and 3 subtypes, with SCA1 as the largest group (Krishna *et al.* 2007). Another study based in southern India state of Tamilnadu focused on the ethnic Tamil community. SCA 1 subtype was also the predominately prevalent hereditary ataxia (Rengaraj *et al.* 2005).

Bahl S et al in 2005 found a founder mutation for SCA subtype 12 in the Haranya region of Northern India. Sixteen percent (20/124) of diagnosed hereditary ataxia in that region were SCA12. Analysis of 20 Indian SCA12 families and ethnically matched normal unrelated individuals revealed one haplotype to be significantly associated with the affected alleles ($P = 0.000$), clearly indicating the presence of a common founder for SCA12 in the Indian population. This haplotype was not shared by the American pedigree with SCA12. Therefore, the SCA12 expansion appears to have originated at least twice (Bahl *et al.* 2005).

Justification of a study on Spinocerebellar Ataxia in a the Sri Lankan population

A study regarding the prevalence of hereditary ataxia has not been conducted in Sri Lanka, and the genetic subtypes of autosomal dominant hereditary ataxias remains unknown.

Earlier reports have ascertained the presence of hereditary ataxia in Sri Lanka. However no prevalence study has been conducted and the genetic type of ataxia remains unknown. Knowledge of the occurrence of hereditary ataxia, of the phenotypes and genotypes present in the country is necessary for health care planning. Provision of appropriate clinical and para – clinical services has been shown to increase the quality of life in patients with neurodegenerative disease.

At the patient level a genetic diagnosis is essential for genetic counseling of both of the affected and non-affected. Such information will assist individuals in making informed choice regarding reproductive options and future career prospects.

Opportunities for research for future treatment options also require a genetic diagnosis. Selection of patients for appropriate clinical trials is an avenue that opens with a proper genetic diagnosis. It leads to a better understanding of pathogenesis and long term clinical outcome of the disease. Identification of the associated genes in spinocerebellar ataxia has also provided insight into the mechanism that could underlie other forms of genetic or non-genetic ataxias

At present there are no published data regarding the genetic characteristics of patients with spinocerebellar ataxia in Sri Lanka. We are yet to answer the questions, do we have the same prevalence of spinocerebellar ataxia seen worldwide and whether 50% are polyglutamine expansion subtypes? Are there founder mutations in our populations like those seen in India

and Japan? Does SCA subtypes distribution mirror those seen in Northern, and eastern India or those of southern India?

At present in Sri Lanka the Human Genetics Unit is the single government center that carries out genetic testing for SCA. From 2009 it has carried out molecular genetic testing for SCA 1, 2, 3, 6, and 7 and of those tested (6/23) 26% has received a positive genetic diagnosis. This indicates the presence of a large patient population with other types of SCAs.

Objectives

The main objective is to investigate patients with ataxia through molecular genetic testing and establish a genetic diagnosis. A second objective is to look at genotype-phenotype correlations and establish the genotype – phenotype spectrum seen in Sri Lanka. The study will provide insight in to the prevalence of SCA subtypes in the Sri Lankan population and may provide additional information regarding the specificities of the described phenotypes. The availability of such information will assist both in the early detection and appropriate management of patients with hereditary ataxia.

Objectives:

1. To clinically phenotype patients with hereditary ataxia based on history, clinical examination, disease progression and investigation results and create a database of Sri Lankan ataxia patients for future clinical and research purposes.

2. To determine the genotype in patients starting with SCA

3. To assess eventual Sri Lankan characteristics of dominant ataxias compared to previous reports

Materials and Methods

2.1 Ethical considerations

The Ethical Review Committee of the Faculty of Medicine, University of Colombo, Sri Lanka, approved the study.

This study was conducted according to the Declaration of Helsinki (2008). The study builds on the collaborative links the Human Genetics Unit of the Colombo Medical Faculty has established with the patients with SCA, local neurologists and foreign experts in the field. As mentioned in the background and justification the study has aimed to identify the clinical phenotype of patients with SCA, their genetic phenotype and the link between these clinical phenotype and genotype of SCA. The study has social value as it is the first portray of the clinical and genetic manifestations of patients in Sri Lanka, and contributes to generalizable knowledge in the field. The study was designed to ensure scientific validity. The study was open to all patients with SCA; this therefore had fair participant selection. Appropriate steps were taken to ensure that consent was obtained in an ethical manner from all study participants.

All volunteers were recruited after obtaining written informed consent using consent forms in Sinhala and Tamil languages. In the case of patients with diminished mental capacity proxy consent was taken from guardians

The patients were interviewed privately in the genetics counseling room and were able to discuss the study privately with the investigators without the presence of others.

The data collection booklet was designed to ensure confidentiality of information gathered. Soon after collecting the personal information, the identification page was removed and filed separately. The only identification number in the rest of the booklet was a coded subject study number which cannot be linked to an individual without the page containing the personal

information which was kept by the principal investigator under lock and key. The electronic database containing the data only had the subject study number thus ensuring confidentiality. The database was password protected. These measures ensured that loss of confidentiality was minimized.

Video recording of gait, speech and neurological examination was performed in patients who gave informed consent. Written informed consent was obtained for recording for research purposes, with full anonymity. Video recording was primarily done to re-evaluate the findings obtained by the principal investigator by a professor in neurology and an expert in the field of movement disorders. This measure was taken to increase the validity of the data. The film obtained is kept in a locked cabinet which is accessible to the principal investigators and supervisors of the research alone.

Minor bruising due to venipuncture was minimized as it was performed under aseptic conditions by a trained nurse.

Direct benefit to patient occurred in those who obtained a genetic diagnosis following testing. In such instances they received specific genetic counseling for their condition. In those who were tested negative by the SCA panel used a direct benefit was not obtained. However they received indirect benefits by excluding the SCA subtypes from their differential diagnosis and by enhancing the generalizable knowledge on hereditary ataxia in Sri Lanka. Future clinical practice may also have benefited by the phenotype- genotype links established.

The samples and data obtained will be stored for future studies in SCA until 2020. Thereafter remaining samples will be anonymised and discarded by the laboratory under the supervision of the investigator. Appropriate consent has been obtained for this purpose and such studies would be subject to ethics review prior to commencement.

2.2 Recruitment of Patients

Identification and recruitment of patients with hereditary ataxia for the study was conducted in the following manner,

1. The archives of the Human Genetics Unit from January 2005 to December 2011 were assessed for referrals of hereditary ataxia. These referrals were by consultant neurologists from tertiary care hospitals.
2. A patient population was identified in an isolated geographical region following information from patients. Twelve patients of the total 46 in the study were recruited from this region. These patients had not been seen by medical professionals previously and were investigated in their homes.

Patients were contacted via phone / mail and those who gave informed consent were recruited into the study. Data for a total of 46 patients were thus included in the study and recorded in the database created for this purpose. This data may be used in a future follow up studies.

Spinocerebellar ataxia for the purpose of recruitment was defined as patients with a clinical diagnosis of Autosomal Dominant Cerebellar Ataxia (ADCA). Asymptomatic individuals with a family history suggestive of SCA were not recruited. Patients were diagnosed with ADCA if they fulfilled the inclusion exclusion criteria listed. (Table 4)

Table 4 Inclusion/ exclusion criteria for ADCA in our study

Inclusion/ exclusion criteria for ADCA in our study	
Inclusion Criteria (1, 2, 3 or 1, 2 or 1, 3)	*Exclusion criteria*
1. Progressive cerebellar ataxia	*1. Secondary ataxias*
2. Other affected family members	
3. Verified molecular diagnosis	

Autosomal recessive cerebellar ataxia was defined as patients with a history of parental consanguinity and/ or sibling affected with a similar disease phenotype.

Sporadic ataxia was defined as those without a family history in which all possible causes of acquired ataxia had been excluded.

2.3 Registration of Patients

Each index subject was registered in the research database. This database was modeled in accordance to the database maintained under Prof C. Tallaksen at the Neurology Department, University of Oslo. Data entered were according to the phenotyping booklet of the patients with spinocerebellar degeneration used in the SPATAX – EUROSCA study (Chantal M.E. Tallaksen 2003) was modified and adopted to collect the phenotyping data of the Sri Lankan patients.

The database included standardized clinical sheets of the patients, geographical origin, consanguinity, the pedigree and additional information such as results from supplementary investigations (blood samples, CT, MRI). The principal investigator filled the clinical data into the database. All index subjects were categorized into 4 groups; autosomal recessive, dominant, X – linked or sporadic form.

2.4 Clinical Evaluation

Complete medical history was obtained from each participant. The family pedigree included familial background up to 3 generations whenever possible. The demographic details and clinical symptomology of ataxia, motor, sensory symptoms, dysarthria, dysphagia was assessed. Clinical assessment of patients and evaluation of investigations was done by the principal investigator. These findings were reassessed by the supervisors.

Scale for the Assessment and Rating of Ataxia (SARA)

The SARA is based on a semi quantitative assessment of cerebellar ataxia and includes eight items (gait, stance, sitting, speech disturbance, finger chase, nose – finger test, fast alternating hand movements, heel – shin slide). It yields a score from 0 (no ataxia) to 40 (very severe ataxia). It is further divided into 3 subgroups: posture and gait (0 - 18), speech (0 -6) and limb kinetic function (0 - 16).

Klockgether T. et al (Schmitz-Hubsch *et al.* 2006) devised this scale and validated it 2006. It was tested it in two trials of 167 and 119 patients with spinocerebellar ataxia. Their results included the mean time to administer SARA in patients, which was 14.2 + 7.5 minutes (range 5 to 40). Interrater reliability, test-retest reliability and internal consistency were high. The study concluded that SARA was a valid and reliable primary outcome measure for ataxia.

Analysis of the usefulness of SARA in assessing spinocerebellar ataxia (Yabe *et al.* 2008) showed inter-rater reliability of the SARA scores between the two neurologists was high. The scores on SARA correlated significantly with the Barthel index and scores on the International Cooperative Ataxia Rating Scale (ICARS). The time taken to review patients by SARA was approximately 4 minutes which was significantly lower than ICARS.

SARA was administered to all the participants by the principal investigator. Findings were video recorded with the informed consent of participants. The findings were further validated by these videos beings assessed by the supervisors. (Appendix 2)

Inventory of Non Ataxia Symptoms (INAS scale)

INAS provides structured information on non-ataxia symptoms in ataxia patients. INAS consists of 30 items, each of which is related to one of the following 16 symptoms or syndromes: areflexia, hyperreflexia, extensor plantar response, spasticity, paresis, amyotrophy, fasciculation, myoclonus, rigidity, chorea, dystonia, resting tremor, sensory symptoms, brainstem occulomotor signs (horizontal and vertical ophthalmoparesis, slowing of saccades), urinary dysfunction and cognitive impairment.

For a semi quantitative assessment of non-ataxia symptoms, the number of non-ataxia symptoms is counted yielding the INAS count, a dimensionless value with a range from 0 to 16. To determine the INAS count, only the presence or absence of one of the 16 symptoms is considered. A symptom is recorded as present if at least one item related to this symptom is positive. Reliability of INAS ratings was tested in two large multi-center trials that served to validate SARA (Schmitz-Hubsch *et al.* 2006). (Appendix 3)

Both SARA and INAS scales were open access research tools freely accessible for research purposes.

Patient Health Questionnaire – 9 (PHQ 9)

The Patient Health Questionnaire 9 (PHQ - 9) was devised as a multipurpose instrument for screening, diagnosing, monitoring and measuring severity of depression. It incorporates DSM IV depression diagnostic criteria with other major depressive symptoms into a major self-report tool.

The items on PHQ – 9 correspond to DSM – IV criteria for depressive disorders and each item is rated from 0 = 'not at all' to 3 = 'nearly every day'. Symptom severity can be described by a sum score (range 0 - 27) using a 4 – step classification (0 – 4, none; 5 – 9, mild; 10 – 14, moderate and ≥ 15, severe depression). For some analyses, scores were transformed into dichotomous variables by using a cutoff score of PHQ≥10 for clinically relevant depressive syndromes. Item level relative frequencies were also documented and relative frequencies noted (percentage of valid data per item) of patients reporting any problem (any rating different from 'not at all') or critical problem (rating of 'more than half the days'). For item 9 (better off dead, hurting oneself) any rating different from not at all was documented (Schmitz-Hubsch *et al.* 2011).

According to Kroenke K, et al (2001) the PHQ – 9 is a criteria-based diagnosis of depressive disorders is a reliable and valid measure of depression severity (Kroenke *et al.* 2001). The diagnostic validity of PHQ – 9 was established using 8 primary care clinics and 3000 patients. Spitzer et al in 2011 found there was good agreement between PHQ diagnoses and those of independent mental health professionals. These characteristics plus its brevity make the PHQ-9 a useful clinical and research tool.

The PHQ-9 was developed by Drs. Robert L. Spitzer, Janet B. W. Williams, Kurt Kroenke and colleagues, with an educational grant from Pfizer Inc. No permission was required to reproduce, translate, display or distribute.

A validated Sinhala translation of PHQ 9 was obtained from the Psychiatry Department, Faculty of Medicine, University of Colombo. (Appendix 4)

Montreal Cognitive Assessment (MoCA)

Montreal Cognitive Assessment (MoCA) Scale was created in 1996 and has been validated in many clinical settings. The MoCA test is a one-page 30-point test administered in approximately 10 minutes. The MoCA assesses several cognitive domains. The short-term task (5 points) involves two learning trials of five nouns and delayed recall after approximately 5 minutes. Abilities are assessed using a clock-drawing task (3 points) and a three-dimensional cube copy (1 point). Multiple aspects of executive functions are assessed using an alternation task adapted from the trail-making B task (1 point), a phonemic fluency task (1 point), and a two-item verbal abstraction task (2 points). Attention, concentration and working memory are evaluated using a sustained attention task (target detection using tapping; 1 point), a serial subtraction task (3 points), and digits forward and backward (1 point each). Language is assessed using a three-item confrontation naming task with low-familiarity animals, repetition of two syntactically complex sentences (2 points), and the aforementioned fluency task. Finally, orientation to time and place is evaluated (6 points).

K. Bürk et al in 2003 studied the neurocognitive features of the most common SCA subtypes SCA1, 2, and 3.The study describes significant impairment in verbal memory and fronto executive tasks in all three SCA subtypes. The finding of intact visuospatial processing and memory is in accordance to other neuropsychological studies in cerebellar patients (Burk *et al.* 2003).

Since the MoCA assesses multiple cognitive domains, it is a useful cognitive screening tool for neurological diseases that affect younger populations, such as Parkinson's disease,

vascular cognitive impairment, Huntington's disease, brain metastasis. In this context usage for spinocerebellar ataxia seemed appropriate.

The validated translation of the MoCA designed by Hanwella R., de Silva V and Karunarathe S. of the Psychiatry department, Faculty of Medicine, University of Colombo was used during the study. The test was administered and results were quantified by the principal investigator. (Appendix 5)

Permission for usage of the MoCA test was obtained from Tina Brosseau, Projects & Development Manager, Center for Diagnosis & Research on Alzheimer's disease (CEDRA) and contact person for MoCA scale administration at www.mocatest.org

2.5 Genetic Testing

DNA extraction

At recruitment, a sample of 5ml of venous blood was collected from each subject into K/EDTA tubes. These blood samples were stored at -80°C, at the Human Genetics Unit until DNA extraction. DNA was extracted from the blood sample and molecular genetic tests performed. DNA extraction was done using Wizard DNA extraction kit from Promega® (Madison, WI USA) according manufacturer's instructions.

Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) was the initial step in many of the experiments performed in this thesis. It is a technique used to amplify single or limited copies of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. The method relies on thermal cycling, consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. Primers (oligonucleotide

fragments) containing sequences complementary to the target region along with a DNA polymerase enzyme are key components to enable selective and repeated amplification. As PCR progresses, the DNA generated is itself used as a template for replication, setting in motion a chain reaction in which the DNA template is exponentially amplified.

The PCR experiment for detection SCA 1, 2, 3, 6, 7 and 8 were performed at the Human Genetics Unit using the ABI 2720 thermal cycler. SCA 12, SCA 17 and Friedreich's ataxia PCR reactions were carried out using Applied Biosystem Veriti 96 well thermal cycler at the Medical Genetics Department, University of Oslo.

Multiplex PCR

It consists of multiple primer sets within a single PCR mixture to produce amplicons of varying sizes that are specific to different DNA sequences. By targeting multiple genes at once, additional information may be gained from a single test run that otherwise would require several times the reagents and more time to perform.

Agarose gel electrophoresis

Electrophoresis through agarose is a method used to separate and identify DNA fragments. Nucleic acid molecules are separated by applying an electric field to move the negatively charged molecules through an agarose matrix. Shorter molecules move faster and farther than longer ones because shorter molecules migrate more easily through the pores of the gel. The location and size of the DNA fragments can be identified directly through staining with a dye that intercalates directly with DNA and fluorescent when examined under a UV

transilluminater and by the use of a DNA ladder alongside the DNA fragments for size differentiation.

Agorose gel was prepared by using 2g of molecular biodegradable agarose (SeaKem® LE agarose Lonza, Rockland, ME, USA) in 100ml of 1 X TAE buffer to obtain the correct percentage gel – 2%. 10µl of SYBR® safe DNA gel stain (Invitrogen ™) was added to each gel. Gels were poured into casting trays with the desired number of wells and set at room temperature to solidify. They were then submerged in 1X TAE buffer, 5µl of PCR product mixed with 1µl of 6 X loading dye (Fermentas 6X loading dye solution #R0611) was pippetted into each well. A Fermentas Gene Ruler ™ DNA ladder was placed in a separate well. A 100V current was applied to the gel for 1 hour. The pattern created by different size DNA fragments was visualised by a UV transilluminater (4000 Pro Gel Logic, Carestream). The size of the fragments was analysed with comparison to the DNA ladder (Fermentas Gel Ruler ™)

Capillary electrophoresis

This is a high-resolution alternative to gel electrophoresis. It is the adaptation of traditional gel electrophoresis into the capillary using polymers in solution to create a molecular sieve also known as replaceable physical gel. This allows analytes having similar charge-to-mass ratios to be resolved by size. This technique is commonly employed in applications of DNA sequencing, genotyping, and fragment analysis.

Molecular Diagnostic Test Selection for HA

The initial testing panel included multiplex PCR and capillary electrophoresis of SCA 1, 2, 3, 6, 7, and 8. Data on world prevalence indicates that SCA 3 is the most common worldwide; SCA 1, SCA2, SCA 6, SCA7 and SCA8 have prevalence of over 2% and the remaining

subtypes are thought to be rare (Schols *et al.* 2004). As Sri Lanka had no previous prevalence figures initial testing with this panel was conducted.

The next step in genetic diagnosis was based on epidemiology and clinical features of the remaining patients. Epidemiology wise the closest country we could compare ourselves with is India. Apart from the main CAG repeat SCA subtypes, SCA 12 and 17 were the SCA subtypes found with evidence of a common founder for SCA 12 (Bahl *et al.* 2005). Clinical phenotypes were assessed and factored into the decision to test for SCA 12 and 17. (Appendix 6)

Apart from the autosomal dominant cerebellar ataxias there were 8 autosomal recessive and 4 sporadic ataxias. 5 of the ARCA and 2 of the sporadic ataxia patients were of the juvenile age group (<20 years). The most common recessive cerebellar ataxia manifesting in children and young adults is Friedreich's ataxia. Therefore molecular testing for this disease was done in these patient groups.

SCA 1, 2, 3, 6, 7, and 8 detection

Initially samples were tested for SCA 1, 2, 3, & 6 using a single multiplex amplification reaction that was analyzed by capillary electrophoresis. Chimeric primers that differed in length and fluorochromes were used to distinguish the four different amplicons (Dorschner *et al.* 2002). SCA 7 & 8 analysis was performed thereafter (Koob *et al.* 1999).

PCR reaction mix of SCA 1, 2, 3, 6:

The multiplex reaction of 20 µl consisted of 0.15 µl of 10µM *SCA1* primers (1.5 pmol each), 0.15 µl of 10µM *SCA2* primers (1.5 pmol each), 0.40 µl of 10µM *SCA3* primers (4.0 pmol each), 0.15 µl of 10µM SCA 6 (*CACNAIA)* primers (1.5 pmol each), 4.00 µmol/L of 2mM dNTPs, 4µl of 5X buffer (50mM Tris HCl, 250mM KCl), 1.4µl of 25mM $MgCl_2$, 0.8 DMSO (4% v/v), 0.5 µl of 5U *Taq* polymerase (2.5U Taq polymerase Gotaq®Flexi, Promega), and 200ng of genomic DNA purified from peripheral blood as described above.

PCR reaction mix of SCA 7 & 8

The multiplex reaction of 20 µl consisted of 0.45 µl of 10µM *SCA7* primers (4.5 pmol each), 0.45 µl of 10µM *SCA8* primers (4.5 pmol each), 2µl of 200 µmol/L of 2mM dNTPs, 4µl of 5X buffer (50mM Tris HCl, 250mM KCl), 1.2µl of 25mM $MgCl_2$, 0.2 DMSO (4% v/v), 0.2 µl of 5U *Taq* polymerase (1U Taq polymerase Gotaq®Flexi, Promega), and 200ng of genomic DNA purified from peripheral blood as described above.

Reactions were performed in an ABI 2720 thermal cycler for initial cycle at 94°C for 5 minutes, 32 cycles of denaturation at 95°C for 1 minute, annealing at 60°C for 2 minutes, and elongation at 68°C for 1.5 minutes, and a final extension at 72°C for 7 minutes.

Samples were prepared for fragment analysis by addition of 0.5 µl PCR product to 9.5µl of Formamide (HiDye formamide, Applied Biosystems Inc.) and 0.5 µl of GS500-LIZ internal molecular weight standard (Applied Biosystems Inc.), denatured at 95°C for 5 minutes, and immediately placed on ice for a minimum of 3 minutes. Samples were injected into an ABI PRISM 3130 Genetic analyzer (Applied Biosystems Inc.) with a 36 cm capillary containing

Performance Optimized Polymer – 7 (POP – 7, Applied Biosystems Inc.). Amplicon length was calculated by comparison with the GS500-LIZ molecular weight standard by the GeneMapper® v4.0 program (Applied Biosystems Inc.).

The multiplex assays were performed using the following fluorescently labeled primers (Sigma - Aldrich®) (Table 5)

Name	*Primer sequence 5' - 3'*	*Amplicon length*
SCA1F	*gcg gtcccaa aag ggtcagt AAC TGG AAA TGT GGA CGT AC*	*162 + (CAG)n*
SCA 1R	*ggtcccaaaagggtcagtCAA CAT GGG CAG TCT GAG*	
SCA 2F	*aaaagggtcagt GGG CCC CTC ACC ATG TCG*	*84 + (CAG)n*
SCA 2R	*caaaagggtcagtCGG GCT TGC GGA CAT TGG*	
SCA 3F	*gcggtcccaaaagggtcagt CCA GTG ACT TTG ATT CG*	*201 + (CAG)n*
SCA 3R	*gcggtcccaaaagggtcagt TGG CCT TTC ACA TGG ATG TGA A*	
SCA 6F	*caaaagggtcagt CAG GTG TCC TAT TCC CCT GTG ATC C*	*126 + (CAG)n*
SCA 6R	*aaagggtcagtTGG GTA CCT CCG AGG GCC GCT GGT G*	
SCA 7F	*gcggtcccaaaagggtcagtTGT TAC ATT GTA GGA GCG GAA*	*314 + (CAG)n*
SCA 7R	*gtcccaaaagggtcagtCAC GAC TGT CCC AGC ATC ACT T*	
SCA 8F	*TTTGAGAAAGGCTTGTGAGGACTGAGAATG*	*309 + (CAG)n*
SCA 8R	*GGTCCTTCATGTTAGAAAACCTGGCT*	

Table 5 Chimeric primers for mutiplex amplification of 6 Spinocerebellar ataxia genes

SCA 12 and 17 detection

Spinocerebellar ataxia type 12 was tested in patients with autosomal dominant cerebellar ataxia without a genetic diagnosis following testing by the SCA 1, 2, 3, 6, 7, and 8 panels. This test was done at the University of Oslo, following the signing of a Material Transfer

Agreement between the Human Genetics Unit and The Medical Genetics Department University of Oslo.

Primers used in the experiment are those stated as by Holmes S. E.(Holmes *et al.* 1999).

Figure 6 illustrates the genomic region amplified by the primers according to the UCSC Genome browser.

Figure 6:Genomic region amplified by the SCA 12 primers, with the CAG repeat region highlighted

>chr5:146258250-146258401 152bp TGCTGGGAAAGAGTCGTG GCCAGCGCACTCACCCTC
TGCTGGGAAAGAGTCGTGgggctgctgacgcggttgggaggagcctcgcc
tttaatgcaccagccgcctccagcctcctgcagcagcagcagcagcagca
gcagcagcagctgcgagtgcgcgcgtgtgggtgtGAGGGTGAGTGCGCTG
GC

Spinocerebellar ataxia type 17 was the final test conducted on the hereditary ataxia patients (Nakamura *et al.* 2001).

Primers used in the experiment are those stated by Nakamura et al.

Forward primer 5' – CCTTATGGCACTGGACTGAC – 3'

Reverse primer 5'- GTTCCCTGTGTTGCCTGCTG – 3'

The area of amplification according to the UCSC Genome browser is depicted below.

Figure 7: Gemonic region amplified by the SCA 17 primers. CAG / CAA repeat region is highlighted

>chr6:170870921+170871165 245bp CCTTATGGCACTGGACTGAC GTTCCCTGTGTTGCCTGCTG
CCTTATGGCACTGGACTGACcccacagcctattcagaacaccaatagtct
gtctattttggaagagcaacaaaggcagcagcagcaacaacaacagcagc
agcagcagcagcagcagcaacagcaacagcagcagcagcagcagcagcag
cagcagcagcagcagcagcagcagcagcagcagcaacaggcagtggcagc
tgcagccgttcagcagtcaacgtccCAGCAGGCAACACAGGGAAC

For both SCA 12 and SCA 17 experiments, approximately 100ng of genomic DNA was amplified in a 25µl reaction volume containing a final concentration of 5µl 360 GC enhancer, 2.5µl 10x Buffer Gold 360, dNTP ABI 0.2mM (2µl), MgCl2 360 1.5mM (1.5µl), Primer mix 10pmol each (2.5µl), Taq Gold 360 0.5U (0.13µl). Samples were denatured at 95°C for 10 minutes followed by 35 cycles of denaturation (95°C, 30 seconds), annealing (55°C, 30 seconds), extension (72°C, 60 seconds), and a final extension of 7 minutes at 72°C in a Applied Biosystems Veriti 96 well thermal cycler (Applied Biosystems, Foster City, CA) (Srivastava *et al.* 2001).

To further quantify the amplicon length the same PCR reaction was done using a florescent forward primer and capillary electrophoresis of PCR product was performed. 1 µl of PCR product was mixed with 12µl MQ water, 12µl Formamide and 0.2µl Size marker 500 (Gene Scan ^{TM}). A 96 well plate was used with PCR mixture loaded in every other row to avoid florescent bleed through; the remaining wells were loaded with MQ water 12µl. Capillary electrophoresis was performed using 3730 x1 DNA Analyser (Applied Biosystems). Analysis of product was done using Gene mapper Generic program.

***FXN* gene detection**

Of the total 46 hereditary ataxia patients 8 had an autosomal recessive history. In an attempt to diagnose these patients we carried out genetic testing for Friedreich's ataxia which is the most common autosomal recessive ataxia worldwide. The protocol was that designed and used in the Diagnostics Laboratory of the Medical Genetics Department, University of Oslo.

The experiment was done to detect homozygous triplet repeat expansion mutation GAA in intron 1 of *FXN* gene. This accounts for 96% of the affected population. A further 4% is due to compound heterozygous mutation for expansion mutation and a second inactivation mutation in the other allele. The detection of this would require sequencing, which we did not perform as a relatively small population of patients is caused by this mutation type and would not be viable option with regard to cost benefit ratios.

Initially repeats were amplified by PCR across the repeat under conditions in which only normal-sized alleles are amplified. Samples with a single peak in the electrophoresis output (either two normal alleles of identical size, or only one normal allele because of resistance of expanded mutant alleles to PCR) were further studied using repeat-primed PCR (RP-PCR).

Initial PCR experiment was performed using the following primers:

Forward: FRDA gaa- F*: 5’ GGG ATT GGT TGC CAG TGC TTA AAA GTT AG 3’

Reverse: FRDA gaa- R: 5’ AGC CTC TGG AGT AGC TGG GAT TAC AGG 3’

(Forward primer is fluorescently marked on the 5’ end)

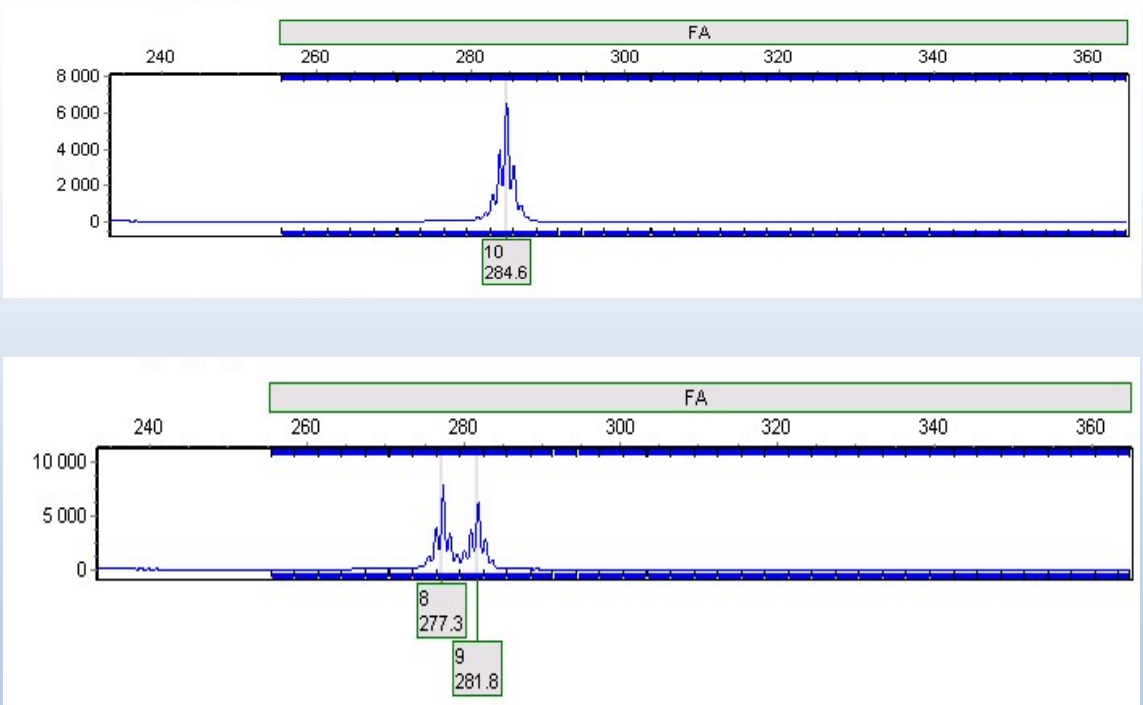
The PCR reaction was as follows:

Approximately 200ng of genomic DNA was amplified in a 25µl reaction volume containing a final concentration of Isis & Go (dNTP, buffer, $MgCl_2$, DMSO and Taq DNA polymerase

mixture) 5µl , 1µl each of forward and reverse FRDA primers of 10pmol/µl and MQ- H_2O 17µl. Samples were denatured at 93°C for 5 minutes followed by 32 cycles of denaturation (91°C, 60 seconds), annealing (68°C, 60 seconds), extension (72°C, 75 seconds), and a final extension of 10 minutes at 72°C in a Applied Biosystems Veriti 96 well thermal cycler

This PCR product was mixed with 12µl HiDi Formamide, 0.2µ LIZ – 500 and 12µl MQ- H_2O and plated in a PCR plate in wells that were spaced to avoid fluorescent bleeding. Plates were then sealed by heat sealing. Capillary electrophoresis was performed by Genescan Fragment analyzer ABI 3730 (Applied Biosystems). Results obtained were analyzed using the program GeneMapper. (Figure 8)

Figure 8: Capillary electrophoresis of PCR products of two sample; 1) homozygous allelea 2) heterozygous alleles



Results were seen as depicted in Figure 8. However with the samples showing a single peak, the possibility of a homozygous alleles or heterozygous alleles with an expanded allele that is large enough not to be detected on the scale is also a possibility. Therefore a second RP PCR is done to prevent confusion and missed detection of large mutant alleles.

Friedreich's ataxia – RP- PCR

The RP – PCR utilized the following primers

Reverse Primer: FRDA – R- Fam* (Reverse primer was fluorescently marked)

5' – GCT GGG ATT ACA GGC GCG CGA – 3'

Forward Primer: FRDAP4 – GAA_F:

5' – TAC GCA TCC CAG TTT GAG ACG GAA GAA GAA GAA GAA GAA GAA-3'

Forward Primer: P3

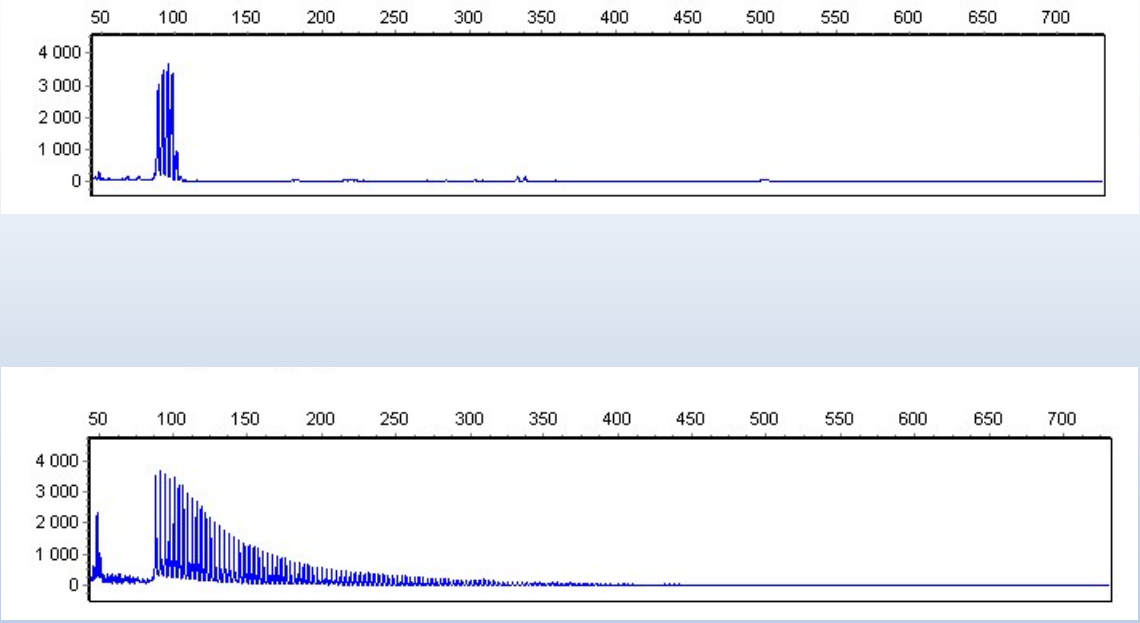
5' – TAC GCA TCC CAG TTT GAG ACG –3'

The PCR reaction was as follows:

Approximately 100ng of genomic DNA was amplified in a 20µl reaction volume containing a MQ- H_2O 9.1µl, 1.6 µl of 0.2mM dNTP, 1.2µl of 25mM $MgCl_2$, 2µl 360 GC enhancer, 2µl of 1x Taq Gold 360 Buffer, 0.1µl of 0.5U Taq Gold 360 DNA polymerase and 1µl each of 10pmol/µl forward and reverse FRDA primers. Samples were denatured at 95°C for 2 minutes followed by 32 cycles of denaturation (95°C, 30 seconds), annealing (60°C, 30 seconds), extension (72°C, 120 seconds), and a final extension of 5 minutes at 72°C in a Applied Biosystems Veriti 96 well thermal cycler.

This PCR product was mixed with 12µl HiDi Formamide, 0.2µ LIZ – 500 and 12µl MQ- H_2O and plated in a PCR plate in wells that were spaced to avoid fluorescent bleeding. Plates were then sealed by heat sealing. Capillary electrophoresis was performed by Genescan Fragment analyzer ABI 3730 (Applied Biosystems). Results obtained were analyzed using the program GeneMapper (Figure 9)

Figure 9: RP- PCR image of 1) non-expanded triplet region region 2) Expanded triplet region



The RP- PCR has two forward primers which anneal to the expanded region of the triplet repeat region. The molecular basis of this is for the second primer to anneal to the amplicon fragment created by the first forward primer and create multiple copies of varied sized triplet

repeat amplicons that appear as the figure above. This confirms a large repeat region and as they are of varied sizes the possibility of missing this due to the region being larger than the detection scale is avoided (Ciotti *et al.* 2004).

2.6 Statistical Methods

In the phenotype analysis, summary values are presented as mean (standard deviation (SD)) with the assumption that data were normally distribution. The $\chi 2$ or Fisher exact tests were used to determine departures from the hypothesized distribution of categorical data. $\chi 2$ goodness – of - fit was used to determine if the distribution of frequency among variables matched a theoretical or reference distribution. Student-t test was used to test for differences between groups in parametric data distributions. Correlation of continuous variable was performed by Pearson correlation coefficient. Relationships of clinical characteristics between patient groups were performed using regression and correlation analyses. Binary variables and continuous variable were analyzed using logistic regression analysis and multiple regression analysis respectively.

3.0 RESULTS

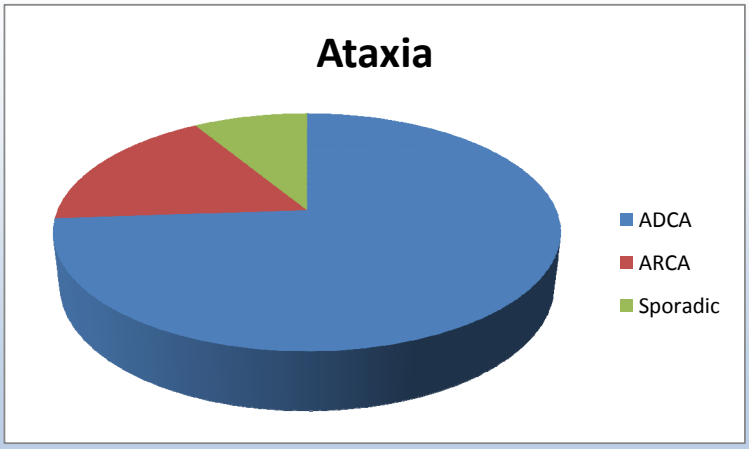
3.1 Overview

Forty six patients; 26 males and 20 females with hereditary ataxia (HA) were included. There were 34 autosomal dominant (ADCA), 8 autosomal recessive (ARCA), and 4 sporadic patients. Spinocerebellar ataxia type 1(SCA1) was seen in 21 patients; SCA 2 in 1 patient, of the total 34 patients with autosomal dominant cerebellar ataxia. This shows a SCA 1 frequency of 61.7% in the autosomal dominant study population. SCA 3, 6, 7, 8, and 12 mutations were not found. The 4 sporadic ataxia patients were also negative for SCA mutations. Friedreich's ataxia was not found in the autosomal recessive or sporadic ataxia population.

Of the total study population 43 were of Sri Lankan Sinhalese origin while the remaining 3 were of Sri Lankan Moor ethnic origin.

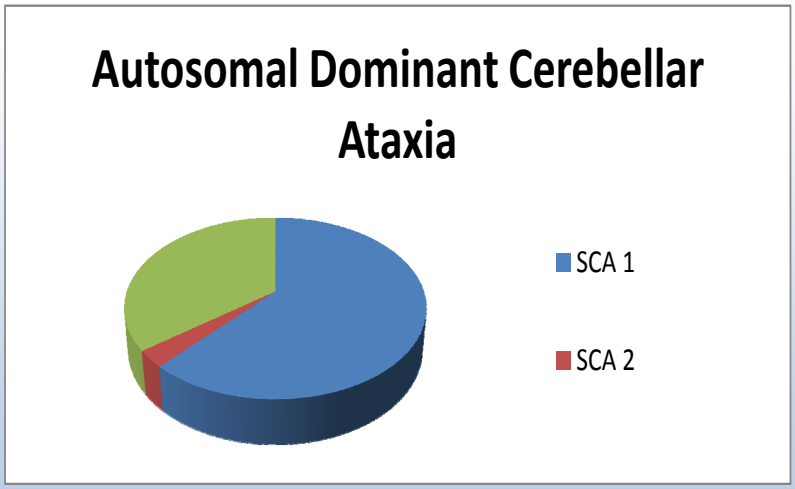
The following charts illustrate the distribution of patient groups (Figure 10, 11)

Figure 10: Distribution of all ataxia patients in study population



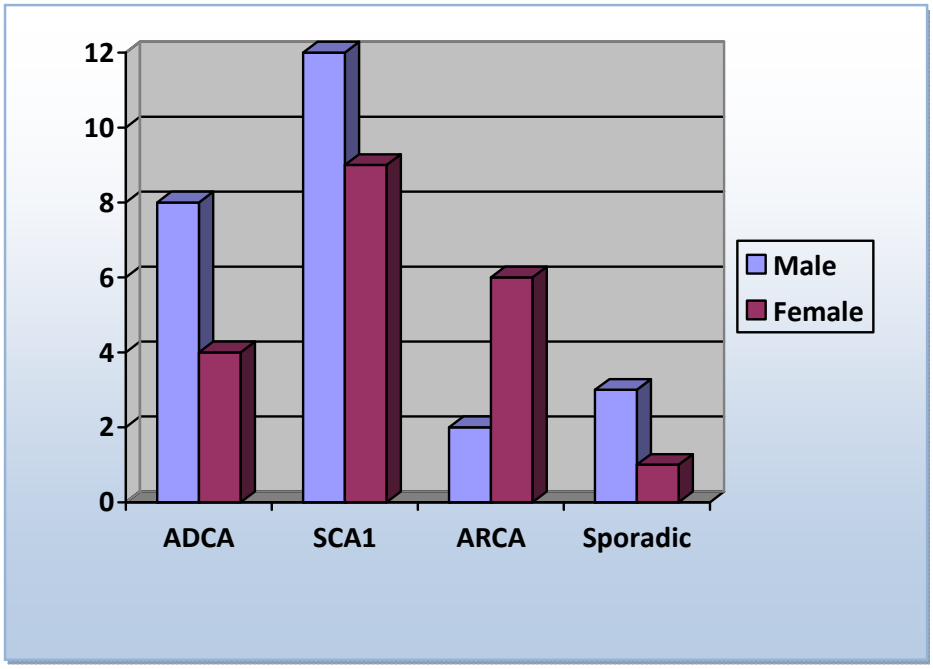
This patient population predominantly had patients with autosomal dominant inherted ataxias (ADCA)(Figure 10). Amongst those with ADCA, SCA 1 was present in 64% of the patient population (Figure 11).

Figure 11: Distribution of Autosomal dominant cerebellar ataxia in the study population



Gender distribution amongst the patient populations showed a male predominance in all patient groups except ARCA. (Figure 12)

Figure 12: Gender distribution amongst the ataxia patient groups



3.2 Geographical distribution

Mapping of the geographical location of patients was done by obtaining the origins of the transmitting parent in the case of autosomal dominant hereditary ataxia. In autosomal recessive and sporadic patients their current geographical location was noted. The southern and western provinces carried the highest number of patient population in all four categories (Table 6 and Figure 13)

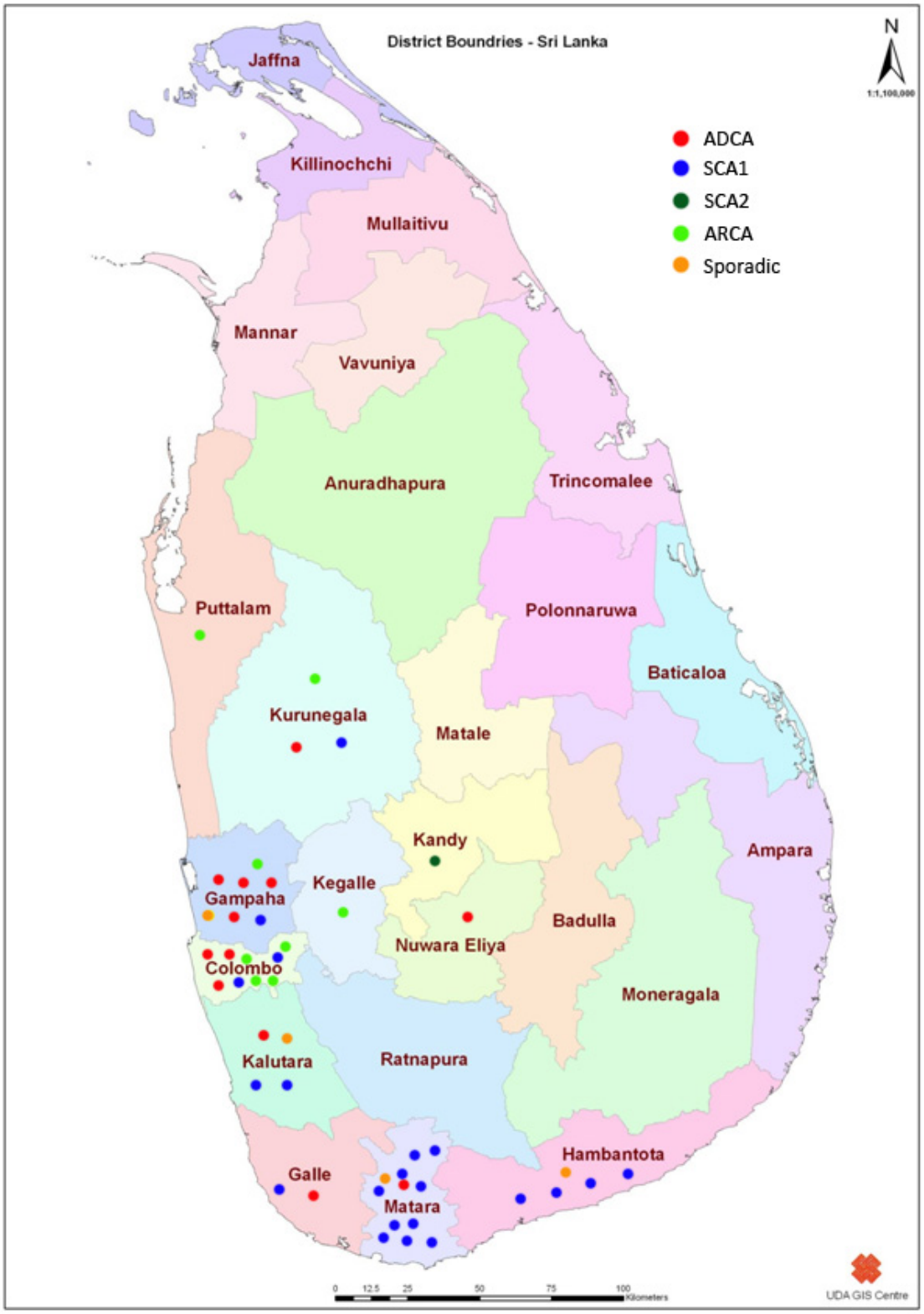


Figure 13: Distribution of all ataxia patients according to district of origin. In the case of sporadic and recessive ataxia the patients' present geographical location was noted

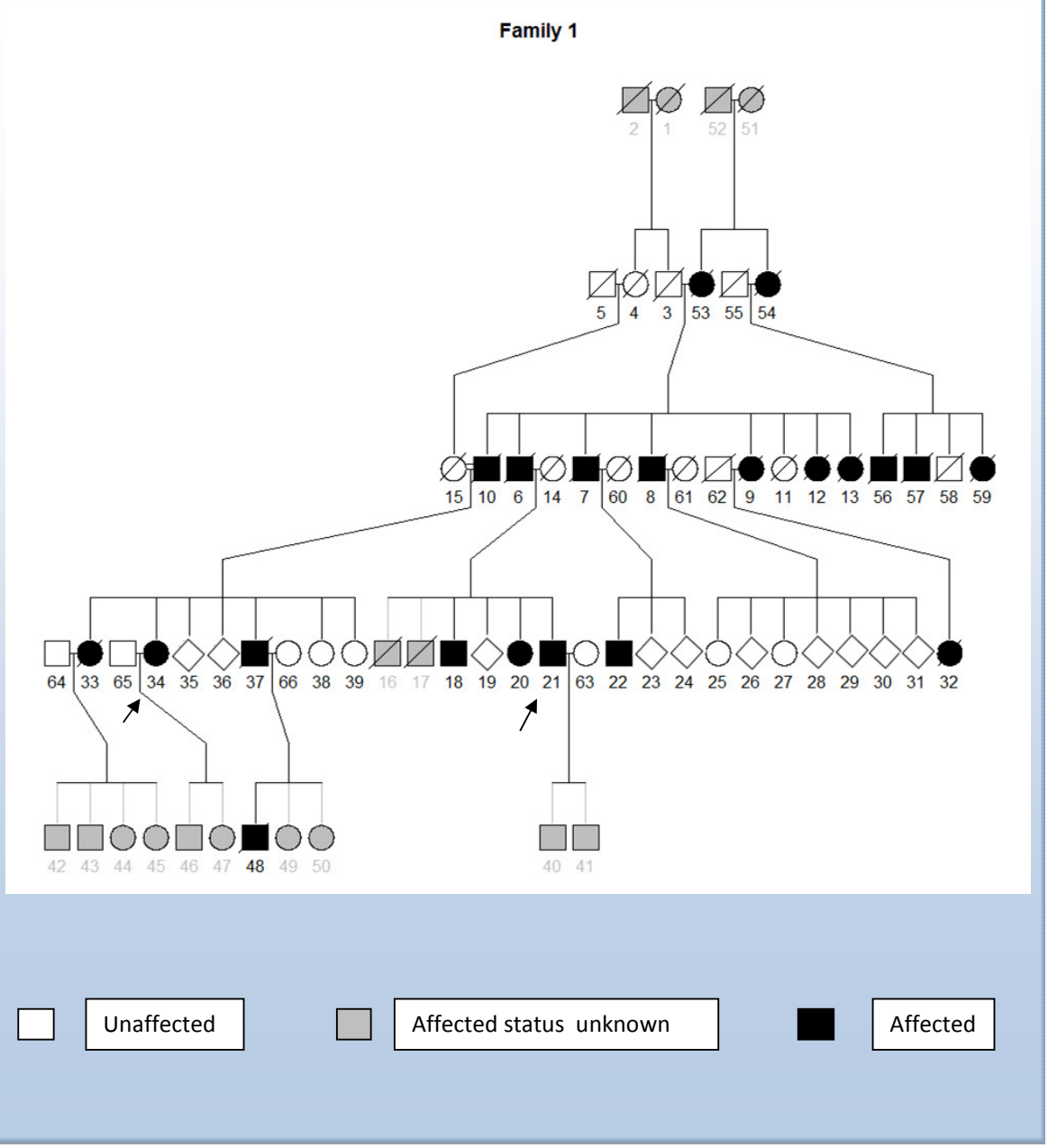
District of Origin	ADCA	SCA 1	SCA 2	ARCA	Sporadic	Total
Colombo	3	2	0	4	0	9
Gampaha	4	1	0	1	1	7
Kaluthara	1	2	0	0	1	4
Matara	1	10	0	0	1	12
Hambanthota	0	4	0	0	1	5
Galle	1	1	0	0	0	2
Kandy	0	0	1	0	0	1
Kegalle	0	0	0	1	0	1
Kurunegala	1	1	0	1	0	3
Nuwaraeliya	1	0	0	0	0	1
Puttalam	0	0	0	1	0	1
	12	21	1	8	4	46

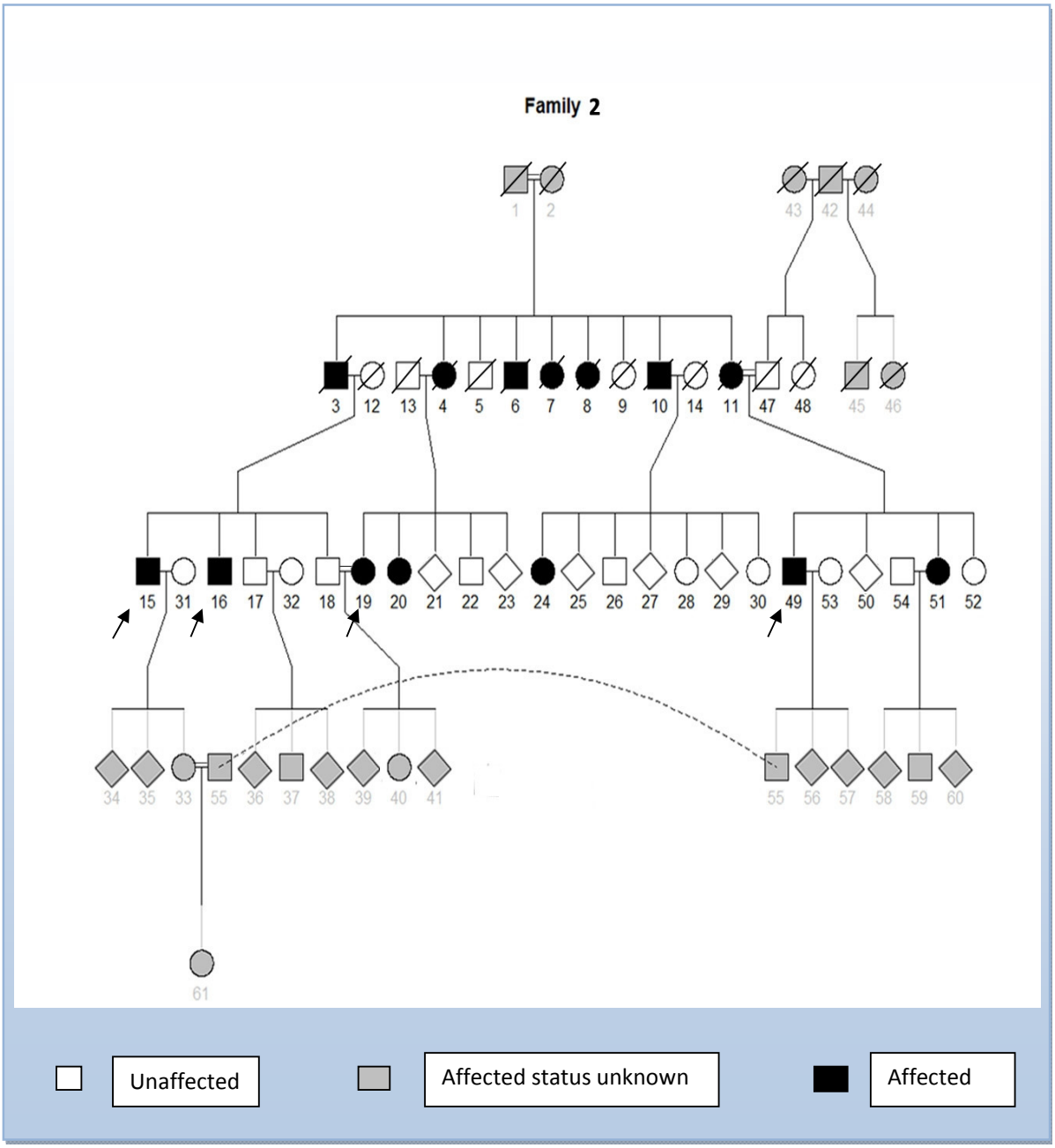
Table 6 Distribution of all ataxia patients according to district of origin

Of the total 21 patients with SCA 1, 15 patients were from a single geographical region in the southern province of Sri Lanka. These villages were situated in the Dickwella, Beliaththa Matara Medical Officer of Health (MOH) division of the Matara Regional Director of Health Services office and Weeraketiya MOH division of the Hambanthota RDHS office. Each MOH division had approximately 2 to 4 affected families.

Two families from this region accounted for 6 of the 15 SCA1 subjects of the study. Patient number 21 and 34 from family 1 and 15, 16, 19 and 49 from family 2 were recruited into the study. There was a high degree of consanguinity in these families. (Figure 14)

Figure 14 Pedigrees of two families with SCA 1 from the Southern province




Family 2
1 2
43 42 44
3 12 13 4 5 6 7 8 9 10 14 11 47 48 45 46
15 31 16 17 32 18 19 20 21 22 23 24 25 26 27 28 29 30 49 53 50 54 51 52
34 35 33 55 36 37 38 39 40 41
55 56 57 58 59 60
61
Unaffected
Affected status unknown
Affected

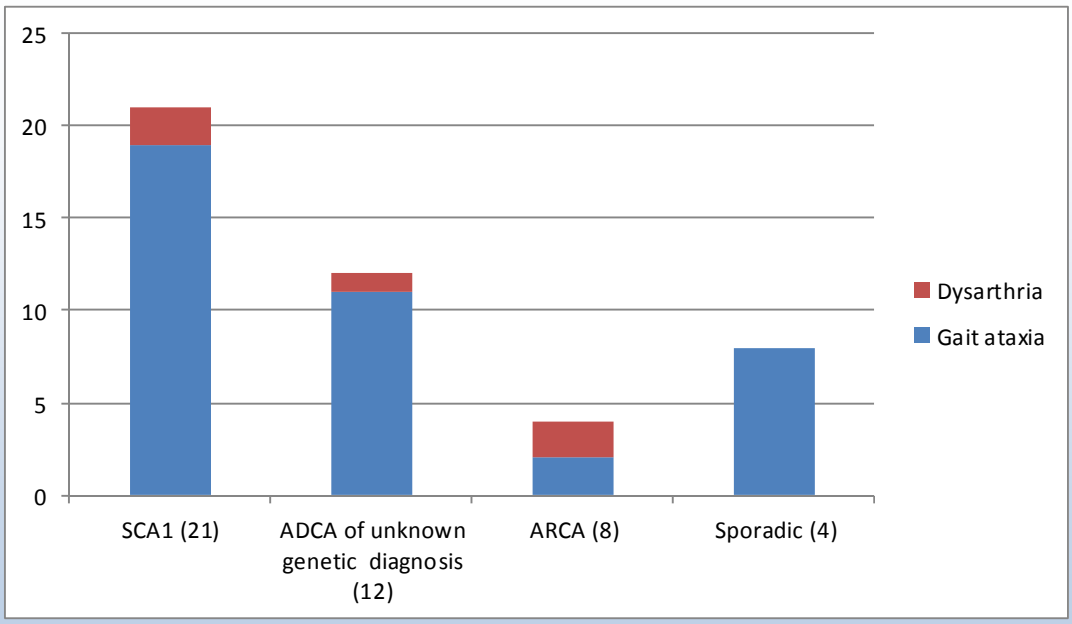
3.3 Clinical Description

Gait ataxia was the most frequent presenting symptom for all ataxia groups (89.1%), followed by dysarthria (10.9%) (Table 7)(Figure 15)

Presenting Symptom	*ADCA**	*SCA1*	*SCA 2*	*ARCA*	*Sporadic*
Dysarthria	*1*	*2*	*0*	*0*	*2*
Gait Ataxia	*11*	*19*	*1*	*8*	*2*
Total	*12*	*21*	*1*	*8*	*4*

Table 7 Presenting symptom in the four ataxia groups

Figure 15: Presenting symptom in the four ataxia groups



Age of onset of symptoms was categorized as shown below. (Table 8, Figure 16) The highest number of SCA 1 patients had their age of onset between 31 – 40 years. In the autosomal dominant ataxia without genetic diagnosis 21 – 30 age groups had the highest number. The majority of patients in the autosomal recessive age group had their age of onset in the juvenile age group. (Table 8, Figure 16)

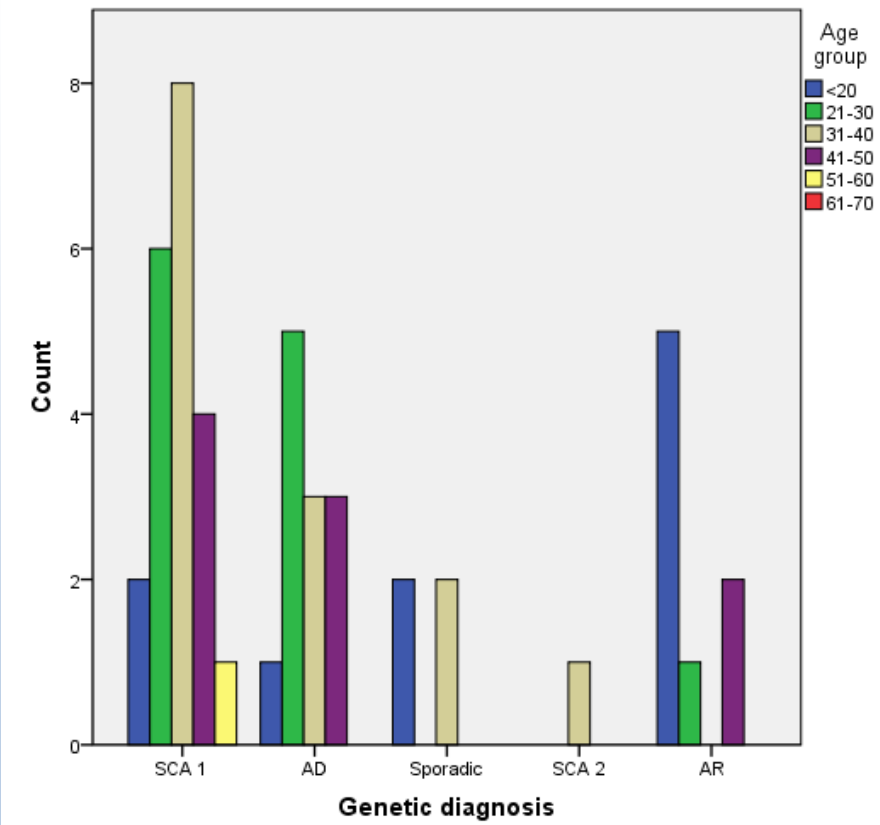
Age group * Genetic diagnosis Cross tabulation

Count

		Genetic diagnoses					
		SCA 1	*ADCA*	*Sporadic*	*SCA 2*	*AR*	*Total*
Age Group	*<20*	*2*	*1*	*2*	*0*	*5*	*10*
	21-30	*6*	*5*	*0*	*0*	*1*	*12*
	31-40	*8*	*3*	*2*	*1*	*0*	*14*
	41-50	*4*	*3*	*0*	*0*	*2*	*9*
	51-60	*1*	*0*	*0*	*0*	*0*	*1*
Total		*21*	*12*	*4*	*1*	*8*	*46*

Table 8 Age of onset of disease in all ataxia groups

Figure 16: Age of onset of disease in all ataxia groups



In SCA1 patients' age of onset ranged from 16 to 55 years, with a mean age of onset of symptoms at 34.8 ± 10 years (range 16 - 55), this included two patients with juvenile onset symptoms (<20 years). Mean ages of onset in ADCA and ARCA patient groups were 32.7±9.8 and 21.2±13.7 years respectively.

In SCA 1 patients there was a significant positive correlation between the duration of disease and disability level ($r = 0.46$, $p<0.05$). In the ADCA with unknown genetic etiology, a similar significant positive correlation was detected ($r = 0.57$, $p = 0.05$). In ARCA and Sporadic ataxia, no significant correlation was detected between the two variables ($r = 0.007$, $p >0.05$ and $r = 0.616$ and $p >0.05$ respectively), which may be due to small sample sizes (Table 9)

SCA 1 and autosomal dominant ataxia of unknown genetic etiology were compared for significant differences in age, age at onset duration and disability level. A significant difference between the two groups was seen only at duration of disease, with autosomal dominant ataxia of unknown genetic etiology having a longer duration.

Autosomal recessive ataxia and autosomal dominant ataxia of unknown genetic etiology were not significantly different in variables age of onset of disease, duration of disease and disability level at presentation.

Sporadic ataxia had a longer duration of disease than the combined group of ADCA (ADCA of unknown genetic etiology + SCA1 + SCA2) ($p < 0.05$). The other two parameters (age of onset and disability level) were not significantly different in the two groups.

	ADCA (12)	*SCA1(21)*	*SCA2 (1)*	*ARCA (8)*	*Sporadic (4)*
Age at presentation (yrs) mean ± SD (range)	*41.4±7.9 (30 - 50)*	*42.1±11.2 (23 - 63)*	*47*	*30.6±12.7 (13 - 50)*	*40.5±14.5 (19 - 50)*
Age at onset (yrs) mean ± SD (range)	*32.7±9.8 (16 - 47)*	*34.8±10 (16 - 55)*	*34*	*21.2±13.7 (6 - 45)*	*26.2±11.7 (16 - 40)*
Disease duration (yrs) mean ± SD (range)	*8.9±5.3 (2 - 17)*	*7.4±3.1 (2 - 13)*	*13*	*9.4±7 (2 - 22)*	*14.2 ± 11.9 (3 -31)*
Disability (0 - 7) mean ± SD (range)	*3.0 ± 2.7 (1 - 6)*	*4±2*	*6*	*4.5±1.5 (2-6)*	*3±0.8 (2-4)*

Table 9 Duration of disease and disease disability of patients

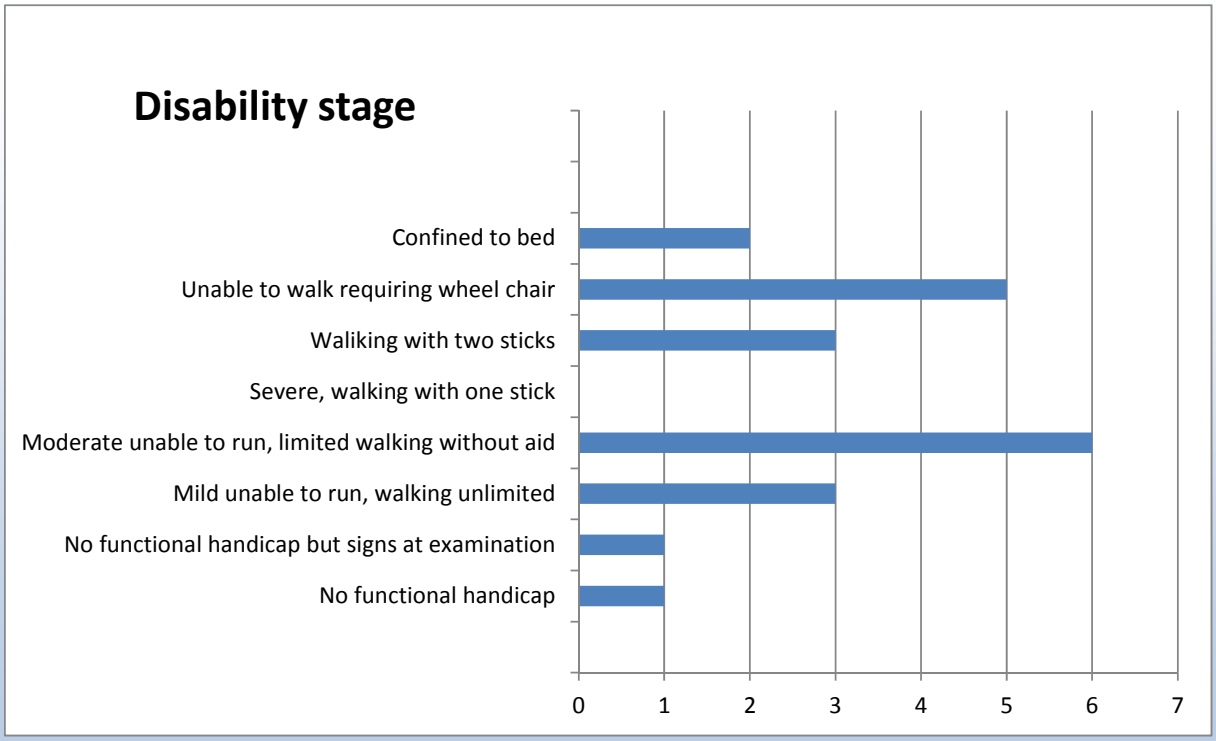
Disability was quantified according to scale shown in Table 10 (Figure 17). The highest frequency of disability in all patient groups (28.6%) was at disability level 3 (moderate unable to run, limited walking without aid), with a mean level of disability of 4 (severe walking with one stick).

Score	*Disabilty level*	*SCA 1*	*SCA 2*	*ADCA*	*ARCA*	*Sporadic*
0	*No functional handicap*	*1*	*0*	*0*	*0*	*0*
1	*No functional handicap but signs at examination*	*1*	*0*	*2*	*0*	*0*
2	*Mild unable to run, walking unlimited*	*3*	*0*	*4*	*1*	*1*
3	*Moderate unable to run, limited walking without aid*	*6*	*0*	*2*	*1*	*2*
4	*Severe, walking with one stick*	*0*	*0*	*1*	*2*	*1*
5	*Walking with two sticks*	*3*	*0*	*2*	*1*	*0*
6	*Unable to walk requiring wheel chair*	*5*	*1*	*1*	*3*	*0*
7	*Confined to bed*	*2*	*0*	*0*	*0*	*0*
	Total	*21*	*1*	*12*	*8*	*4*

Table 10: Quantification of disability level in the 5 ataxia groups

According to Table 10 patients with a disability level that required walking aids or greater assistance was 76%, 50%, 87.5% and 75% in SCA1, ADCA, ARCA and Sporadic ataxia respectively.

Figure 17: Disability levels in SCA 1 patients



Scale for the Assessment and Rating of Ataxia (SARA)

The mean scores for total SARA and the sub scores of SARA were calculated in each subtype of patient. (Table 11)

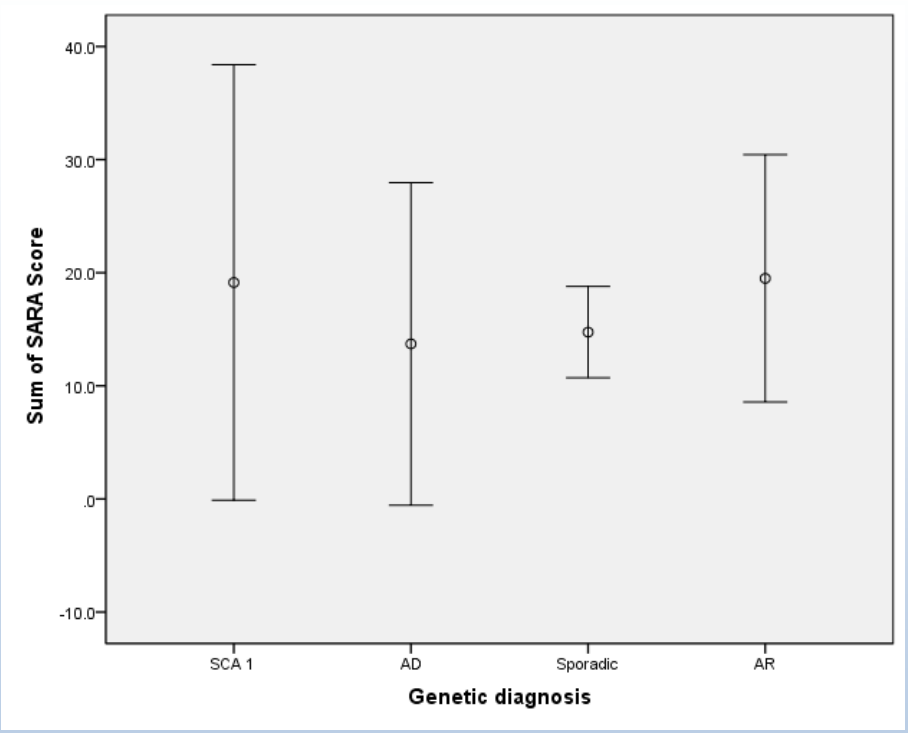
Table 11 SARA score and sub scores in all ataxia patient groups

	ADCA (12)	*SCA 1 (21)*	*SCA 2 (1)*	*ARCA(5)*	*Sporadic (4)*
Sum score - mean ± SD (range)	*13.7±7.1 (4.5 - 27)*	*18.8±9.7 (0 - 32.5)*	*26*	*19.5±5.5 (13 - 29)*	*15±2.2 (13 - 18)*
SARA 1 - 3 posture and gait - mean ± SD (range)	*6.4±4.2 (2 - 15)*	*9.0±5.0 (0 - 16)*	*12*	*10.0±4.2 (5 - 16)*	*6.2±1.7 (4 - 8)*
SARA 4 speech - mean ± SD (range)	*1.8±1.1 (0 -4)*	*2.8±1.5 (0 - 5)*	*4*	*2.0±0.7 (1 - 3)*	*2.5±0.6 (2 - 3)*
SARA 5 - 8 limb kinetic function - mean ± SD (range)	*5.4±2.2 (2.5 - 10)*	*7.0±3.4 (0 - 11.5)*	*10*	*7.8±1.6 (6 - 10)*	*6.0±1.2 (4.5 - 7.5)*

Comparison between the SARA scores of SCA1 patients and ADCA of unknown genetic etiologies showed no significant differences in the sum score of SARA and sub scores, posture and gait and limb kinetic function. However speech sub score was significantly higher in SCA1 compared to ADCA of unknown genetic etiology ($p=0.05$).

Figure 18 shows the mean value and 2SD of the SARA score in the ataxia patient groups.

Figure 18: Mean + 2SD of SARA score vs Genetic Diagnosis



The mean SARA score in SCA 1 achieved was 18.8 ± 9.7 (range 0-32.5).Assessment of correlation between SARA score and CAG repeat length of expanded allele, age , age at onset and disease duration revealed positive correlation between SARA score and age at onset (R^2 = 0.6, $p<0.05$).

Inventory of Non Ataxia Symptoms (INAS)

Table 12 Inventory of non ataxia symptoms in the ataxia patient groups

	ADCA (12)	*SCA 1 (21)*	*SCA 2 (1)*	*ARCA (8)*	*Sporadic (4)*
Sum Score - mean ± SD (range)	*3.8±2.1 (0-7)*	*3.6±2.4 (0 - 9)*	*6*	*3.4±2.4*	*3.0±1.8 (1 - 5)*
INAS 1: Hyperreflexia	*6/12 (50%)*	*16/21 (76.2%)*	*0*	*4/8(50%)*	*2/4(50%)*
INAS 2: Areflexia	*2/12 (16.7%)*	*0/21(0%)*	*1/1*	*1/8 (12.5%)*	*0/4(0%)*
INAS 3: Extensor plantar	*6/12 (50%)*	*8/21 (38.1%)*	*0/1*	*4/8(50%)*	*1/4(25%)*
INAS 4: Spasticity	*8/12 (66.7%)*	*17/21(81%)*	*0/1*	*4/8(50%)*	*2/4(50%)*
INAS 5: Paresis	*4/12 (33.3%)*	*6/21 (28.6%)*	*1/1*	*2/8(25%)*	*2/4(50%)*
INAS 6: Muscle atrophy	*3/12 (25%)*	*8/21 (38.1%)*	*1/1*	*1/8 (12.5%)*	*0/4(0%)*
INAS 7: Fasciculations	*2/12 (16.7%)*	*2/21(9.5%)*	*1/1*	*0/8(0%)*	*0/4(0%)*
INAS 8: Myoclonus	*0/12(0%)*	*0/21(0%)*	*0/1*	*0/8(0%)*	*0/4(0%)*
INAS 9: Rigidity	*0/12 (0%)*	*0/21(0%)*	*0/1*	*0/8(0%)*	*0/4(0%)*
INAS 10: Chorea/Dyskinesia	*0/12 (0%)*	*0/21(0%)*	*0/1*	*0/8(0%)*	*1/4(25%)*
INAS 11: Dystonia	*0/12 (0%)*	*0/21(0%)*	*0/1*	*0/8(0%)*	*0/4(0%)*
INAS 12: Resting tremor	*1/12 (8.3%)*	*1/21(4.8%)*	*0/1*	*1/8(12.5%)*	*1/4(25%)*
INAS 13: Sensory symptoms	*5/12 (41.7%)*	*6/21 (28.6%)*	*1/1*	*3/8 (37.5%)*	*1/4(25%)*
INAS 14: Urinary dysfunction	*3/12(25%)*	*5/21 (23.8%)*	*0/1*	*1/8 (12.5%)*	*1/4(25%)*
INAS 15: Cognitive dysfunction	*2/12 (16.7%)*	*1/21(4.8%)*	*0/1*	*0/8(0%)*	*1/4(25%)*
INAS 16: Brainstem occulomotor signs	*3/12 (25%)*	*6/21 (28.6%)*	*1/1*	*0/8(0%)*	*0/4(0%)*

Spasticity and hyperreflexia were the most prevalent non ataxia features in both the SCA 1 (76% and 81%) and autosomal dominant ataxia of unknown genetic etiology (67% and 50%) groups. Extra pyramidal features such as myoclonus, rigidity, chorea, dyskineasia and dystonia were absent in the two patient populations. (Table 12)

Comparison between ADCA of unknown genetic etiology and SCA 1 with regard to the INAS sum score showed no statistically significant difference.

Correlation between INAS sum score and CAG repeat length of expanded allele, age, age at onset and disease duration variables was not detected.

Correlation between each non-ataxia symptom and variables age, age at onset, disease duration and CAG repeat length were calculated in the SCA 1 patient group. A significant correlation was found with age variable and hyperreflexia, spasticity, urinary dysfunction and cognitive decline. (Table 13)

Table 13 Positive correlations of age variable with non ataxia symptoms

Factor	*Symptom*	*OR (95%)*	*P*
Age	*Hyperreflexia*	*1.031(1.006 - 1.057)*	*0.017*
	Spasticity	*1.038 (1.01 - 1.067)*	*0.008*
	Urinary dysfunction	*0.972 (0.949 - 0.996)*	*0.023*
	Cognitive dysfunction	*0.931 (0.886 - 0.979)*	*0.005*

Cognition level

Cognition was assessed by the administration of the Montreal Cognitive Assessment Scale (MoCA). (Table 14)

Table 14 Cognitive levels according to MoCA scales in ataxia groups and subgrouped according to age, education level, SARA, INAS, Symptom duration and disability level

	ADCA	ADCA	SCA1	SCA1	ARCA	ARCA	Sporadic	Sporadic
	<27	>27	<27	>27	<27	>27	<27	>27
N	7	3	8	0	6	0	3	1
Age Group								
<20	1	0	1	0	3	0	1	1
20 - 29	3	2	3	0	1	0	0	0
30 - 39	1	1	3	0	0	0	2	0
40 - 49	2	0	1	0	2	0	0	0
50 - 59	0	0	0	0	0	0	0	0
60 - 69	0	0	0	0	0	0	0	0
Education								
>12 years	3	2	3	0	5	0	0	0
8 - 12 years	4	1	5	0	0	0	3	1
<8 years	0	0	0	0	1	0	0	0
Gender								
M	4	2	5	0	2	0	3	1
F	3	1	3	0	4	0	0	0
SARA	15.7±5.0	5.8±1.2	14.5±9.9	0	19.5±5.5	0	14.7±2.5	15
INAS	4.3±1.5	2.0±2.0	2.2±1.5	0	4.5±1.5	0	3.3±2.1	3
Sym Duration	9.0±5.1	8.7±2.1	7.8±3.3	0	10.8±7.5	0	18.0±11.4	2
Disability	3.4±1.3	1.3±0.6	3.0±1.9	0	4.7±1.2	0	3.0±1.0	3

Abnormal and normal grouping of cognition was done according to the MoCA scale: ≥ 26 was normal cognition.

All 8 patients (100%) with SCA 1 tested by MoCA showed reduced cognitive levels, while of the 10 patients tested with ADCA of unknown genetic etiology, 7(70%) had abnormal cognition. All patients with ARCA (100%) and 3 / 4 patients with sporadic ataxia (75%) had abnormal cognition.

Within groups step wise multiple regression analysis was performed to assess the level of correlation cognition had with independent variables (age, education level, SARA and INAS). In the ADCA of unknown genetic etiology group significant correlation with cognition was found associated with the mean SARA score ($p<0.05$). In the SCA 1 and ARCA groups no correlation between cognition and independent variable was found

Depression Comorbidity

Prevalence and characteristics of depression was assessed using the Patient Health Questionnaire (PHQ). PHQ is a 9 item self-rating questionnaire. Patients with clinically relevant depression (defined as those with moderate or severe levels of depression) was present in SCA1, ADCA, ARCA and Sporadic ataxia groups in 68.4%, 75%, 80% and 75% respectively. The highest mean sum score was present in the ADCA group. (Table 15)

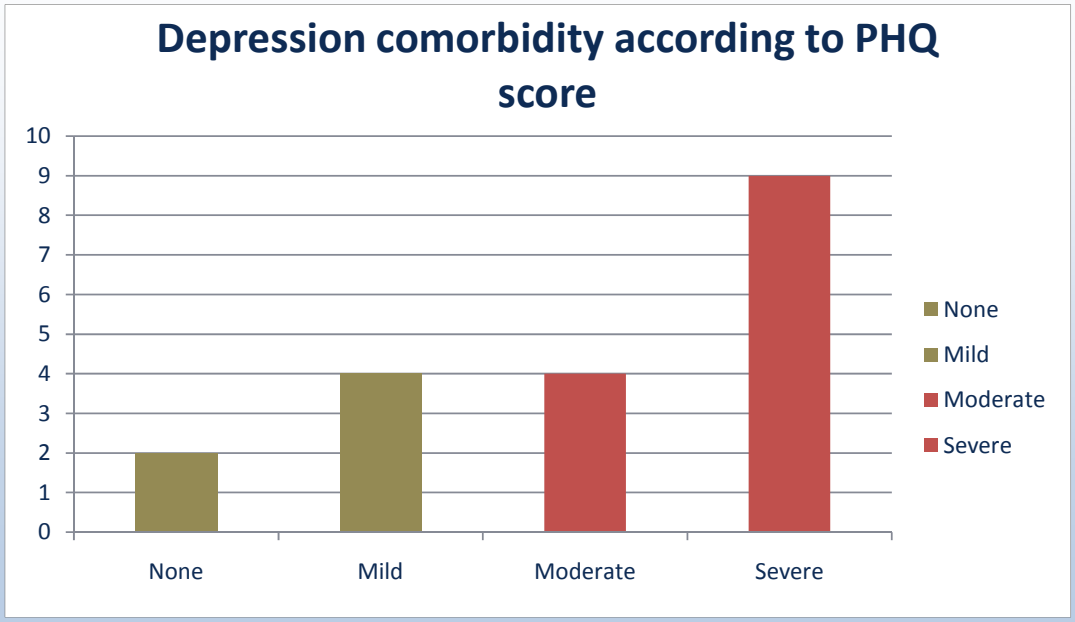
Table 15 PHQ depression score and severity levels according to the ataxia patient groups

	*ADCA * (12)*	*SCA 1 (21)*	*SCA 2 (1)*	*ARCA (8)*	*Sporadic (4)*
N	*12*	*19*	*1*	*5*	*4*
PHQ sum score	*14.4±5.4*	*12±6.3*	*19*	*13.6±6.3*	*11.8±5.9*
PHQ sum score classification (%)					
None	*0(0)*	*2 (10.5)*	*0(0)*	*0 (0)*	*0(0)*
Mild	*3(25)*	*4(21.1)*	*0(0)*	*1 (20)*	*1(25)*
Moderate	*3(25)*	*4 (21.1)*	*0(0)*	*2(40)*	*2 (50)*
Severe	*6(50)*	*9(47.4)*	*1(100)*	*2(40)*	*1(25)*
PHQ sum score (%) clinically relevant depression	*9/12(75)*	*13/19(68.4)*	*1/1(100)*	*4/5(80)*	*3/4(75)*

PHQ score was assessed with level of disability, duration of disease, INAS and SARA, and showed significant correlation with duration of disease in SCA 1 group (p=0.005), which was identified as an independent predictor of depressive status. ADCA of unknown genetic etiology did not show any significant correlations. Statistic regression analysis was not possible in the ARCA and sporadic ataxia groups due to small sample size.

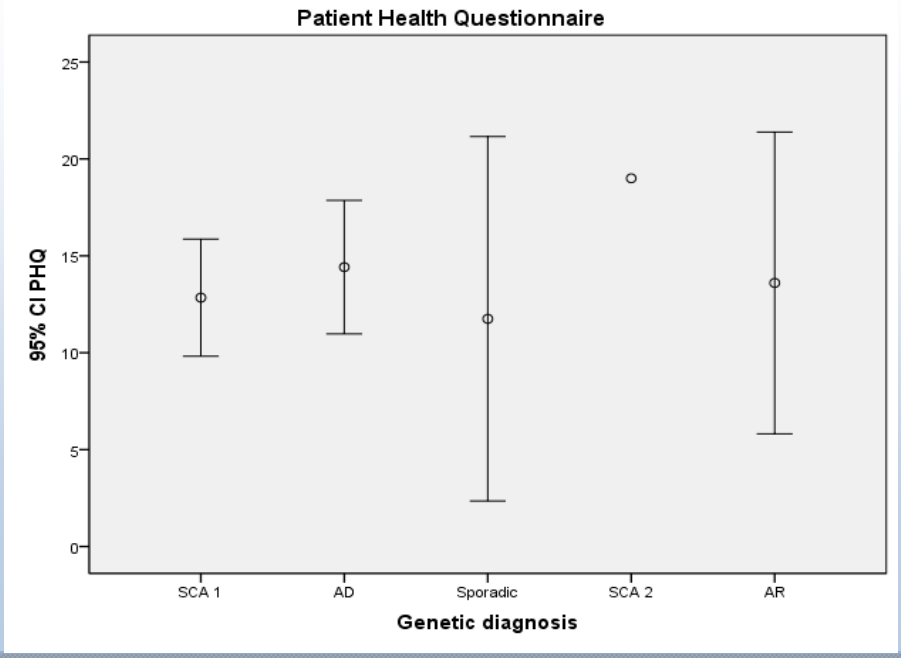
PHQ was answered by 19/21 SCA1 patients. Clinically relevant depression was present in 68.4% of patients (Figure 19)

Figure 19: Distribution of level of depression in SCA 1 patients. Clinically relevant depression in shaded in red



Distribution of PHQ scores in the ataxia patients with 95% confidence distribution is illustrated in Figure 20.

Figure 20: Distribution of PHQ scores in ataxia patient groups with 95% Confidence interval



Sub score analysis of PHQ is listed in Table 16. Sleep disturbance was the most critical problem faced by patients (63.2%).

A total of 8/46 (17.4%) patients were on antidepressants at the time of presentation. The distribution of usage was 2, 1, 1, and 3 in SCA 1, ADCA of unknown genetic etiology, ARCA and Sporadic ataxia respectively.

Table 16 PHQ sub scores in the ataxia patient groups

	Whole sample	*ADCA**	*SCA 1*	*ARCA*	*Sporadic*
	Any problem/ Critical problem	*Any / Critical problem*	*Any / Critical problem*	*Any / Critical problem*	*Any / Critical problem*
N	*41*	*12*	*19*	*5*	*4*
PHQ 1a - Little interest/ pleasure	*17.1/29.3*	*25.0/0*	*15.8/31.6*	*20/40*	*0/75*
PHQ 1b - Feeling down, hopeless	*29.3/48.8*	*50.0/0*	*21.1/47.4*	*40/60*	*0/75*
PHQ 1c - Sleep disturbance	*19.5/65.9*	*25.0/58.3*	*15.8/63.2*	*40/80*	*25/75*
PHQ 1d - Tired or little energy	*22.0/58.5*	*25/75*	*26.3/47.4*	*20/60*	*0/50*
PHQ 1e - Change of appetite	*12.2/36.6*	*16.7/58.3*	*10.5/21.1*	*20/20*	*0/50*
PHQ 1f - Feeling bad about self	*14.6/46.3*	*8.3/41.7*	*10.5/47.4*	*40/40*	*25/50*
PHQ 1g - Trouble concentrating	*9.8/26.8*	*8.3/41.7*	*10.5/31.6*	*20/0*	*0/0*
PHQ 1h - Moving/ speaking slowly	*29.8/39.0*	*33.3/41.7*	*36.8/42.1*	*20/40*	*0/25*
PHQ 1i - Better off dead, hurting oneself	*56.1*	*50*	*47.4*	*80*	*75*

PHQ results at item level given as relative frequencies (percentage of valid data per item) of patientsreporting any problem (any rating different from ‘‘not at all’’) or critical problem (rating of ‘‘more than half the days’’ for PHQ items 1a–1h and any rating different from ‘‘not at all’’ for PHQ item 1i)

3.4 Family History

Family history was obtained and included a 3 generation family pedigree.

Table 17 Parental transmission of triplet repeat mutations, anticipation and CAG repeat in patients

	SCA 1		*ADCA*	
N	*21*		*12*	
	Father	*Mother*	*Father*	*Mother*
Parental Transmission - Father / Mother	*15 (71.4 %)*	*6 (28.6 %)*	*10 (83.3 %)*	*2 (16.7 %)*
	p = 0.05		*P<0.05*	
Age difference between transmitting parent and child -Father / Mother mean ± SD	*12.6±5.4*	*6.8±2.4*	*15.7±6.3*	*14.5±4.9*
	p < 0.05		*p>0.05*	
CAG repeat number - mean ± SD (range)	*52.0 ± 3.8 (47 - 59)*		*N/A*	

From the total autosomal dominant cerebellar ataxia patients 25/34 (73.5%) have received their mutation from their father, with approximately 71.4% transmission seen in the case of SCA1 patients and 83.3% in the ADCA of unknown etiology. In both categories the number of patients with paternal inheritance of mutation was significantly higher compared to maternal ($p<0.05$).

In the SCA 1 patients 12 parent – child pairs were identified that indicated age of onset of disease. In 11 of the 12 pairs, onset of disease in the offspring occurred earlier than in the parent by 11.2±5.3 years, strongly suggesting the presence of anticipation ($\chi 2= 8.33$, $p<0.005$).

For the category of ADCA of unknown genetic etiology 10 parent – child pairs were identified and 9 had an onset of disease earlier than parent by 15.4±5.8 years, which was also significant ($\chi^2= 6.4$, $p<0.05$). A significant proportion of those affected had the mutation

transmitted from their father. However there was no significant difference in the degree of anticipation seen according to the transmitting parent (Table 17).

The age differences in onset of symptom between parent and child was calculated and grouped according to the gender of the transmitting parent.

The effect of parental origin on anticipation in SCA 1 patients was assessed. The mean difference in age of onset in 8 father child pairs (12.6 ±5.4) was not significantly different from the 4 mother child pairs (6.8±2.4) ($t = 2.043$, $p = 0.068$). However those with mutation transmission from their mothers showed a relatively lower age difference compared to those with paternal transmission (Figure 21)

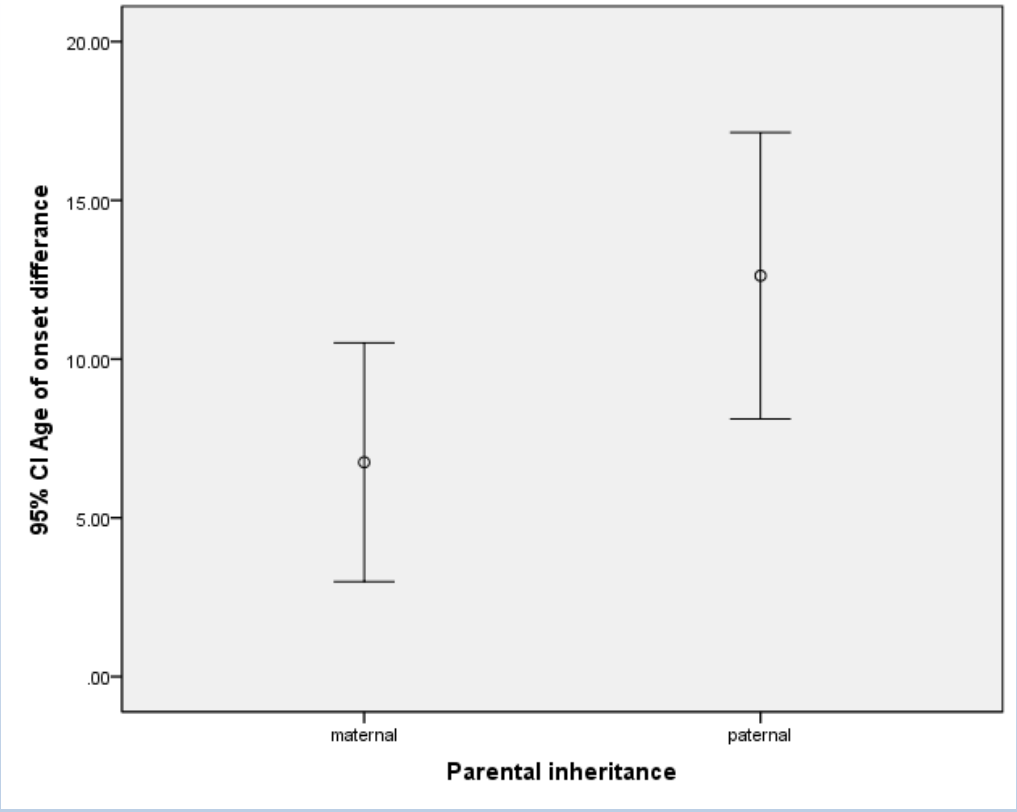


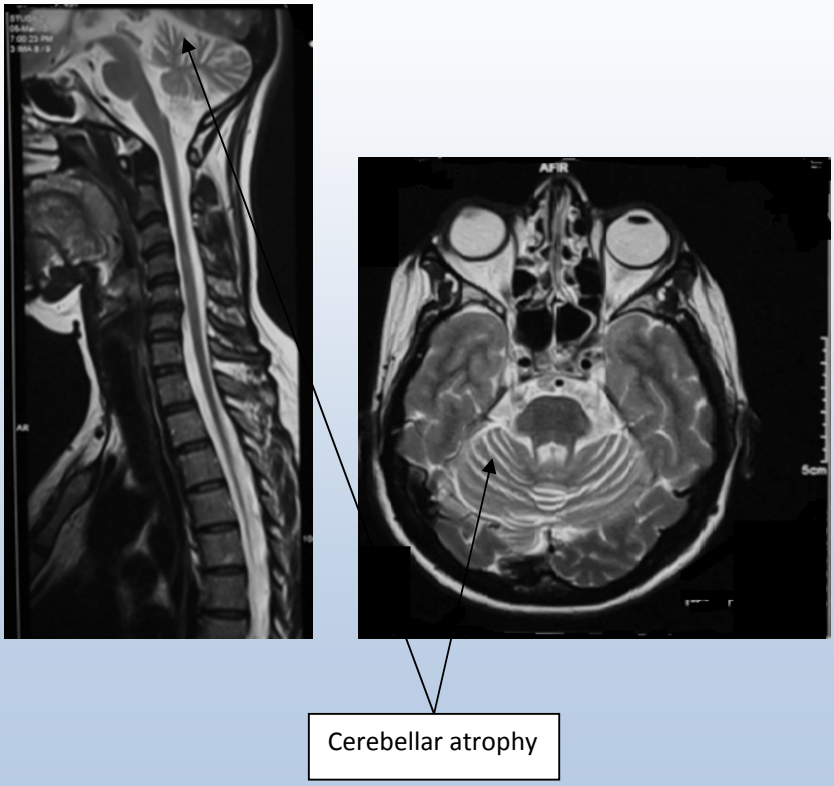
Figure 21: Differences in age of onset between parent and child according to transmitting parent

3.5 Radiological features

SCA1

A typical MRI finding of SCA1 was noted in this patient who was a part of the study population

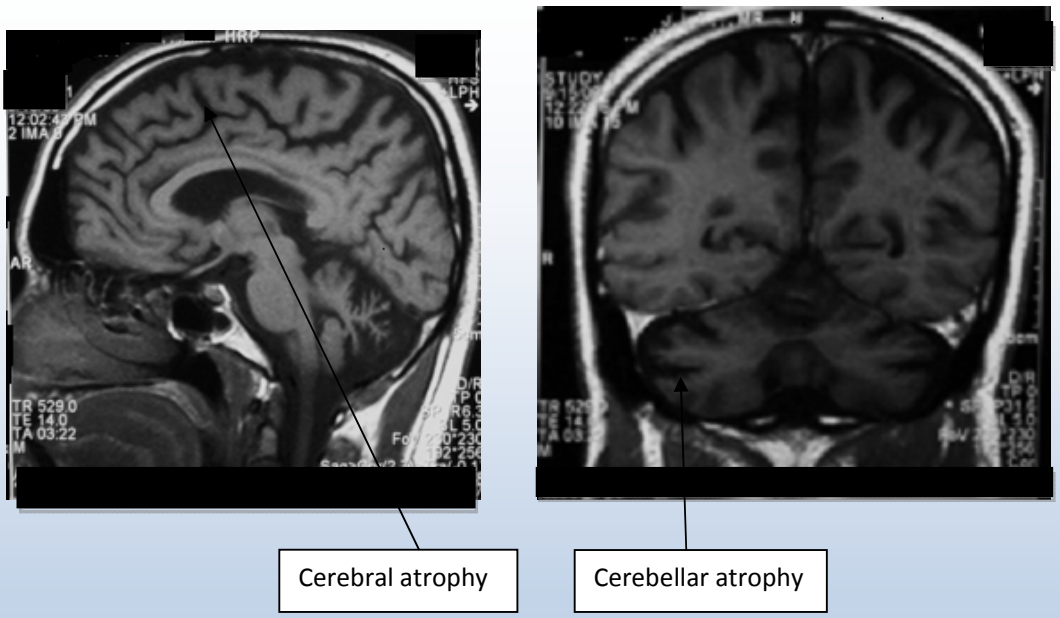
Figure 22: Sagittal and Coronal MRI findings of a patient with SCA 1 in the study group with cerebellar atrophy and thinning of the brainstem at 38 years with disease duration of 8 years



ADCA of unknown genetic etiology

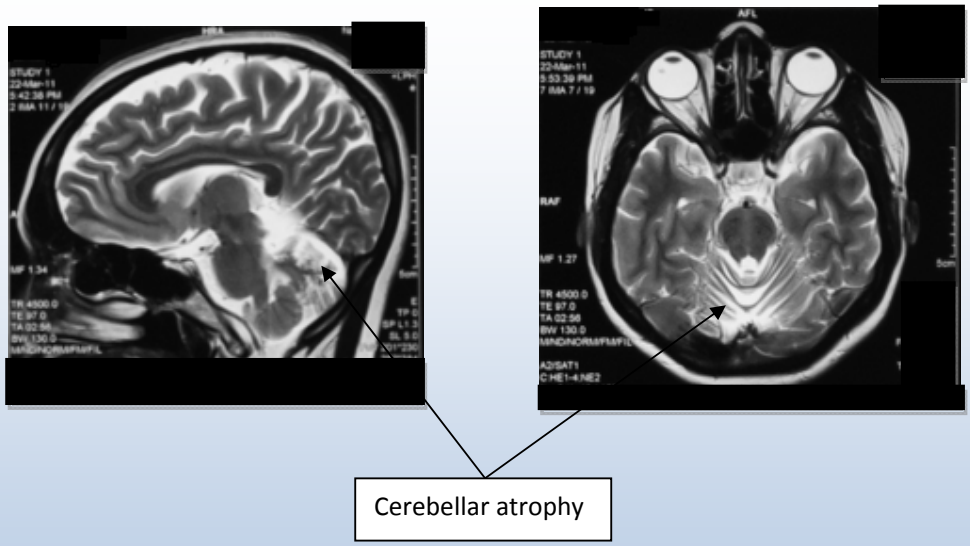
The following MRI was seen in a patient with autosomal dominant cerebellar ataxia without a known genetic diagnosis

Figure 23: MRI showing cerebral and cerebellar atrophy in a 49 year old patient with symptoms for 12 years



Autosomal recessive cerebellar atrophy

Figure 24: Isolated cerebellar atrophy in a patient with ARCA, from our study population



The patient had symptoms from 12 years of age and has had disease duration of 22 years.

3.6 Genetic Results

The mean CAG repeat number in the SCA 1 group was 52±3.8 (range 47 - 59) repeats. Correlations between the age of onset of disease and CAG repeat length, showed inverse correlation (p<0001).

Correlation of the simultaneous effects of CAG repeats length and the parental inheritance (mother or father) on the age of onset of disease in SCA 1 patients was highly significant (p <0.001). However the transmitting parent (independent variable) did not independently correlate significantly with the age of onset (p =0.126) and only CAG repeat length accounted for a significant amount of unique variance of the dependent variable.

Therefore, a bivariant analysis was performed between the age of onset and CAG repeat length. Correlation between the two variables was shown (r = - 0.81,p<0.005).

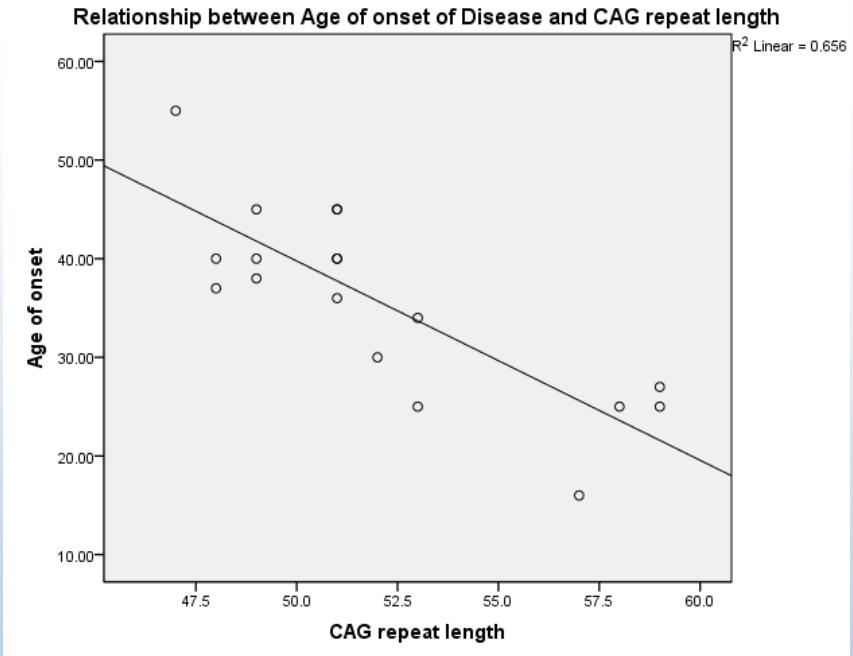
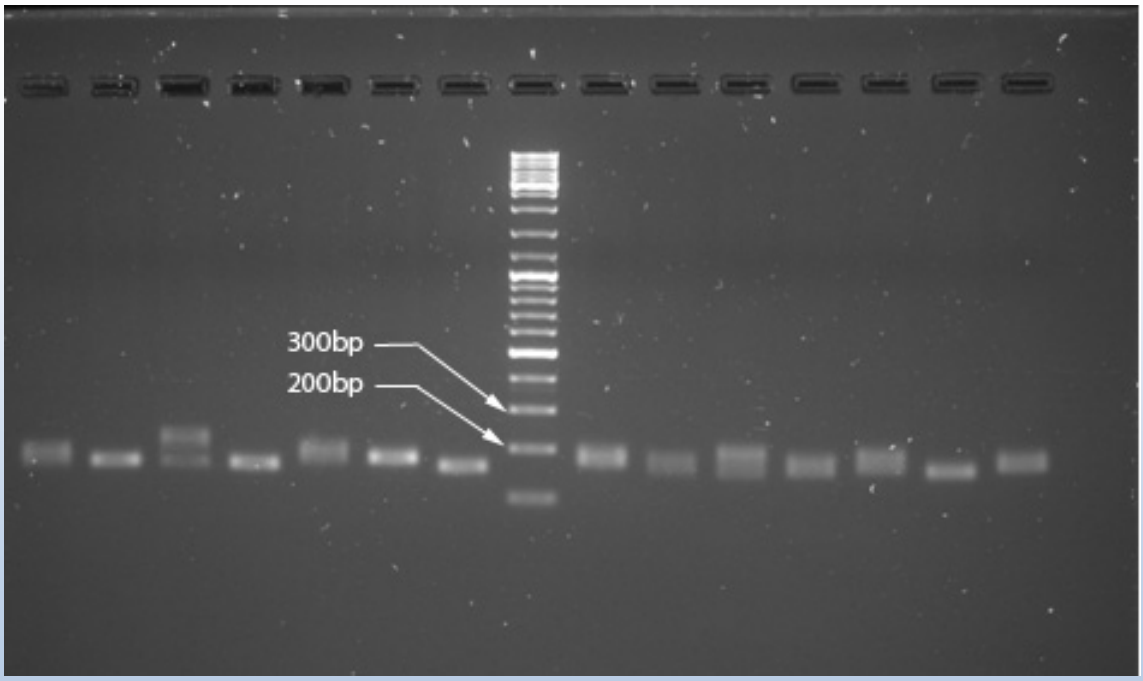


Figure 25: CAG repeat length and age of onset of disease relationship

SCA 12

Patients with autosomal dominant cerebellar ataxia without a genetic diagnosis following SCA 1, 2, 3, 6, 7, and 8 panel assessments were further tested for SCA 12. Expanded alleles contain 55 to 78 repeats compared to 7 to 32 repeats in normal chromosomes.

Figure 26: Agarose gel picture of PCR products for SCA 12 detection



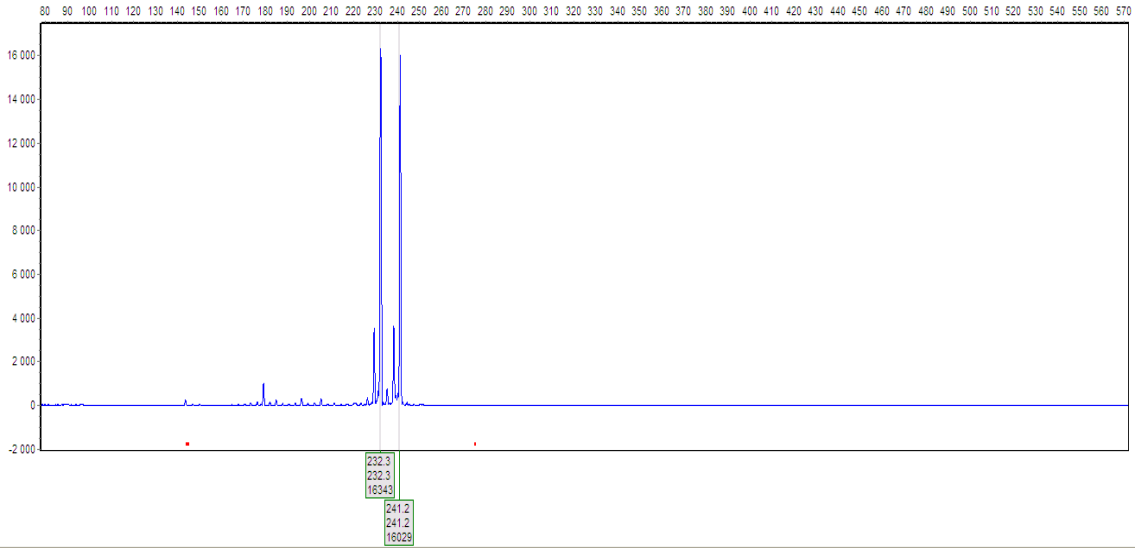
The PCR product amplicon had a normal predicted size of 152 base pairs with 10 CAG repeats. Therefore an expansion of a minimum of 50 repeats would lie in the 300 base pair region and above. None of the samples tested showed a band in that region or above that region, therefore SCA 12 expansion in the patient population was ruled out.

SCA 17

Patients with autosomal dominant cerebellar ataxia without a genetic diagnosis following SCA 1, 2, 3, 6, 7, 8 and 12 panel assessments were further tested for SCA 17. Individuals with normal TBP alleles have between 25 and 44 repeats, whereas SCA17 patients have between 47 and 63 repeats.

The PCR product amplicon had a normal predicted size of 245 base pairs with 30 CAG repeats. Therefore an expansion of a minimum of 50 repeats would lie in the 300 base pair region and above. None of the samples tested showed a band in that region or above that region, therefore SCA 17 expansion in the patient population was ruled out

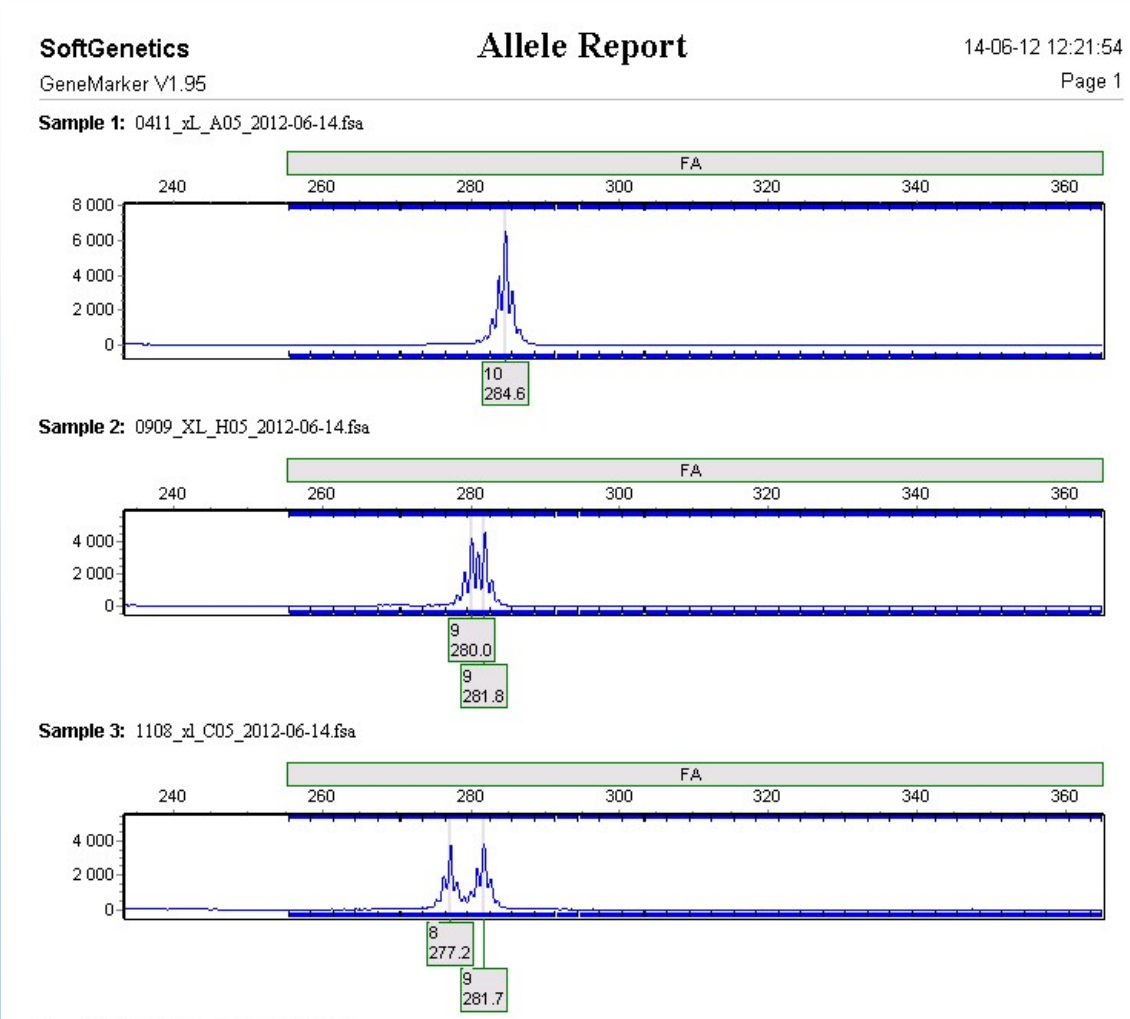
Figure 27: Capillary electrophoresis picture of PCR products for SCA 17 detection



Friedreich's ataxia

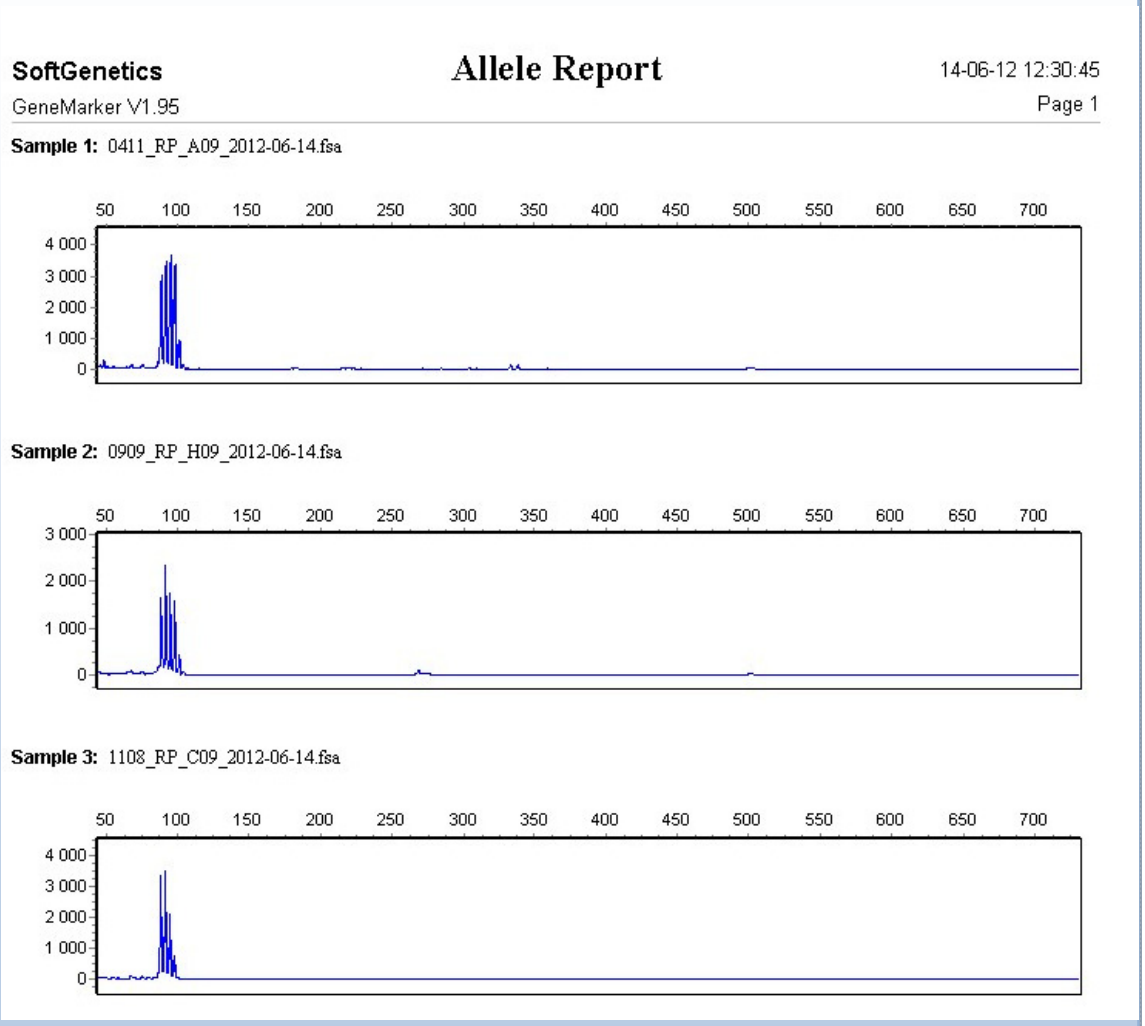
Friedreich's' ataxia was tested for in the 8 autosomal recessive patients and 4 sporadic ataxia patients. Capillary electrophoresis results of the 3 patients PCR and RP- PCR are shown below. None of the patients had a positive result for the genetic tests.

Figure 28: Capillary electrophoresis results of the 3 patients



RP - PCR results of the 12 patients

Figure 29: RP -PCR results of patients



4.0 Discussion

This was the first study in clinical genetics of hereditary ataxia conducted in Sri Lanka. Interestingly, SCA 1 was the most frequently occurring type of SCA identified in the study, accounting for 61.7% of all identified SCA. One SCA 2 family was also found. There were a few recessive ataxias but no specific etiology could be identified. Moreover, the clinical findings suggest a more severe disease course in the SCA 1 patients compared with previous reports. Last but not least this study revealed the presence of isolated pockets of patient groups in the country.

4.1 Demography

Worldwide epidemiology data suggests that SCA 3 is the most common subtype, followed by SCA 2 and SCA 1 and SCA 8 in descending order (Schols *et al.* 2004). Founder effect contributes to the variable prevalence between populations. Therefore the relatively high prevalence of SCA 1 worldwide and a possible founder mutation may have contributed to the 21/22 prevalence of SCA 1 in this patient population.

When comparing the SCA subtype found in Sri Lanka with Indian populations (Table 3), the similarities are more with South Indian data. Apart from the study by Chakravarty, A. et al in the East Bengal region that has included a relatively small patient population, the studies have shown that SCA 1 and 2 are the most prevalent in India. The two studies done in South India by Rengaraj R. et al in 2005 and Krishna N, in 2007 both show the highest prevalence of SCA 1 in their populations.

The geographical and genetic proximity with South India with migratory phenomena may have influenced the epidemiology of SCA in Sri Lanka (Kshatriya 1995; Krishna *et al.* 2007).

South India is the geographically nearest region to Sri Lanka. It is also interesting to note that genetically we may also be closest to this region. A study on the degree of genediversity and genetic admixture among the population groups of Sri Lanka, with the population of of southern, north eastern and north western India, the Middle East and Europe done by Kshatriya, Gautam Kumar in 1995, showed that present day Sinhalese and Tamils of Sri Lanka are closer to Indian Tamils and South Indian Muslims. They are farthest from Veddahs and quite distinct from Gujaratis and Punjabis of northwest India and Bengalis of northeast India. This study on genetic admixture revelaed that Sinhaleses of Sri Lanka have a high contribution from the Tamils of southern India (69.86& +/- 0.61) compared to the Bengalis of northeast India.

In this context the prevalence of a high number of SCA 1 subjects in the Sri Lankan population is geographically and genetically plausible. Further studies are needed to confirm the true epidemiological spread of SCA subtypes and haplotype analysis to confirm founder mutations.

Spinocerebellar ataxia type 1

The majority of patients with SCA 1 mutation were from the western and southern provinces of Sri Lanka. These two provinces are densely populated areas and the inhabitants account for 43% of the total population in the island. Extrapolation of the study population of 20 patients with SCA1 seen with the estimated population in these regions (7,659,468 according to 2001 consensus) results in a estimated SCA1 population of 0.26/100,000, which is significantly below wordwide prevalence figures of 3/100,000. This indicates the presence of a larger patient population. As this is a referred study population, there probably is a recruitment bias, that makes it impossible to give reliable epidemiological data.

SCA 1 familial aggregation

Fifteen patients included in the study came from 6 families living in close proximity to each other. This community lived in the southern province in three MOH division in the Matara RDHS and a single MOH division in the Hambanthota RDHS. Each MOH division had approximately 2 to 4 families with affected patients. Genetic testing confirmed that all these patients were of SCA 1 genetic subtype. However a haplotype analysis of the family members is needed to verify the hypothesis of a common founder.

Spinocerebellar ataxia type 2

A single patient with SCA 2 mutation was found in the study population. He was the only patients seen from the Central Province, Kandy District. A high level of tertiary care facilities exsist within the Central province, and patients are unlikely to travel to Colombo for medical purposes. Therefore there is a high likelihood that many more patients with SCA 2 are present in that region. Geneticits and neurologists should be aware of these findings in this region and test patients further. This also indicates the possible presence of other SCA subtypes scattered throughout the country.

Autosomal dominant cerebellar ataxia of unknown genetic etiology

ADCA of unknown genetic etiology had 8 patients from the western province, with the remaining 4 scattered in the southern and central provinces. The western province is the commercial and economical hub of the country hence many people from across the country migrate to this region. Therefore it is hard to conclude about the true origins of the patients.

To achieve a conclusive genetic diagnosis for these patients, clinical symptoms were analysed and possible differentials are discussed below. The possibilty of testing for these genetic subtypes is outside the scope of the present study and conxtitute an interesting future research endeavour.

Autosomal recessive cerebellar ataxia (ARCA) and Sporadic ataxia

Even though our study focused on autosomal dominant cerebellar ataxia, 8 patients with autosomal recessive and 4 patients with sporadic ataxia were also seen during the course of the study and an attempt was made at diagnosis. With sporadic and ARCA the origin of transmitting parents are near impossible to trace as both parents carry the mutation in ARCA and even if consanguinous, a single geographical location is hard to pin point. Therefore in many instances the patients geographical location was recorded. For ARCA patients geographical location was recorded from the western, southern and sabaragamuwa provinces. Of the 4 sporadic ataxia patients, 2 were from the western province with the remaining 2 from the southern province.

The most common ARCA, Friedreich's ataxia was tested in the patient's group, however all 12 patients tested negative for this mutation

4.2 Clinical Phenotype

A single investigater assessed and rated patients avoiding interrater variabilty. Further validation of assessments was made by supervisors analysing video records of patient examinations.Thus an important effort was made to ensure as many insights as possible in this initial pilot study of the clinical and genetic etiology of HA.

Spinocerebellar ataxia type 1

Clinical phenotype of patients in our study population with SCA1 showed a high prevalence of pyramidal signs with ataxia, which is in accordance with most published series where Spinocerebellar ataxia type 1 is characterized as an autosomal dominant disorder manifesting with cerebellar, pyramidal, and bulbar symptoms (Subramony *et al.* 1993).

The highest frequency of patients (28.6%) belonged to the disability level 3 which is equivalent to moderate disability with inability to run and limited walking without aid. The second highest disability level was 6 (23.8%) which is unable to walk requiring a wheelchair. This indicates that many of the patients in our study population are those dependent on aids or wheel chairs for mobility.

An increase in disability level with duration of disease was present as expected; however the level of disability was greater. Although the disease duration was shorter in our study than in EUROSCA (7.4 ± 3.1vs 9.5±5.5) the mean SARA score of was higher (18.8 ± 9.7 vs. 15.6 ± 9.1) (Jacobi *et al.* 2011)), attesting a more disabling disease in the Sri Lankan patients. This may be related to the higher CAG repeat (52.0 ± 3.8 vs. 47.4± 5.2) and younger age at onset (34.8 ± 10 vs. 37.0±10.6) in the study population, resulting in a rapidly progressive severe

phenotype. In addition a poor level of para-clinical supportive services such as physiotherapy for the neurodegenerative disease as compared to the European population assessed in the EUROSCA study might also be contributing to these clinical discrepancies.

INAS count showed increased pyramidal signs which were similar to previous reports on SCA1. However the mean INAS score was lower than previous studies (3.6±2.4 vs. 5.0±2.3) (Jacobi *et al.* 2011) indicating a phenotype with relatively less extrapyramidal signs. Our non-ataxia symptom analysis showed higher age at presentation being associated with hyperreflexia, spasticity, urinary dysfunction and cognitive impairment. This further confirms the degenerative nature of the disease.

According to the 2 year follow up study on SCA (Jacobi *et al.* 2011) progression of disease is fastest amongst SCA 1 patients with SARA and INAS progression scores paralleling. A follow up study is needed to confirm this in our study population.

In our study population all 8 patients with SCA 1 tested by the MoCA scale showed below normal cognition. All patients had an education level above 8 years of schooling and at the time of assessment were not employed or held blue collar jobs. A subtype specific cognitive impairment in Spinocerebellar ataxia is a debated topic. In a study by Helmstaedter C et al (2010) SCA 1 was shown to preform poorer than controls in 33% of all cognitive test parameters, and at a broader and higher level than SCA 2 and SCA 3 (Klinke *et al.* 2010). The result in our study population has to be further assessed by a case control study to come to a definitive conclusion; however it does indicate that there may be a cognitive impairment component associated with SCA 1 neurodegeneration.

There were a significantly higher number of patients with depression compared to previous reports that used the same scoring system in SCA patients (68.4% vs. 24.5%) (Schmitz-

Hubsch *et al.* 2011). Between the two studies the proportion of patients with no depression, mild, moderate and severe depression was inversely related, with high patient numbers with severe depression in our study population. In our study population there was significantly less social support, financial security and increased disability due to lack of physiotherapy. Identification and treatment of depression was also minimally existent. It is important to note that in chronic neurodegenerative disease it has been shown that physiotherapy and occupational therapy not only slows the progression of neurological deterioration but also has positive effects on the mental health status of patients as does medical interventions for depression (Okamoto *et al.* 2010; Silva *et al.* 2010), highlighting the need for supportive care in this progressive neurological disorder.

Radiological investigations of brain CT and/or MRI were present in 5 patients with SCA1 (2 CT and 3 MRI). Both CT films of patients indicated cerebellar atrophy. Of the MRI films of patients, 1 indicated cerebellar atrophy, 1spinocerebellar atrophy and 1 was reported as a normal brain MRI. The normal brain MRI was reported within the first year of onset of symptoms. In a MRI study SCA 1 and SCA3 presented with severe atrophy throughout the brainstem (midbrain, pons, and medulla), cerebellar hemispheres and cerebellar vermis, putamen and caudate nucleus. In conventional mutation SCAs isolated cerebellar atrophy without brainstem involvement was reported for SCA 5, SCA 11, SCA13 and SCA14. Both the cortex and cerebellum are characteristically affected in SCA 12. Clinical dysfunction has been shown to best correlate with atrophy of the pons in SCA 1 (Durr 2010). Therefore for disease identification and progression brain MRI are important investigative tools and should be available for our patients to a greater extent. It is also important to note that sometimes it may be useful to reconsider an investigation, as radiological signs may become more prominent at later disease stages.

4.3 Genotyping

Spinocerebellar ataxia type 1

Mean CAG repeat in the study was 52.0±3.8 (range 47 - 59). Studies in south Indian populations by Rengaraj et al have documented mean CAG repeats in SCA 1 patients as 43.6 (40 – 48) and by Basu et al the range is documented as 42 – 72 (Basu *et al.* 2000; Rengaraj *et al.* 2005). Therefore this study population appears to have a comparatively large mutant repeat number.

Age of onset shows positive correlation with CAG repeats in the expanded allele in the study population, however it was not in 100% concordance. The patient with the youngest age of onset was 16 years of age and had a CAG repeat of 57 on his expanded allelle. There were 3 patients with larger CAG repeats (59, 59 and 58) who had later ages at onset (25, 27 and 25 years respectively). Therefore though a correaltion does exsist it does not account for 100% of the variance seen in age of onset. A study by Kremer B. P. H et al on the age onset variance have shown that the nonexpanded CAG repeats have significant contribution to age of onset in SCA1 (van de Warrenburg *et al.* 2005). Therefore it would be of interest in future studies to document both the expanded and non expanded allele CAG repeats.

No correlation between CAG repeat lengths SARA or INAS score was present. Correlations between CAG repeat length and severity of SARA and the number, type and severity of INAS was found in the study conducted by Klockgether et al. (Jacobi *et al.* 2011) . This may be due to the relatively smaller size of our sample population and the fact that CAG repeat number showed mild variability with a small standard deviation (52.0±3.8).

A significantly higher contribution by paternal mutant allelles transmission was seen in the study population. This correlates with the findings that when transmitted paternally CAG repeats are more unstable and larger in size in SCA 1 (Dubourg *et al.* 1995)

4.4 Differentials for genetically undiagnosed ataxia groups

Autosomal Dominant Cerebellar Ataxia of unknown genetic etiology

Of the 34 patients with autosomal dominant cerebellar ataxia, 12 did not receive a genetic diagnosis from the test panel done in the study.

In 7 of the 12 patients, disease transmitting parents were from Colombo or Gampaha. The possibilty is that parent migrated from a region which the patient is not aware of is high as Colombo and Gampaha are the main commercial districst of the coutry and peopl from across the country have choosen to live there. Hence the possibilty that they are from a single mutation origin is slim.

Clinically ataxia rating (SARA) were statistically not significantly different to SCA 1 patients with the same sum score, posture and gait and limb kinetic scores. However speech disability score was significantly less in the ADCA group. Non ataxia features (INAS) were statistically similar in the two groups. Therefore this group also consists of patients with a high prevalence of pyramidal features and less extrapyramidal features. However as this consisted of a small patient group findings may be uncertain.

On comparison with the SCA 1 group, age of onset of disease, age and disability level was not significantly different. However duration of disease was significantly higher in ADCA of unknown genetic etiology. It could be concluded that these patients may have a slower progression of disease that would make presentation for diagnosis delayed.

Cognitive assessments revealed that 75% of patients with ADCA had a below average level of cognition. This cognitive level was positively correlated with the SARA score in the patients. Therfore we may conclude that the disease had a effect of on cognition of patients . However many other variable may also have to be considered to come to a definitive conclusion.

Depression is another neuropsychological factor being debated with relation to the neurodegeration of cerebellar ataxia. Of the 12 patients with ADCA 75% have clinically relevent depression. Of the symptom breakdown the highest prevalence of symptoms were tired with no energy, sleep disturbances and change of appetite.

The small sample size does not allow for definite conclusions. For instance, the lack of anticipation and the high paternal inheritance seen may be caused by lack of significant patient numbers.

Autosomal recessive cerebellar ataxia

There was a total of 8 patients with ARCA. All 8 patients had positive family histories for consanguinity or affected siblings. Age of onset divided them into two subgroups. Those included 6 patients with age of onset before 30 years of age and 2 patients with age of onset at 38 and 45 years. Most autosomal recessive ataxias manifest before the age of 30 years (Anheim *et al.* 2012). Therefore the probability of ataxia in the second group of patients being due to aquired causes though familial consanguinity is present, may have to be further investigated into. Examination and investigation findings did not closely relate to Friedreich's ataxia though the most common autosomal recessive cerebellar ataxia worldwide. This was confirmed by absence of Friedreich's ataxia mutation in the study population by molecular testing. Ataxia with vitamin E deficiency, mitochondrial diseases and other autosomal recessive cerebellar ataxia are possible differentials. (Figure 30)

Sporadic ataxia

The most chalenging diagnostic dilemma is an ataxic patient with sporadic onset. In such patients the entire spectrum of genetic and non genetic causes have to be considered before reaching a diagnosis. As we recived referrals for a possible genetic ataxia from neurologists

many of the aquired causes such as structural change, stroke, immunological causes and neoplastic/ paraneoplastic and endocrine causes had been excluded. Our goal was to derive the best fitting hereditary ataxia.

Age of onset is a critical clue in the diagnosis of hereditary ataxia. Two patients had their age of onset below 20 years of age while the other two had a age of onset in the 31 to 40 years age group. For those with an early onset of disease there was pure cerebellar ataxia with no pyramidal signs. There was no sensory ataxia, scoliosis or choreoathetosis. Retinitis pigmentosa and retinal degeneration was not assessed, however there was no history of visual impairment. Both patients had isolated cerebellar atrophy on CT and MRI. Intention tremor was present in one the two patients, however with the early age of onset and no history of X-linked disease, Fragile X Ataxia tremor syndrome seemed unlikely. With a negative genetic diagnosis of Friedreich's ataxia, a tentative possiblity would be Autosomal recessive cerebellar ataxia type 1.

In the 2 patients with age of onset in the 3rd to 4th decade, a diagnostic dilemma occurs as many possible diseases of adultonset sporadic ataxia such as Fragile X ataxia, tremor syndrome and Mutiple systems atrophy (MSA-c) and sporadic adult onset ataxia of unknown etiology (SAOA) have the age of onset set at 50 years and above. However one striking feature was the presence of pyramidal symptoms along with the ataxia symptoms in both patients. Mitrochondrial or metabolic disease such as cerebrotendinous xanthomatosis might be a possibilty but targeted biochemical tests are needed to further the diagnosis.

4.5 Social Aspects

It must be noted that for 12 of the 15 SCA1 patients included in the study, the principal investigater recruited the subjects by travelling to their homes. At the time of recruitment 8 of the 12 patients had no history of medical consultation, though all patients had neurological

signs and symptoms. Their knowledge on the inheritance of the disease, acceptance of it as a familial idiosyncrasy and the absence of a definitive treatment were the main reasons for not seeking medical treatment. However by this action they were also deprived of physiotherapy and other paraclinical treatment modalities which would have improved their quality of life and delayed the progression of the disease.

Surprisingly consanguinity was found in SCA 1 families (Figure 11). It appears to be induced by social convention. It is in part influenced by the inter marriage of individuals within the same caste and by the need to limit the burden of social stigma attached to the disease on the individual by marriage into a similarly affected family. The need of genetic services and health information and education to these regions is clearly emphasized by these findings. This also indicates that further pockets of isolated patient populations may be present within the island.

5.0 Conclusions

This study showed a high prevlence of SCA 1 mutation and a single SCA 2 mutation affected patient. Clinical characteristics of these patients closely correlated to known phenotypes. Finding of high significance was a region with a relatively high density of affected individuals with SCA 1 indicating a possible founder muation and a high prevalence of depression affecting all pateints in the study population.

6.0 Limitations of the study

Patient recruitement was based on a referred patient population. The patients recruited were predominantly from the south and west of the country which accounts for approximately 43% of the islands total population. As a genetics unit situated in Colombo, there was a selection

bias for those situated close to our referal center being overly represented in our patient population. This recruitment bias makes it impossible to give a reliable epidemiological data.

Ataxia patients not followed up by clinicians may have been missed by the study. This is due to the fact that pockets of patient populations, particularly those living in isolated rural areas, coming from extended families where the disease progression and the inability for definitive treatment is known may not be volunteering for health services. Such a patient population was identified during the course of the study, opening the possibilty that further such populations exsist in the island. Therefore this study shows definit defciency with regards to a true epidemiological portrayel of the islands distribution of hereditary ataxia.

7.0 Clinical Recommendations

Diagnosis of Adult onset Cerebellar ataxia is a challenge based on phenotype alone. Accurate family history is of great value and supplementary examinations, investigations are important in excluding sporadic forms. MRI are emerging as an important investigative procedure to differentiate CAG repeat subtypes and conventional mutation subtypes of SCA. Atrophy of the brain stem is seen more often in coding triplet repeat diseases.

Epidemiolgy is also an important indicater as shown by our study. Area of origin played an important role in identifying affected subtype of SCA as in SCA1 patient population from the southern region. It is important that the result of SCA2 is further investigated into as a larger population within the central province is indicated.

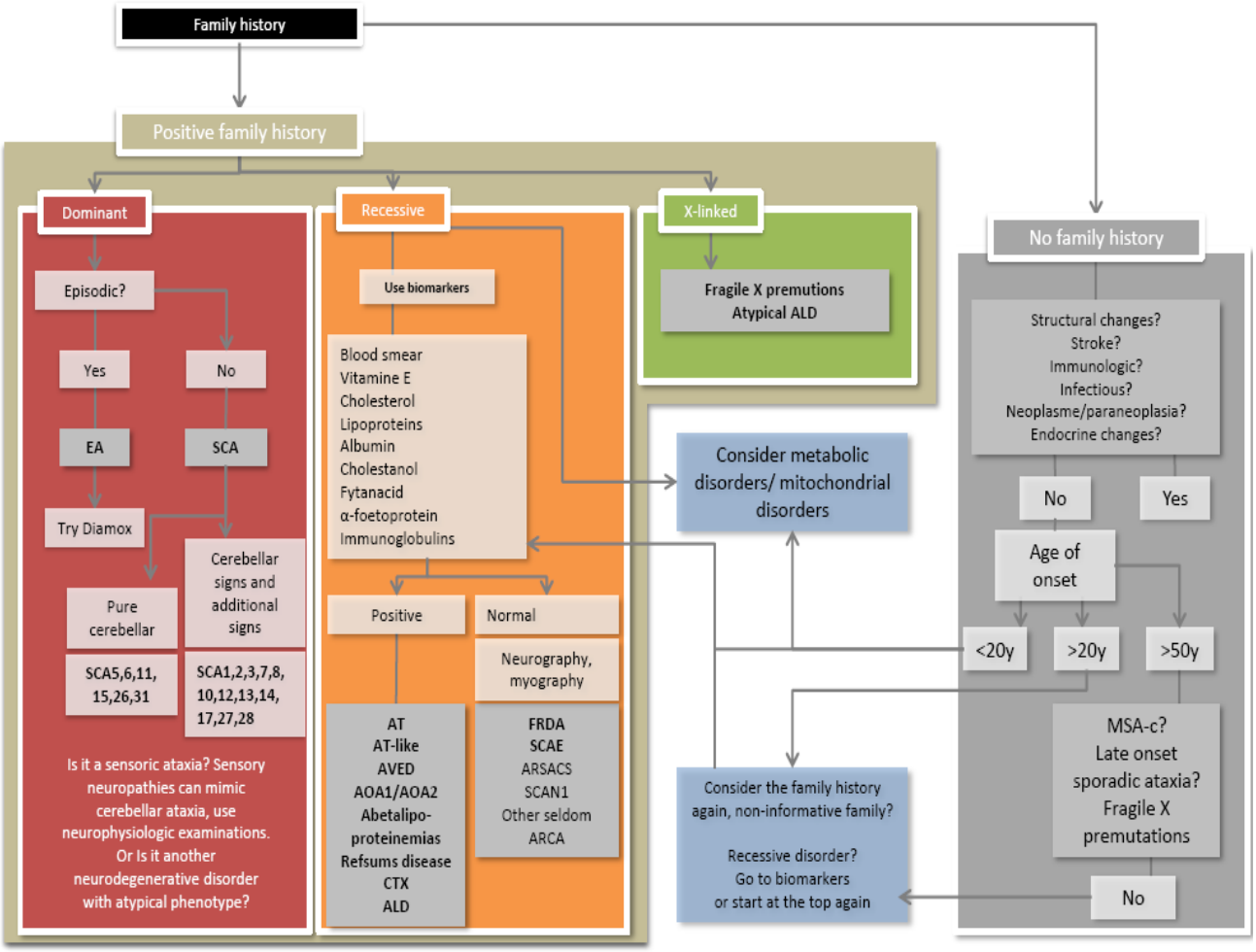
Many different medications have been tried in order to reduce symtoms and stop neurodegeneration. The treatment therefore is primarily supportive. Physical methods such as physiotherapy, different walking assistents (cane, walkers, speech therapy) are essential as well as psychological support and education can in some ways reduce the burden of disease. The proper identification at primary health care level and good collaberation with rehabilitation institutes is important.

8.0 Future research

This preliminary study indicates that Sri Lanka has a population of Spinocerebellar ataxia patients distributed throughout the western, southern and central provinces. A wider epidemiologically sound future study to assess the prevalence throughout the island would be a interesting next step in research in this area.

With this population we identified a familial aggregation in a isolated region of the country. A haplotype analysis to detect a founder mutation would provide important genetic information on the origins and spread of the mutation.

Figure 30: Cerebellar ataxia Differential Diagnosis



Reference: Manto and Marmolino. cerebellar ataxias. Current Opinion in Neurology 2009. 22:419–429

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APPENDIX 1: DOCUMENTS USED FOR SUBJECT RECRUITMENT

This appendix contains the English, Sinhala and Tamil language documents listed below, which were used for subject recruitment.

• Information leaflet for study participants used for the recruitment of patients with Hereditary Ataxia

• Consent form used for recruitment of patients with Hereditary Ataxia

• Data Collection Booklet

INFORMATION SHEET

CLINICAL FEATURES AND GENETIC AETIOLOGY OF SPINOCEREBELLER ATAXIA IN A COHORT OF PATIENTS IN SRI LANKA

This study is conducted by me, Dr Dulika Sumathipala, an MSc student in Clinical Geneticsin the Human Genetics Unit, Faculty of Medicine, Colombo. I would like to invite you to take part in the research study titled "CLINICAL FEATURES AND GENETIC AETIOLOGY OF SPINOCEREBELLER ATAXIA IN A COHORT OF PATIENTS IN SRI LANKA" conducted in collaboration with the University of Oslo, Norway by myself, under the supervision of Prof. Rohan W. Jayasekara and Prof. Vajira H W Dissanayake at the Human Genetics Unit, Faculty of Medicine, University of Colombo and Prof Chantal Tallaksen of the University of Oslo, Norway.

1. Purpose of the study

The purpose of the study is to detect genetic defects causing Spinocerebellar ataxia (SCA) and to correlate clinical features with genetic defects.

2. Voluntary participation

Your participation in this study is voluntary. You are free to not participate at all or to withdraw from the study at any time despite consenting to take part earlier. There will be no loss of medical care or any other available treatment for your illness or condition to which you are otherwise entitled. If you decide not to participate you may withdraw from the study at any time by informing us.

3. **Duration, procedures of the study and participant's responsibilities**

The study will be conducted over 1 year. We require your permission to ask you questions, examine you, have access to your medical records, and video record your speech and gait. We also need your permission to publish the data collected in a scientific journal. We will not mention your name or any other identifiable information about you when we publish the results. We also need to take 5ml of venous blood from you to do the genetic test.

4. **Potential benefits**

Participation in this study will help you to know the genetic defect that has made you develop spinocerebellar ataxia. This will contribute to the increasing of knowledge about spinocerebellar ataxia in Sri Lankan patients with this condition. When we know the common genetic defects causing SCA in Sri Lankan patients it will be possible for us to design a cheap genetic test suitable to be used in Sri Lanka.

5. **Risks, hazards and discomforts**

Blood will be drawn to detect the genetic defect causing SCA. Approximately5ml of blood will be taken for testing from you. The risk to you by participating in the study is the risk of pain, bruising and infection at the needle prick site. These will be minimized by performing blood drawing under aseptic conditions by trained phlebotomist.

6. Reimbursements

There will be no reimbursement for participating in the study, but you will be given a copy of the molecular genetic test results.

7. Confidentiality

Confidentiality of all records is guaranteed and no information by which you can be identified will be released or published. The data collection booklet is designed to ensure confidentiality of information gathered. The electronic database containing the data will have only the subject study number and the database and the computer containing the database would be password protected. These data will never be used in such a way that you could be identified in anyway in any public presentation or publication without your express permission.

8. Termination of study participation

You may withdraw your consent to participate in this study at any time, with no penalty or effect on medical care or loss of benefits. Please notify us as soon as you decide to withdraw your consent However it will not be possible for you to withdraw once the results are sent for publication or once the results are published

9. Clarification

If you have questions about any of the tests / procedures or information please feel free to ask any of the persons listed below by calling 011 2689 545.

Dr Dulika Sumathipala

MSc Student

Human Genetics Unit

Faculty of Medicine

Colombo

Prof Vajira Dissanayake

Medical Geneticist

Human Genetics Unit

Faculty of Medicine

Colombo

CONSENT FORM

CLINICAL FEATURES AND GENETIC AETIOLOGY OF SPINOCEREBELLER ATAXIA IN A COHORT OF PATIENTS IN SRI LANKA

To be completed by the participant/guardian

The participant/ guardian should complete the whole of this sheet himself/herself.

1. Have you read the information sheet? (Please keep a copy for yourself)

YES/NO

2. Have you had an opportunity to discuss this study and ask any questions?

YES/NO

3. Have you had satisfactory answers to all your questions?

YES/NO

4. Have you received enough information about the study? YES/NO

5. Who explained the study to you? ……

6. Do you understand that you are free to withdraw from the study at any time, without having to give a reason and without affecting your medical care?

YES/NO

7. Sections of your medical notes, including those held by the investigators relating to your participation in this study may be examined by other research assistants. All personal details will be treated as STRICTLY CONFIDENTIAL. Do you give your permission for these individuals to have access to your records?

YES/NO

8. Do you give permission for video recording? YES / NO

9. Do you agree to have leftover blood samples and DNA be stored for future research into SCA?

YES/NO

10. Do you agree for the samples to be sent abroad?

YES/NO

10. Have you had sufficient time to come to your decision?

YES/NO

11. Do you agree to take part in this study? YES/NO

Participants'/ Guardian's

signature:……..…….Date……

Name(BLOCKCAPITALS):……

…….

To be completed by the investigator

I have explained the study to the above volunteer and he/ she has indicated her willingness to take part.

Signature of

investigator:……...……Date……

Name

(BLOCKCAPITALS):……

……

Data Collection Form

CLINICAL FEATURES AND GENETIC AETIOLOGY OF SPINOCEREBELLER ATAXIA IN A COHORT OF PATIENTS IN SRI LANKA

Subject Study Number					-		

Name of subject ……

Date of birth ……/……/……

Address ……

……

……

……

Telephone number
(Home) ……

(Mobile) ……

Email ……

Referring Physician ……

Date of Referral ……

Hospital ……**Ward:** ……..

Clinic No/ BHT No ……

Data Protection and Confidentiality

After completion of this page, ensure that the subject study number is entered on **all pages** of this booklet. Then detach this page and store separately from the remainder of the booklet.

Subject Study Number					-		

Date of entry to study Date on consent form

		-			-				

		-			-				

Date of birth

FAMILY TREE

Draw the family tree indicating all illnesses present, document abortions/still births as well.

I
II
III
IV
Consanguinity : Yes / No

Subject Study Number					-		

Additional pedigree information

Location in pedigree	**Clinical or other information**

Subject Study Number					-		

(1)

Age of onset (years)	Gait Disturbances		
	Postural tremor		

(2) Scale for the Assessment and Rating of Ataxia (SARA). See appendix i

SARA Score (/40)		

(3)

Disease progression (SARA score/ disease duration)		

(4) Inventory of Non Ataxia Symptoms (INAS) See appendix ii

INAS Score (/ 16)		

(5) Psychiatric Assessment

Patient Health Questionnaire (PHQ - 9) See appendix iii

PHQ – 9 (/ 27)		

Subject Study Number					-		

(6) Cognitive Assessment

Montreal Cognitive Assessment Scale (MoCA) See appendix iv

MoCA (/30)		

(7) Investigations

Investigation	Date	Comment	
MRI Measurements on T1- and T2-weighted magnetic resonance images [1]		1 anteroposterior diameter of the putamen 2, transverse diameter of the putamen 3, anteroposterior diameter of the midbrain 4, transverse diameter of the midbrain 5, anteroposterior diameter of the pons 6, transverse diameter of the pons 7, width of the middle cerebellar peduncle 8, anteroposterior diameter of the fourth ventricle 9, transverse diameter of the fourth ventricle 10, anteroposterior diameter of the medulla oblongata 11, transverse diameter of the medulla oblongata	Normal / Reduced Normal / Reduced Normal / Reduced Normal / Reduced Normal / Reduced Normal / Reduced Normal / Reduced Normal / Reduced Normal / Reduced Normal / Reduced Normal / Reduced
Nerve Conduction Study [2]		Nerve Conduction Velocity Nerve Conduction Amplitude - Sensory - Nerve Conduction Amplitude – Motor -	Normal / Reduced Normal / Reduced Normal / Reduced

Subject Study Number					-		

Patient Date

		-			-				

	Label	Volume	Storage	Comments
K/EDTA vial	**1.** **2.**	**1.** **2.**	**1.** **2.**	

COMMENTS

Record reasons for missing data and any additional relevant comments. ENSURE THAT ANONYMITY IS PRESERVED.

The booklet should be signed when ALL available data have been entered and cross checked with relevant data recorded elsewhere in this booklet.

Signed…… **Date……**

Investigator/Research Assistant

<u>තොරතුරු පත්‍රිකාව</u>

ශ්‍රී ලංකාවේ මානව ජාන ඒකකයට පැමිණෙන ස්පයිනෙ සෙරිබෙල්ල ඒටැක්සියාව (SCA) සහිත රෝගීන්ගේ ජානමය විවිධත්වය සහ රෝග ලක්ෂණ අධ්‍යයනය

අපගේ පර්යේෂණ කණ්ඩායම විසින් සිදු කරනු ලබන ඉහත සඳහන් පර්යේෂණයට සහභාගී වීම සඳහා ඔබ හට අරාධනා කිරීමට අපි කැමැත්තෙමු. අපගේ පර්යේෂණ කණ්ඩායමේ සාමාජිකයින් වනුයේ;

- මහාචාර්ය වජිර H.W. දිසානායක (අධීක්ෂකවරයා) – කොළඹ විශ්වවිද්‍යාලයේ, වෛද්‍ය පීඨයට සම්බන්ධ, මහාචාර්යවරයෙකි.
- මහාචාර්ය C. ටලක්සෙන් (අධීක්ෂකවරිය) - නෞර්වෙ, ඔස්ලෝ විශ්වවිද්‍යාලයේ මහාචාර්යවරියෙකි
- වෛද්‍ය දුලිකා සුමතිපාල (ප්‍රධාන පර්යේෂකවරිය) – කොළඹ විශ්වවිද්‍යාලයේ, වෛද්‍ය පීඨයට සම්බන්ධ, M.Sc. උපාධි අපේක්ෂකයෙක්.

1) මෙම අධ්‍යයනයේ අරමුණ

මෙම අධ්‍යයනයේ අරමුණ වනුයේ ශ්‍රී ලංකාව තුල SCA රෝගීන් ජානමය වශයෙන් කමන වර්ගීකරනයට අයත්ද යන්න පරීක්ෂා කිරීමය. තවද SCA රෝගීන්ගෙ රෝග ලක්ෂණ සහ SCA ජානමය වර්ගය අතර සබඳතාවයක් තිබෙ දැයි පරීක්ෂා කෙරේ.

2) ස්වේච්ඡා සහභාගීත්වය

මෙම අධ්‍යයනය සඳහා ඔබගේ සහභාගීත්වය ඔබගේ කැමැත්තෙන්ම සිදු කරන්නකි. මෙම අධ්‍යයනය සඳහා ඔබ සහභාගී නොවීමට ඔබට පූර්ණ අයිතිය ඇති අතර, සහභාගී වීමට කළින් කැමැත්ත ප්‍රකාශ කර තිබුණද, මින් ඕනෑම අවස්ථාවක ඔබට ඉවත් වීමට ඔබ හට පූර්ණ අයිතිය ඇත.

3) කාල සිමාව ක්‍රියා පිළිවෙල සහ සහභාගීවන්නන්ගේ වගකීම්

මෙම අධ්‍යයනය අවුරුදු එකක් තුල සිදුකෙරෙ. SCA රෝගීන්ගේ රුධිර සාම්පලයක් ලබාගෙන ජානමය වශයෙන් කුමන SCA වර්ගයට අයත් දැයි පරීක්ෂා කෙරේ. ඔබගේ අවසරය අැතිව රෝග ලක්ෂණ, රෝග වාර්තා අධ්‍යයනයක් කෙරෙන අතර ඔබගේ අැවිදීම, කථනය පටිගත කිරීම සිදුකෙරේ. තවද එකතු කළ දත්ත පකාශයට පත් කිරීමට ඔබගේ අවසරය අවශ්‍ය ය. පර්යේෂණය සඳහා ඒක් වරක් සහභාගී වි දත්ත සහ රුධිර සාම්පල ලබා දිය හැක

4) මින් ලද හැකි ප්‍රතිලාභ

ඔබ මෙම අධ්‍යයනයට සහභාගී වීම නිසා, SCA රෝගයේ ජානමය වර්ගීකරනය පිළිබඳ රෝග විනිශයක් ලැබෙන අතර මෙයින් ලැබෙන තොරතුරු SCA රෝගය සහිත අනෙකුත් පුද්ගලයින්ට අනාගතදි ප්‍රතිලාභ සැලසිය හැක.

5) අවදානම් අනතුරු සහ අපහසුතා

SCA රේගයේ ජානමය වර්ගීකරනය පරීක්ෂා කිරීමට 10ml පමණ රුධිර සාම්පලයක් ලබා ගන්නා අතර මෙම රුධිර සාම්පලය ලබා ගැනීමෙදි යම් අපහසුතාවයක් ැති විය හැක රුධිර සාම්පලය ලබාදීමෙදි ඒන්නත් කටුව නිසා යම් තැල්මක් ඒම ස්ථානයෙ හට ගත හැක මෙය අවම කිරීමට ප්‍රවින හෙදියක් විසින් රුධිර සාම්පලය ලබා ගැනෙ

6) දිමනා

ඔබ මෙම අධ්‍යයනයට සහභාගී වීම නිසා ඔබට දිමනාවක් නොලැබෙ ඒහෙත් ඔබට SCA රේගයේ ජානමය වර්ගීකරනය පිළිබද වාර්තාවක් ලැබෙ.

7) රහසිගත බව

සියලුම තොරතුරු සහිත වාර්තාවන් සහ අධ්‍යයනය මගින් ලබා ගන්නා දත්තයන්ගේ රහස්‍යභාවය තහවුරු කරන අතර, ඔබ හඳුනාගත හැකි ආකාරයේ කිසිවක් ඔබගේ කැමැත්තකින් තොරව හෙළි කිරීමක්, ඉදිරිපත් කිරීමක් හෝ ප්‍රකාශයට පත් කිරීමක් සිදු කරනු නොලැබේ.

8) අධ්‍යයනයට සහභාගී වීම නැවැත්වීම

අධ්‍යයනයට සහභාගී වීමට දුන් කැමැත්ත ඉවත් කර ගැනීම, අධ්‍යයනයේ කුමන හෝ අදියරකදී සිදු කිරීමට ඔබට හැක. එසේ සිදු කරන්නේ නම් එම තීරණය ගත් විගසම ඒ බව පර්යේෂක හට කරුණාකර දැනුම් දෙන්න. නමුත් එකතු කරන ලද දත්ත පකාශයට පත් කිරීමෙන් පසුව ඉවත් කිරීමට නොහැක.

9) වැඩිදුර තොරතුරු

ඔබට මෙහි ක්‍රියා පටිපාටීන් පිළිබඳ කිසියම් ප්‍රශ්නයක් ඇත්නම් හෝ වැඩිදුර තොරතුරු අවශ්‍ය නම්, කරුණාකර පහත නම් සඳහන් වෛද්‍යවරිය අමතන්න.

වෛද්‍ය දුලිකා සුමතිපාල මහත්මිය - දුරකථන – 0112689545

කැමැත්ත ප්‍රකාශ කිරීමේ පත්‍රය

(a) සහභාගී වන්නන් විසින් පිරවීම සඳහාය.

මෙම පත්‍රය සහභාගී වන්නන් /භාරකරුවන් විසින් සම්පූර්ණයෙන් පිරවිය යුතුය.

1. අධ්‍යයනය සම්බන්ධයෙන් තොරතුරු පත්‍රිකාවෙ පැහැදිලි කරන ලද කරුණු ඔබට තේරුනාද? (කරුණාකර තොරතුරු පත්‍රිකාවේ පිටපතක් ඔබ ලබාගන්න) ඔව්/නැහැ

2. මෙම අධ්‍යයනය සම්බන්ධව සාකච්ඡා කිරීමට හා ඒ පිළිබඳව ප්‍රශ්න ඇසීමට ඔබට අවස්ථාවක් ලැබුණා ද? ඔව්/නැහැ

3. ඔබ ඇසූ ප්‍රශ්න සියල්ලටම සෑහීමකට පත්විය හැකි පිළිතුරු ලැබුණාද? ඔව්/නැහැ

4. මෙම අධ්‍යයනය සම්බන්ධයෙන් ප්‍රමාණවත් තොරතුරු ලැබුණාද? ඔව්/නැහැ

5. මෙම අධ්‍යයනය සම්බන්ධයෙන් ඔබට පැහැදිලි කරන ලද්දේ කවුරුන් විසින්ද?

   .....

6. කිසිදු කරුණු දැක්වීමකින් තොරව, මෙම අධ්‍යයනයෙන් ඉවත් වීමට ඔබ හට ඕනෑම අවස්ථාවක හැකියාව ඇති බව පැහැදිලි වූවාද? ඔව්/නැහැ

7. ඔබෙ වෛද්‍ය වාර්තා සහ පර්යේෂණ දත්ත මෙම අධ්‍යයනය සම්බන්ධ සාමාජිකයින් විසින් අධ්‍යයනය කෙරෙ සියළු වාර්තා සහ දත්තවල රහස්‍යභාවය තහවුරු කෙරෙ මෙම අධ්‍යයනය සම්බන්ධ සාමාජිකයින්ට තොරතුරු ලබා දීමට එකඟ වෙනවාද? ඔව්/නැහැ

8. ඔබගේ අැවිදීම, කථනය සහ ඉරියව් අධ යනට විඩියෞ පටිගත කිරීම සිදුකෙරේ. මෙම විඩියෞවල රහස්‍යභාවය තහවුරු කෙරෙන අතර, පර්යේෂක කටයුතු සහ අධයන කටයුතු සඳහා පමණක් යෝදා ගැනේ. මෙම විඩියෞ පටිගත කිරීමට ඔබ එකඟ වෙනවාද?

   ඔව්/නැහැ

9. මෙම අධ්‍යයනයෙන් පසුව ඉතිරි වන රුධිර සාම්පලයක් අැතොත් ඉදිරියෙ කෙරෙන පර්යේෂණය සඳහා භාවිතා කිරීමට ඔබ එකඟ වෙනවාද? ඔව්/නැහැ

10. රුධිර සාම්පල පිටරට යැවීමට එකඟ වෙනවාද? ඔව්/නැහැ

11. මෙම අධ්‍යයනයට සහභාගී වීම සම්බන්ධයෙන් තීරණයකට එලඹීමට ඔබට ඇති තරම් කාලය ලැබුණා ද? ඔව්/නැහැ

12. ඔබ මෙම අධ්‍යයනයට සහභාගී වීමට එකඟ වෙනවාද? ඔව්/නැහැ

සහභාගී වන්නන්ගෙ /භාරකරුවන්

අත්සන:..... දිනය:.....

නම:.....

(b) පර්යේෂක විසින් පිරවීම සඳහාය.

මෙම අධ්‍යයනය සම්බන්ධ කරුණු, මා විසින් අධ්‍යයනයට ස්වේච්ඡාවෙන් සහභාගී වන්නන් හට පැහැදිලි කරන ලදී. ඔහු/ඇ විසින් මෙම අධ්‍යයනයට සහභාගී වීමට කැමැත්ත ප්‍රකාශ කරන ලදී.

පර්යේෂකගේ අත්සන:..... දිනය:.....

නම:.....

தகவல் பத்திரிக்கை

இலங்கை, மனித மரபணு பிரிவுக்கு வருகின்ற நோயாளிகளின் *spinocerebeller ataxia* இன் மருத்துவ இயல்புகள் மற்றும் மரபணு மீடிறன் பற்றிய விரிவான ஆய்வு.

இந்த ஆய்வு கொழும்பு பல்கலைக்கழகத்தின் மருத்துவப்பிரிவு மாணவி துலிகா சுமதிபாலவினால் வைத்திய அதிகாரி வஜிர திஸ்ஸனாயக்கவின் மேற்பார்வையின் கீழ் நடாத்தப்படுகின்றது. உங்களை இந்த ஆய்வில் இணைப்பதற்கு நாங்கள் விரும்புகின்றோம்.

1. ஆய்வின் நோக்கம்.

மரபணு குறைபாடு நோயாளிகளில் *spinocerebeller ataxia* இல் தங்கியிருப்பதனை கண்டுபிடித்தல். பின்பு மருத்துவ வெளிப்படுத்தல்கள் மற்றும் மரபணு குறைபாடுகளுக்கிடையிலான தொடர்பினை அறிதல்.

2. தன்னிச்சையான பங்களிப்பு:

இந்த ஆய்வானது எந்தவித தூண்டுதலுமில்லாததும் உங்கள் சுயவிருப்புடையதுமாகும். இந்த ஆய்விலிருந்து நீங்கள் விரும்பினால் எந்த நேரத்திலும் விலகிக்கொள்ளலாம். இது எந்தவிதத்திலும் உங்கள் மருத்துவத்திலோ அல்லது மருத்துவ பராமரிப்பிலோ பாதிப்பை ஏற்படுத்தாது.

3. ஆய்வின் காலம், படிமுறை மற்றும் ஆய்வாளரின் கடமை:

இந்த ஆய்வின் காலம்மானது 1 வருடம். உங்களை மருத்துவரீதியாக பரிசோதிக்கவும் உங்களது மருத்துவ அறிக்கை மற்றும் மருத்துவ பதிவு என்பவற்றை பார்வையிடவும் உங்கள் உரை மற்றும் நடக்கும் பாணியினை பதிவு செய்யவும் உங்களது அனுமதியினை வேண்டுகின்றோம். அத்துடன் இங்குள்ள ஆய்வுதழ்களிலிருந்து சேகரிக்கப்பட்ட தரவுகளை அடையாளப்படுத்தப்படாத முறையில் வெளியிடவும் உங்களது அனுமதியினை வேண்டுகின்றோம். அத்துடன் ஆய்வில் பயன்படுத்துவதற்காக 10ml குருதி மாதிரி உங்களிடமிருந்து தேவைப்படுகின்றது.

4. ஆய்வின் மூலம் கிடைக்கும் நன்மைகள்.

இந்த ஆய்வில் பங்குகொள்வதன் மூலம் *spinocerebeller ataxia* இனை ஏற்படுத்துகின்ற மரபணு காரணிகளை அறியமுடியும். இது இலங்கையில் *spinocerebeller* பற்றிய பொதுவான அறிவை வளர்ப்பதில் பங்குகொள்கின்றது.

5. பின்விளைவுகளும் பாதிப்புக்களும்.

10ml குருதி உங்களிடமிருந்து எடுக்கப்படும். குருதி எடுக்கப்படும் போது ஊசி குத்துவதால் உபாதை ஏற்படலாம். குருதியை இலகுவாக எடுப்பதற்கும் அதனால் ஏற்படும் உபாதையின் அளவை குறைப்பதற்கும் திறமையும் அனுபவமும் உடைய தாதியால் எடுக்கப்படும்.

6. பிரதிபலன்

இந்த ஆய்வில் பங்கெடுப்பதால் உங்களுக்கு எந்தவிதமான பண உதவியும் வழங்கப்படமாட்டாது. ஆனால் உங்களுக்கு மூலக்கூற்று மரபணு தொடர்பான அறிக்கை கையளிக்கப்படும்.

7. இரகசியம் பேணல்

உங்கள் தொடர்பான தகவல்கள் பதிவு செய்யப்படும். இந்த ஆய்வில் பணியாற்றும் வைத்தியர்கள் மற்றும் ஆராய்ச்சியாளர்கள் மட்டுமே இந்த தகல்களை அறிவர். தேவை ஏற்படின் சில தகவல்கள் விஞ்ஞான ஆய்வித்ழ்களில் வெளியிடப்படும். அப்போது தனிநபர் அடையாளங்கள் பாதுகாக்க நடவடிக்கை எடுக்கப்படும். உங்களைப்பற்றிய தனிப்பட்ட தகவல்கள் உங்கள் அனுமதியில்லாது எந்தவித ஆய்வித்ழ்களிலோ அல்லது காட்சிப்படுத்தல்களிலோ பயன்படுத்தப்படாது.

8. ஆய்விலிருந்து பின்வாங்குதல்

இந்த ஆய்விலிருந்து உங்கள் சுயவிருப்பின் பெயரில் நீங்கள் எந்த நேரத்திலும் பின்வாங்கிக்கொள்ளமுடியும். எந்தவித அபராதமும் விதிக்கப்படமாட்டாது. இருப்பினும் நீங்கள் விலகுவதாக இருந்தால் உடனடியாக ஆய்வுக்குழுவினருக்கு தெரியப்படுத்தவும். ஆனால் முடிவுகள் அனுப்பப்பட்டதன்பின்னரோ அல்லது ஆய்வித்ழ்களில் வெளியிடப்பட்டபின்னரோ நீங்கள் விலகுவது சாத்தியமில்லை.

9. மேலதிக தகவல்களுக்கு

உங்களுக்கு ஏதேனும் மேலதிக தகவல்கள் தேவைப்பட்டால் எந்தவித தயக்கமுமில்லாது 0112689545 என்ற தொலைபேசி இலக்கத்துடன் தொடர்பு கொண்டு பின்வரும் நபர்களில் ஒருவருடன் தொடர்புகொள்ளவும்.

Dr Dulika Sumathipala
Msc student
Human Genetics Unit
Faculty of Medicine
Colombo

Prof Vajira Dissanayake
Medical Geneticist
Human Genetics Unit
Faculty of Medicine
Colombo

ஒப்புதல் படிவம்

இலங்கை, மனித மரபணு பிரிவுக்கு வருகின்ற நோயாளிகளின் *spinocerebeller ataxia* இன் மருத்துவ இயல்புகள் மற்றும் மரபணு மீதான பற்றிய விரிவான ஆய்வு.

பங்குபற்பாளரால்/பாதுகாவலரினால் பூர்த்தி செய்யப்படல் வேண்டும்.

1. நீங்கள் தரப்பட்ட தகவல்களை வாசித்தீர்களா?

ஆம்/இல்லை

2. இந்த ஆய்வு பற்றி கலந்துரையாடவும் உங்கள் சந்தேகங்களை கேட்டறிந்து கொள்ளவும் சந்தர்ப்பம் கிடைத்ததா?

ஆம்/இல்லை

3. உங்களது எல்லா சந்தேகங்களுக்கும் திருப்திப்படுத்தக்கூடிய பதில்கள் கிடைத்தா?

ஆம்/இல்லை

4. உங்களுக்கு இந்த ஆய்வை பற்றிய போதுமான தகவல்கள் தரப்பட்டதா?

ஆம்/இல்லை

5. இவ் ஆய்வு சம்பந்தமாக யாரால் உங்களுக்கு விளக்கமளிக்கப்பட்டது?.....

6. உங்களது எதிர்கால மருத்துவ பராமரிப்பினை பாதிக்காது நீங்கள் எந்த நேரத்திலும் காரணம் ஒன்றும் தராமல் இவ்வாய்விலிருந்து விலகமுடியும் என்பதை விளங்கிக்கொண்டீர்களா?

ஆம்/இல்லை

7. இவ்வாய்வு சம்மந்தமான உங்கள் மருத்துவ அறிக்கைகள் மேற்தரப்பட்ட வைத்தியர்கள் உட்பட இன்னும் சில ஆய்வுக்குழுவினரால் பரிசோதிக்கப்படும். இருப்பினும் உங்களது தகவல்கள் மற்றும் அறிக்கைகள் முழுமையாக இரகசியமாக பேணப்படும். இவர்களுக்கு உங்கள் தகவல் அடங்கிய பதிவுகளை பயன்படுத்த அனுமதி அளிக்கின்றீர்களா?

ஆம்/இல்லை

8. நீங்கள் ஒளிப்பதிவுகளுக்கு அனுமதியளிக்கிறீர்களா?

ஆம்/இல்லை

9. இவ்வாய்வில் பயன்படுத்தப்பட்ட எஞ்சிய குருதிமாதிரிகளை மேற்பார்வையாளரின் நெறிப்படுத்தலின் கீழ் எதிர்கால ஆய்வுகளுக்கு களஞ்சியப்படுத்த சம்மதிக்கிறீர்களா?

ஆம்/இல்லை

10. மாதிரிகளை வெளிநாடுகளுக்கு அனுப்புவதற்கு சம்மதிக்கிறீர்களா?

ஆம்/இல்லை

11. உங்கள் முடிவை எடுப்பதற்கு உங்களுக்கு போதுமான காலம் வழங்கப்பட்டதா?

ஆம்/இல்லை

12. இந்த ஆய்வில் பங்கேற்க சம்மதிக்கிறீர்களா?

ஆம்/இல்லை

....

பங்குகேற்பாளர்/பாதுகாவலரின் கையொப்பம் திகதி

பெயர்:.....

ஆய்வாளரால் பூர்த்தி செய்யப்படல் வேண்டும்

நான் மேலுள்ள ஆய்வில் ஆர்வமுள்ள நபருக்கு ஆய்வினை விளங்கப்படுத்தியுள்ளதுடன் அவர் இவ்வாய்வில் பங்குகொள்ள தான் விருப்பமாகவுள்ளார் என குறிப்பிட்டுள்ளார்.

....

ஆய்வாளர் கையொப்பம் திகதி

பெயர்.....

Rater: _____date: _____patient:_____

Scale for the assessment and rating of ataxia (SARA)

1) Gait

Proband is asked (1) to walk at a safe distance parallel to a wall including a half-turn (turn around to face the opposite direction of gait) and (2) to walk in tandem (heels to toes) without support.

0 Normal, no difficulties in walking, turning and walking tandem (up to one misstep allowed)
1 Slight difficulties, only visible when walking 10 consecutive steps in tandem
2 Clearly abnormal, tandem walking >10 steps not possible
3 Considerable staggering, difficulties in half-turn, but without support
4 Marked staggering, intermittent support of the wall required
5 Severe staggering, permanent support of one stick or light support by one arm required
6 Walking > 10 m only with strong support (two special sticks or stroller or accompanying person)
7 Walking < 10 m only with strong support (two special sticks or stroller or accompanying person)
8 Unable to walk, even supported

Score _____

2) Stance

Proband is asked to stand (1) in natural position, (2) with feet together in parallel (big toes touching each other) and (3) in tandem (both feet on one line, no space between heel and toe). Proband does not wear shoes, eyes are open. For each condition, three trials are allowed. Best trial is rated.

0 Normal, able to stand in tandem for > 10 s
1 Able to stand with feet together without sway, but not in tandem for > 10s
2 Able to stand with feet together for > 10 s, but only with sway
3 Able to stand for > 10 s without support in natural position, but not with feet together
4 Able to stand for >10 s in natural position only with intermittent support
5 Able to stand >10 s in natural position only with constant support of one arm
6 Unable to stand for >10 s even with constant support of one arm

Score _____

3) Sitting

Proband is asked to sit on an examination bed without support of feet, eyes open and arms outstretched to the front.

0 Normal, no difficulties sitting >10 sec
1 Slight difficulties, intermittent sway
2 Constant sway, but able to sit > 10 s without support
3 Able to sit for > 10 s only with intermittent support
4 Unable to sit for >10 s without continuous support

Score _____

4) Speech disturbance

Speech is assessed during normal conversation.

0 Normal
1 Suggestion of speech disturbance
2 Impaired speech, but easy to understand
3 Occasional words difficult to understand
4 Many words difficult to understand
5 Only single words understandable
6 Speech unintelligible / anarthria

Score _____

Rater: _____date: _____patient:_____

5) Finger chase

Rated separately for each side
Proband sits comfortably. If necessary, support of feet and trunk is allowed. Examiner sits in front of proband and performs 5 consecutive sudden and fast pointing movements in unpredictable directions in a frontal plane, at about 50 % of proband´s reach. Movements have an amplitude of 30 cm and a frequency of 1 movement every 2 s. Proband is asked to follow the movements with his index finger, as fast and precisely as possible. Average performance of last 3 movements is rated.

- **0 No dysmetria**
- **1 Dysmetria, under/ overshooting target <5 cm**
- **2 Dysmetria, under/ overshooting target < 15 cm**
- **3 Dysmetria, under/ overshooting target > 15 cm**
- **4 Unable to perform 5 pointing movements**

Score	**R**ight	**L**eft
mean of both sides (R+L)/2		

6) Nose-finger test

Rated separately for each side
Proband sits comfortably. If necessary, support of feet and trunk is allowed. Proband is asked to point repeatedly with his index finger from his nose to examiner's finger which is in front of the proband at about 90 % of proband's reach. Movements are performed at moderate speed. Average performance of movements is rated according to the amplitude of the kinetic tremor.

- **0 No tremor**
- **1 Tremor with an amplitude < 2 cm**
- **2 Tremor with an amplitude < 5 cm**
- **3 Tremor with an amplitude > 5 cm**
- **4 Unable to perform 5 pointing movements**

Score	**R**ight	**L**eft
mean of both sides (R+L)/2		

7) Fast alternating hand movements

Rated separately for each side
Proband sits comfortably. If necessary, support of feet and trunk is allowed. Proband is asked to perform 10 cycles of repetitive alternation of pro- and supinations of the hand on his/her thigh as fast and as precise as possible. Movement is demonstrated by examiner at a speed of approx. 10 cycles within 7 s. Exact times for movement execution have to be taken.

- **0 Normal, no irregularities (performs <10s)**
- **1 Slightly irregular (performs <10s)**
- **2 Clearly irregular, single movements difficult to distinguish or relevant interruptions, but performs <10s**
- **3 Very irregular, single movements difficult to distinguish or relevant interruptions, performs >10s**
- **4 Unable to complete 10 cycles**

Score	**R**ight	**L**eft
mean of both sides (R+L)/2		

8) Heel-shin slide

Rated separately for each side
Proband lies on examination bed, without sight of his legs. Proband is asked to lift one leg, point with the heel to the opposite knee, slide down along the shin to the ankle, and lay the leg back on the examination bed. The task is performed 3 times. Slide-down movements should be performed within 1 s. If proband slides down without contact to shin in all three trials, rate 4.

- **0 Normal**
- **1 Slightly abnormal, contact to shin maintained**
- **2 Clearly abnormal, goes off shin up to 3 times during 3 cycles**
- **3 Severely abnormal, goes off shin 4 or more times during 3 cycles**
- **4 Unable to perform the task**

Score	**R**ight	**L**eft
mean of both sides (R+L) / 2		

Rater: _____date: _____Patient code:_____

Inventory of Non-Ataxia Symptoms (INAS)

***NA**: not assessed / no information available* ***Mod**: moderate*

Part one: clinical findings

Please report the (undoubtful) occurrence of signs also if abnormal findings occur only on one side

Reflexes

1. Biceps (BTR)	❍ normal	❍ hyperreflexia	❍ areflexia	❍ NA
2. Patellar (PTR)	❍ normal	❍ hyperreflexia	❍ areflexia	❍ NA
3. Achilles (ATR)	❍ normal	❍ hyperreflexia	❍ areflexia	❍ NA
4. Extensor plantar reflex	❍ none	❍ unilateral	❍ bilateral	❍ NA

Motor symptoms

5. Spasticity	None	Mild	Mod	Severe	NA
Gait	❍	❍	❍	❍	❍
Upper Limbs	❍	❍	❍	❍	❍
Lower Limbs	❍	❍	❍	❍	❍

6. Paresis	None	Mild	Mod	Severe	NA
Face/tongue	❍	❍	❍	❍	❍
UL proximal	❍	❍	❍	❍	❍
UL distal	❍	❍	❍	❍	❍
LL proximal	❍	❍	❍	❍	❍
LL distal	❍	❍	❍	❍	❍

7. Muscle atrophy	None	Mild	Mod	Severe	NA
Face/tongue	❍	❍	❍	❍	❍
UL proximal	❍	❍	❍	❍	❍
UL distal	❍	❍	❍	❍	❍
LL proximal	❍	❍	❍	❍	❍
LL distal	❍	❍	❍	❍	❍

8. Fasciculations	None	Mild	Mod	Severe	NA
Face/tongue	❍	❍	❍	❍	❍
Upper Limbs	❍	❍	❍	❍	❍
Lower Limbs	❍	❍	❍	❍	❍

9. Myoclonus	None	Mild	Mod	Severe	NA
Face/tongue	❍	❍	❍	❍	❍
Trunk	❍	❍	❍	❍	❍
Upper Limbs	❍	❍	❍	❍	❍
Lower Limbs	❍	❍	❍	❍	❍

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10. Rigidity (should be obvious without movement of opposite limb)	None	Mild	Mod	Severe	NA
Axial	❍	❍	❍	❍	❍
Upper Limbs	❍	❍	❍	❍	❍
Lower Limbs	❍	❍	❍	❍	❍

11. Chorea/Dyskinesia	None	Mild	Mod	Severe	NA
Face/tongue	❍	❍	❍	❍	❍
Neck	❍	❍	❍	❍	❍
Trunk	❍	❍	❍	❍	❍
Upper Limbs	❍	❍	❍	❍	❍
Lower Limbs	❍	❍	❍	❍	❍

12. Dystonia	None	Mild	Mod	Severe	NA
Face/tongue	❍	❍	❍	❍	❍
Neck	❍	❍	❍	❍	❍
Trunk	❍	❍	❍	❍	❍
Upper Limbs	❍	❍	❍	❍	❍
Lower Limbs	❍	❍	❍	❍	❍

13. Resting tremor	None	Mild	Mod	Severe	NA
	❍	❍	❍	❍	❍

Rater: _____date: _____Patient code:_____

Sensory symptoms

14. Impaired vibration sense (tested at malleolus ext)	None (8/8)	Mild (>5/8)	Mod (2-5/8)	Severe (<2/8)	NA
Right foot	❍	❍	❍	❍	❍
Left foot	❍	❍	❍	❍	❍

Ophthalmological findings

Testing of fixation and smooth pursuit

	No	Yes	NA
15. Broken up smooth pursuit	❍	❍	❍
16. Square wave jerks on fixation	❍	❍	❍
17. Downbeat-nystagmus on fixation	❍	❍	❍
18. Gaze evoked-nystagmus on horizontal testing	❍	❍	❍
19. Gaze evoked-nystagmus on vertical testing	❍	❍	❍
20. Ophthalmoparesis on horizontal gaze	❍	❍	❍
21. Ophthalmoparesis on vertical gaze	❍	❍	❍

Testing of fast saccades

	No	Yes	NA
22. Slowing of saccades	❍	❍	❍
23. Hypometric saccades	❍	❍	❍
24. Hypermetric saccades	❍	❍	❍

Testing of visual function

25. Impaired visual acuity (loss of visual acuity <0.6 for binocular sight in distance testing)

No	Yes	NA
❍	❍	❍

Part Two: reported abnormalities

26. Double vision	None ❍	Mild ❍	Mod ❍	Severe/constant ❍	NA ❍
27. Dysphagia	None ❍	Mild ❍	Mod ❍	Severe/ tube feeding ❍	NA ❍
28. Urinary dysfunction	None ❍	Mild ❍	Mod ❍	Severe/ catheter ❍	NA ❍
29. Cognitive impairment (according to examiner)	None ❍	Mild ❍	Mod ❍	Severe ❍	NA ❍

30. Other clinical findings or reported abnormalities

(free text)_____

Rater: _____date: _____Patient code:_____

INAS count

The INAS can be used for clinical description, but is not used as a scale and it is not appropriate to use sum scores.
However, the INAS can be transformed in a set of 16 binary variables

- rated as “present”, if at least one corresponding item or location is rated as mild OR moderate OR severe.
- rated as ”absent” if ALL corresponding items or locations are rated as normal
- rated as missing if at least one corresponding item or location is missing AND other corresponding items or locations rated as normal.

The 16 variables are grouped from the INAS form as follows:

1 Hyperreflexia	items 1, 2, 3
2 Areflexia	items 1, 2, 3
3 Extensor plantar	item 4
4 Spasticity	item 5
5 Paresis	item 6
6 Muscle atrophy	item 7
7 Fasciculations	item 8
8 Myoclonus	item 9
9 Rigidity	item 10
10 Chorea/dyskinesia	item 11
11 Dystonia	item 12
12 Resting tremor	item 13
13 Sensory symptoms	item 14
14 Urinary dysfunction	item 28
15 Cognitive dysfunction	item 29
16 Brainstem oculomotor signs	items 20, 21, 22

These 16 binary variables can be summed up to a simple sum score, the INAS count, that can be used as a semiquantitative variable of extracerebellar involvement in SCA.

PATIENT HEALTH QUESTIONNAIRE-9 (PHQ-9)

Over the last 2 weeks, how often have you been bothered by any of the following problems? *(Use "✔" to indicate your answer)*	**Not at all**	**Several days**	**More than half the days**	**Nearly every day**
1. Little interest or pleasure in doing things	0	1	2	3
2. Feeling down, depressed, or hopeless	0	1	2	3
3. Trouble falling or staying asleep, or sleeping too much	0	1	2	3
4. Feeling tired or having little energy	0	1	2	3
5. Poor appetite or overeating	0	1	2	3
6. Feeling bad about yourself — or that you are a failure or have let yourself or your family down	0	1	2	3
7. Trouble concentrating on things, such as reading the newspaper or watching television	0	1	2	3
8. Moving or speaking so slowly that other people could have noticed? Or the opposite — being so fidgety or restless that you have been moving around a lot more than usual	0	1	2	3
9. Thoughts that you would be better off dead or of hurting yourself in some way	0	1	2	3

FOR OFFICE CODING 0 + _____ + _____ + _____

=Total Score: _____

If you checked off any problems, how difficult have these problems made it for you to do your work, take care of things at home, or get along with other people?

Not difficult at all	**Somewhat difficult**	**Very difficult**	**Extremely difficult**
☐	☐	☐	☐

රෝගීන්ගේ සෞඛ්‍ය පිළිබඳව ප්‍රශ්ණාවලිය

PATIENT HEALTH QUESTIONNAIRE (PHQ-9)

නම/ අංකය ..... දිනය .....

පසුගිය **<u>සති දෙක තුල</u>** පහත සඳහන් දෑ කෙතරම් දවස් ගණනක් ඔබට දැනී ඇත්ද ?

	කවදාවත් නැත	දවස් කිහිපයක්	දවස් 7 කට වඩා	සෑම දවසකම වාගේ
1. යම් දෙයක් කිරීමට උනන්දුව නැති කම හෝ ඉන් සතුටක් නොලැබීම				
2. සිතට දුක හෝ කලකිරීම ඇතිවීම				
3. නින්ද යෑමට ගතවන කාලය වැඩිවීම හෝ නිදියන කාල ප්‍රමාණය අඩුවීම හෝ වැඩිපුර කාලයක් නිදා ගැනීම				
4. නිතරම මහන්සි ගතිය හෝ විඩාව ඇතිවීම හෝ ඇඟට පණනැති ගතිය				
5. කෑමට රුචියක් නැතිබව (කෑමට ආසාවක් නැතිබව) හෝ අධික ලෙස ආහාර ගැනීම				
6. තමන් වැඩකට නැති හෝ අසාර්ථක පුද්ගලයෙකු යැයි සිතීම හෝ තමාගේ හෝ පවුලේ බලාපොරොත්තු ඉටු නොකල පුද්ගලයෙකු යැයි සිතීම				
7. කියවීම, රූපවාහිනිය නැරඹීම, පත්තර කියවීම වැනි දේ වලදී අවධාණය යොමුකිරීමට අපහසුව				
8. අන්අයට වෙනසක් දැනෙන තරමට හෙමින් කතා කිරීම හෝ හෙමින් එහා මෙහා යෑම හෝ එකතැන සිටීමේ අපහසුව හෝ වෙනදාට වැඩියෙන් එහා මෙහා යෑම				
9. ජීවිතය නැති කර ගැනීමට සිතීම (සියදිවි හානිකර ගැනීමට) හෝ ජීවත්වීමෙන් පලක් නැතැයි සිතීම				

10. ඉහත එක ප්‍රශ්ණයකට හෝ ඔබ මුහුණ දී ඇත්නම් ඒ නිසා රුකියාව කිරීමට, ගෙදර වැඩ කිරීමට හෝ අන්අය සමග ආශ්‍රය කිරීමට කෙතරම් අපහසුවක් ඇතිවී ඇත්ද ?

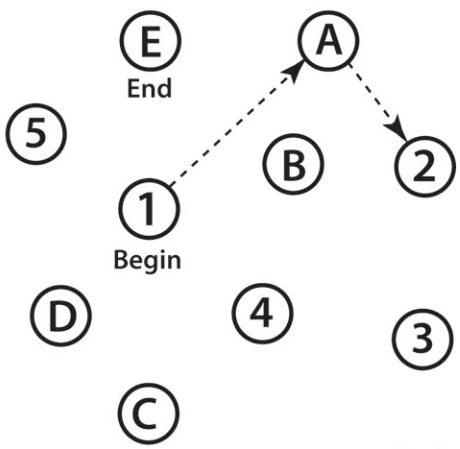
කිසිම අපහසුවක් නැත		යම් අපහසුවක් ඇත		සෑහෙන අපහසුවක් ඇත		අධික අපහසුවක් ඇත	

MONTREAL COGNITIVE ASSESSMENT (MOCA)

Version 7.1 Original Version

NAME :
Education : Date of birth :
Sex : DATE :

VISUOSPATIAL / EXECUTIVE



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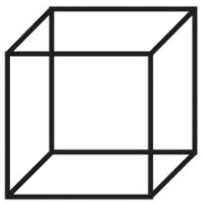


Copy cube

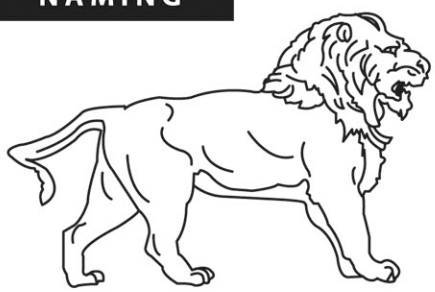
[ ]

Draw CLOCK (Ten past eleven)
(3 points)

[ ] Contour [ ] Numbers [ ] Hands

POINTS ___/5

NAMING



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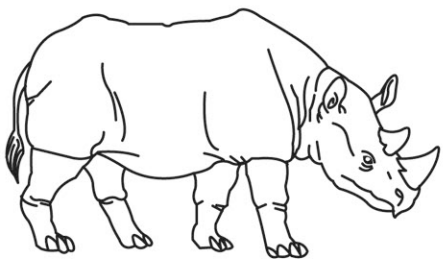

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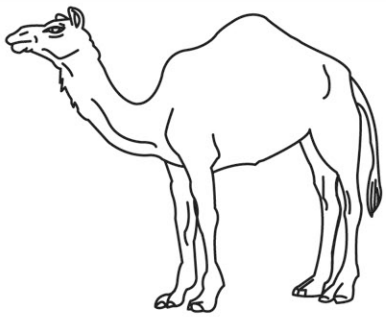


[ ]

___/3

MEMORY

Read list of words, subject must repeat them. Do 2 trials, even if 1st trial is successful. Do a recall after 5 minutes.

	FACE	VELVET	CHURCH	DAISY	RED
1st trial					
2nd trial					

No points

ATTENTION

Read list of digits (1 digit/ sec.). Subject has to repeat them in the forward order [ ] 2 1 8 5 4

Subject has to repeat them in the backward order [ ] 7 4 2

___/2

Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors

[ ] F B A C M N A A J K L B A F A K D E A A A J A M O F A A B

___/1

Serial 7 subtraction starting at 100 [ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65

4 or 5 correct subtractions: **3 pts**, 2 or 3 correct: **2 pts**, 1 correct: **1 pt**, 0 correct: **0 pt**

___/3

LANGUAGE

Repeat : I only know that John is the one to help today. [ ]

The cat always hid under the couch when dogs were in the room. [ ]

___/2

Fluency / Name maximum number of words in one minute that begin with the letter F [ ] _____ (N ≥ 11 words)

___/1

ABSTRACTION

Similarity between e.g. banana - orange = fruit [ ] train – bicycle [ ] watch - ruler

___/2

DELAYED RECALL

Optional

	FACE	VELVET	CHURCH	DAISY	RED
Has to recall words **WITH NO CUE**	[ ]	[ ]	[ ]	[ ]	[ ]
Category cue					
Multiple choice cue					

Points for UNCUED recall only

___/5

ORIENTATION

[ ] Date [ ] Month [ ] Year [ ] Day [ ] Place [ ] City

___/6

www.mocatest.org

Normal ≥ 26 / 30

TOTAL ___/30

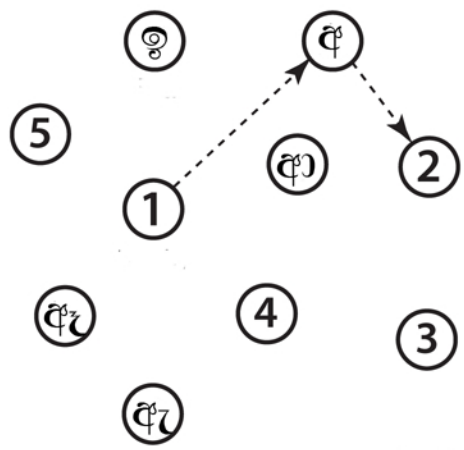
Add 1 point if ≤ 12 yr edu

Administered by: _____

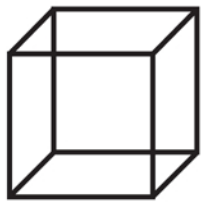
MONTREAL COGNITIVE ASSESSMENT (MOCA)

නම්:
අධ්‍යාපනය: උපන් දිනය
ස්ත්‍රී / පුරුෂ: දිනය:

VISUOSPATIAL / EXECUTIVE



[ ] [ ]



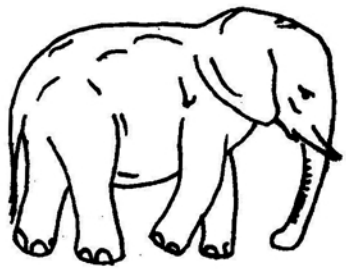
මෙම සණකය අඳින්න.

ඔරලෝසු මුහුණතක් අඳින්න. වේලාව 11 පසුවී මිනිත්තු 10 ඵහි ලකුණු කරන්න.

[ ] Contour [ ] Numbers [ ] Hands

POINTS ___/5

NAMING



[ ] [ ] [ ]

___/3

MEMORY

මෙම වචන ලැයිස්තුව රෝගියා ළඟා දෙවරක් කියවිය යුතුය. (පළමුවාරය නිවැරදිව කියුවත් දෙවාරයක් කියවීම අනිවාර්යය වේ) වචන මතක තබා ගැනීමට උපදෙස් දෙන්න.විනාඩි 5 කින් නැවත රෝගියා ගෙන් මෙම වචන ලැයිස්තුව අසා delayed recall හි සඳහන් කරන්න.

	මුහුණ	කපු	පන්සල	අරලිය	රතු
1st trial					
2nd trial					

No points

ATTENTION

මෙම ඉලක්කම් ලැයිස්තුව කියවන්න. වේගය තත්පරයට ඉලක්කම් 1 වීය යුතුය. රෝගියා විසින් මෙම ඉලක්කම් මුල සිට අගට කියවීම [ ] 2 1 8 5 4

රෝගියා විසින් මෙම ඉලක්කම් අග සිට මුලට කියවීම [ ] 7 4 2

___/2

මෙම අකුරු ලැයිස්තුව කියවන්න "අ" අකුර කියවන සෑම වාරයකම රෝගියා අතින් මේසයට තට්ටු කළ යුතුය

No points if ≥ 2 errors

( ) ප බ අ ක ම න අ අ ජ ක ල බ අ ප අ ක ඩ ඵ අ අ අ ජ අ ම ඔ ප අ අ බ

___/1

Serial 7 subtraction starting at 100 සියයෙන් 7 බැගින් අඩු කරන්න

[ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65

4 or 5 correct subtractions: **3 pts**, 2 or 3 correct: **2 pts**, 1 correct: **1 pt**, 0 correct: **0 pt**

___/3

LANGUAGE

මෙම වාක්‍ය නැවත කියන්න.

1. මා දන්නා පරිදි අද දවසේ අපේ උදව්වට පැමිණ ඇත්තේ නිමල් පමණයි. [ ]
2. බල්ලා බුරන්නට පටන් ගත් වට අපේ ගෙදර සිටින පුසා දිව ගොස් මේසය යට සැඟවෙයි. [ ]

___/2

"ස" අකුරෙන් පටන් ගන්නා වචන හැකි පමන කියන්න [ ] _____ (N ≥ 11 words)

___/1

ABSTRACTION

මෙම දේවල් අතර ඇති සමානකම කුමක්ද? කෙසෙල්-දොඩම් ( ) බයිසිකලය-දුම්රිය( ) ඔරලෝසුව-අඩිකෝදුව( )

___/2

DELAYED RECALL

	Has to recall words WITH NO CUE	මුහුණ [ ]	කපු [ ]	පන්සල [ ]	අරලිය [ ]	රතු [ ]	Points for UNCUED recall only
Optional	Category cue						
	Multiple choice cue						

___/5

ORIENTATION

[ ]දිනය [ ] මාසය [ ]අවුරුද්ද [ ]සතියේ දවස [ ]ස්ථානය [ ]නගරය

___/6

 Version 7.1 **www.mocatest.org** Normal ≥ 26 / 30

Sinhala Version May 2010. Translated by Sanjeewani Karunaratne MD, Varuni de Silva MD, Raveen Hanwella MD

Administered by: _____

TOTAL ___/30

Add 1 point if ≤ 12 yr edu

APPENDIX 6: AUTOSOMAL DOMINANT CEREBELLAR ATAXIA

SCA1 OMIM ID #164400

SCA 1 is the first dominant genetic form of hereditary ataxia discovered (1993). Caused by triplet repeat mutations, CAG repeats greater than 39 is pathogenic. Symptoms may appear at any age but are most often appear after 30 years of age. The initial symptoms are usually gait ataxia. Pyramidal signs are also often seen. MRI reveals atrophy of the brain stem and pons.

SCA2 OMIM ID #183090

This was first described in families from Cuba and the gene isolated in 1993. A triplet repeat mechanism is the genetic basis of the disease. Repeat numbers greater than 35 is pathogenic. Common features are slow eye movements and ophthalmoplegia, neuropathy. Reduced reflexes and memory loss in addition to cerebellar and brainstem atrophy is often seen on brain MRI.

SCA3 OMIM ID #109150

This is also known as Machado – Joseph diseases (MJD). It is caused by a repeat expansion greater than 67. Symptoms besides ataxia include tremor, rigidity, myokimias and neuropathy. SCA 3 is the most prevalent dominant ataxia worldwide.

SCA5 OMIM ID # 600223

SCA5 is caused by a missense mutation in the *SPTBN2* gene. Onset of disease is varied but most often at a young age. Disease progression is slow and is described as “pure”.

SCA6 OMIM ID #183036

SCA6 mutation is found on the same gene as that causing episodic ataxia EA2 and hemiplegic migraine. This is however a CAG repeat expansion mutation. Patients may initially present with episodic ataxia or migraine in addition to the cerebellar signs and symptoms. It is often described as a pure cerebellar disorder.

SCA7 OMIM ID # 164500

What Harding described as ADCA II, with retinopathy. This neurodegenerative disorder is caused by repeat expansions greater than 36. Anticipation is reported giving an aggressive juvenile form.

SCA8 OMIM ID # 608768

Involves poor coordination of extremities, especially lower extremities with gait ataxia and spasticity are reported. Many patients with CAG/ TAG expansions in one allele did not develop the disease due to factors not well understood. An expansion larger than 71 repeats can cause SCA 8.

SCA10 OMIM ID# 603515

SCA 10 is a slowly progressive ataxia described in Mexico and Brazil. In addition to the cerebellar ataxia patients develop epilepsy, weakness and loss of sensation. SCA 10 is caused by an ATTCT repeat expansion of more than 400 repeats in the intron of the *ATXN 10* gene.

SCA 12 OMIM ID #604326

SCA 12 is associated with tremor. Tremor can be the first symptom and later the ataxia is more prominent. In addition Parkinson – like features may also occur and exaggerated tendon reflexes and loss of sensation can be seen. SCA 12 is caused by a CAG repeat expansion. Repeats greater than 51 are known to cause disease.

SCA13 OMIM ID#603259

SCA 13 is often early onset, progressive ataxia. Cognitive decline is often seen, among those with early onset disease. Pyramidal signs are also reported in some patients. The genetic mechanism is missense mutations in a potassium channel gene.

SCA14 OMIM ID # 605361

SCA 14 is found in the Norwegian ataxia population but is rare. It is characterized by a slowly progressive ataxia with often additional pyramidal signs. Many patients are described with myoclonus and cognitive decline.

SCA 15/16 OMIM ID # 606658

This disorder was first described as two different SCA with loci close to each other. A huge deletion in *ITPRI* gene was found in mice models that explained both SCA 15 and SCA16. Missense mutations or deletions are found in the gene in affected patients. The ataxia appears to be a slowly progressive form of ataxia from early childhood.

SCA 17 OMIM ID # 607136

SCA 17 is associated with chorea, dystonia, psychiatric symptoms, memory loss and ataxia. They may also have Parkinson – like features. This is a CAG repeat disorder. If one copy of the gene has 43 – 48 repeats, the individual might develop the disorder. If one copy of the gene has 49 or more repeats the individual will develop SCA 17.

SCA 23 OMIM ID # 610245

SCA 23 has been developed in Dutch families. Symptoms develop in the 40's and 50's. In addition to typical symptoms of ataxia, there are pronounced problems with eye movements. The PDYN gene was recently found to be responsible for this SCA.

SCA 27 OMIM ID # 609307

SCA 27 was originally described in a Dutch family. SCA 27 is caused by a mutation in *FGF14* gene. This SCA is associated with mental impairment, cerebellar dysfunction, neuropathy, tremor exaggerated by physical exercise and stress and in some patients aggressive outbursts and orofacial dyskinesia are reported.

SCA 28 OMIM ID # 610246

SCA28 has an early onset ataxia. It was first described in Italian and German families. The phenotype is an early onset, slowly progressive relatively pure ataxia, but with pyramidal signs in many of the reported patients. SCA 28 is caused by a sequence change in the *AFG3L2* gene.

APPENDIX 7: ABBREVIATIONS

ADCA – **A**utosomal **D**ominant **C**erebellar **A**taxia (In tables with SCA1 separately mentioned, ADCA stands for the patient group of autosomal dominant cerebellar ataxia without genetic diagnosis)

ARCA – **A**utosomal **R**ecessive **C**erebellar **A**taxia

FA – **F**riedreich's **A**taxia

CT – **C**omputed **T**omography

MRI – **M**agnetic **R**esonance **I**maging

DRPLA – **D**entato – **R**ubral – **P**allido – **L**uysian – **A**trophy

HA – **H**ereditary **A**taxia