Frequency of rare recessive mutations in unexplained late onset cerebellar ataxia

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Abstract Sporadic late onset cerebellar ataxia is a well-described clinical presentation with a broad differential diagnosis that adult neurologists should be familiar with. However, despite extensive clinical investigations, an acquired cause is identified in only a minority of cases. Thereafter, an underlying genetic basis is often considered, even in those without a family history. Here we apply whole exome sequencing to a cohort of 12 patients with late onset cerebellar ataxia. We show that 33 % of ‘idiopathic’ cases harbor compound heterozygous mutations in known ataxia genes, including genes not included on multi-gene panels, or primarily associated with an ataxic presentation.

Keywords Ataxia · Whole exome sequencing · Next generation sequencing · Diagnosis

Introduction

Adult onset cerebellar ataxia poses a considerable diagnostic challenge. Initial investigations focus on detecting degenerative, toxic, structural and inflammatory etiologies which together underlie around a third of cases [1]. Thereafter, molecular investigations for a monogenic basis of disease are often undertaken despite 80 % of patients having no relevant family history [2].

Current molecular investigations for sporadic cases echo that of familial forms, beginning with testing for trinucleotide repeat disorders, such as the spinocerebellar ataxias (SCA1, 2, 3, 6, 7 and 17), dentatorubral pallidoluysian atrophy (DRPLA) and Friedreich’s ataxia (FDR) in most centres [1]. However, this approach fails to identify a molecular diagnosis in 87–98 % of late onset sporadic cases [1, 3], and subsequent investigations are undertaken on a gene-by-gene basis, often at considerable time and expense.

The difficulty in establishing monogenic forms of disease using this approach is increasingly challenging given that at least 60 causative ataxia genes are reported [4]. Recent studies have therefore utilized next generation sequencing focusing on infantile or juvenile onset cases [5], or adult onset ataxia with a demonstrable family history [4]. Only two studies have described sub-sets of patients with sporadic onset adult disease, despite it being a major form of ataxia, and suggested that a molecular diagnosis can be reached in ~10 % of cases [4, 6]. Given this, we applied whole exome sequencing to a cohort of individuals with sporadic late onset ataxia.

Methods

Unrelated individuals with sporadic ataxia beginning at 30 years of age or over were identified from routine referrals to our regional neurogenetic service, in Newcastle upon Tyne, England.

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Acquired causes of ataxia were excluded and all participants had negative genetic testing for SCA 1, 2, 3, 6, 7, 17, DRPLA and Friedreich’s Ataxia (FA). In addition, all adult males had negative FMR1 testing.

Blood genomic DNA was fragmented, exome enriched and sequenced (Nextera Rapid Exome Capture 37 Mb and HiSeq 2000, 100 bp paired-end reads). In-house bioinformatic analysis included alignment to UCSC hg19, using BWA as aligner and GATK to detect SNV and INDELS across all samples using standard filtering parameters according to GATK Best Practise Recommendations [7] (see supplementary methods). Further analysis was performed on variants with a minor allele frequency <0.005 in several reference databases and 302 unrelated in-house controls (see supplementary methods). Rare heterozygous, homozygous and compound heterozygous variants were defined, and protein altering and/or putative ‘disease causing’ mutations as predicted by at least three out of four software programmes were included. Pathogenicity was defined in accordance with American College of Medical Genetic guidelines (see supplementary methods). Genes known or suggested to cause ataxia as a primary or secondary phenotype in humans from two suggested clinical panels [4, 8] together with additional genes in which ataxia may result as part of the phenotype (list-supplementary methods) were assessed for variants according to the above criteria, and confirmed by Sanger sequencing (supplementary methods).

Variants were defined using a priori criteria: (1) confirmed pathogenic: dominant disorders—variant previously shown to cause ataxia in humans; recessive disorders—either 2 variants previously shown to cause ataxia in humans; or 1 pathogenic variant with a second variant predicted to affect protein function by at least 3 of 4 prediction algorithms (SIFT, Polyphen2, Mutation Taster, LRT), or through frameshift or truncation. (2) Probable pathogenic: dominant and recessive disorders—variants in known genes causing ataxia in humans and predicted to affect protein function by at least three of four prediction algorithms; (3) uncertain significance: dominant and recessive disorders—variants predicted to affect protein function with weak evidence that gene alteration causes ataxia in humans.

The study was granted ethical approval from a Research Ethics Committee based in the North of England.

Results

Population

Twelve Caucasian individuals of British origin (5 male) with no known consanguinity were included (Table 1). Mean age at disease onset was 46.7 years (SD 11; range 30–70 years). Mean disease duration was 16.6 years (SD 6.9; range 6–30 years). For one patient, the disease duration fell within the range expected for multi-system atrophy [9]. This patient had a normal DaTscan and autonomic function tests. Three individuals had CSF examination with negative oligoclonal bands. Five had nerve conduction studies; two of which were abnormal. Detailed clinical features and the results of clinical investigations are shown in Table 1.

Diagnosis

We identified previously described pathogenic mutations in four of the 12 (33 %) patients in our cohort. All were present on confirmatory Sanger sequencing. No probable pathogenic variants were identified and variants of uncertain significance were found in an additional two cases (17 %). Findings are summarised in Table 2.

Discussion

We identified confirmed or probable pathogenic variants causing sporadic late onset ataxia in four patients (33 %) in our cohort. These findings are comparable to childhood/adolescent ataxia using targeted sequencing panels (40 %) [4] and whole exome sequencing (27 %) [5]. They are also significantly higher than previous data for adult onset cases using either panels or whole exome (both ~10 %) [4, 6].

We detected pathogenic variants in SPG7, SYNE1 and ANO10 (previously published by Balreira et al. [10]). Fogel et al. [6] also identified pathogenic variants in these genes (SPG7 (n = 2), SYNE1 (n = 3) and ANO10 (n = 1). The clinical features of these patients appear relatively homogeneous between their and our study, with pure cerebellar ataxia beginning above the age of 40 for ANO10 and SYNE1 cases, and a more heterogeneous age of onset (<20–50) with additional neurological features including spasticity and a polyneuropathy in SPG7 cases [6]. Therefore, pathogenic mutations in these genes appear to be an important and frequently identified cause of late onset sporadic ataxia.

We used whole exome sequencing (WES) rather than targeted next generation ‘panels’, and it remains a contentious issue as to which is more appropriate in the investigation of neurogenic disorders. WES enables greater genome coverage, and hence detection of pathogenic mutations in genes not considered as having ataxia as a primary phenotype. Our results highlight this as SPG7 was not covered by one ataxia panel [4], SYNE1 by another [8], and ANO10 was not included in either panel. WES however, may result in detection of unexpected findings such as pathogenic mutations predisposing to cancer or neurodegenerative...
| Patient no., sex | Age (years) | Age onset (years) | Disease duration (years) | Presenting symptom | Gait ataxia | Limb ataxia | Ocular signs | Additional neurological features | Other features | MRI | LP | NCS/EMG | Other investigations | Muscle biopsy | Other negative molecular investigations |
|-----------------|-------------|------------------|--------------------------|-------------------|-------------|-------------|-------------|---------------------------------|----------------|-----|-----|---------|-------------------|--------------|---------------------------------------|
| 1, F            | 63          | 40               | 23                       | Slowly progressive midline and appendicular ataxic syndrome | +++          | ++          |            | Early CPEO, dysmetric pursuit, broken saccades | None           | CA  | Normal−OCB | Bilateral CTS (CTS study only) | Normal IHC | Normal−OCB, FMR1 |
| 2, F            | 47          | 30               | 17                       | Slowly progressive spastic ataxic syndrome | ++ (Fr)      | +           |            | CPEO, temporal optic disc pallor, jerky pursuit, hypometric saccades | None           | Mild CA | Normal−OCB | ND                | Mild fibre size variation, nil          |
| 3, F            | 57          | 45               | 12                       | Ataxia developed aged 45 | +++          | ++          |            | Epilepsy aged 7                  | None           | CA and parieto-occipital atrophy | ND | Normal | ND | FMR1 |
| 4, F            | 63          | 40               | 23                       | Slowly progressive midline cerebellar ataxia | ++           | +           |            | GEN, TLE with ongoing infrequent focal seizures, no treatment | Cataracts (age 62) | CA  | ND | ND | POLG, MT-ATP6 & 8 |
| 5, F            | 55          | 35               | 20                       | Slowly progressive spastic ataxic syndrome | +++          | +           |            | GEN, jerky pursuit, hypometric saccades | None           | CA  | ND | ND | SPG7 |
| 6, F            | 76          | 70               | 6                        | Progressive midline and appendicular ataxia | +++          | ++          |            | GEN, up and down beat nystagmus, orthostatic tremor | None           | Mild CA | ND | −DaT | MT-ATP6 & 8 |
| 7, M            | 71          | 60               | 11                       | Slowly progressive midline ataxia | +           | −           |            | GEN, jerky ocular pursuit | None           | None | CA | ND | Normal |
| 8, M            | 58          | 50               | 8                        | Midline ataxia | ++          | +           |            | RAPD, OA, GEN, congenital hearing loss | None           | CA  | ND | SAN | POLG, WFSI, OPA1, MT-ATP6 & 8 |

**Table 1** Clinical features of the 12 patients in the cohort
| Patient no., sex | Age (years) | Age onset (years) | Disease duration (years) | Presenting symptom | Gait ataxia | Limb ataxia | Ocular signs | Additional neurological features | Other features | MRI | LP | NCS/EMG | Other investigations | Muscle biopsy | Other negative molecular investigations |
|-----------------|-------------|------------------|--------------------------|-------------------|-------------|-------------|-------------|-----------------------------|---------------|-----|-----|---------|-------------------|--------------|-------------------------------|
| 9, M            | 70          | 40               | 30                       | Pure midline ataxia | +(stick)    | −           | None        | None                        | None          | CA  | ND  | ND      | Normal IHC               | Normal RCE   | SCA12 mt-DNA LR-PCR          |
| 10, M           | 59          | 44               | 15                       | Pure midline ataxia | +++         | +           | None        | Prominent dysarthria, choking | Brisk reflexes | None | CA  | ND  | ND      | Normal Q10               | Normal RCE   | SPG7 SCA8 SCA12             |
| 11, F           | 65          | 47               | 12                       | Pure midline ataxia | +++ (WhC)   | +           | Oscillopsia | Jerky pursuits and horizontal nystagmus | Hypometric saccades | Cataract, diabetes and short stature | Mild CA; high signal C3, 4 posterior columns; thin cord | −OCB | DRG | ND      | POLG SPG7 POLG2 PEO1 ANT1 mt-DNA LR-PCR |
| 12, M           | 83          | 60               | 23                       | Midscale ataxia    | ++ (stick)  | +           | Jerky pursuit and coarse phasic nystagmus | Normal saccades | None | None | Mild CA | ND  | ND  | Patient declined | Nil          |

Presence or absence of symptoms are indicated by + or − symbol, respectively.

AFTs autonomic function tests, CA Cerebellar atrophy, CPEO chronic progressive external ophthalmoplegia, CTS carpal tunnel syndrome, CVD cerebrovascular disease, DRG dorsal root ganglionopathy, Fr Frame, GEN gaze evoked nystagmus, IHC immunohistochemistry, ND not done, OA optic atrophy, OCB oligoclonal bands, PV periventricular, RAPD relative afferent pupillary defect, RCE respiratory chain enzyme, SVD small vessel disease, TLE temporal lobe epilepsy, WhC wheelchair, WM white matter.
Table 2 Genetic variants of interest identified in the 12 patients

| Pt | Gene | Model | Exome seq identified variant (1) 1000 g | rs# | Variant pathogenicity prediction | Exome seq identified variant (2) 1000 g | MAF variant (1) | MAF variant (2) |
|----|------|-------|---------------------------------|-----|----------------------------------|---------------------------------|--------------|----------------|
| 1  | SPG7 | AR    | c.1529C>T p. Ala510Val          | rs61755320 0.003463 0.0014 c. 1053dupC p. Gly352fs | Pathogenic | rs121918358 0.000077 0 (1) Pathogenic (2) NA |
| 2  | SPG7 | AR    | c.1529C>T p. Ala510Val          | rs61755320 0.003463 0.0014 c.235T>A p. Leu78* | Pathogenic | rs138000380 0.000231 0.0005 c. 132_133insT p. Asp45fs |
| 3  | ANO10| AR    | c.1843G>A p. Asp615Asn          | rs117360770 0.002307 0.0018 c.1762delC p. Leu588fs | Neutral | NA 0.003435 0 (1) NA |
| 4  | SYNE1| AR    | c.9148C>G p. Leu3050Val         | rs138283229 0.002461 0.0009 NA | Neutral | NA 0.000077 0 NA |

Variants of uncertain significance

| 5  | SLC33A1 | AD | c.433G>A p. Gly145Ser          | rs138000380 0.000231 0.0005 c.132_133insT p. Asp45fs | NA | NA 0.003435 0 NA |
| 6  | PLEKHG4| AD | c.2251G>A p. Asp751Asn         | rs121918358 0.000077 0 NA | NA | NA 0.000077 0 NA |

Confirmed pathogenic: dominant disorders—variant previously shown to cause ataxia in humans; recessive disorders—either 2 variants previously shown to cause ataxia in humans; or 1 pathogenic variant with a second variant predicted to affect protein function by at least 3 of 4 prediction algorithms (SIFT, Polyphen2, Mutation Taster, LRT), through frameshift or truncation. Variants of uncertain significance: dominant and recessive disorders—variants predicted to affect protein function with weak evidence that gene alteration causes ataxia in humans.

D pathogenic or deleterious, P polymorphism, NA not applicable N neutral (frameshift mutations considered pathogenic)

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