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Novel genotype-phenotype and MRI correlations in a large cohort of patients with *SPG7* mutations

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Statistical analysis
Dr Channa A,A. Hewamadduma (CAAH) conducted the statistical analysis with the help of the Statistics Department of the University of Sheffield. CAAH is employed with the National Health Service (NHS) (Professional affiliation) and holds an Honorary Senior Clinical Lectureship with the University of Sheffield (Academic affiliation). Dr Nigel Hoggard analysed the statistics pertaining to MRI data. NG is a consultant Neuroradiologist employed by the NHS, professionally and Professor of Neuroradiology at the University of Sheffield, UK. Miss Ruta Segamogaite was an MSc student supervised by CAAH at the University of Sheffield and conducted the data analysis with CAAH.

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Abstract

Objective: To clinically, genetically and radiologically characterize a large cohort of *SPG7* patients

Methods: We have used next generation sequencing panels for ataxias and hereditary spastic paraparesis (HSP) to screen patients attending dedicated ataxia and HSP clinics, and subsequently targeted typical phenotypes for direct genetic testing for variations in *SPG7*. Most patients also underwent MR imaging.

Results: We identified 40 cases with bi-allelic *SPG7* mutations, including six novel mutations, including a large multi-exon deletion, representing one of the largest cohorts so far described. We identified a characteristic phenotype comprising cerebellar ataxia with significant cerebellar dysarthria, mild lower limb spasticity and a waddling gait, predominantly from a cohort of idiopathic ataxia. We report a novel brain MRI finding of dentate nucleus hyper-intensity on T2 sequences. . We confirm that the c.1529C>T allele is frequently present in patients with long-standing British ancestry. Based on the findings of the present study and existing literature we confirm that patients with homozygous mutations involving the M1 peptidase domain of *SPG7* have a younger age of onset compared to individuals with mutations elsewhere in the gene (14 years difference, $p<0.034$), whilst c.1529C>T compound heterozygous mutations are associated with a younger age of onset compared to homozygous cases (5.4 years difference, $p<0.022$).

Conclusions: Mutant *SPG7* is a common cause of ataxia. In patients with British ancestry c.1529C>T allele represents the most frequent mutation. *SPG7* mutations can be clinically predicted by the characteristic hybrid spastic-ataxic phenotype described above, along with T2 hyperintensity of the dentate nucleus on MRI.

Introduction

Hereditary spastic paraparesis (HSP) and hereditary cerebellar ataxias (HCA) are heterogeneous groups of progressive neurodegenerative conditions with significant overlap [1, 2]. HSP can be complicated due to development of cerebellar ataxia, neuropathy, optic atrophy and muscle weakness etc. as can HCA [3]. Moreover the group of ataxias defined as idiopathic cerebellar ataxias (ICA) is often sporadic, relatively late onset with apparently variable symptoms and with no cause identified after extensive metabolic, autoimmune and genetic work-up, can pose a diagnostic challenge. The extensive genetic heterogeneity in HSP with 76 different HSP genetic loci, and more than 36 loci associated with autosomal dominant cerebellar ataxias and a similar number of autosomal recessive genes [4-6] together with the overlapping features of HCA and complicated HSP often cause difficulties in classifying and planning genetic testing.

Next generation sequencing (NGS) is now available for both HSP and HCA patients. However, such panel tests currently remain expensive with a considerable burden to the National health service (NHS) in the UK. Therefore detailed characterisation of the phenotype of currently incurable genetic ataxias and HSP is important in the discovery of underlying molecular factors in addition to help understand the natural history of the disorders to explore potential bio-markers. We highlight the importance of genotype-phenotype correlation beyond the traditional non-specific clinical boundaries of HSP and HCA. We describe our experience in identifying and characterising a large cohort patients with mutations in *SPG7* gene, implicated in HSP, HCA and ICA[7], and a review of all published *SPG7* mutations.

Methods

Patient Cohorts

Within the Sheffield Academic Directorate of Neuroscience we have two large cohorts of patients with cerebellar ataxia (n=1700) and hereditary spastic paraparesis (HSP) (n=336). The Sheffield Ataxia Centre cares for patients with various types of ataxias. The patients are under regular review with the aim of identifying the cause of their ataxia as well as to provide care and support. All cases are assessed clinically and undergo investigations as clinically indicated including MR brain imaging. The severity of ataxia is assessed using the SARA score [8] and the severity of spasticity using Spastic Paraparesis Rating Scale (SPRS). The majority of patients described in this paper were seen at the Sheffield Ataxia Centre, with the remainder being seen in the Sheffield Neuromuscular and Hereditary Spastic Paraparesis clinics.

Genetic testing

Patient consent was obtained for genetic testing and reporting of findings. Libraries of sheared genomic DNA corresponding to panels of either HCA or HSP genes captured using a SureSelect XT custom designed probe set (Agilent, Cheadle, UK) and pair-end sequenced using a HiSeq 2500 instrument (Illumina) were initially used. Raw data were analysed using the Genome Analysis ToolKit [9], (GATK, Broad Institute, Cambridge, MA, USA) according to their best practice guidelines [10, 11]. After initial identification of eleven patients with *SPG7* mutations using the ataxia and HSP gene panels we evaluated the phenotype of these individuals to identify a triad of spastic paraparesis (usually mild), cerebellar ataxia (with prominent cerebellar dysarthria) and a waddling type gait indicative of proximal muscle weakness. Thereafter, the majority of patients who presented with the traditional label of HCA or HSP who had the above triad were analysed by bi-directional Sanger sequencing and dosage analysis (multiplex ligation-dependent probe amplification kit P213-B1 and B2, MRC-Holland) of all 17 exons of the *SPG7* gene. The remainder was identified using either HSP or HCA gene panel testing as before.

Chromatographs were analysed using Mutation surveyor v4.0.8 (http://www.softgenetics.com/ms). Annotation of mutations was carried out in accordance with Human Genome Variation society nomenclature (http://www.hgvs.org/mutnomen), with nomenclature based on the reference sequence NM_003119.3. Novel variants in the *SPG7* gene were assessed for pathogenicity using Alamut Visual version 2.9.0 (Interactive Biosoftware, Rouen, France) and prediction software (Provean, MutPred, SNPS&GO and PolyPhen2). Allele frequencies for novel variants in normal control populations were obtained from the Genome Aggregation Database (gnomAD) [12].

Neuroimaging

Analysis of the imaging was undertaken for all of the patients (N = 38) that underwent brain imaging on the same 3T MR scanner (Ingenia, Philips Medical Systems, Eindhoven, Netherlands) with the same T2 weighted sequence and compared with age and sex matched controls imaged with this sequence (REC reference 09/H1310/79, IRAS 26259). The axial T2 weighted parameters were: TR 3000ms, TE 80, echo train length 15, number of averages 1 and 4mm thick, 512 x 512 matrix. Matching criteria for control subjects were age within 3 years and sex. Relative signal intensity of the dentate nucleus was compared to normal appearing pontine white matter and the red nucleus. A region of interest (area 20mm^2) in these structures, was placed in the region of the dentate nucleus with the lowest signal (by NH). The dentate nucleus signal was then dichotomized by whether the ratio of the signals was less than or more than 1 (i.e. hypo or hyperintense compared to normal appearing white matter in the pons).

Literature review

We reviewed clinical and genetic details of all SPG7 cases thus far reported in the literature (until 30th September 2017). We searched the following terms in Pubmed, Medline, Web of

science and Embase: SPG7, paraplegin, hereditary spastic paraparesis, hereditary spastic paraplegia (mutations), spastic ataxia, ataxia, and selected all the papers reporting *SPG7* and/ or paraplegin mutations and reviewed the phenotype and genotype data published. We excluded publications, which were not in English or where English translation was not available, and papers that did not describe clinical features but only detailed basic experimental science. All mutations described by us and previously reported are depicted in a schematic diagram in relation to functionally important domains (Figure 1).

Statistical analyses

Statistical analysis was performed using Prism GraphPad™ V7.0b and SPSS (2015) statistical software programmes. One-way ANOVA was used for multiple group comparisons and independent samples t-test and the Chi square test were used to compare two groups.

Results

Characterisation of the phenotype

We identified a total of 40 cases positive for pathogenic mutations in both alleles of the *SPG7* gene (Table 1). Initially 11 cases were found to have pathogenic *SPG7* mutations using ataxia or HSP next generation sequencing (NGS) gene panels. More specifically, in four patients the detection was made using the ataxia panel, which consisted of 42 ataxia genes, and in seven patients the detection was made using the HSP panel of 39 genes. In five of the seven HSP panel cases the HSP like phenotype was complicated with cerebellar features and only two of the seven HSP cases had a pure HSP phenotype. Reviewing the phenotype of these 11 cases, we noted that 9 individuals had cerebellar ataxia with prominent slurring of speech, mild spasticity and proximal muscle weakness resulting in a waddling gait.

Direct genetic screening based on the phenotype

Following the clinical characterisation of the initial 11 patients, we undertook direct testing for mutations in the *SPG7* gene in patients who demonstrated the above phenotype of cerebellar ataxia, spasticity (often mild) and waddling gait in a cohort of patients attending the Sheffield Ataxia Clinic and hereditary spastic paraparesis clinics. We identified a further 26 cases (24 from the ataxia clinic and 5 from the neuromuscular clinic) with pathogenic mutations in the *SPG7* gene. The method of genetic testing and basic clinical phenotype are detailed in Table 1. All cases were positive for bi-allelic mutations in the *SPG7* gene (Table 1).

Clinical characteristics of patients with *SPG7* mutations

The clinical characteristics of the 40 probands are summarized in Table 2. There was no history of consanguinity. Eighty-five percent were male. The average age of symptom onset was 41.7 years (SD±11, median age 44 years). Female patients developed symptoms on average 8 years younger than males (38.5 years vs 46.6)(Table 3). The mean duration of the disease at the time of diagnosis was 9 years (SD±4.9, mode 5). Thirty-six out of forty were of long standing British ancestry. Four were UK citizens of Indian, Iranian, German and Bulgarian descent. Of these four cases, three were phenotypically similar to the majority of the cohort, whilst the female patient from Bulgaria had cerebellar ataxia at presentation with very mild spasticity and later developed a waddling gait.

Ninety eight percent of cases (39/40) presented with gait unsteadiness followed by dysarthric speech (29%). Twenty-nine (74%) complained of mild spasticity. Only two patients presented with the typical spastic gait characteristic of HSP (5%) and only 7/40 presented with moderate to severe spasticity related complaints. Eighty two percent of our cohort had mildly increased lower limb tone and more than 93% had brisk reflexes, whilst the Babinski sign was positive in 51%.

At baseline assessment 36/40 cases (90%) were found to have evidence of cerebellar ataxia and 31/40 cases were found to have both mild spasticity and cerebellar ataxia. Despite the cerebellar features, only 2/40 cases were non-ambulant at the time of writing the paper, with a total symptomatic disease duration of 369 patient years. None of the patients could run and 78% of the cases were using some type of walking aid. The severity of the ataxia, as assessed by the SARA score, was less severe (the average SARA score was 10.58 with a median of 10.0) when compared to other inherited ataxias such as SCA 6 (median score 15.0) for the same duration of symptoms.

Sixty four percent (25/40) of cases demonstrated the triad of cerebellar ataxia with dysarthria, spasticity and waddling gait at presentation and 9 others developed the full clinical picture during subsequent follow-up (totalling 87%). Progressive external ophthalmoplegia (PEO) was observed only in one case. In another patient we noted vertical gaze palsy. Nystagmus was present in 38% (15/40). Optic atrophy was seen in one patient. Waddling gait was seen in 87% of our cases.

Although four patients were found to have reduced vibration sense and 3 had reduced pinprick sensation on clinical examination, none of the 19 patients who underwent neurophysiological assessment had evidence of large fibre peripheral neuropathy. The neurophysiological assessments did not suggest evidence of myopathy on EMG.

Mutation analysis

Fifteen cases were homozygous for mutations in the *SPG7* gene (37%) whilst 25 (63%) cases were compound heterozygous. Twelve of the 15 homozygous cases had the common missense mutation in exon 11, c.1529C>T, p.(Ala510Val), whilst the other three cases were homozygous for the c.233T>A, p.(Leu78*) nonsense mutation. 36/40 cases in our cohort who carried the common mutation p.(Ala510Val) in at least one allele were of long standing British

ancestry. The three patients homozygous for the p. (Leu78*) nonsense mutation were second-generation British citizens of Indian, Iranian or Bulgarian descent. The other case, of German descent, was compound heterozygous for the c.1181T>C, p.(Phe394Ser) and c.1045G>A, p.(Gly349Ser) mutations.

The frequency of the c.1529C>T, p.(Ala510Val) mutation in our cohort was 60% (48 out of 80 alleles assessed). The second most common mutant allele (6 alleles), c.233T>A, p.(Leu78*) was seen in 3 patients in the homozygous state, whilst c.1045G>A, p.(Gly349Ser) was seen in five cases in a compound heterozygous state (5 alleles). p.(Ala510Val) and p.Arg485_Glu487del mutations were observed in two thirds of the disease alleles (50/78). In addition to the single case with a large deletion, several small insertions, duplications, deletions and splice site mutations were detected on 7 alleles, 5 of which have been previously described. Most of the pathogenic alleles were missense mutations (60/80) whilst 20 were nonsense mutations (Table 1).

Novel mutations in SPG7

We discovered six novel likely pathogenic mutations in the *SPG7* gene (Table 1) of which five were null mutations, with two frame-shift mutations c.775_781dup p.(Thr261fs) and c.2096dup p.(Met699fs), two nonsense mutations c.754G>T, p.(Gly252*) and c.300T>A, p.(Tyr100*) and a large deletion encompassing at least exons 4 to 9 (c.377-?_1324+?del) was identified using MLPA. The novel missense mutation c.2083C>G, p.(Leu695Val) resulted in substitution of the same amino acid as a previously reported pathogenic mutation c.2084T>C, p.(Leu695Pro) [13]. Predictions by PROVEAN (deleterious), PolyPhen2 (Probably Damaging) and MutPred (Actionable hypothesis) suggested likely pathogenicity, but this was not supported by SNPS&GO (Neutral). This allele is present in the East Asian gnomAD normal control population at a frequency of 0.4626%. Interrogation of the exome sequencing data repository did not show any of the other novel mutations to be present in healthy controls.

MRI Brain imaging

MRI brain imaging was available in 38 cases. Cerebellar atrophy was noted in 92% (35/38). T1 axial sequences indicated cerebellar atrophy (Figure 2A and 2B) and T1 sequences of both dentate nuclei (DN) and the red nuclei (RN) were not distinguishable between controls and *SPG7* cases (Figure 2A and 2C). The same T2 sequence on 3T imaging, as detailed above, was available for 21 patients and these were matched with 17 normal controls. The control group had 15 males, 2 females with a mean age of 52.3 years (range 37 to 68). In sixteen control subjects the dentate nuclei were hypointense compared to normal appearing white matter (Figure 2C), and 1 control subject had dentate nuclei isointense relative to normal appearing white matter. In the patient group there were 18 males and 3 females, mean age 53.8 (range 38 to 71). Three of the 21 patients had hypointense dentate nuclei compared to normal appearing white matter. The dentate nuclei were iso- or hyper-intense compared with normal appearing white matter (T2 imaging) in 18 of the 21 *SPG7* positive cases (Figure 2D). The increase in DN T2 hyperintensity on MRI in *SPG7* cases is significant compared to the controls (Chi square test value 25.76, significant at $p<0.001$)(Figure 2F). Both controls and patients showed no difference in the appearance of the red nuclei (RN), which were hypo-intense compared to normal appearing white matter in the pons (Figure 2E).

Genotype – phenotype correlation from current and other studies

All cases with long standing British ancestry carried at least one allele with the c.1529C>T *SPG7* mutation, making it the most frequent mutational site in this cohort.

After analysing all bi-allelic SPG7 cases reported in the literature we were able to identify some associations. We analysed whether mutations in the different functionally important domains of *SPG7* shown in figure 1 had an impact on the age of onset of symptoms. Patients

who had a homozygous mutation in the M1 peptidase domain had an earlier onset of disease symptoms by 12 years compared to patients with mutations in a non-functionally assigned domain ($p<0.022$)(Figure 3A). Having homozygous, compound heterozygous mutations or the presence of null alleles did not have an impact on age at disease onset. We also noted that patients with the c.1529C>T mutation (which is located in a functionally unassigned domain) when in a compound-heterozygote state developed symptoms 8 years earlier compared to c.1529C>T homozygous cases ($p<0.019$, unpaired t-test)(Figure 3B).

Discussion

We describe a large cohort of 40 unrelated cases with mutations in the *SPG7* gene primarily presenting with cerebellar ataxia and less commonly with features of HSP. To our knowledge our cohort is the second largest in the world so far reported after a large Dutch cohort of 46 unrelated families [14]. We propose the novel observation that the phenotype of cerebellar ataxia (with dysarthria), mild lower limb spasticity and a waddling gait is clinically distinct and should alert clinicians to direct genetic testing for mutations in the *SPG7* gene. Such an approach identified 65% of our cohort of patients, whilst next generation sequencing panels identified the remainder of this cohort of index cases. In addition, it is clear that the *SPG7*-related phenotype is more likely to present as a cerebellar ataxia, whereas only 23% of our cohort was thought to have an hereditary spastic paraparesis phenotype. *SPG7* bi-allelic mutations have historically been associated with HSP, but over the last 12 years since the discovery of pathogenic mutations in this gene, it is now clear that ataxia is the major clinical finding [7, 15, 16]. In another UK based study, *SPG7* accounted for 18.6% of 70 patients with unexplained ataxia with pyramidal signs (Pfeffer et al 2015). We have found SPG7 to be the fourth commonest cause of any genetic ataxia after Friedreich's ataxia (FA), spino cerebellar ataxia 6 (SCA6) and episodic ataxia 2 (EA2), and the second commonest recessive ataxia in the UK after FA based on a large cohort of 1500 patients with various causes of ataxia attending

the Sheffield Ataxia Centre[17]. In support for this finding, 90% of our SPG7 cohort demonstrated gait ataxia with cerebellar dysarthria (a common feature in 74% of the cases), however other cerebellar features were less common (Table 2).

Only 2 out of 40 patients in our cohort were non-ambulant or wheelchair-dependent. In keeping with this observation was the relatively modest median SARA score of 10 indicating that ambulatory loss appears to be relatively rare in *SPG7* cases [16]. This favourable prognostic factor will be useful when counselling *SPG7* patients and their families.

A significant proportion of our cases were male (83%), but interestingly female patients tended to develop symptoms about five years earlier than male patients. The median age of onset of symptoms was 44 years, indicating that SPG7 is a late onset disease. The recessive inheritance almost certainly accounts for the lack of a positive family history and therefore absence of a family history should not deter from *SPG7* testing. Our findings are in keeping with a number of previous reports proposing that SPG7 is a late onset disease [14, 18]. The age of onset however did range between 15 to 60 years. Rarely, therefore SPG7 can present with an early onset ataxia

Progressive external ophthalmoplegia (PEO) was only seen in one of our patients but has been reported in 11% *SPG7* cases worldwide (Supplementary table 1). A previous report by Pfeffer *et al*, 2016 found 9/68 (13%) patients with PEO to be associated with bi-allelic *SPG7* mutations. PEO was also suspected (slow saccades) in one out of five cases in a UK cohort of complex HSP [19]. Nevertheless PEO associated with mutations in the *SPG7* gene was also reported to be rare in other cohorts including a French group of patients with spasticity and optic neuropathy (2/23) [20] and a Dutch HSP cohort (2/46)[14]. A cohort of French Canadian patients did not have any families with PEO (none of the 22 individuals from 12 pedigrees) [16, 21] . This variability in the detection of PEO could be related to several factors such as ascertainment bias, duration of disease or length of follow up. A longitudinal follow-up study on *SPG7* patients from the UK reported a median follow-up duration of 23 years from initial

presentation before detecting PEO [7] as did a Norwegian group (median follow up during which PEO recorded was 24 years) [22]. Taken together, clinicians should be aware that PEO-like features can develop in *SPG7* cases with longer disease duration.

Optic neuropathy was reported in 9.5% of the worldwide *SPG7* cases, compared to our cohort where both PEO and optic neuropathy were only seen in one patient. We did not undertake optical coherence tomography (OCT), which may in part explain the lower frequency of optic neuropathy identified in the present study. In a French cohort of *SPG7* patients with spastic gait, 44% of the patients had evidence of optic neuropathy based on OCT, whilst 40% of the patients with optic neuropathy had normal appearing optic discs on fundoscopy. Two patients were found to have optic neuropathy during a subsequent follow-up period of 3-9 years [20]. Therefore a longer follow-up period and the use of OCT may increase the frequency of optic neuropathy in *SPG7* patients.

The increased T2 signal from the dentate nucleus in *SPG7* cases compared to controls has not been previously described. The dentate nucleus is a site of iron accumulation in normal aging and this is associated with reduced T2 signal. The high signal in the dentate does not appear to be due to a globally reduced brain iron accumulation, with the red nucleus continuing to be hypo-intense relative to normal appearing white matter in the patient group as normally expected. In support of the MRI findings reported here is the post-mortem brain and brainstem analysis from a c.1529C>T homozygous case, that showed neuronal loss in the dentate nucleus [23]. Whilst the exact pathophysiology of the above imaging finding needs further investigation, we propose that dentate nucleus hyperintensity on T2 sequences of the MRI brain could aid in the diagnosis of *SPG7*. MRI findings in the combined worldwide cohort have not been reported consistently. We have found that 92% of our cohort had cerebellar atrophy on MRI.

The waddling gait sign we report here has not been previously highlighted. There are a number of reports that describe muscle weakness over and above the mild pyramidal-type weakness one might usually expect to see in patients with HSP [20]. In keeping with our observations, myopathic features were noted in the cohort of PEO patients described by Pfeffer et al [18]. Furthermore two thirds of the *SPG7* cases from a Dutch cohort were noted to have lower limb muscle weakness, although the pattern of weakness could have been attributed to upper motor neuron involvement [14]. On close scrutiny of the previously published papers, one could recognise the presence of myopathic features that can result in a waddling gait due to proximal leg muscle weakness. This may also account for the rationale of performing muscle biopsies in some cases that have, on occasions, shown evidence of mitochondrial dysfunction [15, 20, 24, 25]. Disordered mitochondrial maintenance has been observed in these patients [18]. We did not find neurophysiological evidence of large fibre peripheral neuropathy or myopathy in our cohort.

At present more than 242 cases of *SPG7* have been described worldwide (supplementary table 1). However only 216 of the 242 cases harbour bi-allelic *SPG7* mutations, whilst 26 cases carry a heterozygous mutation. Analysis of the mutations demonstrates that there is considerable genetic heterogeneity, with more than 96 different mutations in the *SPG7* gene described to date (supplementary table 1). In our cohort we identified six novel mutations. All except one resulted in premature truncation of the paraplegin protein and *in silico* modelling has suggested pathogenicity. The c.1529C>T mutation was present in at least one allele in all of our patients with long standing British ancestry, strongly supporting a previous report of its association with patients with British heritage [15]. The four cases who did not harbour the c.1529C>T mutation, were first generation migrants from Europe or Asia. We have also observed that when all published mutations are analysed, c.1529C>T is the commonest mutation making it the 'hot spot' in the *SPG7* gene. The common allele (c.1529C>T) frequency in our cohort was 60% when compared to 38% of all published *SPG7* mutant alleles

(164/432). The c.1454_1462del mutation is the second most common mutation worldwide, with an allele frequency of 9% (38/432) whilst the third most common allele was c.233T>A, p.(Leu78*) at 7% (31/432 alleles) (supplementary table 1).

In addition to the cohort of 40 cases of bi-allelic *SPG7* mutations described here, we have identified eight cases who were heterozygous for the c.1529C>T common allele with no other *SPG7* variation in the second allele, even after MLPA gene dosage assessment (data not included). In the majority of these cases the clinical phenotype was very suggestive of SPG7 as described above. An autosomal dominant pattern of inheritance has been previously suggested in association with mutations in the *SPG7* gene in individuals carrying the c.1529C>T common allele. However, the allele has a frequency of 3-4% in the British population [25, 26], which poses significant doubt about its pathogenicity when found in the heterozygous state.

We conclude that *SPG7* is a common cause of sporadic ataxia. We recommend direct genetic testing for *SPG7* mutations when cerebellar ataxia with dysarthria is associated with mild lower limb spasticity and a waddling gait. If the patient is of long standing British ancestry, directly testing for the c.1529C>T mutation is highly likely to be diagnostic. MR imaging demonstrating cerebellar atrophy with relative T2 hyperintensity of the dentate nuclei is also strongly supportive of *SPG7*. Taken together, we report the importance of detailed genotype, phenotype and radiological characterisation to understand the natural history of late onset genetic ataxias and hereditary spastic parapareses. In addition, this approach is likely to improve diagnostic efficiency and reduce the expense of NGS based genetic panel tests in patients with the characteristic triad of clinical features and the imaging changes described in this report.

Figure Legends

Figure 1. Schematic diagram of the SPG7 protein with important functional domains and positioning of mutations in the Sheffield cohort and all the published pathogenic mutations in the *SPG7* gene. Mutations described in our cohort of patients are annotated above the SPG7 protein structure, whilst previously published mutations are labelled below. Allelic variation frequency is noted within parenthesis. New mutations detected in our cohort are highlighted in red font. Variations denoted in blue are matching c.DNA sequence of the reported mutations. Some large exon deletions reported are in indicated in the text box. Parentheses from mutations removed to create space. TM1 and TM2: Trans-membrane domain 1 and 2; FtsH: Filamentation temperature sensitive mutant in *E.coli* domain; Coil1 and Coil2: Coiled domain; AAA: ATPases associated with diverse cellular activities. Reference sequence:NM_003119.3.

Figure 2. Magnetic resonance imaging (MRI) of the brain in *SPG7* spastic ataxia cases shows T2 hyperintensity of the dentate nucleus. A) T1 axial image across the dentate nucleus (DN) of a control case. B) T1 axial section through the DN in a patient with c.1529C>T homozygous mutation. C) T2 weighted axial image of the same control and D) T2 axial section through DN in the same patient with c.1529C>T homozygous mutation which demonstrates hyper-intense DN (solid white arrow) compared to the normal appearing white matter. E) T2 weighted axial image of the same patient, which demonstrates the red nucleus (RN). The RN appears hypo-intense compared to normal appearing white matter in all *SPG7* and control cases (solid black arrow with white boarder). F) The observation of hyper-intense T2 signal of the DN was significantly more frequent in the SPG7 patients compared to the control cases ($p<0.001$, Chi square test value 25.7649).

Figure 3. Genotype phenotype correlation in SPG7 mutations and age of onset of symptoms. (A) Association of the position (by the functionally important regions) of the mutation and the age of onset in homozygous SPG7 cases. N terminal = up to first 140aa; FstH = 141-250aa; AAA ATpase = 306-481aa; M1 Peptidase = 544-746aa; The rest = mutations in any other area/s which is/are not described as above. We selected homozygous cases due to the uniformity they create by harbouring two similarly mutated alleles, to compare the effect of the mutation within functionally important domains of the SPG7 protein on the age of onset. One-way ANOVA with multiple comparisons and post hoc Tukey test showed a significantly ($p=0.034$) younger age of onset (14.63 years, SE 5.25, 95% CI 0.82 - 28.4) for those with homozygous mutations in the M1 peptidase domain compared to a mutational position in a functionally undefined domain ("The rest"). **(B)** The c.1529C>T common mutation when in the homozygous state is associated with a significantly later age of onset that when in the compound heterozygous state. c.1529C>T patients provide a degree of mutational homogeneity in that at least one allele is constant allowing comparison between homozygous and compound heterozygous states. Compound heterozygotes developed symptoms on average 5.4 years earlier than the c.1529C>T homozygotes ($p=0.022$, independent samples t-test for equality of means with equal variances assumed).

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Table 1: *SPG7* gene mutations and clinical features at presentation

Pedigree	Gender	Onset Age	Diagnostic delay	Method of genetic testing	Clinical diagnosis prior to genetic testing (HSP- hereditary spastic paraparesis, CA- cerebellar ataxia)	Mutation in cDNA[1]		Predicted Protein change		Cerebellar	Spasticity	Proximal weakness	Dysarthria	PEO	Optic atrophy	Bladder Disturbance
						Allele 1	Allele 2	Allele 1	Allele 2							
1	M	45	5	Direct	CA with spasticity, waddling gait	c.1529C>T	c.1529C>T	p.(Ala510Val)	p.(Ala510Val)	+	+	+	?	-	-	+
2	M	44	5	Ataxia	CA with spasticity, waddling gait	c.1529C>T	c.1937-2A>G	p.(Ala510Val)	p.?	+	+	+	+	-	-	+
3	M	30	10	HSP	CA with spasticity, waddling gait	c.1529C>T	c.1529C>T	p.(Ala510Val)	p.(Ala510Val)	+	++	+	-	-	-	+
4	M	40	6	Direct	CA with spasticity, waddling gait	c.1529C>T	c.1454_1462del	p.(Ala510Val)	p.(Arg485_Glu487 del)	+	++	+	+	-	-	+
5	M	50	6	HSP	CA with spasticity, waddling gait	c.1529C>T	c.1529C>T	p.(Ala510Val)	p.(Ala510Val)	+	+	+	+	-	-	-
6	M	56	5	HSP	HSP- complicated	c.1529C>T	c.1045G>A	p.(Ala510Val)	p.(Gly349Ser)	+	+++	-	+	-	-	-
7	M	15	14	Direct	CA with spasticity	c.1529C>T	c.1529C>T	p.(Ala510Val)	p.(Ala510Val)	+	+++	+	+	-	-	-
8	M	60	5	HSP	HSP- pure	c.1529C>T	c.1045G>A	p.(Ala510Val)	p.(Gly349Ser)	-	+++	-	-	-	-	+
9	F	24	9	Ataxia	CA with spasticity	c.1529C>T	c.1904C>T	p.(Ala510Val)	p.(Ser635Leu)	?	+	-	+	-	-	-
10	M	51	5	Direct	CA with spasticity	c.1529C>T	c.1454_1462del	p.(Ala510Val)	p.(Arg485_Glu487del)	+	+	-	+	-	-	-
11	M	35	6	Direct	CA with spasticity, waddling gait	c.1529C>T	c.1192C>T	p.(Ala510Val)	p.(Arg398*)	+	+	+	-	-	-	+
12	M	30	6	Direct	CA with spasticity	c.1529C>T	c.1529C>T	p.(Ala510Val)	p.(Ala510Val)	+	+	-	+	-	-	+
13	M	44	6	Direct	CA with spasticity, waddling gait	c.1529C>T	c.1529C>T	p.(Ala510Val)	p.(Ala510Val)	+	+	+	?	-	-	?
14	M	37	18	Direct	CA with spasticity, waddling gait	c.233T>A	c.233T>A	p.(Leu78*)	p.(Leu78*)	+	+	+	+	-	+	+
15	M	37	8	Direct	CA with spasticity	c.1529C>T	c.861+2dupT	p.(Ala510Val)	p.(Asn288*)	+	+	-	+	-	-	-
16	M	46	5	Direct	CA with spasticity, waddling gait	c.1529C>T	c.1715C>T	p.(Ala510Val)	p.(Ala572Val)	+	+	+	+	-	-	-

17	M	57	4	Direct	HSP - complicated	c.1529C>T	c.1529C>T	p.(Ala510Val)	p.(Ala510Val)	+	+	-	+	-	-	?
18	M	29	10	Direct	CA with spasticity, waddling gait	c.1529C>T	c.1192C>T	p.(Ala510Val)	p.(Arg398*)	+	++	+	+	-	-	+
19	M	48	6	Direct	HSP - complicated	c.1529C>T	c.1045G>A	p.(Ala510Val)	p.(Gly349Ser)	+	+	-	+	-	-	-
20	M	44	10	Direct	HSP - complicated	c.1529C>T	c.1529C>T	p.(Ala510Val)	p.(Ala510Val)	+	+	-	+	-	-	-
21	F	35	10	Direct	CA with spasticity, waddling gait	c.1529C>T	c.1672 A>T	p.(Ala510Val)	p.(Lys558*)	+	+	+	-	-	-	+
22	M	55	12	HSP	HSP - pure	c.1529C>T	**c.775_781dup**	p.(Ala510Val)	**p.(Thr261fs)**	-	+++		-	-	-	+
23	M	50	7	Direct	CA with spasticity, waddling gait	c.1529C>T	c.1053dupC	p.(Ala510Val)	p.(Gly352fs)	+	+	+	+	-	-	-
24	F	48	20	Direct	CA with spasticity, waddling gait	c.1529C>T	c.861+2dupT	p.(Ala510Val)	p.(Asn288*)	+	+	+	+	-	-	+
25	M	31	10	Direct	CA with spasticity, waddling gait	c.1529C>T	c.1529C>T	p.(Ala510Val)	p.(Ala510Val)	+	+	+	+	-	-	?
26	M	34	10	Direct	CA with spasticity, waddling gait	c.1529C>T	c.1529C>T	p.(Ala510Val)	p.(Ala510Val)	?	N	+	+	-	-	-
27	M	48	7	Direct	CA with spasticity, waddling gait	c.1529C>T	c.1529C>T	p.(Ala510Val)	p.(Ala510Val)	+	+	+	+	-	-	+
28	M	36	5	Ataxia	CA	c.1529C>T	c.1529C>T	p.(Ala510Val)	p.(Ala510Val)	+	N	+	+	-	-	+
29	F	15	20	Direct	CA with spasticity, waddling gait	c.1529C>T	c.1904C>T	p.(Ala510Val)	p.(Ser635Leu)	+	N	+	+	-	-	+
30	F	45	8	Ataxia	CA	c.233T>A	c.233T>A	p.(Leu78*)	p.(Leu78*)	+	+	+	+	-	-	+
31	M	30	20	HSP	HSP - complicated	c.1529C>T	c.1045G>A	p.(Ala510Val)	p.(Gly349Ser)	+	+	+	+	-	-	+
32	M	46	6	HSP	HSP - complicated	c.1181T>C	c.1045G>A	p.(Phe394Ser)	p.(Gly349Ser)	+	+	+	+	-	-	+
33	M	55	7	Direct	CA with spasticity, waddling gait	c.1529C>T	**c.2096dup**	p.(Ala510Val)	**p.(Met699fs)**	+	+	+	-	-	-	-
34	M	42	5	Direct	CA with spasticity	c.1529C>T	c.1529C>T	p.(Ala510Val)	p.(Ala510Val)	+	+	?	+	-	-	?
35	M	46	3	Direct	CA with spasticity, waddling gait	c.1529C>T	**c.377-?_1324+? del (exon 4-9)**	p.(Ala510Val)	**p.?**	+	+	+	+	+	-	?
36	F	58	20	Ataxia	CA	c.1529C>T	**c.2083C>G**	p.(Ala510Val)	**p.(Leu695Val)**	+	N	-	+	-	-	-
37	M	40	12	Direct	CA with spasticity, waddling gait	c.1529C>T	**c.754G>T**	p.(Ala510Val)	**p.(Gly252*)**	+	+	+	+	-	-	+
38	M	43	16	Direct	CA with spasticity and waddling gait	c.233T>A	c.233T>A	p.(Leu78*)	p.(Leu78*)	+	+	+	-	-	-	+

39	M	44	10	HSP	HSP - complicated- ataxia waddling	c.1529C>T	c.1454_1462del	p.(Ala510Val)	p.(Arg485_Glu487del)	+	++	++	-	-	-	-
40	F	45	12	Ataxia	CA and mild spasticity but no waddling	c.1529C>T	**c.300 T>A**	p.(Ala510Val)	**p.(Tyr100*)**	++	+	-	++	-	-	+
Reference sequence: NM_003119.3. (Key: +: mild (or feature present), ; ++: moderate severity, +++: severe, ?: unknown; N: normal; -: reduced (or feature not present). In Bold are new mutations detected.																	

Table 2. Phenotypic description of the patients with SPG7 mutation

Features			Index cases (%)
Male: female ratio			33 : 7
Mean age at onset in years (SD)			41.7 (±11)
Mean age of onset for Males in years (SD)			46.6 (±10)
Mean age of onset for Females in years (SD)			38.5 (±15)
Mean disease duration at examination in years (range)			9.2 (3-20years)
Symptoms at presentation	Impaired balance		39/40 (98%)
	Slurred speech		30/40 (75%)
	Stiffness		30/40 (75%)
	Leg weakness		8/40 (20%)
Other symptoms at presentation	Cognitive disturbance		5/40 (13%)
	Deafness		1/40 (2.5%)
	Bladder disturbance		20/40 (50%)
	Muscle weakness		19/40 (48%)
Cranial nerve examination	Nystagmus		15/40 (38%)
	Vertical gaze palsy		1/40 (2.5%)
	Horizontal gaze palsy and limited vertical gaze		1/40 (2.5%)
	Optic atrophy		1/40 (2.5%)
Cerebellar signs			36/40 (90%)
	Dysdiadochokinesia		16/33 (48%)
	Finger nose test impaired		13/33 (39%)
	Heel shin test impaired		22/33 (67%)
	Cerebellar dysarthria		30/40 (75%)
	Pure spastic gait		2/40 (5%)
	Upper limbs	*Normal*	25/40 (63%)

Muscle tone		*Increased tone*	5/32 (16%)
	Lower limbs	*Normal or reduced*	3/39 (8%)
		Increased tone	37/40 (93%)
Power	Upper limbs	*Normal*	31/34 (91%)
		Reduced	3/34 (9%)
	Lower limbs	*Normal*	31/40 (78%)
		Reduced	20/40 (50%)
Sensation	Vibration	*Normal*	29/40 (73%)
		Reduced	4/33 (12%)
	Pin-prick	*Normal*	30/33 (91%)
		Reduced	3/33 (9%)
	Joint position sense	*Normal*	33/33 (100%)
		Reduced	0/33 (0%)
Tendon reflexes	Upper & lower limbs	*Normal or reduced (1)*	3/40 (8%)
		Brisk	37/40 (93%)
	Babinski	*Positive*	19/37 (51%)
		Negative	18/37 (49%)
Gait	Spastic ataxia		31/40 (78%)
	Pure cerebellar gait		5/40 (13%)
	Spastic waddling gait		4/40 (10%)
MRI Brain	Cerebellar atrophy		35/38 (92%)

Supplementary table 1 (uploaded separately)

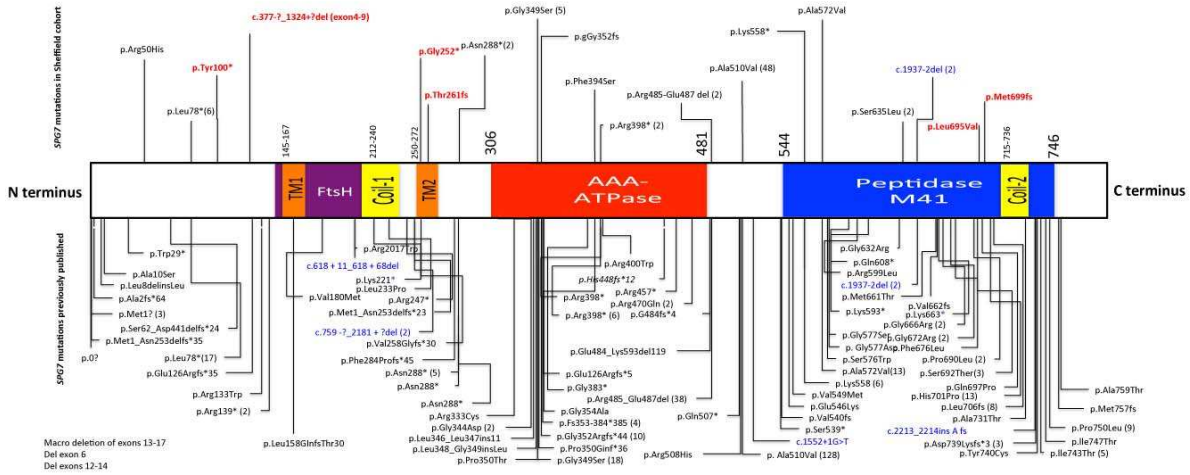
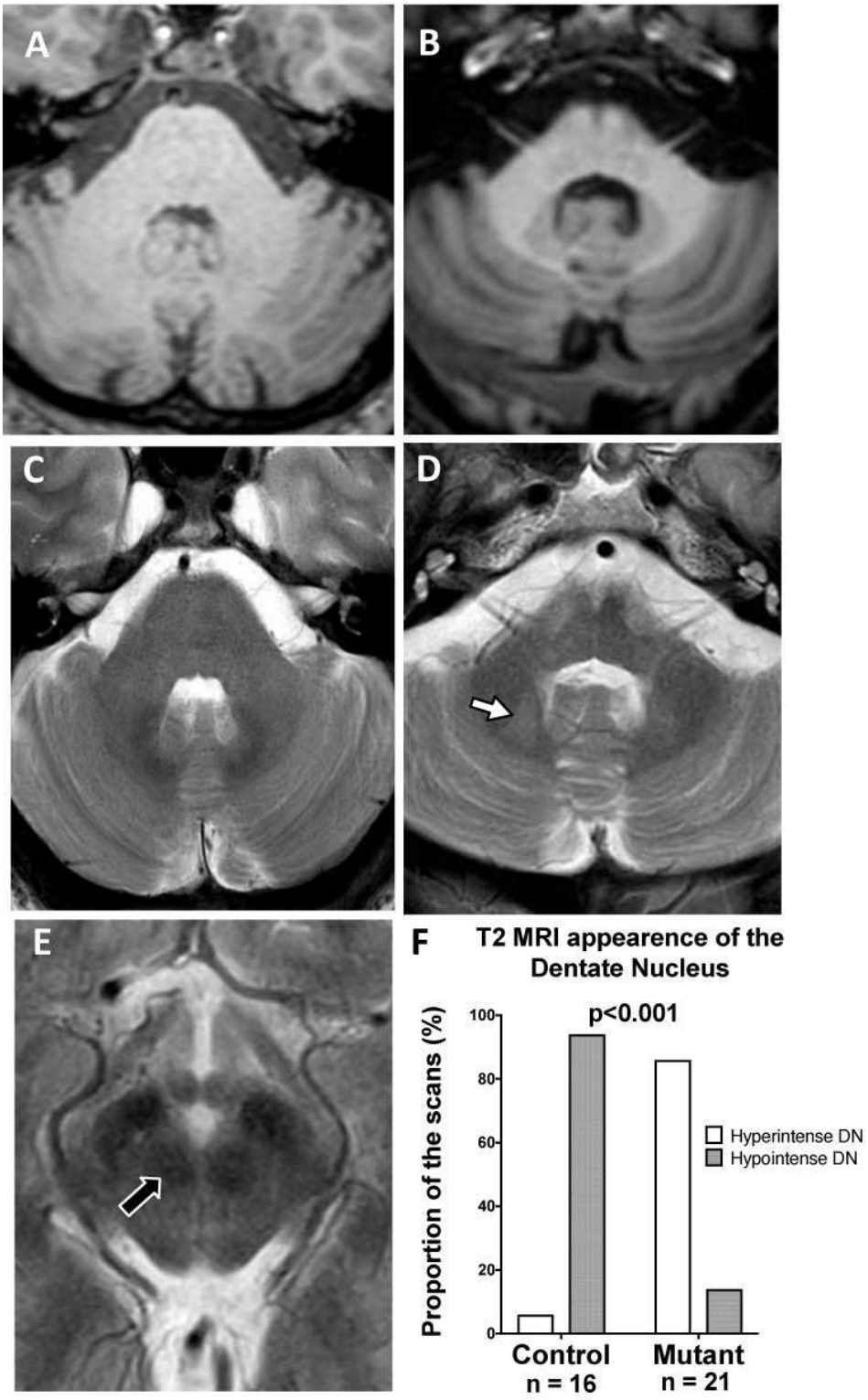

SPG7 mutations in Sheffield cohort
SPG7 mutations previously published
N terminus
C terminus
TM1
FtsH
Coil-1
TM2
AAA-ATPase
Peptidase M41
Coil-2
145-167
212-240
250-272
306
481
544
715-736
746
p.Arg50His
p.Tyr100*
p.Leu78*(6)
c.377-?_1324+?del (exon4-9)
p.Gly252*
p.Thr261fs
p.Asn288*(2)
p.Gly349Ser (5)
p.gGy352fs
p.Phe394Ser
p.Arg485-Glu487 del (2)
p.Arg398* (2)
p.Lys558*
p.Ala572Val
p.Ala510Val (48)
c.1937-2del (2)
p.Ser635Leu (2)
p.Leu695Val
p.Met699fs
p.Trp29*
p.Ala10Ser
p.Leu8delinsLeu
p.Ala2fs*64
p.Met1? (3)
p.Ser62_Asp441delfs*24
p.Met1_Asn253delfs*35
p.0?
p.Leu78*(17)
p.Glu126Argfs*35
p.Arg133Trp
p.Arg139* (2)
p.Leu158GlnfsThr30
p.Arg201Trp
c.618+11_618+68del
p.Lys221*
p.Leu233Pro
p.Val180Met
p.Arg247*
p.Met1_Asn253delfs*23
c.759-?_2181+?del (2)
p.Val258Glyfs*30
p.Phe284Profs*45
p.Asn288* (5)
p.Asn288*
p.Asn288*
p.Arg333Cys
p.Gly344Asp (2)
p.Leu346_Leu347ins11
p.Leu348_Gly349InsLeu
p.Pro350Thr
p.Arg400Trp
p.His448fs*12
p.Arg398*
p.Arg457*
p.Arg470Gln (2)
p.Arg398* (6)
p.G484fs*4
p.Glu484_Lys593del119
p.Glu126Argfs*5
p.Gly383*
p.Arg485_Glu487del (38)
p.Gly354Ala
p.Fs353-384*385 (4)
p.Gly352Argfs*44 (10)
p.Pro350Ginf*36
p.Gly349Ser (18)
p.Gln507*
p.Arg508His
p.Gly632Arg
p.Gln608*
p.Arg599Leu
c.1937-2del (2)
p.Met661Thr
p.Lys593*
p.Gly577Ser
p. Gly577Asp
p.Ala572Val(13)
p.Lys558 (6)
p.Val549Met
p.Glu546Lys
p.Val540fs
p.Ser539*
c.1552+1G>T
p. Ala510Val (128)
p.Val662fs
p.Lys663*
p.Gly666Arg (2)
p.Gly672Arg (2)
p.Phe676Leu
p.Ser576Trp
p.Pro690Leu (2)
p.Ser692Ther(3)
p.Gln697Pro
p.His701Pro (13)
p.Leu706fs (8)
p.Ala731Thr
c.2213_2214ins A fs
p.Asp739Lysfs*3 (3)
p.Tyr740Cys
p.Ala759Thr
p.Met757fs
p.Pro750Leu (9)
p.Ile747Thr
p.Ile743Thr (5)
Macro deletion of exons 13-17
Del exon 6
Del exons 12-14

Figure 1


A
B
C
D
E
F
T2 MRI appearence of the Dentate Nucleus
p<0.001
Proportion of the scans (%)
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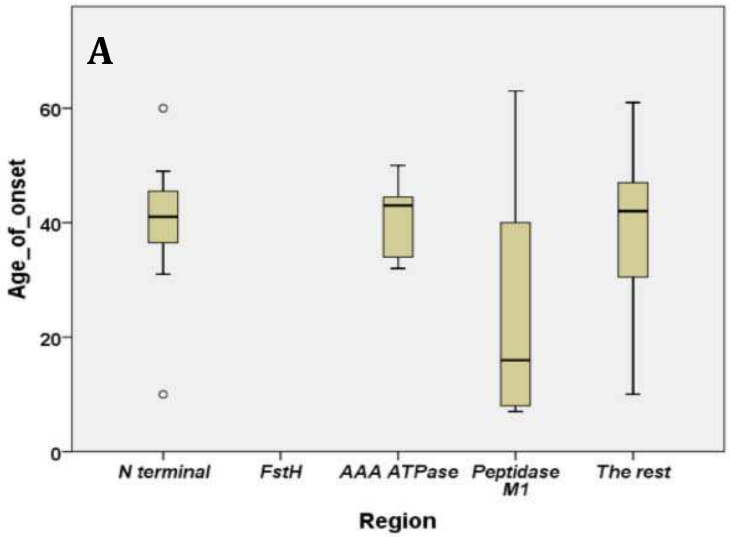
60
40
20
0
Hyperintense DN
Hypointense DN
Control
n = 16
Mutant
n = 21

Figure 2

Figure 03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40
20
0
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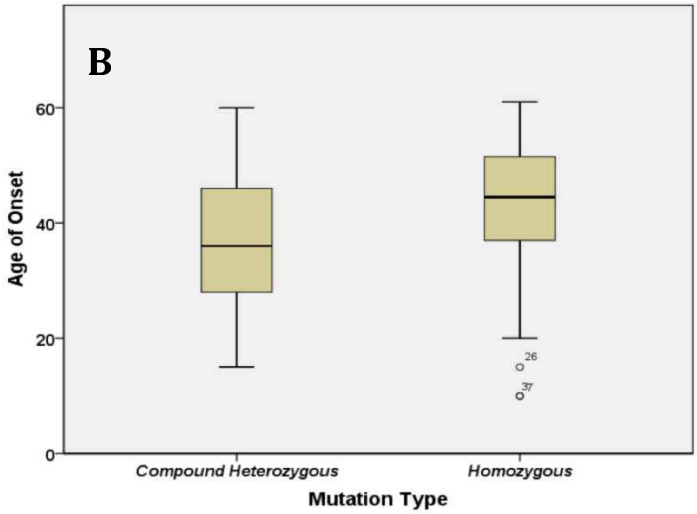
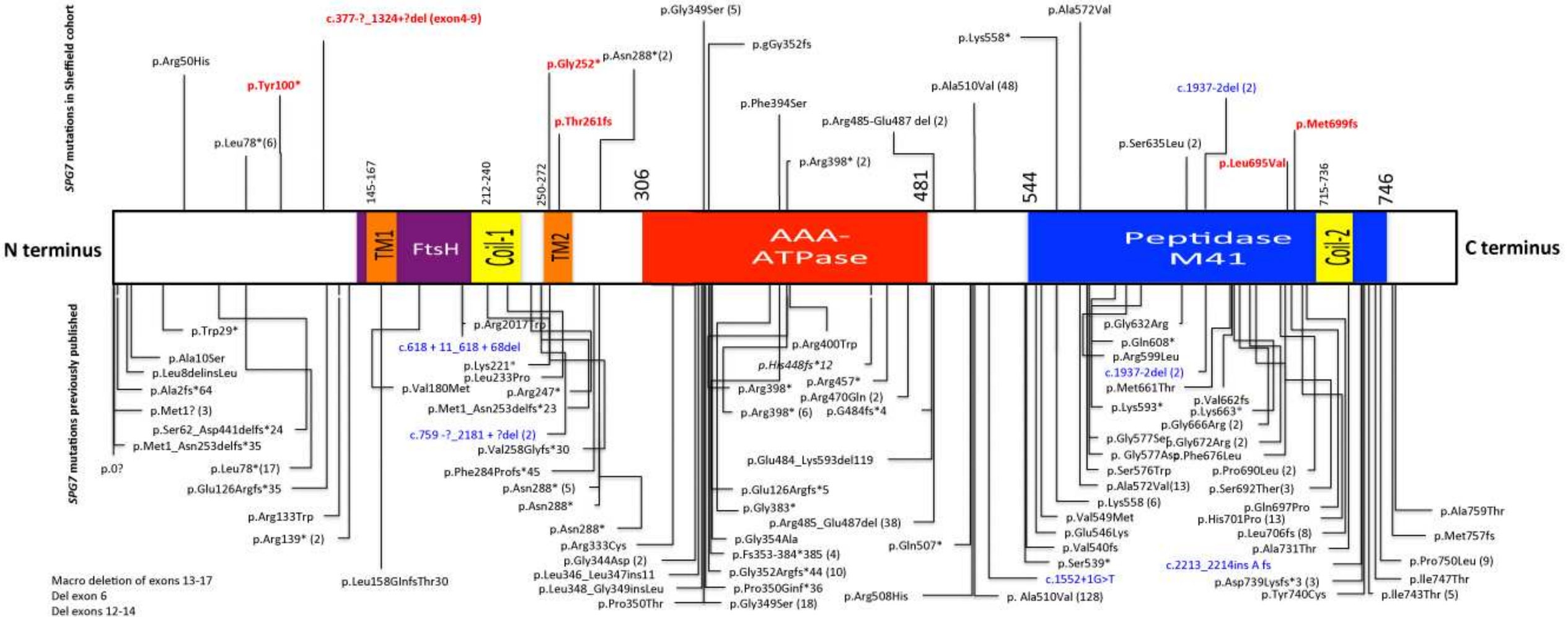
FstH
AAA ATPase
Peptidase M1
The rest
Region


B
Age of Onset
60
40
20
0
26
37
Compound Heterozygous
Homozygous
Mutation Type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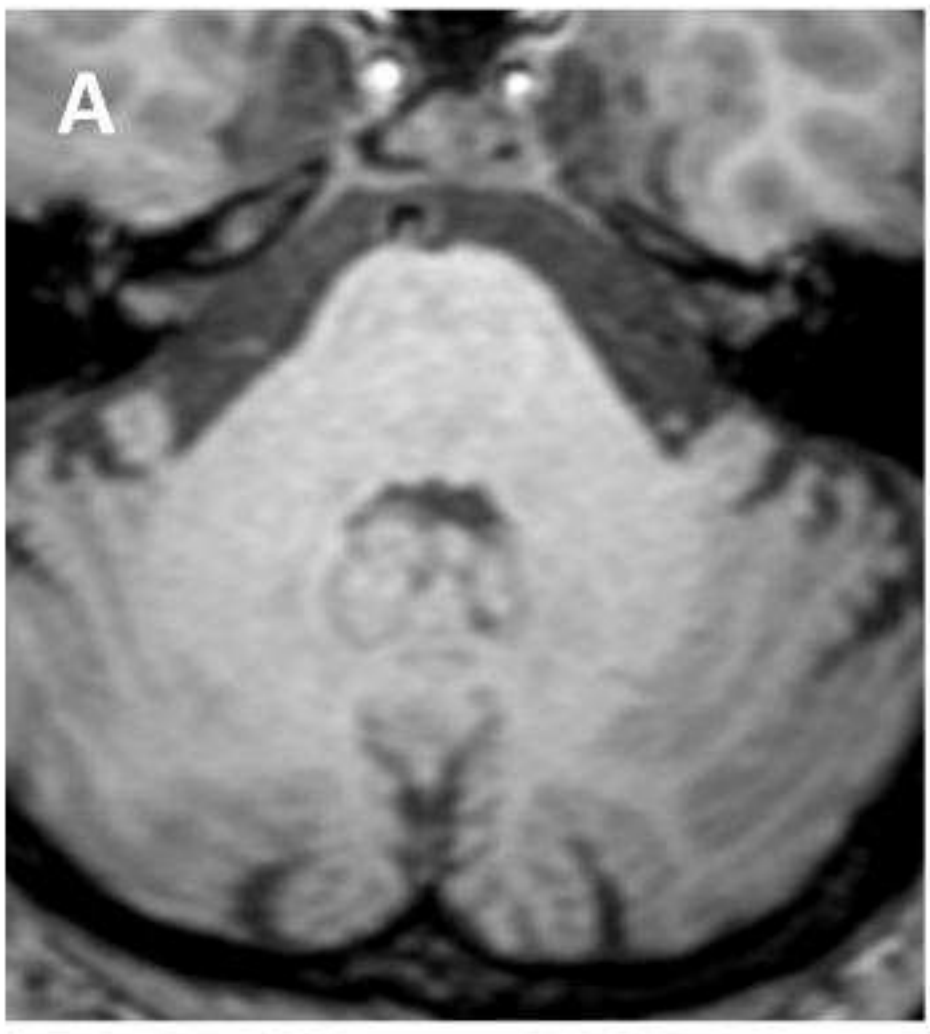
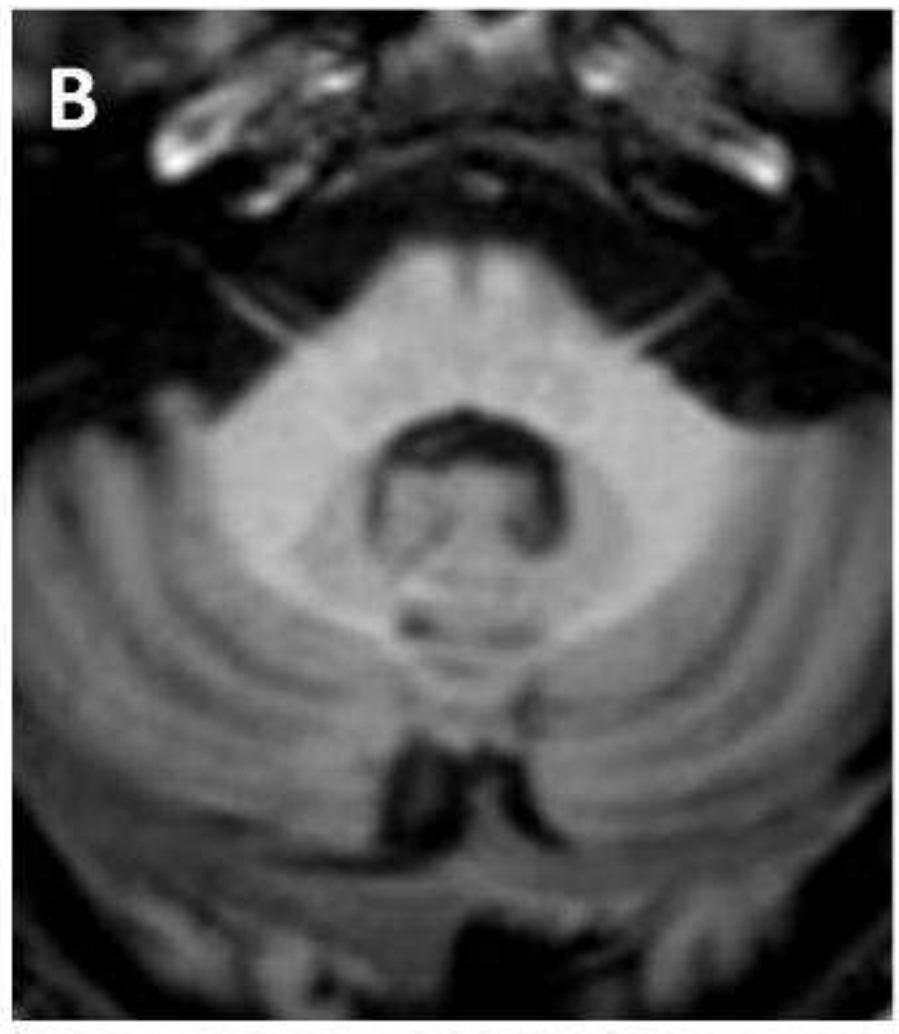
p.Arg398* (2)
p.Arg485-Glu487 del (2)
p.Lys558*
p.Ala510Val (48)
p.Ala572Val
c.1937-2del (2)
p.Ser635Leu (2)
p.Leu695Val
p.Met699fs
p.Trp29*
p.Ala10Ser
p.Leu8delinsLeu
p.Ala2fs*64
p.Met1? (3)
p.Ser62_Asp441delfs*24
p.Met1_Asn253delfs*35
p.0?
p.Leu78*(17)
p.Glu126Argfs*35
p.Arg133Trp
p.Arg139* (2)
p.Leu158GInfsThr30
p.Arg201Trp
c.618 + 11_618 + 68del
p.Lys221*
p.Leu233Pro
p.Val180Met
p.Arg247*
p.Met1_Asn253delfs*23
c.759 -?_2181 + ?del (2)
p.Val258Glyfs*30
p.Phe284Profs*45
p.Asn288* (5)
p.Asn288*
p.Asn288*
p.Arg333Cys
p.Gly344Asp (2)
p.Leu346_Leu347ins11
p.Leu348_Gly349insLeu
p.Pro350Thr
p.Arg400Trp
p.His448fs*12
p.Arg398*
p.Arg457*
p.Arg470Gln (2)
p.Arg398* (6)
p.G484fs*4
p.Glu484_Lys593del119
p.Glu126Argfs*5
p.Gly383*
p.Arg485_Glu487del (38)
p.Gly354Ala
p.Fs353-384*385 (4)
p.Gly352Argfs*44 (10)
p.Pro350Ginf*36
p.Gly349Ser (18)
p.Gln507*
p.Arg508His
p. Ala510Val (128)
p.Gly632Arg
p.Gln608*
p.Arg599Leu
c.1937-2del (2)
p.Met661Thr
p.Lys593*
p.Val662fs
p.Lys663*
p.Gly666Arg (2)
p.Gly577Se
p.Gly672Arg (2)
p. Gly577Asp
p.Phe676Leu
p.Ser576Trp
p.Ala572Val(13)
p.Lys558 (6)
p.Val549Met
p.Glu546Lys
p.Val540fs
p.Ser539*
c.1552+1G>T
p.Pro690Leu (2)
p.Ser692Ther(3)
p.Gln697Pro
p.His701Pro (13)
p.Leu706fs (8)
p.Ala731Thr
c.2213_2214ins A fs
p.Asp739Lysfs*3 (3)
p.Tyr740Cys
p.Ala759Thr
p.Met757fs
p.Pro750Leu (9)
p.Ile747Thr
p.Ile743Thr (5)
Macro deletion of exons 13-17
Del exon 6
Del exons 12-14

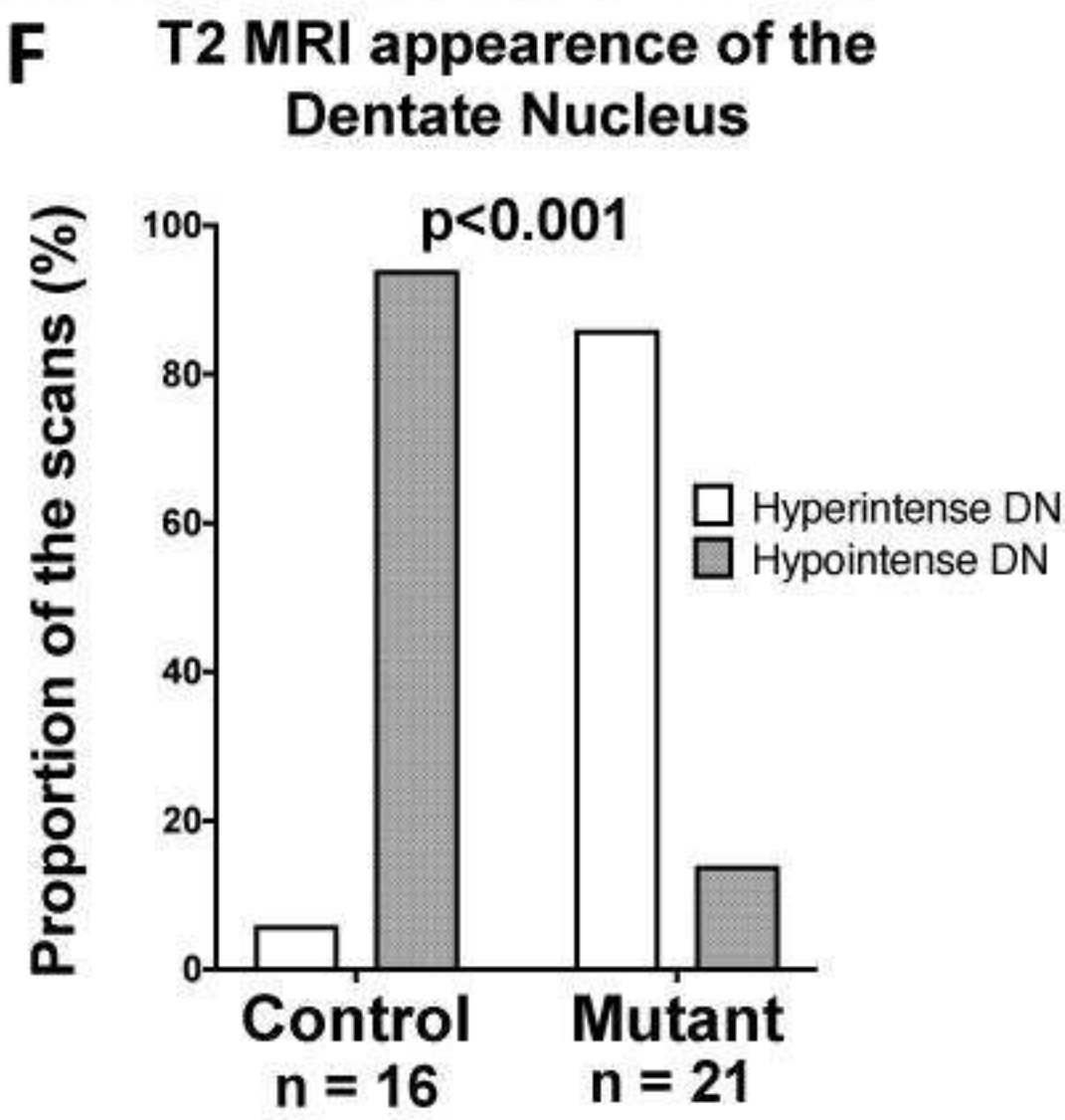

A

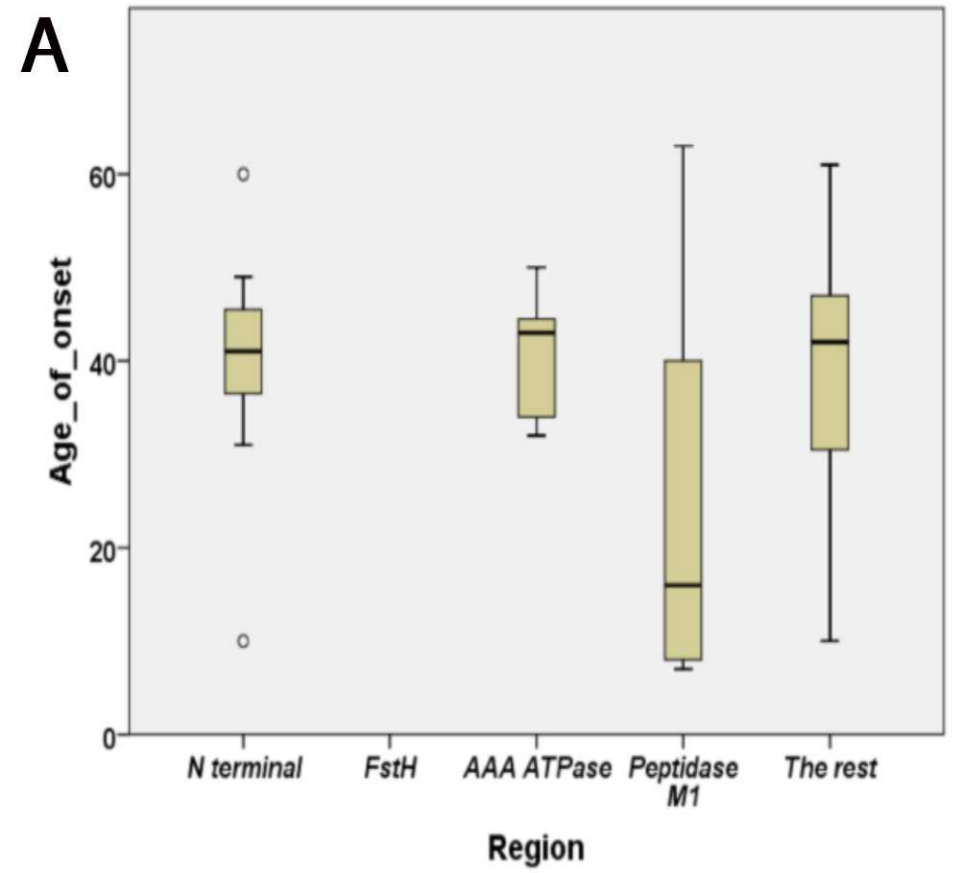

B

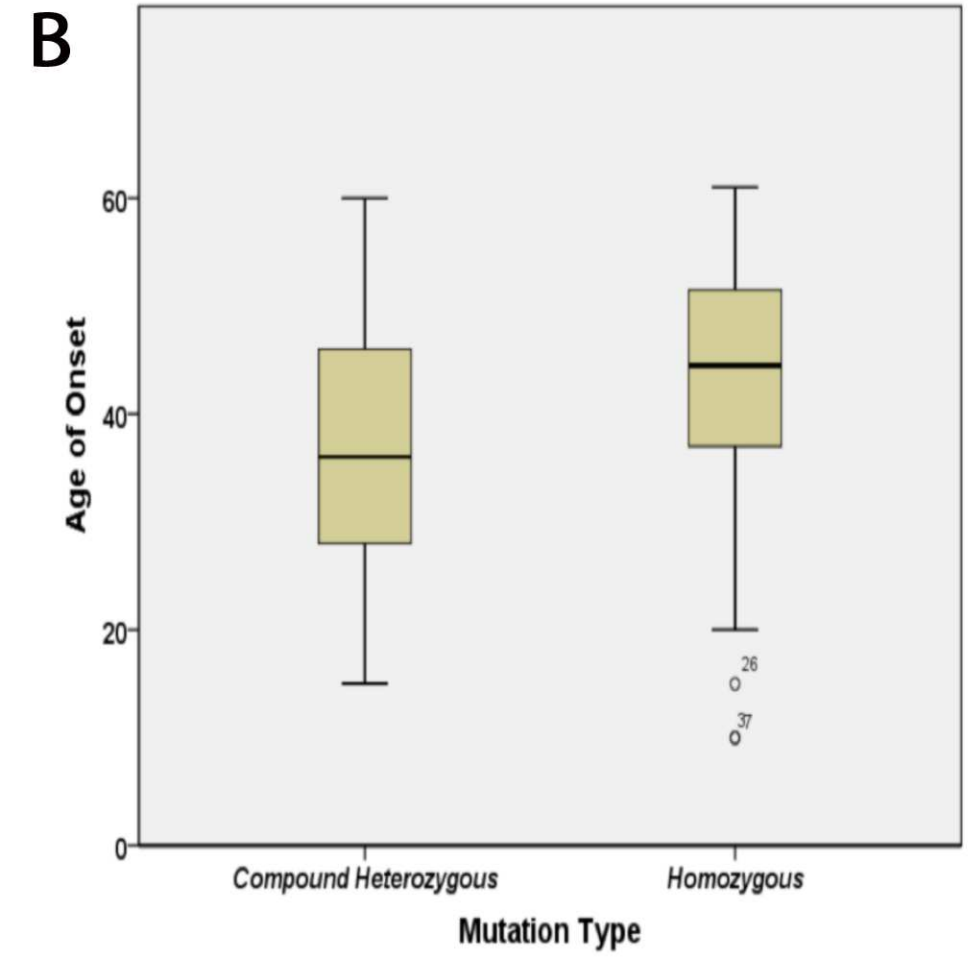

C


D


E


F
T2 MRI appearence of the Dentate Nucleus
p<0.001
Proportion of the scans (%)
100
80
60
40
20
0
Hyperintense DN
Hypointense DN
Control
n = 16
Mutant
n = 21


A
Age_of_onset
60
40
20
0
N terminal
FstH
AAA ATPase
Peptidase M1
The rest
Region


B
Age of Onset
60
40
20
0
26
37
Compound Heterozygous
Homozygous
Mutation Type