Neurogenetics in Argentina: diagnostic yield in a personalized research based clinic

SERGIO ALEJANDRO RODRÍGUEZ-QUIROGA[1,2], MARTA CORDOBA[1],
DOLORES GONZÁLEZ-MORÓN[1,3], NANCY MEDINA[1], PATRICIA VEGA[1],
CECILIA VAZQUEZ DUSEFANTE[1], TOMOKO ARAKAKI[2],
NÉLIDA SUSANA GARRETTO[3] AND MARCELO ANDRES KAUFFMAN[1,4]*

[1]*Consultorio y Laboratorio de Neurogenética, Centro Universitario de Neurología "José María Ramos Mejía" y División Neurología, Hospital JM Ramos Mejía, Facultad de Medicina, UBA, Buenos Aires, Argentina*
[2]*Área de Trastornos del Movimiento, Centro Universitario de Neurología "José María Ramos Mejía" y División Neurología, Hospital JM Ramos Mejía, Facultad de Medicina, UBA, Buenos Aires, Argentina*
[3]*Consultorio de Neurooftalmología, Centro Universitario de Neurología "José María Ramos Mejía" y División Neurología, Hospital JM Ramos Mejía, Facultad de Medicina, UBA, Buenos Aires, Argentina*
[4]*IBCN "Eduardo de Robertis", Facultad de Medicina, UBA-CONICET, Buenos Aires, Argentina*

Summary

As a whole neurogenetic diseases are a common group of neurological disorders. However, the recognition and molecular diagnosis of these disorders is not always straightforward. Besides, there is a paucity of information regarding the diagnostic yield that specialized neurogenetic clinics could obtain. We performed a prospective, observational, analytical study of the patients seen in a neurogenetic clinic at a tertiary medical centre to assess the diagnostic yield of a comprehensive diagnostic evaluation that included a personalized clinical assessment along with traditional and next-generation sequencing diagnostic tests. We included a cohort of 387 patients from May 2008 to June 2014. For sub-group analysis we selected a sample of patients whose main complaint was the presence of progressive ataxia, to whom we applied a systematic molecular diagnostic algorithm. Overall, a diagnostic mutation was identified in 27·4% of our cohort. However, if we only considered those patients where a molecular test could be performed, the success rate rises to 45%. We obtained diagnostic yields of 23·5 and 57·5% in the global group of ataxic patients and in the subset of ataxic patients with a positive family history, respectively. Thus, about a third of patients evaluated in a neurogenetic clinic could be successfully diagnosed.

1. Introduction

The increasing availability of molecular diagnostic tools has allowed for many advances in neurogenetics that have contributed to a better understanding of the role of genes in different diseases, even in those that classically were considered as non-genetic; e.g. the discovery of LRRK2 as a cause of late-onset typical Parkinson´s disease (Corti *et al.*, 2011). Moreover, disorders traditionally considered and classified as unitary, as a consequence of the advances in neurogenetics, are now unfolded in dozens of individual diseases, such as spinocerebellar ataxias (SCAs) (Matilla-Duenas *et al.*, 2012) and hereditary spastic paraparesis (Schule & Schols, 2011).

However, the recognition and molecular diagnosis of these disorders is not always straightforward. Besides the high cost of genetic studies that are particularly relevant in less developed countries such as Argentina, the inherent clinical complexity of these disorders make a common theme of diagnostics odysseys that last for many years and that frequently end with non-diagnostic results. Although, this situation is well acknowledged, there is a surprising paucity of empirical data regarding the utility and diagnostic yield of the comprehensive work that could be accomplished in neurogenetic clinics.

We performed a prospective, observational, analytical study of the patients seen in a neurogenetic clinic

* Corresponding author: Consultorio de Neurogenética – División Neurología, Hospital JM Ramos Mejía, Urquiza 609 (1221), Buenos Aires, Argentina, 541141270280. E-mail: marcelokauffman@marcelokauffman.info

at a tertiary medical centre in Buenos Aires, Argentina. We assessed the diagnostic profile of this unselected cohort of subjects affected with diverse neurological conditions, which presumably were considered to have a genetic etiology by the referring physician, as well as the diagnostic yield of a comprehensive diagnostic evaluation, which included a personalized clinical assessment along with traditional and next-generation sequencing diagnostic tests.

2. Materials and methods

(i) *Patients*

We prospectively included a cohort of 387 patients that were referred to our neurogenetic clinic in a tertiary neurology service at a public hospital in Buenos Aires, Argentina, from May 2008 to June 2014. All of them gave their informed consent to freely participate in this research study. The institutional ethics committee of our institution approved this study. We used a structured clinical interview in order to register demographic characteristics, familial history and the clinical features of the disease that motivated their consultation. For sub-group analysis we selected a sample of patients whose main complaint was the presence of progressive ataxia of non-structural etiology, defined as the absence of space-occupying lesions, vascular malformations, or ischemic or hemorrhagic injuries in the brainstem or cerebellum that could reasonably explain their symptoms. We applied a systematic molecular diagnostic algorithm in this sub-group.

(ii) *Molecular studies*

Different strategies were used for studying molecular genetic alterations in each individual case according to the clinical presentation observed. Although it is not always possible to systematize the complex decision-making process involved in the diagnostic approach of complex and rare disorders such as neurogenetic diseases, we tried to guide the individual approaches on the basis of algorithms and guidelines proposed for the evaluation of each one of the diverse neurological conditions (Fogel & Perlman, 2007; England *et al.*, 2009; Finsterer *et al.*, 2009; Harbo *et al.*, 2009; Gasser *et al.*, 2010; Burgunder *et al.*, 2011; Siskind & Shy, 2011; Patterson *et al.*, 2012; Kauffman, 2013; Fogel *et al.*, 2014). The molecular studies included DNA fragment sizing using capillary electrophoresis for trinucleotide-repeat disorders, Sanger sequencing of candidate genes, massively-parallel pyrosequencing for mitochondrial diseases and whole-exome sequencing for more genetically heterogeneous disorders. When the main complaint was progressive ataxia, we systematically investigated the presence of pathological alterations in FXN, ATXN1, ATXN2, ATXN3, CACNA1A, ATXN8 and TBP/SCA17 genes through fragment sizing using capillary electrophoresis. Details of each individual reaction are available on request. We used the following bioinformatic tools and databases for the characterization of the obtained sequences and inference of pathogenicity: ENSEMBL (Flicek *et al.*, 2012), Mutation@A Glance (Hijikata *et al.*, 2010), SIFT (Sim *et al.*, 2012), POLYPHEN2 (Zou *et al.*, 2011) and Mutation Taster (Schwarz *et al.*, 2010).

3. Results

(i) *Global analysis*

During a 6-year period, we evaluated 387 patients in our neurogenetics clinic. The average age of our cohort was about 41 years, involving a wide range of ages that spanned from children to the elderly (<1 to 86 years old). A similar number of females and males attended our clinic. The mean time from symptom onset until the first evaluation in our centre was of 12·5 years (newborn to 77 years); if we only considered those patients where a molecular test could be performed, this lapse is reduced to an average of 9 years (newborn to 36 years). We were able to identify a genetic cause in 106 patients, which gives an overall diagnostic success rate of 27·4% (Table 1); if we only considered the group of patients where genetic tests could be performed because they were available in our laboratory, the success rate rises to 45% (106 confirmed diagnoses in 235 studied patients).

(ii) *Chronic and progressive ataxias program*

We implemented a program with the purpose of systematizing the assistance of chronic and progressive ataxic patients. We included a total of 140 patients. A summary of the clinical and demographic features is presented in Table 2. About a third of them had a positive family history for a similar condition. The vast majority of families were compatible with an autosomal dominant pattern of inheritance (86%). We identified the genetic cause in 33 patients (23·5%); if we only analyze the population with a positive family history the success rate to reach a definitive diagnosis increases up to 57·5% (Table 2). The most frequent causes of ataxia were SCA2, SCA3 and Friedreich ataxia. We also identified abnormalities in SCA1 and SCA7 genes as genetic etiologies. We were able to diagnose two cases of familial Gerstmann-Sträussler-Scheinker, Huntington's disease in a patient where his first symptom was the presence of ataxia, Niemann Pick type C in a 29 year old woman with 8 years of ataxia associated with psychiatric symptoms and cognitive impairment, and ataxia with oculomotor apraxia type 1 (AOA-1) in a 10 year old child with symptom onset when she was 3 years old.

Table 1. *Patients with molecular confirmatory diagnosis.*

Patient (n)	Age	Sex	Family history	Inheritance	Evolution of the disease until the diagnosis (years)	Diagnosis	Gene	Mutation
1	10	F	No	Sporadic	7	Ataxia – AOA1	APTX	NM_175069·1:c.879G > A; p.Trp293X
2	21	M	Yes	AR	9	Ataxia – Friedreich ataxia	FXN	Abnormal GAA repeat
3	18	F	Yes	AR	6	Ataxia – Friedreich ataxia	FXN	Abnormal GAA repeat
4	18	M	Yes	AR	2	Ataxia – Friedreich ataxia	FXN	Abnormal GAA repeat
5	14	F	No	Sporadic	6	Ataxia – Friedreich ataxia	FXN	Abnormal GAA repeat
6	22	M	No	Sporadic	17	Ataxia – Friedreich ataxia	FXN	Abnormal GAA repeat
7	36	F	No	Sporadic	19	Ataxia – Friedreich ataxia	FXN	Abnormal GAA repeat
8	18	M	No	Sporadic	4	Ataxia – Friedreich ataxia	FXN	Abnormal GAA repeat
9	32	M	No	Sporadic	9	Ataxia – Friedreich ataxia	FXN	Abnormal GAA repeat
10	37	F	Yes	AR	24	Ataxia – Friedreich ataxia	FXN	Abnormal GAA repeat
11	39	F	Yes	AD	11	Ataxia – SCA1	ATXN1	Abnormal CAG trinucleotide repeat expansion (32/54 repeats)
12	30	F	Yes	AD	1	Ataxia – SCA1	ATXN1	Abnormal CAG trinucleotide repeat expansion (31/48 repeats)
13	29	F	Yes	AD	1	Ataxia – SCA1	ATXN1	Abnormal CAG trinucleotide repeat expansion (32/53 repeats)
14	44	F	Yes	AD	6	Ataxia – SCA1	ATXN1	Abnormal CAG trinucleotide repeat expansion (30/50 repeats)
15	39	M	Yes	AD	9	Ataxia – SCA1	ATXN1	Abnormal CAG trinucleotide repeat expansion (30/48 repeats)
16	55	M	Yes	AD	10	Ataxia – SCA2	ATXN2	Abnormal CAG trinucleotide repeat expansion (20/38 repeats)
17	30	M	Yes	AD	7	Ataxia – SCA2	ATXN2	Abnormal CAG trinucleotide repeat expansion (22/43 repeats)
18	30	M	No	Sporadic	13	Ataxia – SCA2	ATXN2	Abnormal CAG trinucleotide repeat expansion (22/41 repeats)
19	32	M	Yes	AD	9	Ataxia – SCA2	ATXN2	Abnormal CAG trinucleotide repeat expansion (22/43 repeats)
20	14	F	Yes	AD	13	Ataxia – SCA2	ATXN2	Abnormal CAG trinucleotide repeat expansion (22/51 repeats)
21	43	F	Yes	AD	10	Ataxia – SCA2	ATXN2	Abnormal CAG trinucleotide repeat expansion (22/39 repeats)
22	25	F	Yes	AD	2	Ataxia – SCA2	ATXN2	Abnormal CAG trinucleotide repeat expansion (22/40 repeats)
23	31	F	Yes	AD	6	Ataxia – SCA2	ATXN2	Abnormal CAG trinucleotide repeat expansion (22/44 repeats)
24	45	M	Yes	AD	10	Ataxia – SCA3[a]	ATXN3	Abnormal CAG trinucleotide repeat expansion (23/64 repeats)
25	54	M	Yes	AD	3	Ataxia – SCA3[a]	ATXN3	Abnormal CAG trinucleotide repeat expansion (19/63 repeats)

Table 1. (*Cont.*)

Patient (n)	Age	Sex	Family history	Inheritance	Evolution of the disease until the diagnosis (years)	Diagnosis	Gene	Mutation
26	32	F	Yes	AD	3	Ataxia – SCA3[a]	ATXN3	Abnormal CAG trinucleotide repeat expansion (23/71 repeats)
27	46	F	Yes	AD	10	Ataxia – SCA3	ATXN3	Abnormal CAG trinucleotide repeat expansion (31/62 repeats)
28	65	M	Yes	AD	13	Ataxia – SCA3	ATXN3	Abnormal CAG trinucleotide repeat expansion (19/62 repeats)
29	66	F	Yes	AD	3	Ataxia – SCA6	CACNA1A	Abnormal CAG trinucleotide repeat expansion (9/20 repeats)
30	32	F	Yes	AD	5	Ataxia – SCA7	ATXN7	Abnormal CAG trinucleotide repeat expansion (7/66 repeats)
31	24	M	No	Sporadic	7	Ataxia – STUB1[b]	STUB1	NM_005861·2:c.[612 + 1 G > C]; [823C > G]; p.[?]; [Leu275Val]
32	43	F	Yes	AD	4	Ataxia – Gerstmann-Sträussler-Scheinker disease	PRNP	NM_000311·3:c.305C > T; p.Pro102Leu
33	47	F	Yes	AD	1	Ataxia – Gerstmann-Sträussler-Scheinker disease	PRNP	NM_000311·3:c.305C > T; p.Pro102Leu
34	39	F	Yes	AD	20	Autosomal dominant CPEO	C10orf2 (PEO1)	NM_021830·4:c.1001G > A; p.Arg334Gln
35	64	M	Yes	AD or maternal	20	Autosomal dominant CPEO	C10orf2 (PEO1)	NM_021830·4:c.1433 T > G; p.Phe478Cys
36	53	F	Yes	AD	32	CADASIL	NoTCH3	NM_000435·2:c.635:G > T; p.Cys212Phe
37	21	M	No	AR (consanguinity)	21	Cerebrotendinous xanthomatosis	CYP27A1	NM_000784·3:c.1234C > T; p.Arg405Trp
38	51	M	Yes	AR	49	Cerebrotendinous xanthomatosis	CYP27A1	NM_000784·3:c.1183C > T; p.Arg395Cys
39	55	F	Yes	AD	5	CMT1A	PMP22	Duplication
40	33	M	Yes	AR	20	Developmental delay/dystonia[c]	GRIK2	NM_021956·4:c592C > T; p.Arg198X
41	34	F	Yes	AR	34	Developmental delay/dystonia[c]	GRIK2	NM_021956·4:c592C > T; p.Arg198X
42	45	F	No	Sporadic	1	Duchenne muscular dystrophy	DMD	NM_004006·2:c.1149 + 1C > A
43	10	M	Yes	X-linked (recessive)	4	Emery Dreifuss	EMD	NM_000117·2:c.2 T > C; p.Met1X
44	41	M	Yes	X-linked (recessive)	2	Emery Dreifuss	EMD	NM_000117·2:c.461_465dup; p.Tyr155_Gly156InsCTfsX82
45	59	F	Yes	AR	10	Familial amyloid neuropathy	TTR	NM_000371·3:c148G > A; p.Val30Met
46	65	M	Yes	AD	1	Familial Creutzfeldt–Jakob disease	PRNP	NM_000311·3:c.598G > A; p. Glu200Lys
47	8	M	Yes	AD	1	Familial focal epilepsy with variable foci	DEPDC5	NM_001242896:c.[4718 T > C]; p.[Leu1573Pro]
48	34	M	Yes	AD	3	Huntington's disease	HTT	Abnormal CAG trinucleotide repeat expansion (17/50 repeats)
49	36	F	Yes	AD	5	Huntington's disease	HTT	Abnormal CAG trinucleotide repeat expansion (20/48 repeats)

50	48	M	Yes	AD	6	Huntington's disease	HTT	Abnormal CAG trinucleotide repeat expansion (17/44 repeats)
51	55	M	No	Sporadic	3	Huntington's disease	HTT	Abnormal CAG trinucleotide repeat expansion (18/42 repeats)
52	55	F	Yes	AD	10	Huntington's disease	HTT	Abnormal CAG trinucleotide repeat expansion (16/44 repeats)
53	57	F	Yes	AD	10	Huntington's disease	HTT	Abnormal CAG trinucleotide repeat expansion (16/42 repeats)
54	38	F	Yes	AD	6	Huntington's disease	HTT	Abnormal CAG trinucleotide repeat expansion (32/47 repeats)
55	31	F	Yes	AD	3	Huntington's disease	HTT	Abnormal CAG trinucleotide repeat expansion (18/50 repeats)
56	48	M	Yes	AD	6	Huntington's disease	HTT	Abnormal CAG trinucleotide repeat expansion (18/42 repeats)
57	86	F	No	Sporadic	4	Huntington's disease	HTT	Abnormal CAG trinucleotide repeat expansion (20/32 repeats)
58	46	F	No	Sporadic	5	Huntington's disease	HTT	Abnormal CAG trinucleotide repeat expansion (18/45 repeats)
59	22	M	Yes	AD	7	Huntington's disease	HTT	Abnormal CAG trinucleotide repeat expansion (15/51 repeats)
60	27	F	No	AD	1	Huntington's disease	HTT	Abnormal CAG trinucleotide repeat expansion (16/51 repeats)
61	53	F	Yes	AD	2	Huntington's disease	HTT	Abnormal CAG trinucleotide repeat expansion (19/44 repeats)
62	34	M	Yes	AD	1	Huntington's disease	HTT	Abnormal CAG trinucleotide repeat expansion (18/46 repeats)
63	36	M	Yes	AD	1	Huntington's disease	HTT	Abnormal CAG trinucleotide repeat expansion (19/51 repeats)
64	22	M	Yes	AD	5	Huntington's disease	HTT	Abnormal CAG trinucleotide repeat expansion (19/72 repeats)
65	40	F	Yes	AD	1	Huntington's disease	HTT	Abnormal CAG trinucleotide repeat expansion (16/45 repeats)
66	48	F	Yes	AD	9	Huntington's disease	HTT	Abnormal CAG trinucleotide repeat expansion (13/42 repeats)
67	33	M	Yes	AD	3	Huntington's disease	HTT	Abnormal CAG trinucleotide repeat expansion (18/50 repeats)
68	40	M	No	Sporadic	11	Huntington's disease	HTT	Abnormal CAG trinucleotide repeat expansion (16/44 repeats)
69	42	M	Yes	Father affected	1	Huntington's disease[d]	HTT	Abnormal CAG trinucleotide repeat expansion (20/39 repeats)
70	24	F	Yes	AD	Presymtomatic	Huntington's disease	HTT	Abnormal CAG trinucleotide repeat expansion (8/43 repeats)
71	28	F	Yes	AD	Presymtomatic	Huntington's disease	HTT	Abnormal CAG trinucleotide repeat expansion (18/44 repeats)
72	53	M	Yes	Sporadic	2	Huntington's disease	HTT	Abnormal CAG trinucleotide repeat expansion (13/44 repeats)
73	10	M	Yes	AD	7	Hypokalemic periodic paralysis	CACNA1S	NM_000069·2:c.3716G > A; p.Arg1239His

Table 1. (*Cont.*)

Patient (n)	Age	Sex	Family history	Inheritance	Evolution of the disease until the diagnosis (years)	Diagnosis	Gene	Mutation
74	72	M	Yes	X-linked (recessive)	2	Kennedy disease	AR	Abnormal CAG trinucleotide repeat expansion (43 repeats)
75	18	M	No	Sporadic	15	Lafora disease	NHLRC1	NM_198586·2:c.122C > T; p.Pro41L
76	15	M	No	Sporadic	1	Leber hereditary optic neuropathy	ND1	NC_012920:m.3460G > A
77	20	M	No	Sporadic	2	Leber hereditary optic neuropathy	ND4	NC_012920:m.11778G > A
78	36	M	Yes	AD or maternal	17	Leber hereditary optic neuropathy	ND4	NC_012920:m.11778G > A
79	18	M	No	Sporadic	1	Leber hereditary optic neuropathy	ND4	NC_012920:m.11778G > A
80	31	M	No	Sporadic	1	Leber hereditary optic neuropathy	ND4	NC_012920:m.11778G > A
81	28	M	No	Sporadic	8	Leber hereditary optic neuropathy	ND1	NC_012920:m.3460G > A
82	16	M	No	Sporadic	1	Leber hereditary optic neuropathy	ND1	NC_012920:m.3460G > A
83	45	F	No	Sporadic	1	MELAS	MT-TL1	NC_012920:m.3243A > G
84	26	F	No	Sporadic	18	MELAS	MT-TL1	NC_012920:m.3243A > G
85	31	F	No	Sporadic	<1	MELAS	MT-TL1	NC_012920:m.3243A > G
86	37	M	No	Sporadic	36	Mitochondrial myopathy	MT-TK	NC_012920:m.8344A > G
87	42	F	No	Sporadic	32	Myotonic dystrophy	DMPK	Abnormal CTG trinucleotide repeat expansion in the non-coding region
88	23	M	Yes	AD	5	Myotonic dystrophy	DMPK	Abnormal CTG trinucleotide repeat expansion in the non-coding region
89	20	M	Yes	AD	8	Myotonic dystrophy	DMPK	Abnormal CTG trinucleotide repeat expansion in the non-coding region
90	46	M	No	Sporadic	7	Myotonic dystrophy	DMPK	Abnormal CTG trinucleotide repeat expansion in the non-coding region
91	32	F	Yes	AD	15	Myotonic dystrophy	DMPK	Abnormal CTG trinucleotide repeat expansion in the non-coding region
92	44	F	No	Sporadic	11	Myotonic dystrophy	DMPK	Abnormal CTG trinucleotide repeat expansion in the non-coding region
93	23	M	Yes	AD	17	Myotonic dystrophy	DMPK	Abnormal CTG trinucleotide repeat expansion in the non-coding region
94	30	F	Yes	AD	14	Myotonic dystrophy	DMPK	Abnormal CTG trinucleotide repeat expansion in the non-coding region
95	40	M	Yes	AD	5	Myotonic dystrophy	DMPK	Abnormal CTG trinucleotide repeat expansion in the non-coding region
96	29	F	No	Sporadic	7	Niemman Pick-C	NPC1	NM_000271·4:c.[2932C > T]; [3410A > C]; p. [Arg978Cys]; [Asn1137Ser]
97	21	F	No	Sporadic	13	Niemman Pick-C	NPC1	NM_000271·4:c. [3019C > G]; [3104C > T]; p. [Pro1007Ala]; [Ala1035Val]
98	25	F	No	Sporadic	8	Niemman Pick-C	NPC1	NM_000271·4:c. [3134 T > A]; [3451G > A]; p. [Leu1045Pro]; [Ala1151Thr]
99	6	M	No	Sporadic	1	Niemman Pick-C	NPC1	NM_000271·4:c. [3343G > T]; [3532A > G]; p. [Val1115Phe]; [Ser1178Gly]

100	25	M	No	Niemman Pick-C	NPC1	NM_000271-4:c.[1201C > A]; [3019C > G]; p.[Pro401Thr]; [Pro1007Ala]
101	19	M	No	Niemman Pick-C	NPC1	NM_000271-4:c.[2292G > A]; [2872C > T]; p.[Ala764Ala]; [Arg958X]
102	23	F	No	Pol III-Related leukodystrophies	POLR3A	NM_007055-3:c.[378I G > A]; [3014G > A]; p.[Glu1261Lys]; [Arg1005His]
103	23	F	No	Spastic paraplegia 11	SPG11	NM_025137:c.[6763insA]; [6726A > T]; p.[Leu2255HisfsX83]; [Gln2242His]
104	22	M	No	Spinal muscular atrophy	SMN1	Homozygous deletion of exons 7 and 8
105	24	F	No	Vanishing white matter leukodystrophy	EIF2B5	NM_003907-2: c.[1030C > T]; [1010A > G]; p.[Arg344X]; [His337Arg]
106	Newborn	M	No	XLAG	ARX	NM_139058-2:c.1034G > C; p.Arg345Pro

a Rodriguez-Quiroga et al. (2013).
b Cordoba et al. (2014).
c Cordoba et al. (2015).
d Rodriguez-Quiroga et al. (2013).
AD, autosomal dominant; AR, autosomal recessive; CADASIL, cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy; CPEO, chronic progressive external ophthalmoplegia; F, female; M, male; MELAS, mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes; XLAG, X-linked lissencephaly with abnormal genitalia.

(iii) Illustrative cases

Here we describe three cases that are illustrative of different clinical scenarios where a molecular diagnostic confirmation test showed its usefulness.

(a) Case 1. New presentation of a well-known genetic disorder

An 86 year old woman without remarkable past medical or familial history was referred to our centre because she had been presenting non-stereotyped and irregular involuntary movements in upper and lower limbs for the last 4 years. These movements had progressively increased in frequency and intensity. Neurological exam revealed the presence of severe cognitive impairment, widespread pyramidal signs and choreic involuntary movements in the face, neck, upper and lower limbs. A brain CT-scan, blood chemistry and endocrinological tests were unremarkable. A peripheral blood smear did not show acanthocytes. A molecular analysis looking for abnormal CAG repeats in exon 1 of the Huntingtin gene showed a normal allele of 20 repetitions and an abnormally expanded allele of 32 repetitions. These results were confirmed in a second sample taken and analysed independently. Consequently, the clinical picture was interpreted as late-onset Huntington's disease with the presence of a CAG expansion in the unstable or intermediate range.

(b) Case 2. Diagnostic certainty: contribution of molecular diagnosis in leukoencephalopathies

A 25 year old woman was brought to our centre for the study of a disorder characterized by recurrent neurological deficits triggered by traumatic events that she had been suffering for the last 20 years. Her deficits had lasted for less than a week without complete recovery after their pousses. They included diverse symptoms and manifestations such as ataxia, motor weakness and seizures. Her family history was unremarkable. At the time of consultation, her neurological exam showed left homonymous hemianopia, left-sided hemiparesis and diffuse signs of pyramidal dysfunction. Remarkable diffuse white matter abnormal signals along cavitated areas at the left frontal lobe and thinning of the corpus callosum on MRIs allowed us to suspect a diagnosis of childhood ataxia with central nervous system hypomyelination/vanishing white matter disease. Therefore, we sequenced the EIF2B5 gene confirming this diagnosis by finding two new mutations: NM_003907·2; c.1032C > T; p.R344X and c.1012A > G; p.H337R.

(c) Case 3. Implementing genomic medicine in the clinic: exome sequencing in muscle diseases

A 45 year old woman, who was under treatment with statins, presented asymptomatic hyper-CK-emia

Table 2. *Chronic and progressive ataxia program.*

Demographic features	
Total number of patients	140
Sex (%)	
Male	42·9%
Female	57·1%
Age (range)	43·9 (11–81)
Family history (%)	35·7%
Inheritance pattern	
AD	86%
AR	14%
Median age of first symptom-years (range)	33·08 (3–68)
Time in years from symptoms onset to evaluation in our neurogenetic Centre in years (range)	10·5 (0–60)

Pedigrees with confirmatory molecular diagnosis	
• Pedigrees with autosomal dominant inheritance Confirmatory molecular diagnosis	33 families 18/33 (58·3%)
• Pedigrees with autosomal recessive inheritance Confirmatory molecular diagnosis	8 families 5/8 (62·5%)
• Total number of pedigrees with positive family history Confirmatory molecular diagnosis	41 families 23/41 (57·5%)
• Patients with sporadic ataxia Confirmatory molecular diagnosis	86 patients 8 patients (9·3%)

AD, autosomal dominant; AR, autosomal recessive.

(1500 to 2000 UI) that failed to improve after discontinuation of the therapy. A few months later, she complained of loss of strength that predominantly affected her lower limbs. At neurological examination, she presented proximal weakness in upper limbs and in pelvic girdle muscles. Electromyography showed myopathic changes, whereas the muscle biopsy revealed a complex picture with denervation and myopathic signs. Owing to these inconclusive findings, exome sequencing was performed. After selecting rare and potentially deleterious variants (maximum population frequency of 0·01, potentially affecting protein sequence and predicted deleterious impact by at least three bioinformatic tools) in a list of well-known genes causing myopathies (Kaplan & Hamroun, 2014), only one variant was highlighted in the gene coding for dystrophin (DMD) (NM_004006·2:c.1149 + 1C->A). This variant was absent in population databases and affects a canonical splice site. Furthermore, this same splice site was previously compromised in a patient suffering from Duchenne muscular dystrophy that is registered in the UMD-DMD database (Tuffery-Giraud et al., 2009). Considering the female sex of our patient, we concluded that her hyper-CK-emia could be caused by her DMD mutation.

4. Discussion

The results of our study show the diagnostic yield of the combined work of a clinic and a research based laboratory focused on clinical neurogenetics at a large tertiary care facility. Although there has been significant progress in the field of medical genetics during the last few years, it is still quite difficult to establish definitive molecular diagnoses in certain settings with less developed access to state of the art technologies. However, we think that the implementation of a clinic and a laboratory specialized in neurogenetics, which make use of their own resources within a framework of research, allowed us to obtain this high yield of definitive diagnoses by means of a systematized program. This program reduced the complexity inherent to low prevalence diseases and provides a framework for clinical research without economic and financial constraints that private care settings may have.

In addition, our figures of diagnostic yield are similar to other reports where systematized programs in the field of neurogenetics were evaluated. Edlefsen et al. (2007) retrospectively studied the diagnostic yield in a subspecialty centre in the United States, reporting a definitive molecular diagnosis in 30·2% of the studied population. A recent study implemented by the National Institutes of Health in the United States, which made use of next-generation sequencing techniques, obtained a diagnostic yield of 24% (Gahl et al., 2012). However, we were not able to offer next-generation sequencing or chromosomal microarray based diagnostics to the majority of our patients, precluding us to analyze the impact in diagnostic yield that a widespread use of these techniques could

have. A review of 18 programs aimed at the diagnosis of spinocerebellar ataxias in different regions of the world showed a mean diagnostic yield of 56% (Durr, 2010); a figure not dissimilar to our rate of successful etiological identification in the 56% of the families with spinocerebellar ataxias and dominant inheritance that we described here.

Furthermore, we described three cases that highlight the process of establishing a definitive molecular diagnosis for suspected neurogenetic disorders. Some neurogenetic diseases are beginning to be recognized and detected in elderly persons. This situation, noticed by some authors as an oxymoron (Bird *et al.*, 2008), is well illustrated in the Huntington's disease diagnosis made in patient 1. The identification of the molecular basis of various disorders of the nervous system has permitted us to split diseases where phenotypic similarities classically led us to consider them as individual entities (Bonnemann, 2011). Leukodystrophies and diseases caused by EIF2B5 mutations are a good example of this situation (Matsukawa *et al.*, 2011). The utility of next-generation sequencing is highlighted in the third case, where combining phenotypic and genomic information allowed us to arrive at a plausible explanation of the etiology of the condition affecting a patient for quite a heterogeneous disorder such as hyper-CK-emia.

In conclusion, we showed that applying a research based systematic framework in the field of neurogenetics allowed for a high diagnostic yield to be obtained in an area traditionally considered complex.

We would like to thank to our patients and their families.

Declaration of interest

References

Bird, T. D., Lipe, H. P. & Steinbart, E. J. (2008). Geriatric neurogenetics: oxymoron or reality? *Archives of Neurology* **65**, 537–539.

Bonnemann, C. G. (2011). The collagen VI-related myopathies: muscle meets its matrix. *Nature Reviews. Neurology* **7**, 379–390.

Burgunder, J. M., Schols, L., Baets, J., Andersen, P., Gasser, T., Szolnoki, Z., Fontaine, B., Van Broeckhoven, C., Di Donato, S., De Jonghe, P., Lynch, T., Mariotti, C., Spinazzola, A., Tabrizi, S. J., Tallaksen, C., Zeviani, M., Harbo, H. F., Finsterer, J. & EFNS (2011). EFNS guidelines for the molecular diagnosis of neurogenetic disorders: motoneuron, peripheral nerve and muscle disorders. *European Journal of Neurology* **18**, 207–217.

Cordoba, M., Rodriguez, S., González Morón, D., Medina, N. & Kauffman, M. A. (2015). Expanding the spectrum of Grik2 mutations: intellectual disability, behavioural disorder, epilepsy and dystonia. *Clinical Genetics* **87**, 293–295.

Cordoba, M., Rodriguez-Quiroga, S., Gatto, E. M., Alurralde, A. & Kauffman, M. A. (2014). Ataxia plus myoclonus in a 23-year-old patient due to STUB1 mutations. *Neurology* **83**, 287–288.

Corti, O., Lesage, S. & Brice, A. (2011). What genetics tells us about the causes and mechanisms of Parkinson's disease. *Physiological Reviews* **91**, 1161–1218.

Durr, A. (2010). Autosomal dominant cerebellar ataxias: polyglutamine expansions and beyond. *The Lancet. Neurology* **9**, 885–894.

Edlefsen, K. L., Tait, J. F., Wener, M. H. & Astion, M. (2007). Utilization and diagnostic yield of neurogenetic testing at a tertiary care facility. *Clinical Chemistry* **53**, 1016–1022.

England, J. D., Gronseth, G. S., Franklin, G., Carter, G. T., Kinsella, L. J., Cohen, J. A., Asbury, A. K., Szigeti, K., Lupski, J. R., Latov, N., Lewis, R. A., Low, P. A., Fisher, M. A., Herrmann, D. N., Howard, J. F. Jr, Lauria, G., Miller, R. G., Polydefkis, M., Sumner, A. J. & American Academy of Neurology (2009). Practice parameter: evaluation of distal symmetric polyneuropathy: role of laboratory and genetic testing (an evidence-based review). Report of the American Academy of Neurology, American Association of Neuromuscular and Electrodiagnostic Medicine, and American Academy of Physical Medicine and Rehabilitation. *Neurology* **72**, 185–192.

Finsterer, J., Harbo, H. F., Baets, J., Van Broeckhoven, C., Di Donato, S., Fontaine, B., De Jonghe, P., Lossos, A., Lynch, T., Mariotti, C., Schöls, L., Spinazzola, A., Szolnoki, Z., Tabrizi, S. J., Tallaksen, C. M., Zeviani, M., Burgunder, J. M., Gasser, T. & European Federation of Neurological Sciences (2009). EFNS guidelines on the molecular diagnosis of mitochondrial disorders. *European Journal of Neurology* **16**, 1255–1264.

Flicek, P., Amode, M. R., Barrell, D., Beal, K., Brent, S., Carvalho-Silva, D., Clapham, P., Coates, G., Fairley, S., Fitzgerald, S., Gil, L., Gordon, L., Hendrix, M., Hourlier, T., Johnson, N., Kähäri, A. K., Keefe, D., Keenan, S., Kinsella, R., Komorowska, M., Koscielny, G., Kulesha, E., Larsson, P., Longden, I., McLaren, W., Muffato, M., Overduin, B., Pignatelli, M., Pritchard, B., Riat, H. S., Ritchie, G. R., Ruffier, M., Schuster, M., Sobral, D., Tang, Y. A., Taylor, K., Trevanion, S., Vandrovcova, J., White, S., Wilson, M., Wilder, S. P., Aken, B. L., Birney, E., Cunningham, F., Dunham, I., Durbin, R., Fernández-Suarez, X. M., Harrow, J., Herrero, J., Hubbard, T. J., Parker, A., Proctor, G., Spudich, G., Vogel, J., Yates, A., Zadissa, A. & Searle, S. M. (2012). Ensembl 2012. *Nucleic Acids Research* **40**, D84–D90.

Fogel, B. L., Lee, H., Deignan, J. L., Strom, S. P., Kantarci, S., Wang, X., Quintero-Rivera, F., Vilain, E., Grody, W. W., Perlman, S., Geschwind, D. H. & Nelson, S. F. (2014). Exome sequencing in the clinical diagnosis of sporadic or familial cerebellar ataxia. *JAMA Neurology* **71**, 1237–1246.

Fogel, B. L. & Perlman, S. (2007). Clinical features and molecular genetics of autosomal recessive cerebellar ataxias. *The Lancet. Neurology* **6**, 245–257.

Gahl, W. A., Markello, T. C., Toro, C., Fajardo, K. F., Sincan, M., Gill, F., Carlson-Donohoe, H., Gropman, A., Pierson, T. M., Golas, G., Wolfe, L., Groden, C., Godfrey, R., Nehrebecky, M., Wahl, C., Landis, D. M., Yang, S., Madeo, A., Mullikin, J. C., Boerkoel, C. F., Tifft, C. J. & Adams, D. (2012). The National Institutes of Health Undiagnosed Diseases Program: insights into rare diseases. *Genetics in Medicine* **14**, 51–59.

Gasser, T., Finsterer, J., Baets, J., Van Broeckhoven, C., Di Donato, S., Fontaine, B., De Jonghe, P., Lossos, A., Lynch, T., Mariotti, C., Schöls, L., Spinazzola, A., Szolnoki, Z., Tabrizi, S. J., Tallaksen, C. M., Zeviani, M., Burgunder, J. M., Harbo, H. F. & EFNS. (2010). EFNS guidelines on the molecular diagnosis of ataxias and spastic paraplegias. *European Journal of Neurology* **17**, 179–188.

Harbo, H. F., Finsterer, J., Baets, J., Van Broeckhoven, C., Di Donato, S., Fontaine, B., De Jonghe, P., Lossos, A., Lynch, T., Mariotti, C., Schöls, L., Spinazzola, A., Szolnoki, Z., Tabrizi, S. J., Tallaksen, C., Zeviani, M., Burgunder, J. M., Gasser, T. & EFNS. (2009). EFNS guidelines on the molecular diagnosis of neurogenetic disorders: general issues, Huntington's disease, Parkinson's disease and dystonias. *European Journal of Neurology* **16**, 777–785.

Hijikata, A., Raju, R., Keerthikumar, S., Ramabadran, S., Balakrishnan, L., Ramadoss, S. K., Pandey, A., Mohan, S. & Ohara, O. (2010). Mutation@A Glance: an integrative web application for analysing mutations from human genetic diseases. *DNA Research* **17**, 197–208.

Kaplan, J. C. & Hamroun, D. (2014). The 2015 version of the gene table of monogenic neuromuscular disorders (nuclear genome). *Neuromuscular Disorders* **24**, 1123–1153.

Kauffman, M. A. (2013). Tres preguntas y una respuesta: algoritmo diagnóstico molecular en enfermedades mitocondriales. *Neurología Argentina* **5**, 19–26.

Matilla-Duenas, A., Corral-Juan, M., Volpini, V. & Sanchez, I. (2012). The spinocerebellar ataxias: clinical aspects and molecular genetics. *Advances in Experimental Medicine and Biology* **724**, 351–374.

Matsukawa, T., Wang, X., Liu, R., Wortham, N. C., Onuki, Y., Kubota, A., Hida, A., Kowa, H., Fukuda, Y., Ishiura, H., Mitsui, J., Takahashi, Y., Aoki, S., Takizawa, S., Shimizu, J., Goto, J., Proud, C. G. & Tsuji, S. (2011). Adult-onset leukoencephalopathies with vanishing white matter with novel missense mutations in EIF2B2, EIF2B3, and EIF2B5. *Neurogenetics* **12**, 259–261.

Patterson, M. C., Hendriksz, C. J., Walterfang, M., Sedel, F., Vanier, M. T. & Wijburg, F. (2012). Recommendations for the diagnosis and management of Niemann-Pick disease type C: an update. *Molecular Genetics and Metabolism* **106**, 330–344.

Rodríguez-Quiroga, S. A., González-Morón, D., Arakaki, T., Garreto, N. & Kauffman, M. A. (2013). [The broad phenotypic spectrum of SCA-3: hereditary spastic paraplegia]. *Medicina* **73**, 552–554.

Rodríguez-Quiroga, S. A., Gonzalez-Morón, D., Garretto, N. & Kauffman, M. A. (2013). Huntington's disease masquerading as spinocerebellar ataxia. *BMJ Case Reports* **2013**, pii: bcr2012008380.

Schule, R. & Schols, L. (2011). Genetics of hereditary spastic paraplegias. *Seminars in Neurology* **31**, 484–493.

Schwarz, J. M., Rodelsperger, C., Schuelke, M. & Seelow, D. (2010). MutationTaster evaluates disease-causing potential of sequence alterations. *Nature Methods* **7**, 575–576.

Sim, N. L., Kumar, P., Hu, J., Henikoff, S., Schneider, G. & Ng, P. C. (2012). SIFT web server: predicting effects of amino acid substitutions on proteins. *Nucleic Acids Research* **40**, W452–W457.

Siskind, C. E. & Shy, M. E. (2011). Genetics of neuropathies. *Seminars in Neurology* **31**, 494–505.

Tuffery-Giraud, S., Beroud, C., Leturcq, F., Yaou, R. B., Hamroun, D., Michel-Calemard, L., Moizard, M. P., Bernard, R., Cossée, M., Boisseau, P., Blayau, M., Creveaux, I., Guiochon-Mantel, A., de Martinville, B., Philippe, C., Monnier, N., Bieth, E., Khau Van Kien, P., Desmet, F. O., Humbertclaude, V., Kaplan, J. C., Chelly, J. & Claustres, M. (2009). Genotype-phenotype analysis in 2,405 patients with a dystrophinopathy using the UMD-DMD database: a model of nationwide knowledgebase. *Human Mutation* **30**, 934–945.

Zou, M., Baitei, E. Y., Alzahrani, A. S., Parhar, R. S., Al-Mohanna, F. A., Meyer, B. F., & Shi, Y. (2011). Mutation prediction by PolyPhen or functional assay, a detailed comparison of CYP27B1 missense mutations. *Endocrine* **40**, 14–20.