Identification of a Novel Mutation in ADRBK1 in a Kindred with a Autosomal Dominant Cerebellar Ataxia

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This thesis is presented for the degree of Master of Philosophy

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Abstract

A novel mutation in the gene ADRBK1 (OMIM 109365) was identified in a kindred with autosomal dominant cerebellar ataxia (ADCA). An Australian family was found to have a unique phenotype of ADCA characterized by a slowly progressive cerebellar ataxia and dysarthria without oculomotor abnormalities. Ten family members (five symptomatic, four asymptomatic and one obligate carrier) participated. All subjects underwent clinical examination, MRI scanning of the brain and exome sequencing. Cerebellar atrophy without brainstem involvement was identified on MRI in both symptomatic and some asymptomatic subjects. DNA was extracted, sequenced and aligned with reference genetic information. A mutation in ADRBK1 was identified in nine subjects: all of the symptomatic and some of the asymptomatic subjects. The mutation in ADRBK1 is predicted to produce an amino acid change from Isoleucine (ATT) to Methionine (ATG) at position 140. This novel mutation in ADRBK1 requires further study to prove biological relevance in ADCA.

Statement of Candidate

I certify that the work in this thesis entitled "Identification of a Novel Mutation in ADRBK1 in a Kindred with a Autosomal Dominant Cerebellar Ataxia" has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree to any other university or institution other than Macquarie University.

I also certify that the thesis is an original piece of research and it has been written by me. Professor Dominic Rowe and Professor Mark Connor have critiqued my work. Any help and assistance that I have received in my research work and the preparation of the thesis itself have been appropriately acknowledged.

In addition, I certify that all information sources and literature used are indicated in the thesis.

The research presented in this thesis was approved by Macquarie University Ethics Review Committee: reference number 5201200039 on 28th March 2012.

Ronald Chong Hing Siu (Student ID: 42803454) 1st August 2014

Acknowledgments

I would like to thank my supervisor and mentor, Professor Dominic Rowe for his inspiration, encouragement and patience. This project could not be successful without his support. I wish to acknowledge Professor Mark Connor for his guidance for this project.

I thank Dr Carolyn Orr for her mentorship, kindness and friendship.

I would like to express gratitude to Dr Bryan Traynor, Dr Andrew Singleton andd Ms Monia Ben Hamed Hammer at National Institute of Health, Bethesha for their hospitality during my stay in Bethesha, and their teaching and laboratory support for the whole exome sequencing and DNA analysis.

I would like to thank Professor Carolyn Sue and Mr Luke Carroll at Kolling Institute of Medical Research, Sydney for their teaching and allowing me to perform DNA extraction at their laboratory.

I thank all my colleagues at Macquarie Neurology for their support, advice and friendship.

Lastly, I would like to thank my wife, Dr Alice Lee for her encouragement and understanding, especially during stressful and patience-grinding times.

General Introduction

The cerebellum controls balance and coordinates movements by its complex cellular architecture. Cerebellar disorders are divided into hereditary and acquired forms. Autosomal dominant cerebellar ataxia is a form of hereditary cerebellar disorder. It is characterised by progressive cerebellar dysfunction, radiological changes and a positive family history. The common clinical manifestations are ataxia, dysarthria and nystagmus. At present, there are more than 35 types of autosomal dominant cerebellar ataxia and their genetic pathophysiologies are known. This project studied a family originating from Scotland and migrating to Australia in the early 20th century. They demonstrate a form of cerebellar ataxia characterised by slow progression and the absence of nystagmus, not previously described in the literature. Clinical assessment, radiological findings and family history confirmed the diagnosis of autosomal dominant cerebellar ataxia. Whole exome sequencing and deoxyribonucleic acid (DNA) analysis were performed to identify the responsible mutant gene.

This thesis starts with the basics of cerebellar anatomy and its function, followed by a literature review of the cerebellar disorders and basic genetics. The details of this project are described in the latter chapters, followed by results and discussion.

Cerebellum Introduction

Anatomy

Structure and Function

The adult human cerebellum weighs 150g and constitutes 10% of the brain volume in an average adult (1, 2). Anatomically, it is dorsal to the brain stem and caudal to the cerebral hemispheres in the posterior cranial fossa. The cerebellum connects to the brain through the dorsal aspect of the brainstem via the cerebellar peduncles (1, 2). There are three pairs of tracts in the cerebellar peduncles: the superior, middle and inferior cerebellar peduncles (3). It is divided into the anterior, posterior and flocculonodular lobes by two transverse fissures. Longitudinally, the cerebellum is divided into three mediolateral regions by two longitudinal furrows: the vermis in the midline and two cerebellar hemispheres on either side. The cerebellar hemispheres are further divided into the intermediate and lateral regions.

Figure 1 Cerebellar lobes

A. Cerebellar lobes shown on an axial T2-weighted MRI scan. The right anterior lobe is demonstrated in red and the right posterior lobe is in green.

B. Cerebellar lobes shown on a sagittal T2 FLAIR MRI scan at the midline. The anterior lobe is outlined in yellow, the posterior lobe in light turquoise and the flocculonodular lobe in green. The black arrow marks the primary fissure and the white arrow the posterolateral fissure.

A

B

13
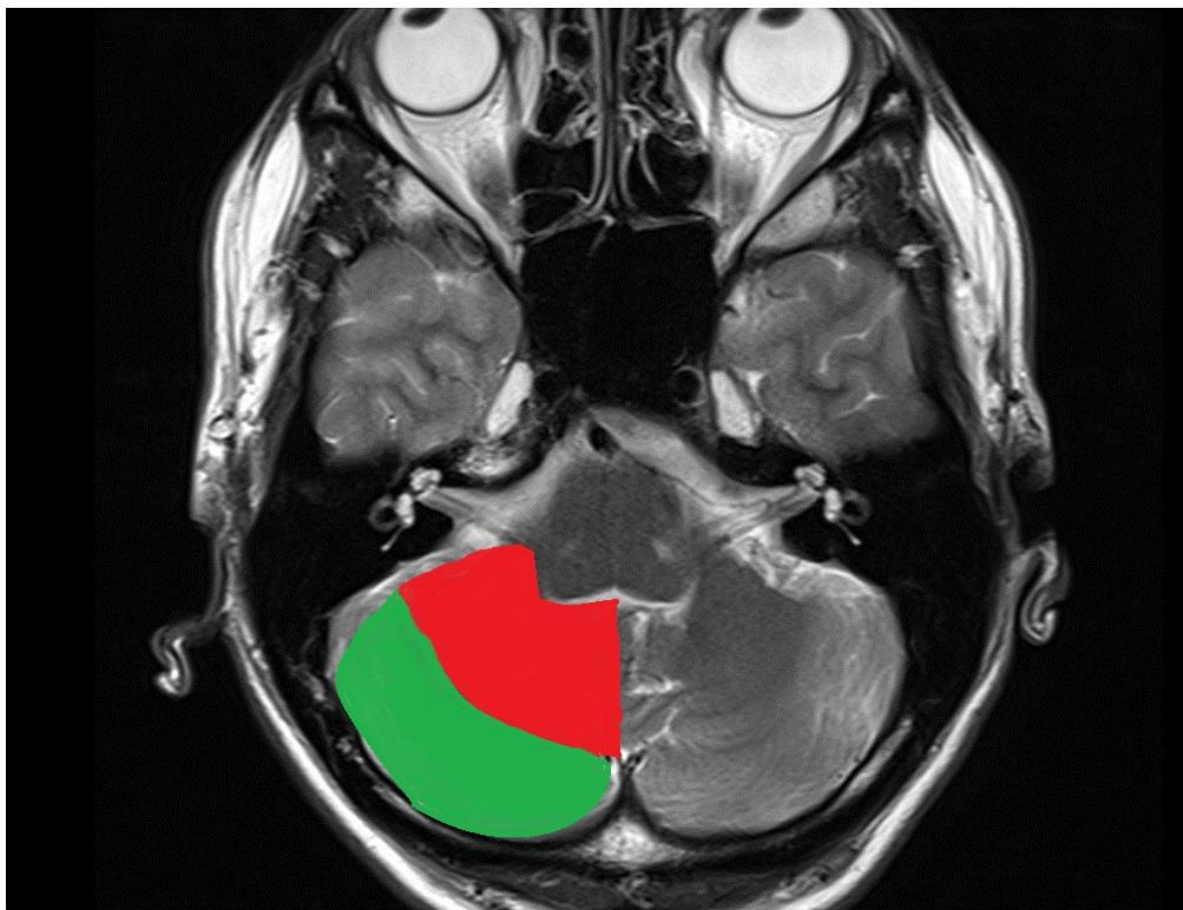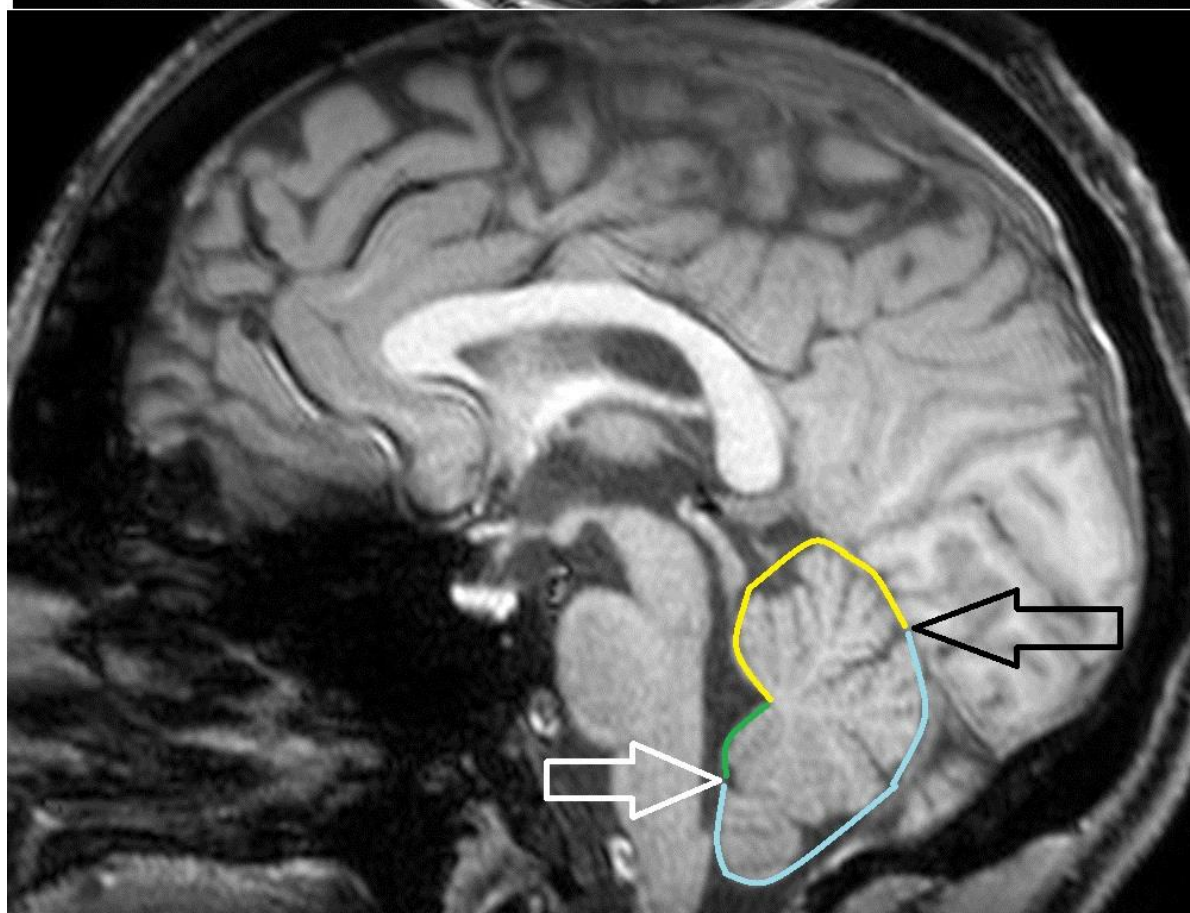
Blood supply

Three pairs of arteries supply the cerebellum: the superior, anterior inferior and posterior inferior cerebellar arteries (3). The posterior inferior cerebellar arteries originate from the vertebral arteries, whereas the anterior inferior and the superior cerebellar arteries originate from the basilar artery(4). A common variation of the vascular anatomy of the cerebellar arteries can occur, where the proximal branches of the cerebellar arteries also supply the lateral part of the brainstem. Therefore, in these patients with an infarction of the cerebellum due to embolism or thrombosis of the major cerebellar arteries, other neurological symptoms as a result of involvement of the brainstem may occur. Furthermore, the anterior inferior cerebellar artery supplies a branch of the internal auditory artery, meaning that hearing loss is common in patients with a cerebellar stroke involving ipsilateral anterior inferior cerebellar artery (5)

Pathways within the cerebellar peduncles

There are different tracts running via the cerebellar peduncles connecting the cerebellum and the brainstem (6). The superior cerebellar peduncle contains both afferent and efferent fibers: the efferent fibers arise from the deep cerebellar nuclei and are termed the cerebellorubral, dentatothalamic and fastigioreticular fibers. The afferent fibers within the superior cerebellar peduncle comprise two tracts: the anterior spinocerebellar and tectocerebellar tracts (6). The middle cerebellar peduncle only comprises afferent fibers arising from the contralateral pons and is known as the pontocerebellar tract. The inferior cerebellar peduncle contains multiple afferent and efferent fibers. Afferent fibers include the dorsal spinocerebellar tract fibers arising from the ipsilateral spinal cord, the cuneocerebellar tract fibers arising from ipsilateral accessory cuneate nucleus, the olivocerebellar tract fibers arising from the contralateral inferior olive in the brain stem and the vestibulocerebellar tract fibers arising from the vestibular ganglion and nuclei (6). The efferent fibers that run in the inferior cerebellar peduncle are the cerebello-olivary, cerebello-vestibular and cerebello-reticular tracts. Table 1 summarises the afferent and efferent tracts that travel in the different cerebellar peduncles.

TABLE 1 THE AFFERENT AND EFFERENT TRACTS THAT RUN IN DIFFERENT CEREBELLAR PEDUNCLES

	Afferent	Efferent
Superior cerebellar peduncle	Anterior spinocerebellar tract Tectocerebellar tract	Cerebellorubral fibers Dentatothalamic fibers Fastigioreticular fibers
Middle cerebellar peduncle	Pontocerebellar tract	Nil
Inferior cerebellar peduncle	Dorsal spinocerebellar tract Cuneocerebellar tract Olivocerebellar tract Vestibulocerebellar tract	Cerebello-olivary tract Cerebello-vestibular tract Cerebello-reticular tract

After passing through the cerebellar peduncles, the afferent input fibers are distributed to different regions in the cerebellum: the vestibulocerebellum, spinocerebellum and cerebrocerebellum. These regions receive multiple different inputs including vestibular, visual, auditory and somatic inputs.

The vestibulocerebellum receives afferent inputs from the vestibular nuclei via the vestibulocerebellar tract and visual input from the superior colliculi. It also has efferent output to the medial and lateral vestibular nuclei. The main function of the vestibulocerebellum is controlling balance.

The spinocerebellum consists of the vermis and the intermediate region of the cerebellar hemispheres. The vermis receives afferent input including visual, auditory, vestibular and somatic inputs from the head and proximal parts of the body via the dorsal spinocerebellar and cuneocerebellar tract fibers. The vermis coordinates posture and locomotion, including gaze, by controlling the proximal muscles of the body and limbs via the medial descending systems that connect the vermis with the cortex and brain stem The other region that receives somatosensory input from the limbs is the intermediate region of the cerebellar hemispheres. Similar to the vermis, it receives somatosensory input via the dorsal spinocerebellar and cuneocerebellar tract fibers (6). Its efferent output project to the interposed nucleus, then indirectly transmits signals to the lateral corticospinal and rubrospinal systems to control distal muscles of the limbs and digits. As all somatosensory afferent inputs from these regions are received via the spinal cord, it is collectively termed the spinocerebellum (7).

The remainder of the cerebellum, namely the lateral region of the hemispheres, is known as the cerebrocerebellum (8). This region of the cerebellum is involved in planning and mental rehearsal of all complex motions. It assesses movement errors by receiving afferent input from the cerebral cortex and projecting efferent output back to the cerebral cortex in the motor, premotor and prefrontal cortices (8).

Layers of cerebellum

The cellular arrangement of the cerebellum is highly organized. There are five types of neurons in the cerebellar cortex: the inhibitory stellate, basket, Purkinje, Golgi and granule neurons. They form three different layers that constitute the cerebellar cortex (9)

Figure 2 Cerebellar Layers

Granule cell

Parallel fiber

Glomerulus

molecular layer

Purkinje cell layer

Granular layer

white matter

Climbing fiber

Purkinje cell

Mossy fiber

Golgi cell

Basket cell
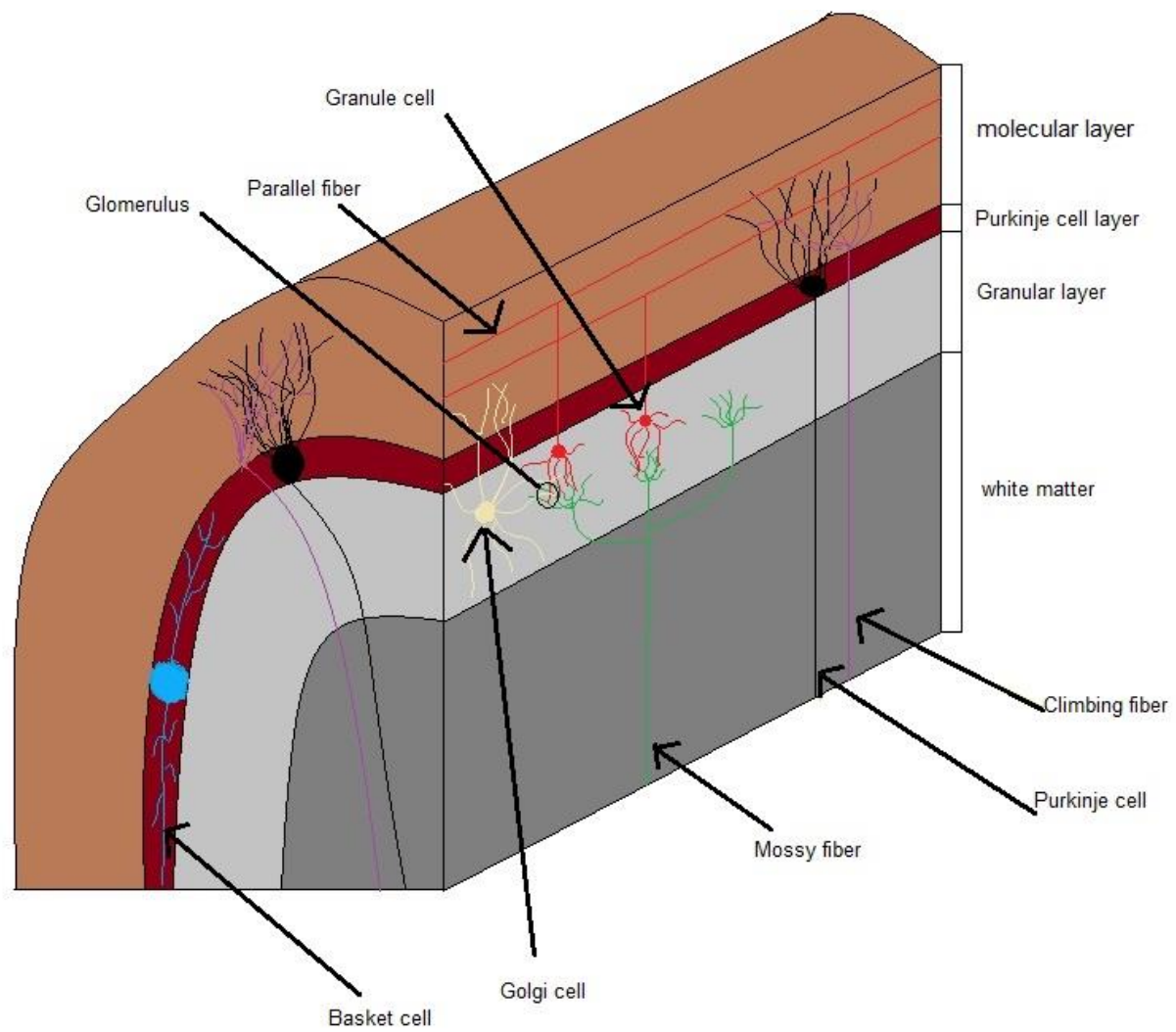
Molecular layer (outermost layer)

Inhibitory stellate and baskets neurons are both inhibitory interneurons located in the molecular layer of the cerebellar cortex. These two classes of neurons are positioned among the axons of excitatory granule neurons and the dendrites of the inhibitory Purkinje neurons (10). However, the cell bodies of the granule and Purkinje neurons are located in deeper layers. The granule neurons project their axons into the molecular layer forming parallel fibers that run parallel to the long axis of the folia. These parallel fibers form synapses perpendicularly with the dendrites of the Purkinje neurons.

Purkinje cell layer (middle layer)

The middle layer is a single cell layer that consists of Purkinje cell bodies. Axons of the basket neurons project to Purkinje cell bodies and terminate in this layer. The output of the Purkinje neurons is inhibitory (11). Their axons either project into the deep white matter of the cerebellum or become efferent outputs projecting to the vestibular nuclei. Lugaro cells lie underneath the Purkinje and it provides an interconnections of multiple neurons in different cerebellar layers by its long dendrites (12) .

Granular layer (innermost layer)

The cell bodies of granule and Golgi neurons are locate in the granular layer, the innermost layer of the cerebellar cortex. There are relatively fewer Golgi neurons compared with the granule neurons which number in the millions in the

granular layer (13), although much larger in size. This layer is an important layer receiving the majority of the afferent inputs from multiple parts of the body via the mossy fibers; therefore it plays an important role in coordinating balance. Mossy fibers terminate in this layer and form a synaptic complex called cerebellar glomeruli with the granule and Golgi neurons. Unipolar brush cell was recognised as a new cell type in the Granular layer in the cerebellum in 2008 by the Federative International Committee on Anatomical Terminology, which is a subcommittee of the International Federation of Associations of Anatomists (14). It functions as a signal amplifier for the mossy fibers to activate granule neurons.

Fibers projecting to cerebellum

Mossy and climbing fibers are the two major afferent inputs to the cerebellum and both are excitatory. The mossy fibers transmit signals to the cerebellum from the cerebral cortex, brain stem and spinal cord. They terminate in the granule layer forming two types of excitatory synapses: the first is formed with the granule and the Golgi neurons and is termed the cerebellar glomerulus, and the other is formed with dendrites of Purkinje neurons (6).

Each climbing fiber wraps around one Purkinje neuron and forms multiple synapses with the dendrites of the Purkinje neuron. Climbing fibers transmit signals from their contralateral inferior olivary nuclei in the brainstem, thought to be involved in the process of learning movement. Despite each Purkinje neuron only receiving excitatory input from one climbing fiber, as the climbing fiber forms multiple synapses with the dendrites, the excitatory response is extremely strong. Excitation produces an action potential with an initial large amplitude spike followed by a high frequency burst of smaller amplitudes; known as a complex spike (15).

Purkinje neurons receive inhibitory signals via feedback inhibition from Golgi cells, and via feed forward inhibition from both basket and stellate neurons. Once a granule neuron is excited, it projects a signal to Purkinje neurons via the parallel fibers in the molecular layer. The parallel fibers not only produce an excitatory signal to Purkinje neurons, but also activate the dendrite of the Golgi neuron in the molecular layer at the same time. When a Golgi neuron is activated,

it projects inhibitory signal to the granule neuron at the cerebellar glomerulus. By this feedback inhibition, Golgi neurons can reduce the excitatory signal from granule neurons to Purkinje neurons. Whereas, once the basket neurons and the stellate neurons are activated by the parallel fibers, they then directly inhibit the Purkinje neurons by feed forward inhibition(16).

Regions of cerebellum

Vestibulocerebellum

The semicircular canals and the otolith organs in the ears sense the motion of the head and its position relative to the gravitational field. The signals from these organs are transferred to the vestibulocerebellum via the mossy fibers that arise in the vestibular nuclei. The vestibulocerebellum also receives visual input indirectly from the superior colliculi. The efferent outputs consist of signals to the neurons in the medial vestibular nuclei and the reticular formation to control the oculomotor function; and to control head and eye co-ordination via the medial longitudinal fasciculus. These efferent outputs also inhibit lateral vestibular nuclei to control the axial muscles and limb extensors via the vestibulospinal tract. Therefore, the vestibulocerebellum plays an important role in balance during stance and gait by controlling axial muscles and limb extensors (17).

Spinocerebellum

There are two important afferent input pathways projecting to the spinocerebellum: the ventral and dorsal spinocerebellar tracts. These tracts originate from the spinal gray matter and terminate as mossy fibers in the vermis or intermediate cortex in the cerebellum. They provide somatic sensory input from the muscles and proprioceptors in the lower limbs. The afferent input pathways projecting to the spinocerebellum from the upper limbs relay similar sensory information but are less important in controlling balance and stance. The afferent pathways arising from the spinal cord first form synapses

with the precerebellar nuclei in the reticular formation in the brain stem before transmitting signals to the spinocerebellum. Different somatic inputs project to different areas in the spinocerebellum; there have been mapping studies of the spinocerebellum since the 1940s (17).

The Purkinje neurons in the spinocerebellum either project to the reticular formation in the brain stem or directly to the spinal cord to control the descending motor pathways. The efferent outputs of the spinocerebellum proceed through the superior cerebellar peduncle to the reticular formation, then transmit signals to the spinal motor neurons via the rubrospinal, rubroreticular and reticulospinal tracts. There are also efferent outputs to the motor cortex through the thalamus. The main function of the spinocerebellum is to control muscle tone and to coordinate the actions of the antagonistic muscle groups that participate in stance and gait.

Cerebrocerebellum

The cerebrocerebellum receives afferent inputs from the cerebral cortex and olive via the corticopontine and olivocerebellar tracts respectively (18). Efferent outputs exit the cerebellum via the superior cerebellar peduncle and terminate in either the contralateral ventrolateral thalamus or parvocellular red nucleus. The output that terminates in the contralateral ventrolateral thalamus forms further connections to the premotor and motor cortex. This entire circuit is termed the dentatothalamocortical pathway. Conversely, the output that terminates in the contralateral parvocellular red nucleus projects back to the

cerebrocerebellum, functioning as a feedback loop called the dentate-rubro-olivo-cerebellar neural feedback loop. These complex circuits ensure the precise coordination of all movements by receiving real-time information from the periphery and correcting errors during voluntary movements. They also store information of all the different types of movement one learns throughout life. There is some evidence that the cerebrocerebellum also has a role in cognition (18).

Table 2 summarises the regions of the cerebellum, their input and functions.

TABLE 2 REGIONS OF CEREBELLUM, THEIR INPUTS AND FUNCTIONS

	Input	Functions
Vestibulocerebellum	Semicircular canals and otolith organs in the ears	• Control head and eye movement co-ordination • Control axial muscles and limb extensors
Spinocerebellum	Muscle and joint proprioceptors in the limbs	• Control muscle tone and coordinate actions of antagonistic muscle groups that participate in stance and gait
Cerebrocerebellum	Cerebral cortex	• Coordinate and correct errors during voluntary movement • Store movement information • Role in cognition

Clinical manifestation of cerebellar disease

Different regions of the cerebellum receive different afferent inputs and project different efferent outputs. Therefore, patients with lesions in different parts of the cerebellum may have different clinical manifestations (19-21). For example, patients with lesions in the spinocerebellum will have difficulties with stance and gait as the spinocerebellum is the primary center for receiving somatic inputs from muscle and proprioceptors and project efferent outputs to the axial muscles to coordinate actions of antagonistic muscle groups that participate in stance and gait.

There are many clinical signs associated with cerebellar dysfunction. The most common clinical manifestations are ataxia, intention tremor, dysarthria, gait and stance disturbance, and hypotonia (22).

Ataxia and intention tremor

Ataxia is also known as incoordination. It is the most common manifestation of cerebellar disease. In clinical examination, it is described as dyssynergia, dysmetria and dysdiadochokinesis (20, 23). The cerebellum plays an important role in real-time correction of movements to ensure that actions are smooth and precise by correcting their velocity and force. In cerebellar disease, the movement may terminate prematurely. In the physical examination, it is most prominent in the finger-nose test. The patient is instructed to use his index finger to touch his own nose and the examiner's finger alternatively. The affected patient's finger is able to reach the target, either the examiner's finger or

his own nose, by a series of jerky movements. These jerky movements are perpendicular to the path of the movement, and are nearly always horizontal. Clinically this is termed intention tremor. Alternatively, the patient's finger can miss the target by overshooting. This is due to the delayed activation of the opposing muscles and with correct by a series of secondary movements. This is known as dysmetria (24) .

There are a number of other forms of tremor associated with cerebellar disease. Wing-beating tremor, also known as Holmes' tremor, is a coarse, irregular, wide range tremor that is elicited by holding the arms out to the sides with elbows bent (25). Titubation is a rhythmic tremor of the head or upper trunk in the anteriopresterior plane at rest and is often associated with midline cerebellar disease.

If the entire vestibulocerebellum or its afferent or efferent outputs are involved in cerebellar disease, then extraocular movements are affected (25). Patients with lesions involving this region of the cerebellum are not able to hold their gaze in the eccentric position, which results in rapid repetitive saccades when looking eccentrically. Jerky movements also occur in voluntary conjugate gaze. Pursuit eye movements are slow and the eyes may overshoot the target, with the gaze eventually corrected after multiple jerks, similar to dysmetria in the limbs. Skew deviation (vertical displacement of one eye), ocular flutter (the inability to fix gaze in the horizontal plane) and opsoclonus (inability to fix gaze in the horizontal and vertical planes) are also associated with cerebellar disease, although are more common with pontine gaze centre disorders.

Dysarthria

Speech disturbance is another common clinical manifestation of cerebellar disease. Scanning dysarthria is exclusive to cerebellar disease and is characterized by words broken into syllables, poor articulation and hypoprosody (lack of normal speech rhythm, stress and pitch). Furthermore, some patients may have explosive speech. This is characterized by completing a sentence more forcefully when a syllable is interrupted involuntarily.

Gait and stance disturbance

Patients with cerebellar disease can have disturbance of both stance and gait. The cerebellar ataxic gait is also known as the drunken gait, with a wide base, unsteadiness, irregular cadence of steps and lateral veering. When severe, the patient may even have difficulty sitting in a chair without assistance. On the other hand, in patients with mild forms of cerebellar ataxia, it is only detected clinically when the patient loses balance when walking heel to toe. Patients may also have difficulty when standing with their feet together with or without eyes closed (25, 26) .

Hypotonia

Tone is the normal resistance to passive manipulation in muscles, and in cerebellar dysfunction this is reduced. It is more apparent with acute rather than chronic cerebellar lesions. There are a few ways to demonstrate hypotonia clinically: When the wrist of the affected side is tapped on the outstretched arm,

it will displace through a wider range than normal. This is a result of hypotonic muscles in the shoulders that fail to fixate the arm. The second test is to shake the relaxed upper limbs, during which the flapping movements display a larger excursion in the affected limb. The third test is to swing the arms at the shoulders where the hypotonic arm will have an increased range and duration of swinging. (25, 26).

Other clinical signs

Patients with cerebellar disease have defects in movement that require alternation or rapid change in direction of movement. During the physical examination, patients are asked to pronate and supinate their hand on the dorsum of the other hand as quickly as possible. The term dysdiadochokinesis is used when the movement is slow, fragmented and clumsy (25, 26). The normal rhythm of these movements is interrupted by irregularities of force and velocity that require a precise co-ordination of the agonist and antagonist muscle groups by the cerebellum.

Initiation of movement is slower in patients with cerebellar disease. There is electrophysiological evidence showing problems in the initiation of both slow and fast movements. Neurophysiologically, the initial compound motor action potential is prolonged and the peak force produced by the motor unit is also reduced (27).

Inherited and acquired causes of cerebellar disease

Cerebellar diseases are classified into inherited and acquired causes. (20, 23, 28, 29).

Inherited causes of cerebellar disease

The prevalence of autosomal dominant hereditary cerebellar ataxia ranges from 0.0-5.6 per 100,000 population. The most common form is spinocerebellar ataxia (SCA) 3, followed by SCA 2 and SCA 6. The prevalence of autosomal recessive hereditary cerebellar ataxia ranges from 0.0-7.2 per 100,000 population, with the most common form Friedreich's ataxia followed by ataxia with oculomotor apraxia (30).

SCA is characterised by progressive cerebellar dysfunction (31, 32), cerebellar and brainstem atrophy (31) and a positive family history. To date, more than thirty five different types of SCAs are identified (33).

Genetically, spinocerebellar ataxias are divided into coding polyglutamine expansion SCAs, non-coding polyglutamine expansion SCAs and conventional mutation SCAs (31). Table 3 shows the different types of SCAs classified by groups. The genetic pathogenesis is discussed in the latter chapter.

TABLE 3 CLASSIFICATION OF SPINOCEREBELLAR ATAXIA (SCA) ACCORDING TO THEIR MUTATION TYPE [31]

Coding polyglutamine expansion SCAs	Non-coding expansion SCAs	Conventional mutation SCAs
SCA 1	SCA 8	SCA 5
SCA 2	SCA 10	SCA 11
SCA 3	SCA 12	SCA 13
SCA 6	SCA 31	SCA 14
SCA 7		SCA 15/16
SCA 17		SCA 20
DRPLA		SCA 27
		SCA 28

Note: SCAs that are not listed in this table have unknown genetic mutations

In general, there are pathological, clinical and genetic classifications for hereditary cerebellar ataxia. The genetic classification separates different classes according to their pathogenetic mechanism, as listed in Table 4 (34)

Mitochondrial

Pathologies under this category are defined by defect in mitochondrial oxidative metabolism due to defective proteins coded by nuclear or mitochondrial DNA. Friedreich's ataxia is the most common form of early onset autosomal recessive ataxia under this category. About 98% of patients carry a triple expansion of guanine-adenine-adenine (GAA) and the remainder have either a missense or truncating mutation in frataxin (FXN) gene. FXN gene encodes the mitochondrial protein frataxin. This is deposited in many tissues but mainly in the spinal cord and dorsal root ganglia, and participates in assembly of iron-sulfur cluster. GAA expansion causes reduction of frataxin which leads to mitochondrial iron overload and damages proteins. The clinical signs of Friedreich's ataxia include progressive ataxia, dysarthria, absent tendon reflexes, positive Babinski sign, reduced vibratory sensation, and sensory axonal peripheral neuropathy (35). There are extra-cerebellar manifestations of Friedreich's ataxia since frataxin also deposits in other tissue in the body, including left ventricular hypertrophy, diabetes mellitus and skeletal deformities such as scoliosis and pes cavus. The number of repeat triplets of GAA is closely related to the severity of the peripheral neuropathy and the existence of left ventricular hypertrophy (35). However, there is as yet no effective treatment to slow neurological progression (36, 37).

TABLE 4 CLASSIFICATION OF HEREDITARY ATAXIA ACCORDING TO THEIR PATHOGENETIC MECHANISM (34)

- Mitochondrial
- Metabolic
- Defective DNA repair
- Abnormal protein folding and degradation
- Channelopathies
- Other

Neuropathy, ataxia and retinitis pigmentosa (NARP), myoclonic epilepsy with ragged-red fibers (MERRF) syndrome and Kearns-Sayre Syndrome are conditions caused by point mutations and deletions in the mitochondrial genome (38, 39). NARP is an early onset disorder with extracerebellar manifestations of sensory neuropathy, muscle weakness and vision loss. However, the pathogenesis of the defect of the ATP synthesis causing the clinical manifestations in NARP is still not fully understood. Myoclonic epilepsy with ragged-red fibers (MERRF) syndrome is a very rare cause of cerebellar ataxia with extracerebellar features of myoclonic epilepsy, short stature, hearing loss and poor night vision. Kearns-Sayre Syndrome is an uncommon, early onset and severe form of chronic progressive cerebellar ataxia with external ophthalmoplegia. In addition to the cerebellar ataxia and chronic progressive external ophthalmoplegia, patients with Kearns-Sayre syndrome also develop proximal muscle weakness, deafness and endocrinopathies, most commonly type 2 diabetes mellitus.

Metabolic ataxia

Metabolic disturbances are rare causes of hereditary cerebellar ataxia. Most are early onset and demonstrate autosomal recessive inheritance. They can cause either intermittent ataxia or progressive ataxia. The mechanism of the metabolic ataxia is due to a defect in the urea cycle; metabolism of amino acids, pyruvate, vitamin E or lipids; storage disorder; or peroxisomal disease. The most common form of metabolic ataxia is an x-linked inherited disorder with a deficiency of ornithine transcarbamylase in the urea cycle. Due to this deficiency, patients

develop a hyperammonaemic state during acute intermittent attacks. The two common precipitants of the acute intermittent attacks are a high protein diet or infection. In the heterozygous female, symptoms during the acute attack include irritability, episodic vomiting, lethargy and even coma, whereas chronically, patients may have developmental delay, mental retardation and epilepsy (40). The mainstay of treatment is to prevent acute attacks by maintaining a low protein diet and by treating infections promptly. During an acute attack, treatments include arginine supplementation and intravenous hydration. The other inherited metabolic diseases that cause intermittent ataxia are intermittent branched-chain ketoaciduria, Hartnup disease, late onset multiple carboxylase deficiency and pyruvate dehydrogenase deficiency.

The most common metabolic disease that causes progressive ataxia is Ataxia with Isolated Vitamin E Deficiency (AVED). It is a rare autosomal recessive disorder caused by missense or truncating mutations in the Tocopherol Transfer Protein Alpha (TTPA) gene (41). Patients with AVED have a defect in the alpha-tocopherol transport protein which leads to low serum vitamin E, with normal absorption of vitamin E. In addition to the clinical manifestations of Friedreich's ataxia, patients with AVED may also develop head titubation and dystonia. The treatment for AVED is vitamin E administration to normalise serum Vitamin E.

Niemann-Pick disease type C is a lysosomal storage disorder usually presenting before ten years of age, although some patients may present up to the sixth decade (42). NPC1 gene mutation is responsible for about ninety-five percent of cases, with NPC2 gene mutations responsible for the rest. Both of the genes

encode a protein involving the transportation system of the endosomal-lysosomal system. The defect in the protein leads to accumulation of cholesterol and glycolipids in lysosomes that leads to structural changes in the nervous system. The clinical manifestations are similar to the Friedreich's ataxia. Extracerebellar manifestations are athetosis, dystonia, dementia, seizures, supernuclear gaze palsy and splenomegaly.

Other rare metabolic ataxia causes of progressive ataxia are listed in Table 5 (34).

TABLE 5 RARE CAUSES OF METABOLIC ATAXIA CAUSING PROGRESSIVE ATAXIA (34)

- Krabbe Disease

- Hexosaminidase A deficiency

- Kufs Disease

- Cholestanolosis

- Refsum disease

- Adrenomyeloneuropathy

Ataxias associated with defective DNA repair

There are three important mechanisms of DNA repair - sensing DNA damage and excising and repairing DNA (43, 44). These mechanisms detect and repair errors in DNA and any defect in these mechanisms may lead to production of the defective protein by the mutated gene.

Ataxia telangiectasia is a rare autosomal recessive, neurodegenerative disorder caused by truncating mutations in the Ataxia Telangiectasia Mutated (ATM) gene on chromosome 11. There are more than 270 mutations described (45, 46). Under normal circumstances, the ATM protein encoded by the ATM gene is responsible for DNA repair by sensing DNA double-strand breaks. When DNA double-strand breaks are detected, the ATM protein induces cell cycle arrest and activates other proteins to repair the defective DNA or in severe cases, activates programmed cell death. Patients with ataxia telangiectasia develop progressive loss of axons in the posterior columns and cerebellar degeneration. However, the exact mechanism of how ATM gene mutations produce ataxia telangiectasia is not fully understood. Since the ATM gene is responsible for DNA repair, patients with ataxia telangiectasia have a higher than population of developing solid and haematologic malignancies. Other extracerebellar manifestations are a large fiber sensory neuropathy, reduced or absent tendon reflexes, telangiectasia on skin and sclera, and recurrent respiratory tract infections due to immunodeficiency. Life expectancy is significantly reduced in ataxia telangiectasia , with the majority of patients dying in their second or third decade of life from infection or malignancy (45, 46).

With the exceptions of immunodeficiency and telangiectasia, patients with ataxia with oculomotor apraxia share similar clinical manifestations with ataxia telangiectasia (47-49). In addition, patients with ataxia with oculomotor apraxia type 1 may also develop axonal motor neuropathy, mild cognitive impairment, chorea and myoclonus. Missense and truncating mutations in the aprataxin (APTX) gene are the causes of ataxia oculomotor apraxia type 1. On the other hand, ataxia with oculomotor apraxia type 2 is caused by a truncating mutation in the senataxin (SETX) gene. Both of these mutations lead to defects in DNA repair and are autosomal recessive disorders. Chorea and myoclonus usually gradually disappears in patients with ataxia with oculomotor apraxia type 1, whereas in patients with ataxia with oculomotor apraxia type 2, chorea and myoclonus persists.

Table 6 lists the other rare DNA repair defect disorders causing progressive cerebellar ataxia (34).

TABLE 6 DNA REPAIR DEFECTS CAUSING PROGRESSIVE ATAXIA [34]

- Xeroderma pigmentosum

- Cockayne Syndrome

Channelopathies

Channelopathies cause autosomal dominant and early onset episodic ataxias. Episodic ataxia type 1 is caused by missense mutations of the potassium voltage-gated channel, shaker-related subfamily, member 1 (KCNA1) gene, which is responsible for the potassium voltage-gated channel subfamily A member 1 (Kv1.1) (50). Kv1.1 is located in all tissues but predominantly in the cerebellum and peripheral nervous system. The mutation causes a reduction in the potassium current amplitude and changes the voltage-dependent kinetics that leads to neuronal excitability. Patients usually become symptomatic in late childhood or early adolescence. Clinical manifestations are brief episodic ataxic attacks triggered by either emotional or physical stress and sudden movements. Episodic ataxia type 2 is caused by truncating mutations in the calcium channel, voltage-dependent, P/Q type, alpha 1A subunit) (CACNA1A) gene, which is responsible for the alpha 1 A subunit of P/Q type voltage-gates calcium channel (51). It demonstrates incomplete penetrance with symptom onset in childhood or early adolescence. Clinical manifestations include cerebellar ataxia, vertigo, diplopia, nystagmus, nausea, and migraine headaches. Emotional or physical stress, caffeine or alcohol may trigger symptoms. The cerebellar ataxic signs are usually slowly progressive. Acetazolamide, a carbonic anhydrase inhibitor, is the treatment for both episodic ataxia types 1 and 2 and may act by altering inter/intracellular pH (52).

Abnormal protein folding and degradation

The spinocerebellar ataxias are a group of autosomal dominant disorders leading to progressive ataxia. Different types of spinocerebellar ataxia have different pathogenesis due to different genetic mutations (28, 53). At present, there are more than 35 different types of SCA identified (33). Some of the pathogenesis and the functions of the mutated genes are poorly understood.

Coding polyglutamine expansion SCAs

The genetic pathogenesis of spinocerebellar ataxias 1, 2, 3, 6, 7, 17 and dentatorubral-pallidoluysian atrophy (DRPLA) are due to the same mutation, which is the polyglutamine expansion of the cytosine-adenine-guanine (CAG) triplet sequence (28, 31, 48, 53-55). Different types of cerebellar ataxia have different thresholds before the repeated triplet number becomes clinically significant. All the above spinocerebellar ataxia require around 40 repeat triplets of CAG to become symptomatic, with the exception of spinocerebellar ataxia 6, which only requires about twenty repeat CAG triplets (56). In general, the number of triplet repeats has an inverse correlation with the age of onset.

ATXN is the gene responsible for SCAs 1, 2, 3, and 7(31). The ATXN gene encodes a protein called ataxins, which form intranuclear inclusions. However, the exact mechanism of how the intranuclear inclusion causes cerebellar ataxia is not fully understood. In SCA 6, the mutated gene responsible is CACNA1A (56), whereas the TPB gene (which encodes transcription factors) is responsible for SCA 17 (57). DRPLA is caused by a mutated ATN1 gene (58, 59). In addition to

cerebellar signs, patients with SCA may have extracerebellar signs: some are more specific to a particular spinocerebellar ataxia and others are nonspecific.

Clinically, patients with SAC 1 have corticospinal signs including increased knee jerk, spasticity, and positive Babinski signs. Bulbar signs are also a predominant feature in this type of SCA (60).

In patients with SCA 2, slow saccadic eye movements are the distinguishing feature when compared with SCA 1(60). Other clinical signs in patients with SCA 2 are sphincter disturbance, cognitive impairment and posterior column dysfunction. Additionally, patients may have perioral fasciculations, cramps, amytrophy, and parkinsonism (60).

SCA 3 is the most common form of spinocerebellar ataxia. It is also known as Machado-Joseph disease and accounts for about twelve percent of spinocerebellar ataxia in Australia (61). The extracerebellar manifestations include slow saccades, extrapyramidal signs, muscle cramps, fasciculations, cognitive impairment and autonomic dysfunction. One form of SCA 3 presents in a manner clinically indistinguishable to motor neurone disease (62).

In patients with SCA 6, cerebellar signs are the main clinical feature. They may also present with horizontal and vertical nystagmus as well as abnormal vertibuloocular reflexes (60). The age of onset varies from the second to sixth decade of life and is inversely proportional to the number of repeat CAG triplets.

Visual loss is a unique manifestation in patients with SCA 7 and, depending on the age of onset, other clinical manifestations are different (63). With early onset disease, the extracerebellar features include seizures, myoclonus and cardiac involvement, whereas patients with adult onset SCA 7 develop pigmentary macular degeneration causing visual loss before the development of ataxia.

SCA 17 is described in four Japanese families (64). Patients present between age twenty to thirty with dementia, psychiatric features and cerebellar manifestations including dysmetria and dysdiadochokinesis. Epilepsy may also develop.

In DRPLA, different numbers of repeat CAG associate with different clinical manifestations: progressive myoclonus, epilepsy and dementia are associated with longer repeats, whereas choreic movements and neuropsychiatric symptoms are likely to associate with smaller repeats (58, 59).

Non-coding expansion SCAs

In non-coding SCAs (types 8, 10, 12, and 31), it is believed that the disease is caused by a gain of function mutation (31). Unlike the coding polyglutamine SCAs, they do not share the same mutation.

SCA 8 is caused by CTG expansion and the responsible gene is ATXN8. However there is no relationship between the number of repeat CTG and the penetrance.

Therefore, it is controversial to use CTG repeats to diagnose SCA 8 genetically (65, 66).

In SCA 10, the responsible gene is ATXN10, and its mutation is caused by repeat expansion of ATTCT repeats (67). The clinical manifestation is slowly progression cerebellar ataxia without brainstem dysfunction (67).

SCA 12 is exclusively identified in Indian people (31, 68). It is caused by CAG triplet repeats, causing a mutated PPP2R2B gene. Its extracerebellar manifestations are tremor and cognitive impairment (69, 70).

SCA 31 is caused by insertion of the pentanucleotide repeat, TGGAA, in thymidine kinase 2 (TK2). The mean age of onset is the sixth decade and is associated with hearing impairment (71).

Conventional mutation SCAs

The conventional mutation SCAs include SCAs 5, 11, 13, 14, 15/16, 20, 27 and 28. They are caused by missense, in-frame deletion, frameshift, deletion or duplication (31). Table 7 summaries the conventional mutation SCA and their responsible genes.

TABLE 7 CONVENTIONAL MUTATION SPINOCEREBELLAR ATAXIAS (SCA) AND THEIR MUTATED GENE (31)

Type	Mutated gene	Type of mutation
SCA 5	SPTBN2	Missense, in-frame deletion
SCA 11	TTBK2	Frameshift
SCA 13	KCNC3	Missense
SCA 14	PRKCG	Missense
SCA 15/16	ITPR1	Deletion
SCA 20	------	Duplication
SCA 27	FGF14	Missense, frameshift
SCA 28	AFG3L2	Missense

------ = gene not known

SCA 5 is caused by missense and in-flame deletion mutations in Spectrin, beta, non-erythrocytic 2 (SPTBN2) gene. It was identified in a large American family. It demonstrates slow progression and radiologically, the brainstem is relatively spared (72, 73).

SCA 11 was identified in a British family with the responsible mutated TTBK2 (tau tubulin kinase 2) gene caused by frameshift mutation. Similar to SCA 5, the course of progression is slow (74, 75). Interestingly, there was another unrelated family carrying the same mutated gene, also showing very slow progression (76).

SCA 13 is caused by a missense mutation in the KCNC3 (potassium voltage-gated channel, Shaw-related subfamily, member 3) gene (77) and is associated with developmental delay, low IQ and seizures if symptoms start in early life (78).

Missense mutations in the PRKCG (protein kinase C, gamma) gene is responsible in SCA 14. It is associated with slowly progressive cerebellar ataxia, myoclonus, facial dystonia and cognitive impairment (79-82).

SCA 15 and SCA 16 are the same disease (83) with pure cerebellar ataxia. It is caused by deletions in the ITPR1 (inositol 1,4,5-trisphosphate receptor, type 1) gene (83).

The phenotype of SCA 20 is dysphonia and palatal myoclonus with age of onset ranging from 19 to 64 years. It is likely caused by duplication of chromosome 11q12 (84).

A missense and a nonsense mutation in the FGF14 (fibroblast growth factor 14) gene were identified in SCA 27. Again, it is associated with slowly progressive cerebellar ataxia with low IQ and executive dysfunction (85, 86).

Lastly, a mutated AFG3L2, AFG3-like AAA ATPase 2, gene causes SCA 28, presenting as lower limb hyper-reflexia, ophthalmoplegia and ptosis (87).

Table 8 summaries all known SCAs, mean age of onset and their extracerebellar signs.

TABLE 8 TYPES OF SPINOCEREBELLAR ATAXIA, THEIR AGE OF ONSET AND EXTRACEREBELLAR MANIFESTATIONS (20, 23, 28, 29, 34, 48, 51, 53-55, 64, 71, 88-95)

Type	Age of onset (decade)	extracerebellar signs
1	4th	extrapyramidal, cognitive impairment, peripheral neuropathy, dysautonomy
2	3rd -4th	extrapyramidal, cognitive impairment, peripheral neuropathy, dysphagia
3	4th	extrapyramidal, peripheral neuropathy, lower motor neuron dysfunction, dysautonomy, REM sleep behavioual, neuropsychiatric symptoms
4	4th-7th	Peripheral neuropathy, deafness, areflexia at ankles
5	3rd-4th	Nil
6	5th- 6th	extrapyramidal, peripheral neuropathy
7	3rd-4th	retinopathy
8	3rd	neuropsychiatric symptoms, sensory impairment, myoclonus
9	Adult onset	extrapyramidal and posterior column signs
10	3rd	epilepsy, cognitive impairment
11	3rd	pyramidal
12	3rd	tremor, cognitive impairment
13	childhood	pyramidal, developmental delay, seizure, low IQ
14	2nd	facial myokimia myoclonus, reduced vibratory sensation
15	3rd	cognitive impairment
17	childhood- 3rd	parkinsonism, dystonia, cognitive impairment, myoclonus, seizures
18	2nd-3d	sensorimotor neuropathy with pes cavus
19	3rd	cognitive impairment, myoclonus
20	4th	pyramidal, extrapyramidal, dysphonia, palate myoclonus
21	childhood- 3rd	extrapyramidal, cognitive impairment
23	5th-6th	slow saccades, hyperreflexia
25	childhood	sensory neuropathy
27	childhood- 2nd	extrapyramidal, cognitive impairment
28	early – mid-life	lower limb hyper-reflexia, ophthalmoplegia, ptosis
30	late onset	pyramidal
31	6th	hearing impairment
DRPLA		epilepsy

There are differences between polyglutamine expansion SCAs and conventional mutation SCAs in presentation and radiological findings (31). Clinically, polyglutamine expansion SCAs have a more variable age of onset, whereas conventional mutation SCAs have an earlier age of onset. Radiologically, in polyglutamine expansion SCAs, the brainstem is usually involved. Converserly, this is relatively spared in conventional mutation SCAs (31). Table 9 summarize the differences between polyglutamine and conventional mutation SCAs.

TABLE 9 DIFFERENCES BETWEEN POLYGLUTAMINE EXPANSION SPINOCEREBELLAR ATAXIA (SCA) AND CONVENTIONAL MUTATION SCAS (31)

	Polyglutamine expansion SCAs	Conventional mutation SCAs
Clinical	• Relatively faster progression • Early onset cases are usually associated with more severe course	• Relatively slower progression • Early onset cases are usually not associated with more severe course
Radiological	• Brainstem usually affected	• Brainstem usually spared

Others

The underlying pathogenesis of the disorders classified under this category are not well understood. They are rare and usually have multiple extracerebellar manifestations.

Congenital ataxia syndrome is a rare, mainly autosomal recessive disorder. X-linked and autosomal dominant causes are also found in some cases (96). The pathogenesis is unknown. Extracerebellar manifestations are optic atrophy, skin abnormality and mental retardation.

Infantile-onset spinocerebellar ataxia is an autosomal recessive condition (97), presenting as a subacute, early onset ataxia with rapid progression. It also has multiple extra-cerebellar manifestations including epilepsy, athetosis, optic atrophy, ophthalmoplegia, hearing loss, sensory neuropathy, primary hypogonadism in the female and reduced mental capacity.

Early onset cerebellar ataxia with retained tendon reflexes is an autosomal recessive disorder similar to Friedreich's ataxia, except for the absence of severe skeletal abnormalities, cardiomyopathy or diabetes mellitus, but has preserved knee reflexes (97).

Cerebellar ataxia with hypogonadism is an autosomal recessive disorder (97, 98) with variable clinical manifestations. Most patients have secondary

hypogonadism. Other extracerebellar manifestations may include corticospinal signs, dementia, peripheral axonal neuropathy and deafness.

Marinesco-Sjogren Syndrome is an autosomal recessive disorder with multiple extracerebellar manifestations including mental retardation, cataracts, short stature, delayed sexual development due to secondary hypogonadism and demyelinating peripheral neuropathy (99).

Acquired causes of cerebellar ataxia

There are many acquired causes of cerebellar ataxia. They are usually caused by insults to the cerebellum or the connecting pathways. However, pathologies in other parts of the central nervous system can also cause cerebellar ataxia. The most common causes of acquired cerebellar ataxia are vascular, toxic, infectious or autoimmune.

Vascular

Cerebellar infarction accounts for about 3% of ischaemic strokes (4, 26, 100-105). The mean age for cerebellar stroke is sixty five and more than sixty percent are male (21, 106) (107). Patients with well-established cardiovascular risk factors include hypertension, diabetes mellitus, cigarette smoking, dyslipidaemia, atrial fibrillation and history of stroke and transient ischaemic attack have a high risk for cerebellar stroke (108). The commonest causes of cerebellar stoke are cardio-embolism or small and large vessel atherosclerosis, (21, 106, 109) but artery to artery embolism from vertebral dissection is also an important cause (110). Twenty-seven percent of patients less than age forty suffer from cerebellar stroke due to vertebral artery dissection (107) and eighty percent of patients with vertebral artery dissection develop stroke in the posterior circulation territory (111). The posterior inferior cerebellar artery is most commonly involved, followed by the superior cerebellar artery and the anterior inferior cerebellar artery (101-103). Given that the normal variance of the vascular anatomy for the cerebellar circulation is broad, the clinical manifestations of cerebellar stroke vary. Presentations may range from

asymptomatic to dysarthria, ataxia and nystagmus. It may be difficult to distinguish cerebellar stroke from other disorders of the peripheral vestibular system. Apart from the typical cerebellar symptoms and clinical signs, patient with cerebellar stroke may develop other non-specific symptoms including headache, nausea and vomiting.

Toxic

There are a number of toxins that cause cerebellar damage. The most common and major toxin is alcohol. Patient with chronic excessive alcohol intake may develop progressive gait disturbance associated with a mild speech disturbance and ocular movement disturbance (25). Imaging typically shows atrophy at the vermis with relative sparing of the cerebellar hemispheres but the degree of the atrophy does not necessarily correlate to the clinical symptoms and signs. Some illicit drugs such as cocaine and heroin, are known to cause cerebellar damage. Furthermore, excessive exposure to certain environmental toxins, for example, lead and mercury may also cause cerebellar damage (112) .

Medications

5- Fluorouracil (5-FU) is a pyrimidine analogue that induces cell cycle arrest and apoptosis by inhibiting the DNA synthesis. It is typically used in the therapy of gastroenterological cancers. 5-FU may cause cerebellar ataxia if the patient has a dihydropyrimidine dehydrogenase deficiency, which cause abnormalities in pyrimidine metabolism (113).

The commonly used anticonvulsant, phenytoin, causes cerebellar dysfunction in the context of acute overdose as well as with chronic use (113, 114). Phenytoin is a voltage-dependent sodium channel blocker that limits the propagation of seizure discharge in the cerebral cortex. The exact mechanism of cerebellar ataxia caused by phenytoin is not fully understood. In the setting of acute phenytoin overdose, it can produce neurotoxic symptoms such as drowsiness, tremor and cognitive impairment, as well as cerebellar signs including ataxia and dysarthria. There is some evidence that suggests a relationship between long-term phenytoin use with cerebellar degeneration and sensory peripheral neuropathy (115).

Infection

Patients with human immunodeficiency virus infection complicated by lymphomas, chronic meningeal infection, progressive multifocal leukoencephalopathy and toxoplasmosis have a higher risk of developing central nervous system dysfunction including cerebellar ataxia (116). In addition, HIV positive individuals may develop cerebellar ataxia due to granule neuron degeneration in the cerebellum. This is usually revealed on magnetic resonance imaging of the brain as pan-cerebellar atrophy.

Although Creutzfeldt-Jakob disease (CJD) typically causes rapidly progressive dementia by accumulating mutant prion protein, it may also cause ataxia (25, 117). The most common form of CJD is sporadic (eighty five to ninety five percent), followed by familial (five to fifteen percent) and iatrogenic (less than

one percent). The prion protein is formed by post-translational modification of the normal prion protein. About seventeen percent of patients with Creutzfeldt-Jakob disease have ataxia. Extracerebellar manifestations of Creutzfeldt-Jakob disease are minor behavioural symptoms, upper motor neuron signs, myoclonus and dementia.   The diagnosis of Creutzfeldt-Jakob disease is based on biochemical testing for the presence of 14-3-3 protein in cerebrospinal fluid, radiological changes on MRI and neuropathological changes.

Gerstmann-Straussler-Scheinker syndrome (GSS) is a rare prion disease with an autosomal dominant pattern of inheritance. Clinical manifestations include progressive cerebellar ataxia, dementia and proximal lower limb weakness (118). It is diagnosed only by detection of the PRNP gene mutation on chromosome 20 and prognosis is usually only five years after diagnosis (119-122). Similar to CJD, there is no effective treatment to date.

Paraneoplastic cerebellar degeneration

Paraneoplastic cerebellar degeneration is an uncommon disorder associated with malignancy; in particular, small cell lung carcinoma, gynaecological, breast carcinoma and some forms of lymphoma. There are two auto-antibodies, anti-Yo and anti-Tr, that are highly specific for paraneoplastic cerebellar degeneration, whereas, other antibodies are associated with other neurological disorders. (123).

The anti-Yo antibody is also termed the Purkinje cell antibody type 1. The associated malignancies for anti-Yo antibody are breast and gynaecological cancers. Rarely, anti-Yo antibody is associated with adenocarcinoma. The target antigen of the anti-Yo antibody is the cdr protein on the Purkinje neuron. The anti-Tr antibody is most commonly associated with Hodgkin's lymphoma. Its target antigen is located in the cytoplasm of the Purkinje neurons. The antibodies not only trigger the antibody-mediated immune responses, it also triggers cytotoxic T-cell responses, leading to marked degeneration of the Purkinje neurons with inflammation in the cerebellar cortex, deep cerebellar nuclei and the inferior olivary nuclei. Patients with paraneoplastic cerebellar degeneration usually first present with non-specific symptoms including dizziness, nausea, and vomiting. This is then followed by more cerebellum-specific symptoms (gait disturbance, diplopia, and dysarthria). Symptoms usually become stable after several weeks of deterioration (123). Patients may also develop other extracerebellar manifestations: for example, patients with small cell lung carcinoma may develop encephalomyelitis and Lambert-Eaton Syndrome, a rare disturbance of neuromuscular transmission. About twenty percent of patients do not have a history of malignancy at the time the diagnosis of paraneoplastic cerebellar degeneration is considered, thus a careful search for occult malignancy in adult onset cerebellar degeneration is warranted (124).

The diagnosis of paraneoplastic cerebellar degeneration depends on a combination of the history, clinical findings and investigation results. If the patient has a background history of malignancy (in particular, small cell lung carcinoma, breast cancer, gynaecological cancer and Hodgkin's lymphoma) and

develops an acute onset of cerebellar ataxia, the diagnosis of paraneoplastic cerebellar degeneration is likely. The approach to diagnose the paraneoplastic cerebellar degeneration is to exclude other neurological pathologies including malignant metastases in the central nervous system (124). Clinicians should consider checking serum auto-antibodies for paraneoplastic syndrome but note that a negative result does not exclude the diagnosis.

Basic genetics underpinning this study

Genetic disorders are caused by abnormalities in genes and are either caused by a single gene abnormality (monogenic) or abnormalities in multiple genes (polygenic). In single gene disorders, the common modes of inheritance are autosomal dominant, autosomal recessive, X-linked dominant, X-linked recessive and mitochondrial (125).

Autosomal dominant

An individual only requires inheritance of one affected gene from one parent in order to acquire disease. Both males and females are affected equally and the chances of passing the affected gene to their offspring is fifty percent (125).

Autosomal recessive

A person must inherit two affected genes from each parent in order to develop disease. If a person only inherits one affected gene from a single parent, he/she becomes a carrier of the disorder, also termed a heterozygote. If both parents are carriers, the chance for a child to develop disease is one in four, with the odds of becoming a carrier one in two. The chance of a child not inheriting any affected gene is one in four (125).

X-linked

If the abnormal gene is located on the X chromosome, this defines an X-linked genetic disorder. There are two types of X-linked genetic disorders: X-linked

dominant and X-linked recessive. Similar to the autosomal dominant and autosomal recessive modes of inheritance, an individual only requires one abnormal X chromosome in order to develop the disorder in an X-linked dominant condition. Regardless of whether the disease is X-linked recessive or dominant, males have fifty percent chance of receiving the abnormal gene from the mother and develop disease. Whereas in the female, it is more complicated due to a process known as x-linked inactivation, in which there is a random inactivation of one of the two X chromosomes in females. In other words, in the X-linked dominant condition, if the father has the disease, all his female children will have the disease. On the other hand, if the mother is the affected parent, female offspring have a fifty percent chance of inheriting the condition. In an X-linked recessive condition, female offspring have a fifty percent chance of carrying the abnormal gene if the mother is a carrier (125).

Mitochondrial Inheritance

The mitochondrial genome is located inside the mitochondria, within the cytoplasm of the cell. During fertilization, none of the mitochondria of the sperm is introduced into the ovum. Therefore, all mitochondrial genetic disorders are inherited maternally. If the father has the inherited disorder, none of his offspring will inherit the affect mitochondrial gene. Conversely, if the mother carries the affected gene, all her offspring will be affected, although with considerable clinical variability due to the mosaicism of the mitochondrial distribution in embryogenesis (125).

Multiple gene abnormalities

There are disorders that are caused by multiple gene abnormalities rather than a single gene abnormality. In this scenario, an individual would need to inherit multiple affected genes for the condition in order to develop disease. The likelihood of acquiring the inherited disorder with multiple gene abnormalities disorder is directly proportional to the number of the affected genes inherited (125).

Whole exome sequencing

Whole exome sequencing is one of the applications of Next Generation Technology/ sequencing which is used to identify the mutated gene in Mendelian (monogenic) disorders. It can sequence more than ninety five percent of the exons in the whole genome (126) and is used to determine the variations of the exons and the coding regions of the gene of any currently known genes. Exons only constitute about one percent of the whole genome; however, they contain about eighty five percent of the mutations in Mendelian disorders (127, 128). Compared to the previous method of using linkage analysis in families, whole exome sequencing is a faster method to identify the mutated gene in Mendelian disorders. The principle of the whole exome sequencing to identify the potential mutated gene is to collect as many DNA samples as possible in the affected family from both symptomatic and asymptomatic members, then perform the whole exome sequencing on all available DNA samples. The next step is to identify the potential genes which only present in the clinically symptomatic subjects and obligate carriers but not in the asymptomatic subjects. Thee align the sequenced

exome with a reference genome database, either local or international database to identify all mutated genes and all known normal variants are excluded from the analysis. The procedural details of the whole exome sequencing for this study is discussed in a later chapter.

A study of the genotype and phenotype of a kindred with a novel autosomal dominant cerebellar ataxia

This study describes a kindred with autosomal dominant cerebellar ataxia with a previously unrecognised phenotype. This study describes the proband and the examination and investigations of the relatives of the proband that were able to be studied. None of the previously described genetic causes of this autosomal dominant cerebellar ataxia were identified. The proband and her relatives were then subjected to exome sequencing in collaboration with the National Institutes of Health, Bethesda, USA. One novel mutation identified by Sanger sequencing co-segregates with the disease. This mutation in the gene termed ADRBK1 has not previously been identified. The physiological consequence of this mutation and how it produces cerebellar ataxia is as yet unknown, and is the target of further studies.

Background

The proband was a fifty four year old Australian born female when first examined. She was medically well until her first pregnancy at age thirty one, when she developed mild dysarthria during the third trimester. Admitted to hospital for further investigation, it was initially thought that she had suffered an ischaemic stroke. Due to her pregnancy, limited investigations were performed. Her dysarthria improved and her speech subsequently returned to normal over several weeks. Post confinement, her dysarthria relapsed and she developed

cerebellar ataxia. She consulted a neurologist, where the diagnosis of spinocerebellar ataxia was made after clinical assessment and magnetic resonance imaging of the brain. The dysarthria and ataxia continued to progress for about five years, then slowed down. Genetic investigations at the time excluded SCA 1, 2, 3 and 6 as causes of her presentation, although her phenotype was atypical for any of these genetic syndromes. She was referred for further assessment and ongoing management of her spinocerebellar ataxia some ten years after her initial presentation. She was subsequently diagnosed with papillary carcinoma of the thyroid and underwent thyroidectomy in 2000. Since then, thyroxine replacement was her only medication.

As part of the workup, a detailed family history of cerebellar ataxia was obtained from the proband and the pedigree is showed in Figure 3.

Figure 3 Pedigree of the studied family

The pedigree of the studied family demonstrates an autosomal dominant inheritance pattern. The first generation (I) immigrated to Australia from Scotland in the early twentieth century and subject I-1 was believed to have cerebellar ataxia, with problems with coordination. There was only one clinically symptomatic subject in generation II (subject II-1), whilst subject II-6 was believed to be an obligate carrier who lived in a low level care nursing home and mobilised with a four-wheeled walker. She did not have any signs of cerebellar disease. There were 6 symptomatic subjects in generation III, subjects III-4, III-5, III-13, III-17, III-19 and III-20. In generation IV, the proband (subject IV-21) was the only symptomatic subject and there were no symptomatic subjects in generation V at the time of this study.

Ten subjects were recruited in this study: the proband and subjects II-6, III-4, III-5, III-13, III-19, III-21, IV-7, IV-8, and IV-20.
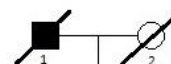
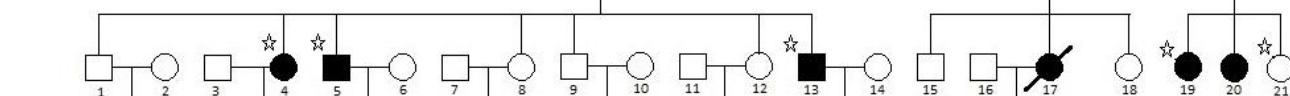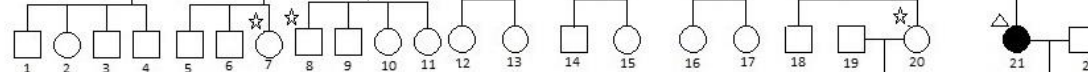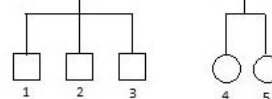= asymptomatic male; ■ = symptomatic male; ○ = asymptomatic female; ● = symptomatic female; ◉ = obligate carrier; / = deceased

☆ = studied subjects; △ = proband

The family history of cerebellar ataxia in the studied family showed an autosomal dominant pattern

Given the family history was consistent with an autosomal dominant condition, the proband and her mother were referred to a clinical geneticist at the Department of Clinical Genetics in Sydney West Area Health Service for further investigation and management as part of her clinical workup. All the known inherited spinocerebellar ataxias were screened on the both the proband and her mother and none of the known mutations were identified. In addition, on physical examination the proband was found to have cerebellar dysarthria and ataxia but no nystagmus was present. Furthermore, the progression of the cerebellar ataxia slowed down after several years. Nystagmus is found in most of the known spinocerebellar ataxias, with the ataxia usually progressive. Therefore, it raised the hypothesis that the form of inherited spinocerebellar ataxia seen in this family was possibly secondary to a novel mutation not previously described.

Method

Approval from the human research committee at Macquarie University was obtained before the commencement of this study (Ethics Reference number: 5201200039).

Details of the study including aim, procedures involved, and purpose of use of information were explained to the proband before detailed family history and the contact details of all contactable family members were obtained.

This project was designed to study this novel mutation: therefore, contacting all family members was necessary to determine and identify the responsible gene.

All subjects included in this study were recruited via personal contact between family members. Formal initial contact for this study was conducted by telephone, email or mail.

The project information sheet was given to the potential studied subjects and written informed consent obtained. The information sheet and consent are attached to the appendix.

Selection criteria

Inclusion criteria

1) 18 years or above
2) Biologically related to the proband
3) Consent to participate in this study

Collection of information

II-6, III-4, III-5, III-13, III-19, III-21, IV-7, IV-8, IV-20 and the proband consented to participate to this study.

All subjects were either interviewed at Macquarie Neurology at Macquarie University Hospital clinic, or at a convenient location of their choosing. Detailed past medical histories were obtained from the subjects, followed by neurological examination and blood collection. All subjects were also invited to have a MRI scan of the brain at Macquarie Medical Imaging at Macquarie University Hospital.

Neurological examinations were performed either by Professor Dominic Rowe or Dr Ronald Siu, who were both registered medical practitioners, and the examination was video-recorded. Blood was collected from all consented subjects by aseptic technique in Ethylenediaminetetraacetic acid (EDTA) tubes. No complications resulted following venepuncture. All blood samples were kept in a locked refrigerator at Macquarie Neurology before sending to Kolling Institute of Medical Research in Sydney for DNA extraction which was done by Ronald Siu under guidance of Professor Carolyn Sue. DNA samples were then sent to the Neurogenetics Laboratory, National Institute of Health, Bethesda, USA for whole exome sequencing which was done and analysed by Ronald Siu under the guidance of Dr Bryan Traynor and Dr Andrew Singleton.

Deoxyribonucleic Acid Extraction

Blood was collected by Ronald Siu from all contactable family members who agreed to participate in the study. About ten mililitres of blood was collected from each subject into an EDTA tube. The blood samples were kept cool on ice during transport between sites. All blood samples were sent to Kolling Institute of Medical Research for DNA extraction. The blood samples were centrifuged to

separate leucocytes from whole blood. QIAamp DNA Blood Mini Kit manufactured by QIAGEN was used to extract DNA from whole blood (QIAGEN).

The QIAamp DNA Blood Mini Spin protocol was followed for DNA purification:

1. Pipet 20 microliters QIAGEN protease into the bottom of a 1.5mL microcentriuge tube

2. 200 microliters of whole blood was added to the microcentrifuge tube.

3. Buffer AL (included in the kit) was added to the sample and mixed by pulse-vortexing for 15 seconds.

4. The mixed sample was incubated at 56 degree C for 10 minutes.

5. The mixed sample was then briefly centrifuged to remove drops from the inside of the lid.

6. 200 microliters of ethanol (100%) was added to the sample and mixed by pulse-vortexing for 15 seconds followed by briefly centrifuge to remove drops from the inside of the lid.

7. The mixture was then transferred to the QIAamp Mini spin column and then centrifuged at 8000rpm for 1 minute.

8. 500microliters of Buffer AW1 (included in the kit) was added, then centrifuged at 8000rpm for 1 minute.

9. 500microliters buffer AW2 was added followed by centrifuging at 14000rpm for 3 minutes.

10. The QIAamp Mini spin column was placed in a clean 1.5ml microcentrifuge tube and the collection tube containing the filtrate was discarded.

11. 200microliters of distilled water was added to the QIAamp Mini spin column and incubated at room temperature for 1 minute followed by centrifuging at 8000rpm for 1 minute.

The extracted DNA samples were kept at Kolling Institute of Medical Research before posting to National Institute of Health, Bethesda, for whole exome sequencing with Illumina HiSeq 2000 v3.

Whole exome sequencing

The DNA sample of each family member was allocated a unique identity number before further processing for confidentiality and privacy. Whole exome sequencing was performed by Illumina HiSeq 2000 v3 and the samples were prepared according to the Illumina TruSeq DNA Sample Preparation Guide.

DNA Analysis

The sequenced exome of each individual underwent several bioinformatic filtering steps to identify potential variants. Firstly, the sequenced exomes were aligned against the local genome database at National Institute of Health and reference human genome 19 (Genome Reference Consortium GRCh37) (http://www.genomereference.org) (129). Then those potential variants that were previous identified in the 1000 Genome Project (130) were excluded in the second filtering step. Those that were non-synonymous, stop loss or gain or within essential splice sites were prioritized for analysis. Thirdly, the remaining potential variants were then visually inspected manually by using Integrative

Genomics Viewer (131), and variants which were artefacts of the sequencing process were excluded. The fourth filtering step was to identify the variants present in the known symptomatic subjects and absent in the asymptomatic subjects. Figure 4 shows the filtering steps of the DNA analysis.

All remaining potential variants were validated by Sanger sequencing (132) and their protein coding effects predicted using SeattleSeq Annotation (http://snp.gs.washington.edu).

Figure 4 Filtering steps of the DNA analysis

There were five filtering steps in DNA analysis performed in this study. All available DNA underwent whole exome sequencing. The first filtering step was to align each individual exome sequence with the reference Human Genome 19 to identify potential variants. All variants previously indentified in the 1000 genome project database or known as a normal variant were excluded in filtering step two. In filtering step three, all potential variants were then manually visualised by using Integrative Genomics Viewer to exclude artifact variants. After filtering step three, all potential variants in all subjects were gathered to identify any variant present in symptomatic subjects but not in asymptomatic subjects. The final step of the DNA analysis was to use Sanger Sequencing to validate the potential variant.

Whole exome sequencing of all available subjects

First filter

Aligned with reference Human Genome 19 (Genome Reference Consortium GRCh37)
(http://www.genomereference.org)

Second filter

Variants were previously identified in 1000 Genome Project database

Third filter

Potential variants was then visually inspected by using Integrative Genomics Viewer

Fourth Filter

Identified those variants in symptomatic subjects and not in asymtomatic subjects

Fifth filter

Sanger sequencing to validate the variant

Results

Analysis of the family history

The family history of cerebellar ataxia in the study family demonstrated an autosomal dominant pattern of inheritance (Figure 3). They originally migrated from Scotland to Australia in the early 20th Century.

I-1 was the first generation in Australia. He was a ship captain and, although there was no documented evidence of ataxia, according to the proband, I-1 was believed to have dysarthria and incoordination in later life. He was not able to milk a cow in his farm in his latter years. It was unclear whether he had any siblings.

II-1 was the only child of I-1 that was clinically symptomatic. He had progressive dysarthria and ataxia which was not investigated, because it was believed that alcohol was the underlying aetiology. He was deceased at age sixty three in 1978 from lung cancer.

II-6 was ninety three years old and included in this study. It was believed that she was an obligate carrier. She lived in a low-level care nursing home and mobilized with a four wheeled walker at the time of this study. On physical examination, there was no evidence of cerebellar ataxia nor dysarthria.

III-4 was seventy one years old with no significant medical history. Similar to the proband, she became symptomatic during her third pregnancy at age thirty three in 1974, with sudden onset of dysarthria followed by slowly progressive ataxia.

III-5 was sixty nine years old with no significant medical history. He developed dysarthria at age forty five in 1988 but did not develop ataxia until ten years later. The progression of both dysarthria and ataxia were slow.

III-13 was sixty two years old and developed dysarthria and ataxia in his late fifties. He had a past medical history of hypertension and deep vein thrombosis.

III-17 was the proband's mother and died at age seventy three in 2012 from carcinoma of unknown origin. She started with dysarthria and ataxia during her first pregnancy and her symptoms continued to progress slowly.

III-19 was fifty eight years old with a medical history of hypertension. Similar to the proband, her dysarthria started during her first pregnancy at age thirty five and did not demonstrate ataxia until several years later. Both symptoms were slow to progress.

III-20 did not consent to participate in this research project.

The proband (IV-21) was the only symptomatic member in generation IV and none of the family members in generation V was symptomatic at the time of this project.

Neurological examination

Structured neurological examination was performed on the proband and the consenting affected family members. The proband had a wide-based gait and was unable to walk heel-to-toe due to unsteadiness, consistent with a cerebellar gait. Her speech examination revealed cerebellar dysarthria with poor articulation and hypoprosody but without any language deficit. Both intraocular and extraocular movements were normal without evidence of nystagmus. Other cranial nerves were normal. Upper and lower limb examination revealed dysdiadochokinesis and dysmetria on nose to finger testing. Tone, power, and deep tendon reflexes were normal. Sensation was normal in all modalities, including testing light touch sensation by using cotton wool balls; pain sensation by using pinprick; vibratory sensation by using a tuning fork and proprioception at the metatarsophalangeal joint of the big toes bilaterally. The clinical examination was recorded and stored on the USB stick attached to this thesis.

Neurological examination findings of all symptomatic subjects were similar to the proband.

Magnetic resonance imaging scans

MRI scans of the brain were performed on both symptomatic (III-4, III-5, III-13, III-19 and the proband IV-21) and asymptomatic subjects (IV-7, IV-8, IV-20). Non contrast T1-weighted spin echo, T2-weighted spin echo, diffusion weighted and Fluid Attenuated Inversion Recovery (FLAIR) images were acquired on a

Siemans Intera 3.0T Magnetic Imaging Scanner. All MRI scan images are saved in the USB stick attached.

The MRI scans of all symptomatic subjects showed varying degrees of cerebellar atrophy with a normal brainstem, which is consistent with currently known mutations in spinocerebellar ataxia (Figures 5, 6, 7, 8 and 9).

The MRI scans of IV-7 and IV- 20, who were both asymptomatic, demonstrated prominence of the cerebellar sulci, which may represent mild or early cerebellar atrophy with a normal brainstem (Figures 10 and 11).

The MRI scans of subject IV-8 (asymptomatic) did not demonstrate any evidence of cerebellar atrophy. (Figure 12).

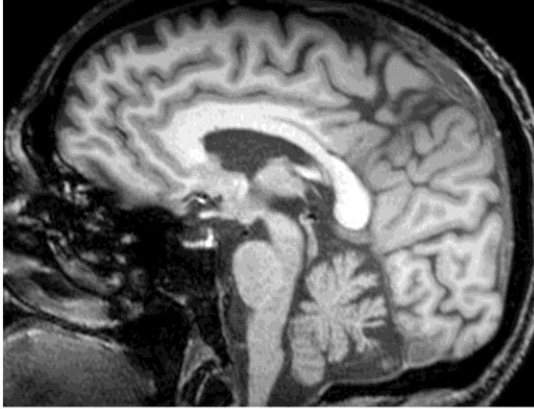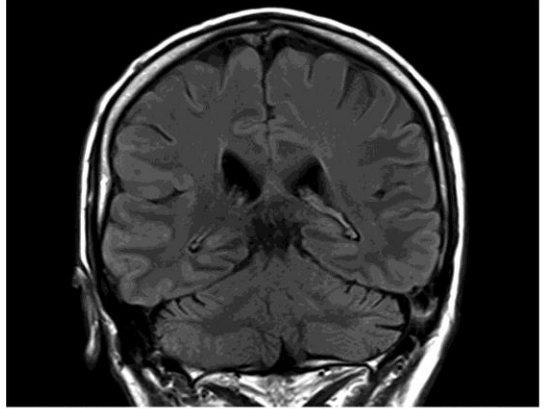# Figure 5 MRI brain scan images of subject III-4

A. T1-weighted sagittal MRI brain scan showed severe cerebellar atrophy, preserved brainstem and cerebral cortex.

B. T2-weighted FLAIR coronal MRI scan showed severe lateral cerebellar atrophy affecting the upper and lower lobes.

C. T2-weighted axial MRI scan of the mid cerebellum showed thicken cerebellar fissure.

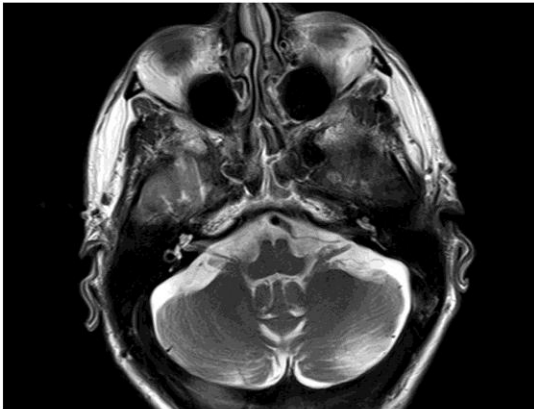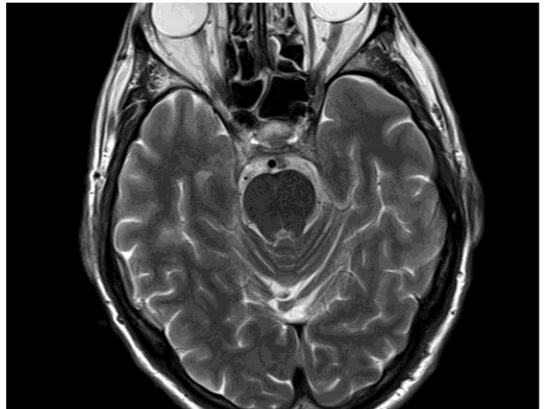D. T2-weighted axial MRI scan of the upper cerebellum showed normal brainstem and normal upper cerebellum.
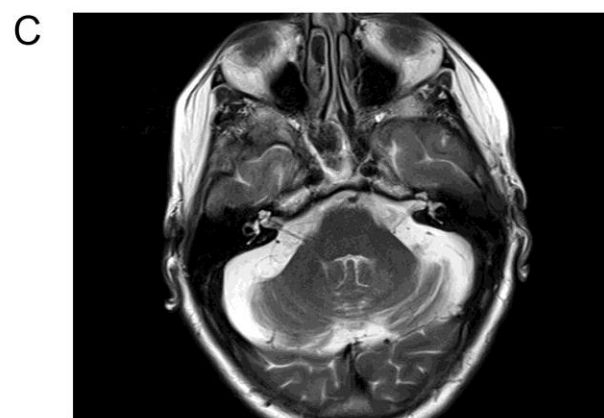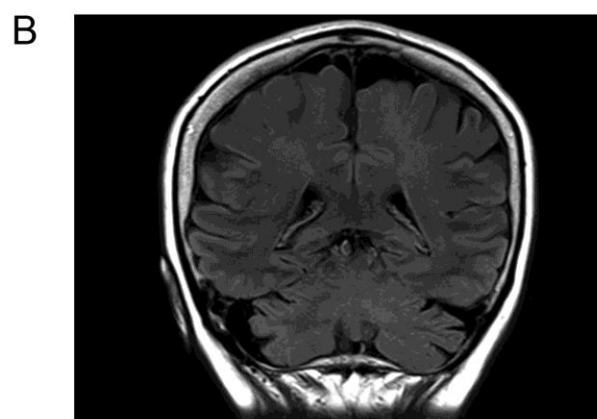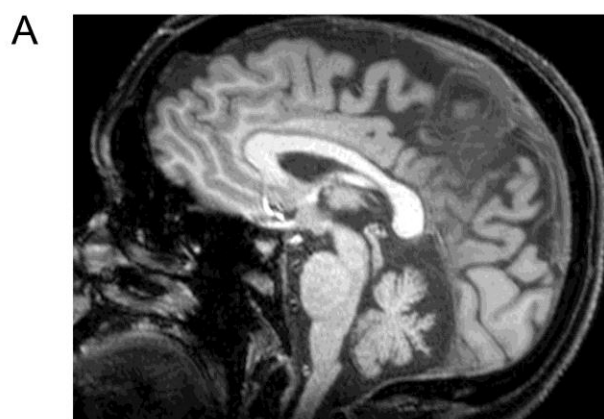
Figure 6 MRI brain scan images of subject III-5

A. T1-weighted sagittal MRI brain scan showed severe cerebellar atrophy, preserved brainstem and cerebral cortex.

B. T2-weighted FLAIR coronal MRI scan showed moderate lateral cerebellar atrophy affecting the upper and lower lobes more on the left.

C. T2-weighted axial MRI scan of the mid cerebellum showed thicken cerebellar fissure.

D. T2-weighted axial MRI scan of the upper cerebellum showed normal brainstem and normal upper cerebellum.

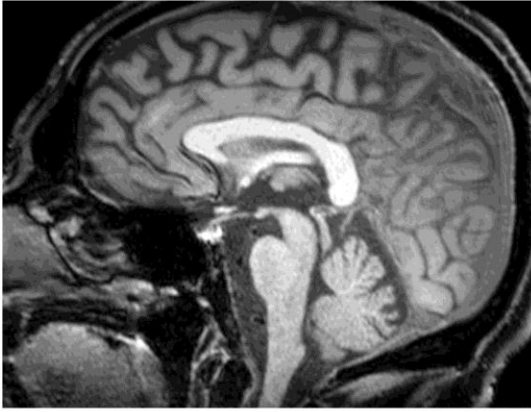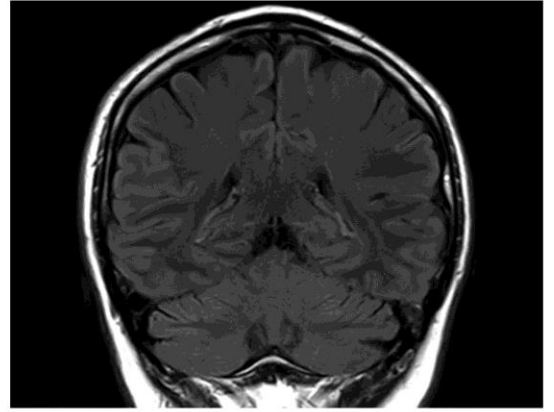# Figure 7 MRI brain scan images of subject III-13

A. T1-weighted sagittal MRI brain scan showed moderate cerebellar atrophy with increased cerebrospinal fluid, preserved brainstem and cerebral cortex.

B. T2-weighted FLAIR coronal MRI scan showed moderate lateral cerebellar atrophy affecting the upper and lower lobes.

C. T2-weighted axial MRI scan of the mid cerebellum showed thicken cerebellar fissure.

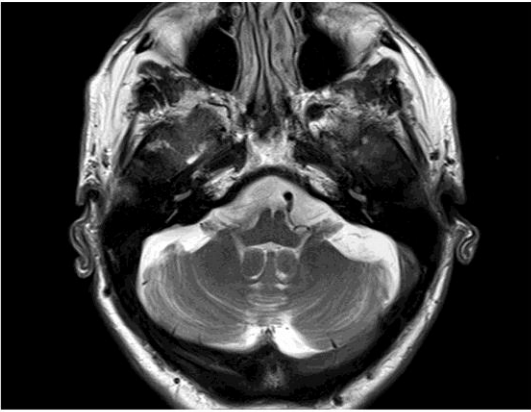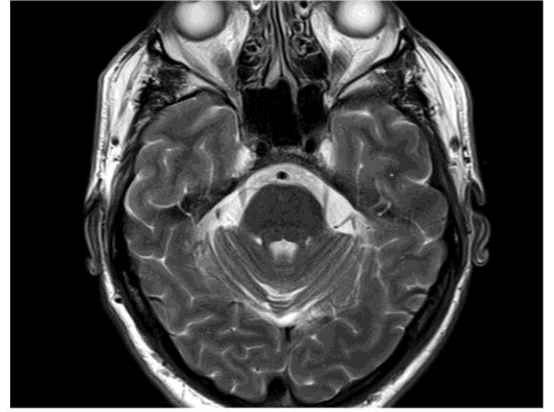D. T2-weighted axial MRI scan of the upper cerebellum showed normal brainstem and normal upper cerebellum

Figure 8 MRI brain scan images of subject III-19

A. T1-weighted sagittal MRI brain scan showed severe cerebellar atrophy with preserved brainstem and cerebral cortex.

B. T2-weighted FLAIR coronal MRI scan showed severe lateral cerebellar atrophy affecting the upper and lower lobes.

C. T2-weighted axial MRI scan of the mid cerebellum showed thicken cerebellar fissure.

D. T2-weighted axial MRI scan of the upper cerebellum showed normal increased cerebrospinal fluid and a normal brainstem.
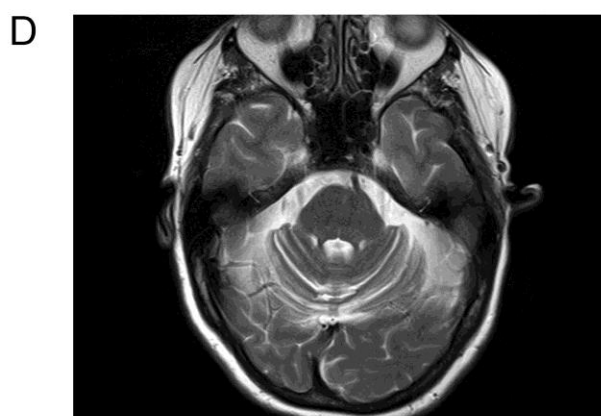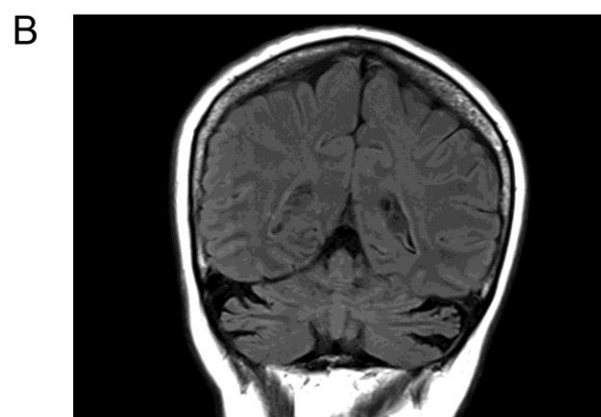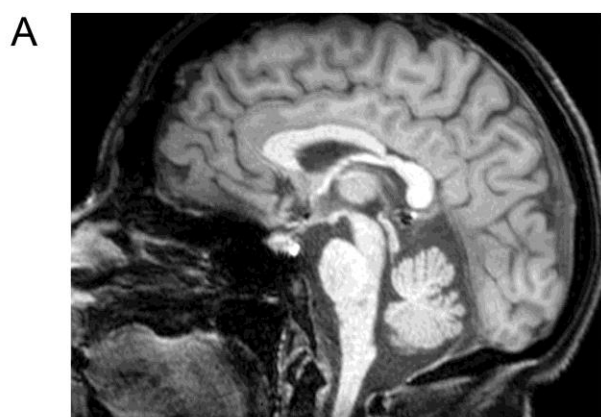
Figure 9 MRI brain scan images of subject III-13 (proband)

A. T1-weighted sagittal MRI brain scan showed severe cerebellar atrophy with preserved brainstem and cerebral cortex.

B. T2-weighted FLAIR coronal MRI scan showed severe lateral cerebellar atrophy affecting the upper and lower lobes.

C. T2-weighted axial MRI scan of the mid cerebellum showed thicken cerebellar fissure.

D. T2-weighted axial MRI scan of the upper cerebellum showed normal increased cerebrospinal fluid and a normal brainstem.

Figure 10 MRI brain scan images of subject IV-7

A. T1-weighted sagittal MRI brain scan showed prominence of cerebellar sulci, normal brainstem and cerebral cortex.

B. T2-weighted FLAIR coronal MRI scan showed prominence of cerebellar sulci with increased cerebrospinal fluid.

C. T2-weighted axial MRI scan of the mid cerebellum showed normal cerebellar fissure.

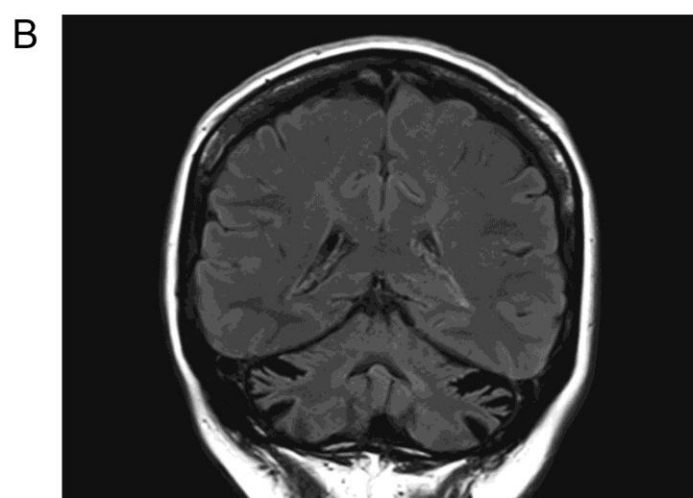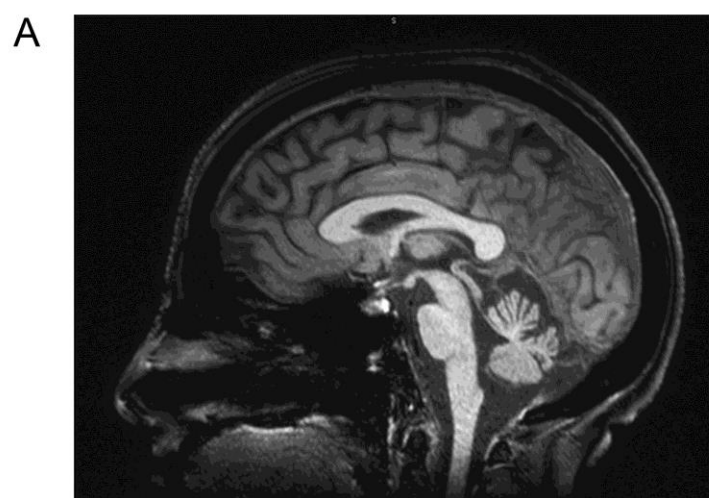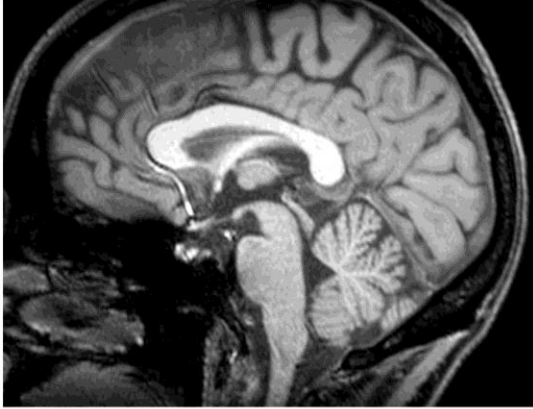D. T2-weighted axial MRI scan of the upper cerebellum showed normal brainstem and upper cerebellum.
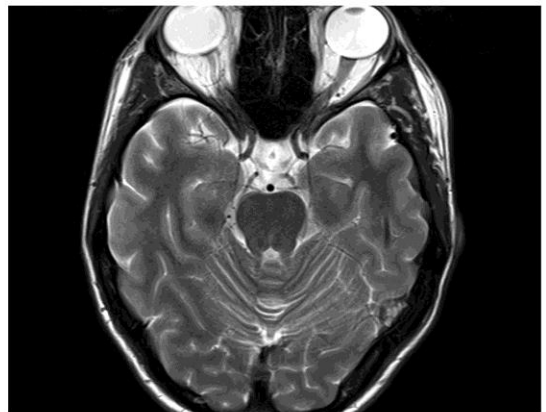
Figure 11 MRI brain scan images of subject IV-20

A. T1-weighted sagittal MRI brain scan showed prominence of cerebellar sulci, normal brainstem and cerebral cortex.

B. T2-weighted FLAIR coronal MRI scan showed prominence of cerebellar sulci with increased cerebrospinal fluid.

C. T2-weighted axial MRI scan of the mid cerebellum showed normal cerebellar fissure.

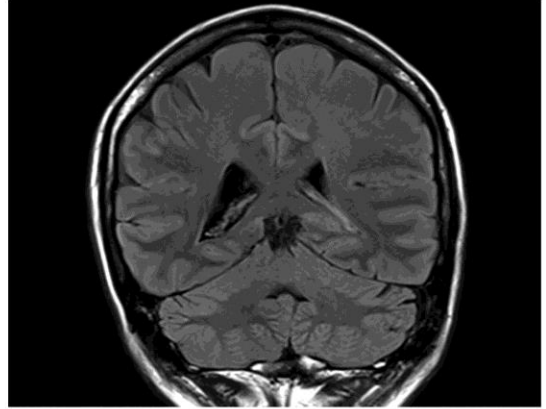D. T2-weighted axial MRI scan of the upper cerebellum showed normal brainstem and upper cerebellum.
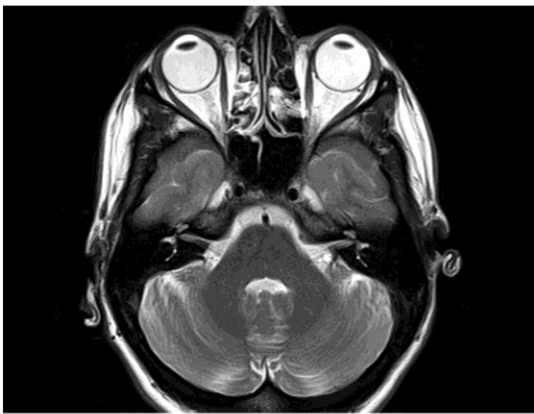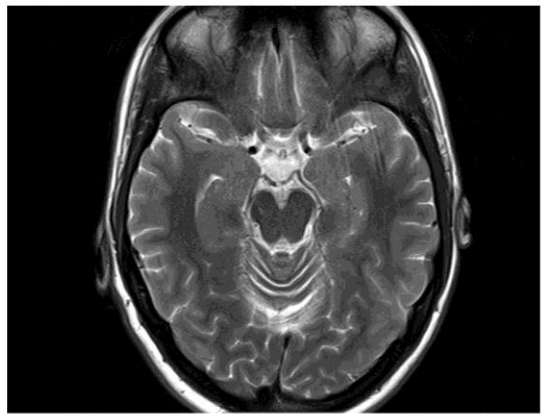
Figure 12 MRI brain scan images of subject IV-8

A. T1-weighted sagittal MRI brain scan showed a normal cerebellum, normal brainstem and cerebral cortex.

B. T2-weighted FLAIR coronal MRI scan showed a normal cerebellum.

C. T2-weighted axial MRI scan of the mid cerebellum showed normal cerebellar fissure.

D. T2-weighted axial MRI scan of the upper cerebellar showed normal upper cerebellar

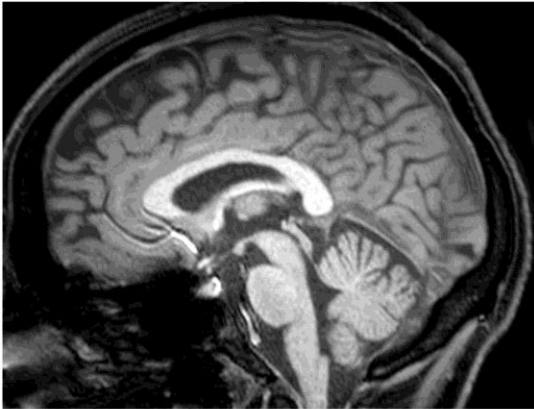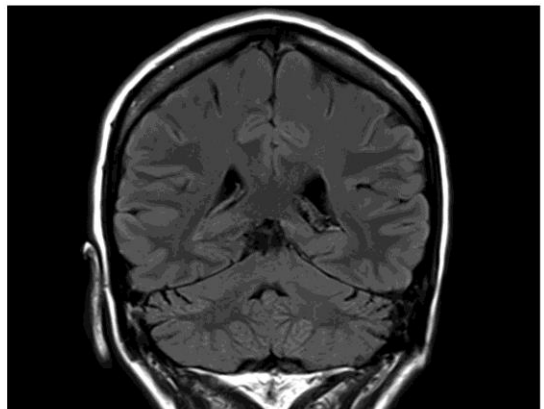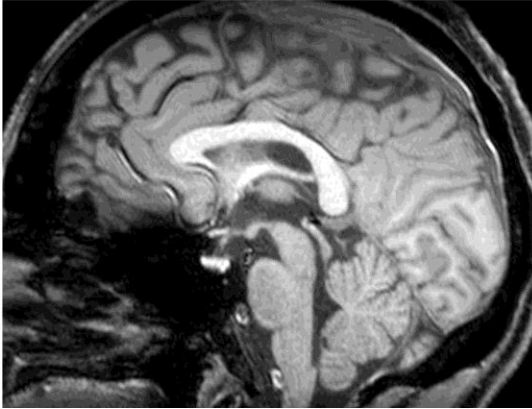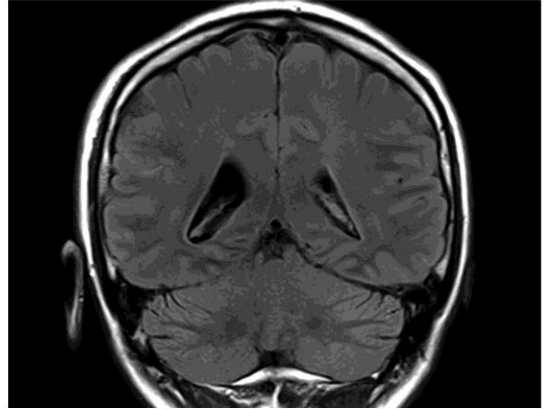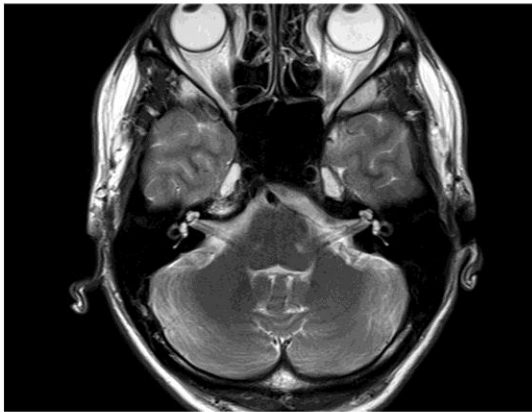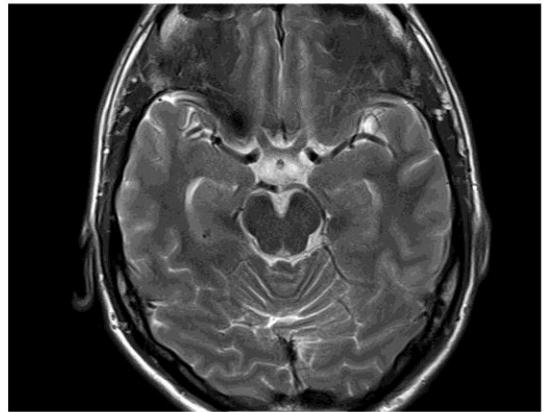After applying all filtering steps for DNA analysis as described above, a mutation of adrenergic receptor, β, kinase-1 (ADRBK1) on chromosome 11 was the only potential variant present in all symptomatic subjects, obligate carriers and some asymptomatic subjects but absent in only one asymptomatic subject IV-8 (Figure 13).

Sanger sequencing was also performed on all available DNA samples for segregation testing and validation. It confirmed that ADRBK1 segregated with the disease in the family. Sanger sequencing also identified the mutation was due to an amino acid change from ATT (Isoleucine) to ATG (Methionine) at amino acid position 140 (Figure 14).

Lastly, ADRBK1 was further verified to exclude polymorphism by comparing with Human Genome Diversity Project (HGDP) database (http://www.hagsc.org/hgdp/).

Figure 13 Fourth filtering step of the DNA analysis

Fourth filtering step of DNA analysis confirmed that the mutated ADRBK1 presented in all symptomatic subjects (III-4, III-5, III-13, III-19and IV-21), obligate carrier (II-6) and some asymptomatic subjects (III-21, IV-7 and IV-20) but it was not present only in one asymptomatic subject (IV-8)

Subjects	Clinical Status	ADRBK1
III-5	Affected	wt/mt
II-6	Obligate carrier	wt/mt
IV-7	Not affected	wt/mt
III-19	Affected	wt/mt
III-21	Not affected	wt/mt
IV-8	Not affected	wt/wt
III-13	Affected	wt/mt
IV-20	Not affected	wt/mt
III-4	Affected	wt/mt
IV-21	Proband	wt/mt

wt= wild type; mt= mutant; ADRBK1= adrenergic receptor, beta, kinase-1

Figure 14 Sanger sequencing of the mutated ADRBK1

A. Sequencing of the proband, II-6 (obligate carrier), III-5 (symptomatic), III-13 (symptomatic), III-19 (symptomatic), III-21 (asymptomatic), IV-20 (asymptomatic)

B. Sequencing of subjects III-4 (symptomatic) IV-7 (asymptomatic)

C. Sequencing of subject IV-8 (asymptomatic)

D. Reference from Human Genome Diversity Project

The Sanger Sequencing confirmed the mutated ADRBK1 gene is due to amino acid change from ATT (Isoleucine) to ATG (Methionine) at amino acid position 140.

A
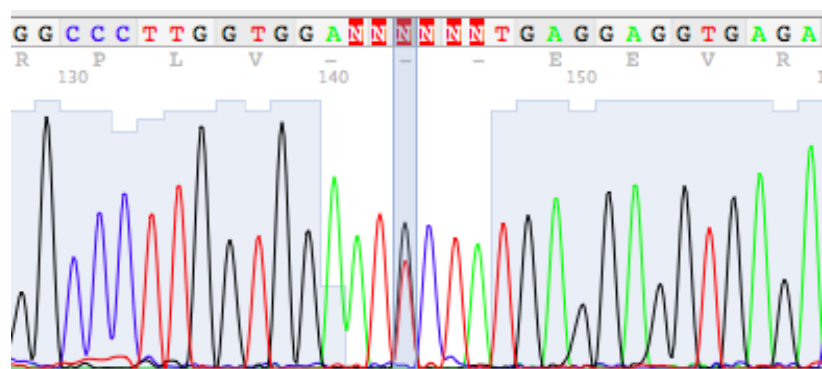
B
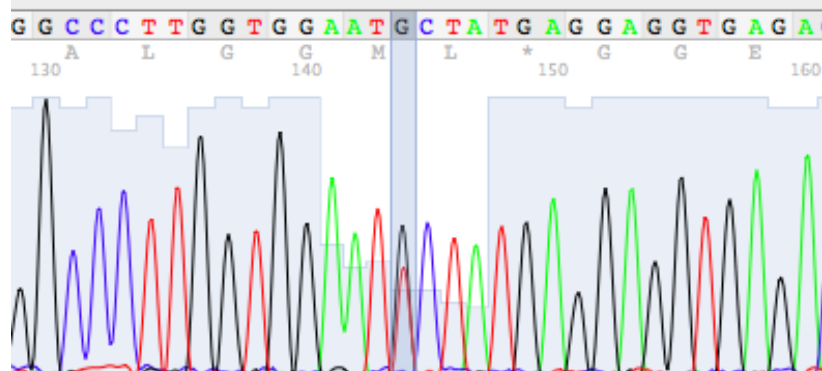
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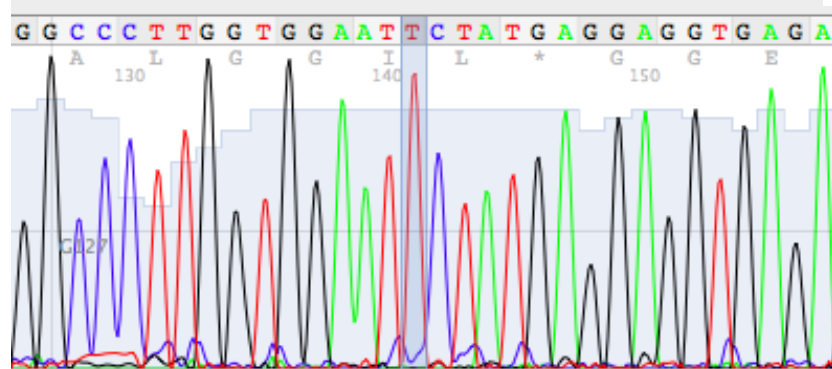
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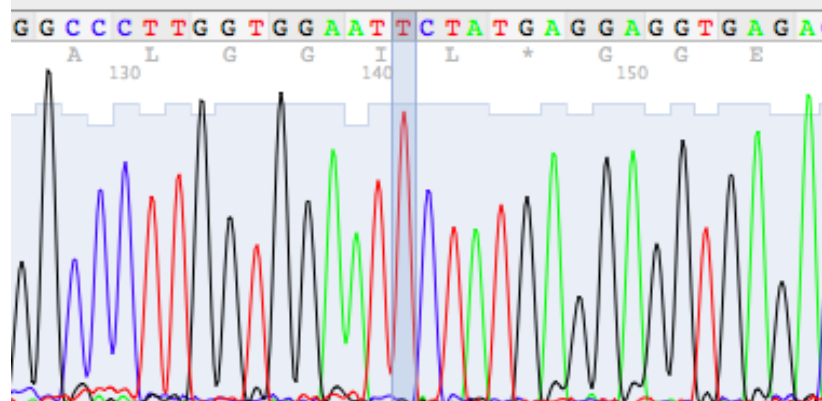
Discussion

This study revealed that mutation of ADRBK1, also as known as G-protein coupled receptor kinase 2 (GRK2), is likely responsible for the autosomal dominant cerebellar ataxia in this family. This gene was not previously described to cause any cerebellar ataxia. Dysregulation of GRK2 is commonly found and plays an important role in chronic cardiac failure (133). Further cardiac investigations including echocardiogram to assess cardiac function may identify subclinical cardiac dysfunction in the studied family. Conversely, investigating those patients with cardiac failure associated with ADRBK1 mutations may identify subtle cerebellar dysfunction clinically or subclinical cerebellar atrophy on MRI scan.

It was assumed that II-6 is a carrier. However, clinically she was asymptomatic. She was in her nineties and had started to use a four wheeled walker for mobilising. It was difficult to establish whether her reduced mobility was secondary to subtle cerebellar dysfunction or other pathology. Furthermore, she was not able to undergo MRI scanning due to logistic difficulties. MRI scan may have identified cerebellar atrophy, which would support the above assumption.

According to the segregation result, three out of four clinically asymptomatic family members (III-20, IV-7 and IV-20) have the mutated ADRBK1 gene and the MRI scan of IV-20 showed prominence of cerebellar sulci, which could represent early cerebellar atrophy. It is likely in these family members, especially subject IV-20, to become symptomatic in the future if the mutated ADRBK1 is the

responsible gene. On the other hand, if subject IV-8 develops cerebellar ataxia, this would not be consistent with an ADRBK1 mutation as she does not possess the mutation. Therefore, long term observation of this family is warranted.

Furthermore, if both II-6 and III-21 had MRI scans confirming cerebellar atrophy, it would support an ADRBK1 mutation causing cerebellar atrophy radiologically, although this may not necessarily symptomatic. The other hypothesis of the above is that this autosomal dominant cerebellar ataxia is possibly incomplete penetrance.

The mutation identified in the ADRBK1 gene in this study is classified as a conventional mutation. Similar to the other conventional mutation SCAs, symptomatic subjects had slow progression of cerebellar dysfunction with preserved life expectancies. Subject II-6 was in her nineties at the time of this study and did not have severe mobility disturbance, which was consistent with a convention mutation SCA.

III-4, III-17, III-19 and proband became symptomatic during their pregnancies. It raises the possibility of pregnancy as a triggering factor. However, II-6 and IV-20 both had three children and but they remained asymptomatic, therefore, pregnancy was not an absolute triggering factor for this unique SCA.

This study discovered a novel ADRBK1 mutation that may cause a unique autosomal dominant cerebellar ataxia. Identifying second kindred with the same

phenotype and genotype or further study of the functional property of this novel

mutation is required to further evaluate this finding.

General Discussion

Spinocerebellar ataxia is a heterogeneous group of dominantly inherited disorders. In recent years, there are several new mutated genes identified. The usual clinical manifestation of the SCA is progressive cerebellar dysfunction, including cerebellar ataxia, dysarthria and nystagmus. Different types of SCA have different clinical presentations including age of onset and co-existing extracerebellar manifestations. The family studied in this project had an unusual phenotype, whereby slowly progressive cerebellar dysfunction was not associated with nystagmus.

ADRBK1 is also known as GRK2. There is recent literature showing that it may also have a significant role in neurological disorders due to its effect on G-protein coupled receptors (GPCR) signalling in neurons and astrocytes (134, 135). GPCR functions as a signal messenger transferring extracellular signals intracellularly via a second messenger at the plasma membrane. There are six different classes of G-protein coupled receptors and Group 1 metabotropic glutamate receptors (mGluR) are considered as class C GPCR. Glutamate is an agonist for mGluR which plays an important role in glutamate homeostasis. Dysregluation of mGluR an important trigger for neuro-inflammation and toxicity. When tissue injury occurs, glutamate is released and activates N-methyl-D-aspartate receptors, which leads to an influx of calcium from the extracellular space into the cytoplasm. The mGluR is also activated by glutamate, which leads to release of calcium from endogenous stores by activation of phospholipase Cb1 (PLCb1). GRK2 provides negative feedback by desensitization of the mGluR, limiting the

release of calcium from its endogenous stores. Therefore, dysregulated or dysfunctional GRK2 would lead to excessive calcium release from endogenous stores, resulting in cell death(134, 135). In the mouse model, overexpression of GRK2 has a neuroprotective effect. Conversely, neurons in mice with GRK2 deficiency are more susceptible to injury (134).

Despite recent evidence that dysregulation of GRK2 is associated with neuronal death, there is no literature to date linking mutations in ADRBK1 with any neurological disorders in humans. Therefore, a functional study of the mutated ADRBK1 gene to further evaluate its relationship with cerebellar ataxia is indicated.

Conclusion

A novel mutation in ADRBK1 was found in this family, who present with a unique phenotype of autosomal dominant cerebellar ataxia. Recent studies have identified that GRK2 is neuroprotective and has an important role in controlling intracellular glutamate and calcium homeostasis. Functional studies of ADRBK1 to establish its role in spinocerebellar ataxia and further follow-up of this family are recommended.

Appendix 1

Information sheet and consent form

MACQUARIE UNIVERSITY

Australian School of Advanced Medicine
MACQUARIE UNIVERSITY NSW 2109 AUSTRALIA

Phone +61 (0)2 9812 3500
Fax   +61 (0)2 9812 3600

Investigator: Ronald Siu

Supervisor: Professor Dominic Rowe
                 Professor of Neurology

Information and Consent Form

Genotype and Phenotype Assessment of a Kindred with a Novel Autosomal Dominant Cerebellar Ataxia (Genetic assessment of ADCA)

You are invited to participate in a study of identifying a new gene in autosomal dominant cerebellar ataxia. Autosomal dominant means that a person only needs to get one abnormal gene from one of his/her parent in order to have the disease. There are a number of genes that have been identified in autosomal dominant cerebellar ataxia and patients present with different clinical picture (phenotype) with different gene (genotype). There is a new, unique phenotype that has been identified in your family, therefore the purpose of this study is to investigate if a new gene be involved in this new and unique clinical presentation of autosomal dominant cerebellar ataxia. If a new gene is identified, this raises a possibility of treatment to slow the progress of the disease in the future.

The study is being conducted by Ronald Siu (Telephone 02 9812 3720, email ronald.siu@students.mq.edu.au) of the Australian School of Advanced Medicine, Macquarie University, to meet the requirement of Master of Philosophy under the supervision of Professor Dominic Rowe (Telephone 02 9812 3720, email dominic.rowe@mq.edu.au) of the Australian School of Advanced Medicine, Macquarie University.

If you decide to participate, you will be interviewed by Ronald Siu who will obtain a medical history, perform a clinical examination followed by a blood collection. Videorecording will be carried out during the clinical examination. A magnetic resonance imaging (MRI), and a diffuse tensor imaging (DTI) of the brain will be performed at Macquarie Medical Imaging to look for any abnormality. You are required to lie flat for about 30 minutes during the MRI scan and you will need to fill out a standardised questionnaire for the MRI safety. There are minor risks from blood collection including bleeding and bruising. You will be observed by Ronald Siu or Professor Dominic Rowe, who are registered medical practitioners, for at least 15 minutes after the blood collection to ensure the bleeding stops. If bleeding persists, we will refer you to appropriate medical attention. Your blood sample will be processed and your

DNA sent to the National Institute of Health in Maryland, USA for further DNA testing. You are not required to pay any fee for any of the investigations involved in this study.

Any information or personal details gathered in the course of the study are confidential (*except as required by law*). No individual will be identified in any publication of the results. You will be allocated with an individual number/initial. Only your allocated number/initial will be kept together with the data. Only the researchers have access to the date. Either Ronald Siu or Professor Dominic Rowe will contact you once the summary of result is available and further support or medical referral will be provided if necessary. Only the DNA result that is related to ADCA will be disclosed to you. If a new gene is identified, the result and data will be used in publication and no individual participant will be identified.

Participation in this study is entirely voluntary: you are not obliged to participate and if you decide to participate, you are free to withdraw at any time without having to give a reason and without consequence.

If you do not wish to participant in this study, please tick the DO NOT WISH TO PARTICIPATE box below and return either by email to ronald.siu@students.mq.edu.au or by post with the enclosed envelope. If you do not response within the next 4 weeks, you may receive a follow up phone call from one of the researchers to clarify if you wish to participate. Except the investigator, no one will know you refuse to be involved in this study.

I do not wish to participate in this study

☐

I, _____ have read *(or, where appropriate, have had read to me)* and understand the information above and any questions I have asked have been answered to my satisfaction. I agree to participate in this research, knowing that I can withdraw from further participation in the research at any time without consequence. I have been given a copy of this form to keep.

Participant's Name:_____
  (Block letters)

Participant's Signature: _____ Date:_____

Investigator's Name:_____
  (Block letters)

Investigator's Signature: _____ ___ Date:_____

The ethical aspects of this study have been approved by the Macquarie University Human Research Ethics Committee. If you have any complaints or reservations about any ethical aspect of your participation in this research, you may contact the Committee through the Director, Research Ethics (telephone (02) 9850 7854; email ethics@mq.edu.au). Any complaint you make will be treated in confidence and investigated, and you will be informed of the outcome.

(INVESTIGATOR'S [OR PARTICIPANT'S] COPY)

Appendix 2

Ethics Approval

From: Ethics Secretariat <ethics.secretariat@mq.edu.au>
To: Professor Dominic Rowe <dominic.rowe@mq.edu.au>
Cc: ronald.siu@students.mq.edu.au
Subject: Approved- Ethics application- Rowe (Ref: 5201200039)
Date: 28 March 2012 13:01:54 NZDT

Dear Professor Rowe

Re: "Genotype and phenotype assessment of a kindred with a novel autosomal
dominant cerebellar ataxia" ¬†(Ethics Ref: 5201200039)

Thank you for your recent correspondence. Your response has addressed the
issues raised by the Human Research Ethics Committee and you may now
commence your research.

The following personnel are authorised to conduct this research:

Chief Investigator- Professor Dominic Rowe
Co-Investigators- Prof Carolyn M. Sue, Dr Bryan Traynor, Dr Carolyn Orr, Dr
Ronald Chong Hing Siu & Prof Mark Connor

NB. ¬†STUDENTS: ¬†IT IS YOUR RESPONSIBILITY TO KEEP A COPY OF THIS APPROVAL
EMAIL TO SUBMIT WITH YOUR THESIS.

Please note the following standard requirements of approval:

1.   The approval of this project is conditional upon your continuing
compliance with the National Statement on Ethical Conduct in Human Research
(2007).

2.   Approval will be for a period of five (5) years subject to the provision
of annual reports. Your first progress report is due on 28 March 2013.

If you complete the work earlier than you had planned you must submit a
Final Report as soon as the work is completed. If the project has been
discontinued or not commenced for any reason, you are also required to
submit a Final Report for the project.

Progress reports and Final Reports are available at the following website:

http://www.research.mq.edu.au/for/researchers/how_to_obtain_ethic
s_approval/
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3.   If the project has run for more than five (5) years you
cannot renew
approval for the project. You will need to complete and submit a
Final
Report and submit a new application for the project. (The five
year limit
on renewal of approvals allows the Committee to fully re-review
research in
an environment where legislation, guidelines and requirements are
continually changing, for example, new child protection and
privacy laws).

4.   All amendments to the project must be reviewed and approved
by the
Committee before implementation. Please complete and submit a
Request for
Amendment Form available at the following website:

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5.   Please notify the Committee immediately in the event of any
adverse
effects on participants or of any unforeseen events that affect
the
continued ethical acceptability of the project.

6.   At all times you are responsible for the ethical conduct of
your
research in accordance with the guidelines established by the
University.
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Yours sincerely
Dr Karolyn White
Director of Research Ethics
Chair, Human Research Ethics Committee

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