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ORIGINAL PAPER

High Degree of Genetic Heterogeneity for Hereditary Cerebellar Ataxias in Australia

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Abstract

Genetic testing strategies such as next-generation sequencing (NGS) panels and whole genome sequencing (WGS) can be applied to the hereditary cerebellar ataxias (HCAs), but their exact role in the diagnostic pathway is unclear. We aim to determine the yield from genetic testing strategies and the genetic and phenotypic spectrum of HCA in Australia by analysing real-world data. We performed a retrospective review on 87 HCA cases referred to the Neurogenetics Clinic at the Royal North Shore Hospital, Sydney, Australia. Probands underwent triplet repeat expansion testing; those that tested negative had NGS-targeted panels and WGS testing when available. In our sample, 58.6% were male (51/87), with an average age at onset of 37.1 years. Individuals with sequencing variants had a prolonged duration of illness compared to those with a triplet repeat expansion. The detection rate in probands for routine repeat expansion panels was 13.8% (11/80). NGS-targeted panels yielded a further 11 individuals (11/32, 34.4%), with WGS yielding 1 more diagnosis (1/3, 33.3%). NGS panels and WGS improved the overall diagnostic rate to 28.8% (23/80) in 14 known HCA loci. The genetic findings included novel variants in *ANO10*, *CACNA1A*, *PRKCG* and *SPG7*. Our findings highlight the genetic heterogeneity of HCAs and support the use of NGS approaches for individuals who were negative on repeat expansion testing. In comparison to repeat disorders, individuals with sequencing variants may have a prolonged duration of illness, consistent with slower progression of disease.

Keywords Hereditary cerebellar ataxia · Spinocerebellar ataxia · Genetics · Triplet repeat expansion · Next-generation sequencing · Whole genome sequencing

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Introduction

Hereditary cerebellar ataxias (HCAs) are a group of neurodegenerative disorders that predominantly result in the progressive worsening of cerebellar function. Phenotypic manifestations are inconsistent across the group, but most cases reveal wide-base uncoordinated gait, dysarthria and limb dysmetria [1]. Other disease manifestations may also include pyramidal signs, cognitive dysfunction and peripheral neuropathy [2]. A clinical indication of HCA can be clarified by a genetic diagnosis. HCAs can have autosomal dominant (known as spinocerebellar ataxia, or SCA), autosomal recessive, X-linked or mitochondrial modes of inheritance [3].

A previous study that screened for HCA triplet repeat disorders [SCAs 1, 2, 3, 6, 7, and dentatorubral-pallidoluysian atrophy (DRPLA)] in Australians found that SCA types 1 and 6 were the most common dominant variants in those of Anglo-Celtic origin (the predominant ethnicity in the Australian population) [4]. Friedreich's ataxia (FRDA) is reported to be the commonest autosomal-recessive HCA loci in Caucasians worldwide [5]. As SCA types 1, 2, 3, 6, and 7 and FRDA are amongst the most common types of HCA [6], a repeat expansion testing panel of these loci is currently available in Australia as part of routine clinical diagnostic investigations.

HCA demonstrates genetic heterogeneity, with more than 100 genes and loci known to be associated with the variable clinical phenotypes (Supp Table 1) [3]. As more causative genes are discovered, newer sequencing approaches, such as next-generation sequencing (NGS) panels, whole exome sequencing (WES) and whole genome sequencing (WGS), are being used to improve the diagnostic yield of rarer HCA variants. For example, one study used NGS panels to capture 58 known human ataxia genes and found an overall diagnostic rate of 18% [7]. Another study found a much higher diagnostic rate of 64% in undiagnosed hereditary and sporadic ataxias using exome sequencing [8]. A recent study identified relevant genetic variations in up to 15% of autosomal dominant cases after exclusion of repeat expansion disorders [9]. A further study found a very probable or definite diagnosis for 22.6% using exome-targeted capture [10].

Current recommendations are to first screen for triplet repeat expansions in cases of chronic adult-onset ataxia with a dominant family history [11]. However, there are no clear guidelines on how to integrate NGS into the diagnostic pipeline for HCAs. In this study, we performed a retrospective review of clinical notes and genetic investigations. We aim to determine the yield from genetic testing strategies and the genetic and phenotypic spectrum of HCA in Australia using real-world data.

Materials and Methods

The study was approved by the Northern Sydney Local Health District Human Research Ethics Committee (HREC/15/HAWKE/434), and all participants provided written informed consent. Individuals who underwent WGS had pre-test and post-test genetic counselling with a genetic counsellor regarding the impact and consequences of the result. Patients were referred to the Neurogenetics Clinic at Royal North Shore Hospital (RNSH) for tertiary consultative care by general practice physicians or neurologists.

All data is stored on clinically secure databases at RNSH and at respective genetic testing facilities. WGS data is securely stored and can be accessed in the future if required.

Retrospective Case Note Review

We performed a retrospective clinical case note review of individuals with suspected HCA referred to the Neurogenetics Clinic at Royal North Shore Hospital (RNSH), Sydney, Australia, over a 15-year period from 2002 to 2017. The Neurogenetics Clinic primarily services the state of New South Wales but also sees patients from other Australian states. All medical records from the Neurogenetics Clinic were searched. Cases were included based on the following criteria: (i) the individual was clinically diagnosed with HCA according to published criteria [3]; (ii) acquired causes of hereditary ataxia had been excluded following appropriate investigations [12]; (iii) individuals had undergone testing by at least one modality of genetic analysis—that is, triplet repeat expansion testing, NGS-targeted panel or WGS. To note, some individuals had further genetic testing via Centogene (Rostock, Germany), which includes a NGS-targeted panel along with a more comprehensive repeat expansion testing panel (including SCA8, SCA31, SCA36 and DRPLA)(Fig. 1). The American College of Medical Genetics and Genomics (ACMG) Class 4 (likely pathogenic) and Class 5 (pathogenic) variants were considered as relevant to the genetic diagnosis [13].

Investigators (C.K. and K.R.K.) reviewed case files to ensure that the clinical signs recorded were consistent with the diagnosis of HCA according to published criteria [2, 3, 11].

Routine Triplet Repeat Expansion Testing

Routine triplet repeat testing for SCA1, 2, 3, 6, 7, 12 and 17 and Friedreich's ataxia (FRDA) was performed at Sydney South West Pathology Service (Concord Repatriation General Hospital) using PCR and fragment analysis. FRDA was analysed via long range PCR, fragment analysis, repeat primed PCR and sequencing analysis of the coding region of the *FXN* gene.

NGS-Targeted Panels

The spinocerebellar ataxia panels were performed at Centogene, Germany and Oxford Molecular Genetics Laboratory, United Kingdom (see Supp. Table 1 for genes

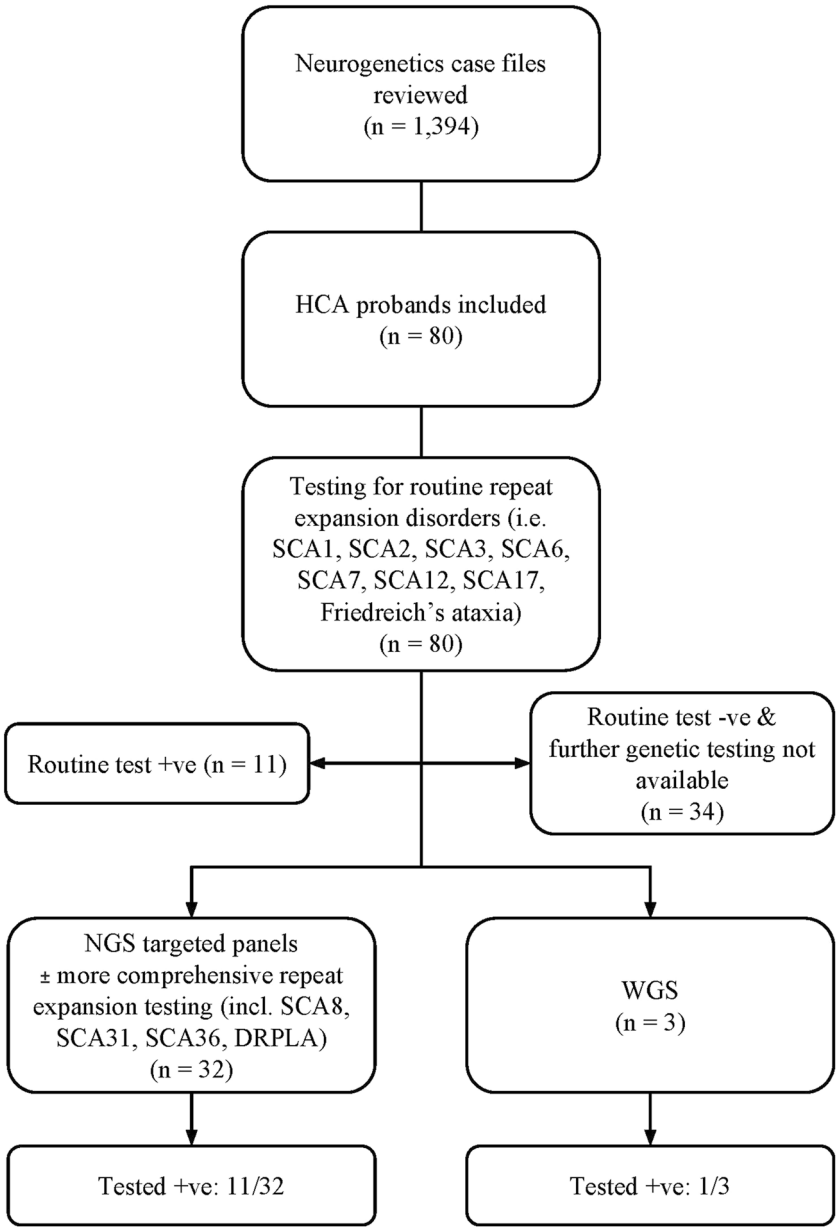

Fig. 1 Flowchart representing retrospective review of HCA cases: *HCA* hereditary cerebellar ataxia, *SCA* spinocerebellar ataxia, *+ve* positive, *-ve* negative, *NGS* next-generation sequencing, *DRPLA* dentatorubral-pallidoluysian atrophy, *WGS* whole genome sequencing

tested). For the tests performed at Centogene, sequencing variants were detected using PCR and next-generation sequencing of both DNA strands along exons and exon-intron splice junctions. PCR based amplicon library capture was utilised. Centogene also offered more comprehensive repeat expansion testing (SCA1, 2, 3, 6, 7, 8, 10, 12, 17, 31, 36 and DRPLA). Triplet repeat expansion variants were analysed via PCR and capillary electrophoresis. Repeat primed assay was performed on genes with large expansion potential.

For the test performed at Oxford Molecular Genetics Laboratory, a custom design HaploPlex Target Enrichment from Agilent Techologies was used. Sequencing was performed on the Illumina MiSeq and data was analysed on a validated in-house pipeline.

WGS and Filtering

Patient DNA samples were sequenced on Illumina HiSeq X sequencers located at the Kinghorn Centre for Clinical Genomics (KCCG, Sydney, Australia). Sequencing libraries were prepared using TruSeq Nano DNA Library Preparation kit with extracted patient DNA. Sequencing analysis followed a BWA/GATK best practices pipeline [14], as previously described [15].

Filtering of variants was performed using the in-house Seave platform [16]. Variants were filtered according to the pattern of inheritance in the patient's family, the mode of inheritance and disease frequency of a given variant. If phenotype-specific analyses were needed, disease gene

specific filtering from Orphanet and OMIM was applied. From this, variants with evidence for causing or contributing to disease phenotype were reported.

It should be noted that we did not search for novel genes, and patients were not tested for copy number variants or structural variants, as these services were not clinically approved at the time.

Statistical Analysis

Statistical analysis was performed with IBM SPSS Statistics (Version 24, 2016) software. Individuals with triplet repeat expansion and sequencing variants were compared. Mean age at examination, age at onset, disease duration and scores on the Scale for the Assessment and Rating of Ataxia (SARA) were compared using independent Student's *t*-test. Gender, family history, pyramidal signs and cerebellar atrophy on MRI variables were compared using Fisher's exact test. We used a *P* value less than 0.05 for the level of significance.

Results

In this study, we considered 80 probands, with 7 additionally referred family members. Overall, Australia's population is predominantly from an Anglo-Celtic ancestry, with those of English, Australian, Scottish or Irish heritage accounting for 89.9%, followed by Chinese (5.6%) and Italian ancestry (4.6%) [17]. Recording of patient ethnicities was not done routinely in our Neurogenetic Clinic reviews. Nevertheless, assuming ancestries from the surnames of the probands, 71/80 (88.8%) were of Anglo-Celtic ancestry, 3/80 (3.8%) of Italian ancestry, and another 6 probands from other ancestries. We would conclude that our cohort was representative of the Australian population.

There was a predominance of males in our sample (M/F: 51/36), 96.6% of individuals had an ataxic gait, and 41.4% of individuals had an appreciable degree of cerebellar atrophy on magnetic resonance imaging (MRI) according to the reporting radiologist. Individuals with sequencing variants had a longer disease duration to date (from onset to 2017; mean: 27.3 ± 16.2 years) compared to individuals with triplet repeat expansions (mean: 14.1 ± 12.9 years, $p = 0.041$). This is also the case when comparing duration from disease onset to genetic diagnosis (sequencing variant mean: 25.2 ± 17.1 years; triplet repeat expansion mean: 10.1 ± 12.9 years, $p = 0.030$) (Table 1). In terms of severity as measured by SARA scores, there was no significant difference in individuals with triplet repeat disorders in comparison to those with sequencing variants ($p = 0.698$).

Probands had been tested for triplet repeat expansions. SCA1, 2, 3, 6, 7 and FRDA were part of the most routinely offered panel. According to availability, 35 individuals who tested negative to triplet repeat expansion went on for NGS targeted panels ($n = 32$) or WGS (n = 3).

From a total of 80 probands, 23 (28.8%) had pathogenic or likely pathogenic variants (Table 2), 13 (16.3%) probands had variants of uncertain significance (Supp Table 2), and 44 (55.0%) had no disease-relevant variants found. There were 3 cases of SCA6 (13.0%). SCA2, 3, 7, 8, 17, autosomal recessive spinocerebellar ataxia type 10 (SCAR10), and Friedreich's ataxia each had 2 cases (2/23, 8.7%). SCA14, 22, ataxia/familial hemiplegic migraine (FHM), autosomal dominant cerebellar ataxia with deafness and narcolepsy (ADCADN), Niemann-Pick disease type C, and spastic paraplegia 7 (SPG7) each had 1 case (1/23, 4.3%). See Table 3 and Supp Table 3 for clinical manifestations of all 80 probands and 7 referred family members. Overall, the diagnostic rate for routine repeat expansion panels was 11/80 (13.8%), with NGS targeted panels yielding 11/32 (34.4%) and WGS 1/3 (33.3%).

Triplet Repeat Expansions

Of the 14 probands with triplet repeat disorder: all (100%) had cerebellar ataxia, 7 (50.0%) had pyramidal signs, 10 (71.4%) had dysarthria, 10 had nystagmus (horizontal and/or vertical) and 8 (57.1%) had limb ataxia (upper or lower limb dysmetria and/or dysdiadochokinesis). Eleven cases were identified via routine triplet repeat expansion panels, 3 via the more comprehensive repeat expansion panel.

Several cases had distinctive clinical features, including severe dementia in a proband with SCA17, and retinal dystrophy in individuals with SCA7. We also identified two probands with SCA8, detected using the more comprehensive repeat expansion panel. For pt 32, the SCA8 expansion segregated with disease in two brothers (Table 2)a.

Of note, a *BEAN1* expansion was identified in one proband of Caucasian background (pt 1), raising the possibility of SCA31. Sequencing part of the expanded region revealed at least 100 pure TACAA repeats that could be reflective of the prevalent expansions reported within the Caucasian population. It has been shown that the known Caucasian motif expansion (TACAA)n, (GAAAA)n does not segregate with the disease [25, 26], and so we concluded that the *BEAN1* expansion found in pt 1 was not pathogenic.

Sequencing Variants on NGS-Targeted Panels

For the eight probands who had sequencing variants detected using NGS targeted panels, all had cerebellar ataxia, five had pyramidal signs, and three had nystagmus.

Of interest, we detected a novel variant in *CACNA1A* [NM_023035.2: c.4415C>T; p.(Ser1472Leu)] in man with progressive ataxia (pt 58), which segregates with disease in the family (pt 75) (Tables 2b and 3).

A proband who presented with a progressive cerebellar disturbance had a sister with a complicated hereditary spastic

Table 1 Overall demographics and clinical manifestations of individuals with suspected HCA

Variable	All patients	Triplet repeat +ve	Sequencing variant +ve	*p* value[a]
Total/probands	87/80	16/14	11/9	
Gender (M/F)	51/36	10/6	7/4	0.517
Age at onset (years)[b]	37.1 ± 18.9 (2–75)	35.5 ± 16.5 (8–61)	31.1 ± 15.8 (8–55)	0.533
Duration: ongoing (years)[b,c]	20.3 ± 15.7 (4–65)	14.1 ± 12.9 (4–55)	27.3 ± 16.2 (4–54)	0.041
Duration: from onset to initial presentation	15.0 ± 16.0 (0[d]–51)	10.1 ± 12.9 (0–51)	22.7 ± 17.1 (1–51)	0.059
Duration: from onset to diagnosis	16.2 ± 16.7 (1–53)	10.5 ± 13.4 (1–53)	25.2 ± 16.9 (1–53)	0.030
Duration: from initial presentation to diagnosis[e]	1.8 ± 3.4 (0–13)	0.9 ± 0.8 (0–2)	2.6 ± 4.7 (0–13)	0.249
Family history (%)	41 (47.1)	8 (50.0)	7 (63.6)	0.696
Gait ataxia (%)	84 (96.6)	16 (100.0)	11 (100.0)	1.000
Pyramidal signs (%)	42 (48.3)	7 (43.8)	6 (54.5)	0.704
Cerebellar atrophy on MRI (%)	36 (41.4)	8 (50.0)	5 (45.5)	1.000

+*ve*: positive

[a] Analysis comparing triplet repeat positive patients and sequencing variant positive patients

[b] Mean ± SD (range)

[c] Ongoing duration means disease duration from onset to 2017

[d] 0 years. duration from initial presentation to diagnosis indicates that initial presentation to Neurogenetics Clinic and genetic diagnosis occurred in same year

[e] 4 triplet repeat expansion positive patients are removed from this analysis as they are tested from outside RNSH's Neurogenetics Clinic

paraplegia (HSP)/spastic-ataxia phenotype. The proband and his sister were found to have biallelic variants in the *SPG7* gene, including a known mutation [NM_003119.3: c.1529C>T; p.(Ala510Val)] and a novel variant [NM_003119.3: c.1745G>A; p.(Gly582Asp)], consistent with the diagnosis of SPG7.

We also detected a novel homozygous variant in *ANO10* [NM_018075.3: c.1A>T; p.(0?)] in a man with a spastic-ataxia phenotype. Software analyses [27] suggest that the next in-frame putative initiation codon would result in deletion of 120 amino acids, and other putative initiation codons would result in alternate reading frames and complete loss of normal protein product. Therefore, this variant is likely to be pathogenic, and overall highly suggestive of a diagnosis of SCAR10.

A woman with a slowly progressive cerebellar disturbance was found to have novel compound heterozygous variants in *ANO10* [a known frameshift variant NM_018075.3: c.132dup; p.(Asp45fs) and a novel canonical splice variant NM_018075.3: c.1219-1G>T].

Pathogenic/Likely Pathogenic Variants on WGS

In a man (Pt 66) with progressive cerebellar disturbance and a family history consistent with autosomal dominant inheritance (Fig. 2), WGS was used to detect a heterozygous missense variant in *PRKCG* [NM_002739.3: c.448T>C; p.(Cys150Arg)], the gene causatively associated with SCA14 [28]. Sanger sequencing of the proband's father (II:1) confirmed the same variant in *PRKCG*. Although the variant is not a known pathogenic allele, another allele of this codon [NM_002739.3: c.449_450delGCinsTT; p.(Cys150Phe)] has been reported in an Australian family [29].

From the three individuals undergoing WGS, there were no incidental finding reported.

Sequencing Variants Detected from Phenotype-Directed Genetic Testing

In two cases, genetic testing was guided by the clinical phenotype and investigations using a phenotype-directed genetic testing approach. In a woman (Pt 80) with cerebellar ataxia, the presence of additional features of vertical supranuclear gaze palsy and cognitive impairment prompted testing for Niemann-Pick Type C. The biomarker lyso-sphingomyelin 509 was elevated (1.6 units, reference range < 0.5), and sequencing of *NPC1* revealed two previously reported mutations [22, 23] (Table 2b).

The distinctive phenotype of cerebellar ataxia, narcolepsy and deafness in a woman with a family history consistent with autosomal dominant inheritance prompted genetic testing of the *DNMT1* gene. This revealed a previously reported mutation NM_001130823.2: c.1709C>T; p.(Ala570Val) [20] in keeping with the diagnosis of autosomal dominant cerebellar ataxia with deafness and narcolepsy (ADCADN).

Discussion

From our multi-tiered diagnostic approach, we have demonstrated a high degree of genetic heterogeneity in our HCA Australian cohort, with 14 genetic loci identified. We report

Table 2 Genetic variants identified in probands with pathogenic or likely pathogenic variants

(a) Triplet repeat expansions

Pt	Gene	Mode	Diagnosis	Repeats reported	Affected range	Reference transcript
6	*ATXN2*	AD	SCA2	38	≥33	NM_002973.3
10	*ATXN2*	AD	SCA2	39	≥33	NM_002973.3
25	*ATXN3*	AD	SCA3	73	52–86	NM_004993
40	*ATXN3*	AD	SCA3	65	52–86	NM_004993
71	*ATNX7*	AD	SCA7	49	38–130	NM_000333
77	*ATNX7*	AD	SCA7	45	38–130	NM_000333
15	*ATXN8OS*	AD	SCA8	80	≥80	NG_016173
32	*ATXN8OS*	AD	SCA8	86	≥80	NG_016173
5	*CACNA1A*	AD	SCA6	22	≥20	NM_023035.2
42	*CACNA1A*	AD	SCA6	22	≥20	NM_023035.2
4	*FXN*	AR	FRDA	800	66–1700	NM_000144
51	*FXN*	AR	FRDA	1000	66–1700	NM_000144
22	*TBP*	AD	SCA17	49	≥49	NM_003194
39	*TBP*	AD	SCA17	47	≥47	NM_003194

(b) Pathogenic/likely pathogenic sequencing variants

Pt	Gene	Mode	Diagnosis	Coding change	Protein change	Reference	Previously reported
86	*ANO10*	AR	SCA10	c.132dup	p.(Asp45fs)	NM_018075.4	Yes [18]
87	*ANO10*	AR	SCAR10	c.1A>T	p.(0?)	NM_018075.3	Novel
30	*CACNA1A*	AD	Ataxia/FHM1	c.1748G>A	p.(Arg583Gln)	NM_023035.2	Yes [19]
58	*CACNA1A*	AD	SCA6	c.4415C>T	p.(Ser1472Leu)	NM_023035.2	Novel (Segregates with disease)
81	*DNMT1*	AD	ADCADN	c.1709C>T	p.(Ala570Val)	NM_001130823.1	Yes [20]
63	*KCND3*	AD	SCA22	c.1034G>T	p.(Gly345Val)	NM_004980.4	Yes [21]
80	*NPC1*	AR	NPC	c.3289G>A	p.(Asp1097Asn)	NM_002310.5	Yes [22]
	NPC1	AR	NPC	c.3263A>G	p.(Tyr1088Cys)	NM_000271.4	Yes [23]
66	*PRKCG*	AD	SCA14	c.448T>C	p.(Cys150Arg)	NM_002739.3	Novel
79	*SPG7*	AR	SPG7	c.1529C>T	p.(Ala510Val)	NM_003119.3	Yes [24]
	SPG7	AR	SPG7	c.1745G>A	p.(Gly582Asp)	NM_003119.3	Novel (Segregates with disease, *in trans* with known pathogenic variant)

Pt patient, *SCA* spinocerebellar ataxia, *FRDA* Friedreich's ataxia, *FHM* Familial hemiplegic migraine, *SPG* Spastic paraplegia, *ADCADN* autosomal dominant cerebellar ataxia with deafness and narcolepsy, *NPC* Niemann-Pick disease type C, *AD* autosomal dominant, *AR* autosomal recessive, *aa* amino acid. Part (a) details mutations of triplet repeat expansion origins; *italics*: yielded from larger NGS expansion panels. Part (b) details pathogenic or likely pathogenic sequencing variants from NGS & WGS testing; underline: result yielded from WGS testing

a genetic diagnostic rate of 23/80 (28.8%) probands with HCA. The detection rate for routine repeat expansion panels was 11/80 (16.3%), with NGS-targeted panels yielding 11/32 (34.4%) and WGS 1/3 (33.3%). Of the 23 individuals with a genetic diagnosis, 14 had repeat disorders (SCA2, SCA3, SCA6, SCA7, SCA8 and SCA17 and Friedreich's ataxia), and 9 had sequencing variants (in the *ANO10*, *CACNA1A*, *DNMT1*, *KCND3*, *NPC1*, *PRKCG* and *SPG7* genes). The diagnostic rate of our study compares favourably to previous studies: 18% detection rate reported by Nemeth et al. [7] and up to 15% for Coutelier et al. [9]. However, the diagnostic yield for HCA may be lower than the overlapping disorder of HSP according to published studies from our institution [30, 31].

HCA is known to be a disease with high clinical and genetic heterogeneity. Occasionally, phenotypic features can be suggestive of a particular genetic diagnosis (such as central visual loss in SCA7 [32] or cognitive impairment and vertical supranuclear gaze palsy in Niemann-Pick disease type C [33]). However, it is apparent that the phenotypic features cannot reliably predict the genotype. Therefore, NGS techniques are appropriate for interrogating the many ataxia genes associated with HCAs in the absence of robust phenotype to genotype correlations.

We also report that individuals with sequencing variants have a longer disease duration in comparison to individuals with triplet repeats (sequencing variants 27.3 ± 16.2 years; triplet repeat 14.1 ± 12.9 years). A significant difference was

Table 3 Clinical manifestations of individuals with pathogenic variants

Pt no., sex	Age of onset (year)	Duration (year)	Gait ataxia	Limb ataxia	Dysarthria	Pyramidal signs	Other neuro signs	SARA score	MRI	Genetic test type
Pathogenic variants										
Pt 4, M	9	10	+	–	–	LL Spast. & HyR	UL sensorimotor neuropathy	6	n	1
Pt 5, M	55	8	+	UL	+	–	Horizontal & torsional nystagmus	12	Right pons capillary telangiectasia	1
Pt 6, M	49	13	+	UL	+	LL HyR	Chin tremor	13	Severe CA	1
Pt 10, M	38	4	+	–	+	–	Horizontal nystagmus, diplopia, postural tremor	6	n	1
Pt 15, F	49	6	+	UL & LL	+	UL & LL HyR	Vertigo, UL paresthesia	20	n	2
Pt 22, F	48	19	+	–	–	UL & LL HyR	Grasp reflex +	32	Severe CA	1
Pt 25, F	29	12	+	LL	–	–	Horizontal nystagmus	6	Mild CA (vermis)	1
Pt 30, M	22	28	+	UL & LL	+	UL & LL HyR Babinski +	Intention tremor	4	Moderate CA	2
Pt 31, M	48	21	+	UL	+	–	Horizontal nystagmus	19	Mild CA	Family study
Pt 32, M	40	21	+	–	+	–	Horizontal nystagmus, titubation, UL & LL paresthesia	19	Severe CA	2
Pt 39, M	38	10	+	UL	+	UL & LL HyR	Horizontal nystagmus, cognitive impairment, epilepsy	13	Severe CA & brainstem atrophy	1
Pt 40, M	8	55	+	UL & LL	+	–	Horizontal & vertical nystagmus	15	n	1
Pt 42, F	61	5	+	UL & LL	+	UL & LL HyR	Horizontal nystagmus	10	Moderate CA	2
Pt 51, M	18	18	+ (WC)	–	–	–	Horizontal nystagmus, central vision loss	28	Mild CA	1
Pt 58, M	44	33	+	–	+	–	Horizontal & vertical nystagmus	28	n	2
Pt 63, M	28	34	+	UL & LL	+	LL HyR	–	21	n	2
Pt 66, M	10	43	+	UL & LL	+	–	–	13	Moderate CA	3
Pt 71, F	25	10	+	UL & LL	+	UL & LL Spast. & HyR	Dysphagia, bilateral optic disc atrophy, omni-directional ophthalmoplegia	17	n	1
Pt 73, F	36	5	+	UL & LL	–	UL HyR	Horizontal nystagmus, decreased downward gaze, cognitive impairment	12	Mild frontal atrophy, moderate hippocampal atrophy	Family study
Pt 75, M	30	15	+	–	–	–	Vertigo	11	n	Family study
Pt 77, M	30	4	+	–	+	–	Horizontal nystagmus	8	n	1
Pt 78, F	33	5	+	UL & LL	+	UL & LL	Horizontal nystagmus	8	Moderate CA	Family study
Pt 79, M	40	6	+	UL & LL	–	LL HyR	Bilateral ptosis, pes cavus	4	n	2
Pt 80, F	8	54	+	–	+	–		4.5	n	2

Table 3 (continued)

Pt no., sex	Age of onset (year)	Duration (year)	Gait ataxia	Limb ataxia	Dysarthria	Pyramidal signs	Other neuro signs	SARA score	MRI	Genetic test type
							Bilateral upward gaze palsy, UL myoclonic jerk, cognitive impairment			
Pt 81, F	55	4	+	–	–	–	Deafness, narcolepsy, bilateral optic nerve cupping	6	n	2
Pt 86, F	32	17	+	UL & LL	+	LL HyR	Horizontal nystagmus	20	Severe CA	2
Pt 87, M	41	27	+	–	+	LL HyR	Vertical nystagmus, dysphagia, urinary incontinence	20.5	Severe CA	2

+: presence, –: absence

CA cerebellar atrophy, *HyR* hyperreflexia, *HyT* hypertonia, *L* left, *LL* lower limb, *n* normal, *Spast.* spasticity, *UL* upper limb, *WC* wheelchair. Bold data indicate referred family members

Genetic test type: 1, routine triplet expansion repeat testing; 2, NGS-targeted panel +/− more comprehensive expansion testing; 3, whole genome sequencing

not found for initial presentation to genetic diagnosis, suggesting that this was not only due to a prolonged diagnostic odyssey for individuals with sequencing variants. It is possible that the difference in disease duration is due to relative differences in awareness of disease onset. However, from our data, triplet repeat expansion and sequencing variant have comparable family history of cerebellar ataxia (50% vs. 63.6% respectively). Furthermore, both have comparable disease severity as measured by SARA scores, albeit measured at examination, not at disease onset (triplet 14.8 ± 7.6; sequencing 13.5 ± 8.4, $p = 0.698$). It is important to note that 'repeat disorders' and 'sequencing variants' are heterogenous, varied disorders and it might also be important to assess disease progression for individual genetic forms. Additionally, to more accurately discern the rate of disease progression, longitudinal studies would be required. However, given that individuals with sequencing variants were found to have disease duration almost twice of patients with triplet repeats, this is suggestive of a genuine difference that may be relevant to providing prognostic information in the clinic. For the repeat expansion disorders, longer CAG repeats may be associated with faster disease progression, although this was only shown in SCA1 from a large longitudinal cohort study [34]. Furthermore, gene-gene interactions between different repeat expansion genes may also modify disease progression [35]. However, the exact reason for the relative difference in disease duration between repeat disorders and sequence variants is uncertain and may warrant further investigation.

One limitation of our study is selection bias for the individuals referred to our clinic as a tertiary referral centre. Another caveat is that individuals referred with HCA were tested in a non-uniform manner. There were no clear criteria regarding which method of NGS technology to choose in individuals who tested negative for routine triplet repeat expansion panels. For example, while we detected a *PRKGC* variant on WGS, this could have been detected by targeted resequencing of SCA genes. Furthermore, not all individuals who tested negative to triple repeat expansion testing had further NGS-targeted panels or WGS testing due to the commercial availabilities of these investigations. Prospective studies with a more uniform approach to genetic testing would help further define the role of NGS-targeted panels in diagnosing HCAs.

Nevertheless, 35 probands in this study who tested negative to routine triplet repeat expansion panels were able to have NGS-targeted panels or WGS testing. From which, 12 probands tested positive, expanding the genetic heterogeneity of our sample.

It has been discussed in the literature that WES has a higher diagnostic rate compared to NGS-targeted panels and has been suggested as a first-line test for HCA [36]. However, no cases were investigated with WES due to limited accessibility of this test at the time. Also, compared to WES, WGS may have more uniform coverage of the genome as well as the

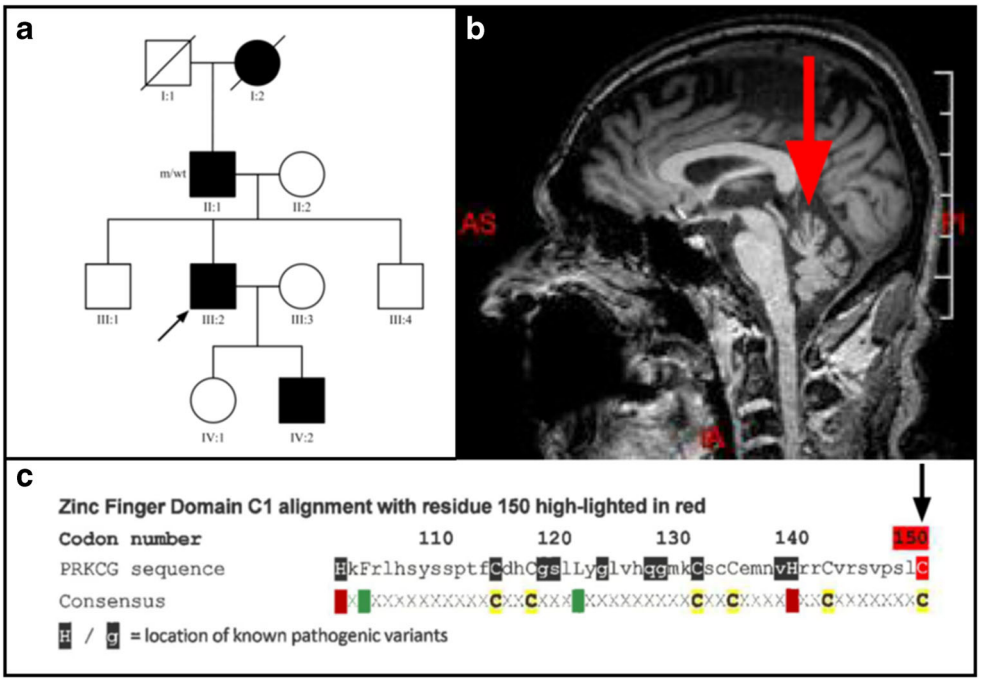

Fig. 2 Case study of PRKCG variant detected on WGS: **a** A pedigree showing autosomal dominant mode of inheritance of SCA14, and segregation of mutation in *PRKCG* from II:1 to III:2; *blacked circle or box*: presence of symptoms; *m/wt*: mutation/wild-type. **b** Moderate atrophy of the cerebellum on sagittal T1-weighted MRI brain (*red arrow*). **c** Pathogenic variants to *PRKCG* gene in Zinc finger domain schema

potential to detect coding and non-coding variants and has been used for similar conditions such as HSP [15]. With cost of WGS reducing over time, it may become more clinically practical and accessible to utilise this test.

Conclusion

We provide real-world evidence to show that HCA is genetically heterogenous in an Australian sample. Furthermore, our data suggests that individuals with repeat expansion disorders may have a more rapid disease progression in comparison to individuals with disease-relevant sequencing variants. Moreover, we provide support for performing NGS approaches such as multi-gene panels or WGS in individuals presenting with HCA who are negative following routine expansion testing. The benefits of NGS panels or WGS for HCA could be further explored with cost-effectiveness studies.

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Compliance with Ethical Standards

Disclosure of Conflict of Interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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