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

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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

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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 spaceoccupying 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 decisionmaking 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 trinucleotiderepeat 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	Atavia AOA1	ADTY	NM 175060.1: a 870G > A: n Trn203X
2	21	M	Ves	AR	9	Ataxia = AOA1 Ataxia = Friedreich ataxia	FXN	Abnormal GAA repeat
$\frac{2}{3}$	18	F	Ves	AR	6	Ataxia - Friedreich ataxia	FXN	Abnormal GAA repeat
4	18	M	Ves	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	Ves	AD	11	Ataxia $-$ SCA1	ATXN1	Abnormal CAG trinucleotide repeat expansion
11	57	1	105	n D	11	Huxiu Serri	7117111	(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	М	Yes	AD	9	Ataxia – SCA1	ATXN1	Abnormal CAG trinucleotide repeat expansion (30/48 repeats)
16	55	М	Yes	AD	10	Ataxia – SCA2	ATXN2	Abnormal CAG trinucleotide repeat expansion (20/38 repeats)
17	30	М	Yes	AD	7	Ataxia – SCA2	ATXN2	Abnormal CAG trinucleotide repeat expansion (22/43 repeats)
18	30	М	No	Sporadic	13	Ataxia – SCA2	ATXN2	Abnormal CAG trinucleotide repeat expansion (22/41 repeats)
19	32	М	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	М	Yes	AD	10	Ataxia – SCA3 ^a	ATXN3	Abnormal CAG trinucleotide repeat expansion (23/64 repeats)
25	54	М	Yes	AD	3	Ataxia – SCA3 ^a	ATXN3	Abnormal CAG trinucleotide repeat expansion (19/63 repeats)

			Family		Evolution of the disease until the			
Patient (n)	Age	Sex	history	Inheritance	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	М	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	М	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	М	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 $000435 \cdot 2:c.635:G > T: p.Cvs212Phe$
37	21	M	No	AR	21	Cerebrotendinous xanthomatosis	CYP27A1	NM 000784.3:c $1234C > T$: p Arg405Trp
5,		1.1	110	(consanguinity)			0112/11	1 <u>-</u> 000,012012010 1, pillg10011p
38	51	м	Vec	ΔP	10	Cerebrotendinous vanthomatosis	CVP27A1	NM 000784.3:c $1183C > T$: n Arg395Cys
20	55		Vas		5	CMT1A	DMD22	Duplication
39	22	I' M	Vac		20	CIVITIA Developmental delay/dystenia ^{c}	CDIV2	NM 021056 $4_{12}502C > T_{1} = A_{12}108V$
40	22		I es		20	Developmental delay/dystoma	GRIK2	$NM_021950.4.0592C > 1; p.Aig196A$
41	34	Г	Yes	AK	34	Developmental delay/dystoma	GKIK2	$NM_0219364:C392C > 1; p.Arg198A$
42	45	F	No	Sporadic		Duchenne muscular dystrophy	DMD	$NM_{004006} \cdot 2:c.1149 + 1C > A$
43	10	М	Yes	X-linked (recessive)	4	Emery Dreifuss	EMD	NM_00011/-2:c.2 1 > C; p.Met1X
44	41	М	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	Μ	Yes	AD	1	Familial Creutzfeldt–Jakob disease	PRNP	NM_000311.3:c.598G > A; p. Glu200Lys
47	8	М	Yes	AD	1	Familial focal epilepsy with variable foci	DEPDC5	NM_001242896:c.[4718 T > C]; p.[Leu1573Pro]
48	34	М	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	М	Yes	AD	6	Huntington's disease	HTT	Abnormal CAG trinucleotide repeat expansion (17/44 repeats)
51	55	М	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	(10/42 repeats) Abnormal CAG trinucleotide repeat expansion
55	31	F	Yes	AD	3	Huntington's disease	HTT	(12/47) repeats) Abnormal CAG trinucleotide repeat expansion
56	48	М	Yes	AD	6	Huntington's disease	HTT	(18/30 repeats) Abnormal CAG trinucleotide repeat expansion (18/42 repeats)
57	86	F	No	Sporadic	4	Huntington's disease	HTT	(10/42 repeats) Abnormal CAG trinucleotide repeat expansion
58	46	F	No	Sporadic	5	Huntington's disease	HTT	(18/45 repeats) Abnormal CAG trinucleotide repeat expansion
59	22	М	Yes	AD	7	Huntington's disease	HTT	(15/45 repeats) Abnormal CAG trinucleotide repeat expansion
60	27	F	No	AD	1	Huntington's disease	HTT	(15/51 repeats) Abnormal CAG trinucleotide repeat expansion
61	53	F	Yes	AD	2	Huntington's disease	HTT	(10/31 repeats) Abnormal CAG trinucleotide repeat expansion
62	34	М	Yes	AD	1	Huntington's disease	HTT	(19/44 repeats) Abnormal CAG trinucleotide repeat expansion
63	36	М	Yes	AD	1	Huntington's disease	HTT	(18/46 repeats) Abnormal CAG trinucleotide repeat expansion
64	22	М	Yes	AD	5	Huntington's disease	HTT	(19/51 repeats) Abnormal CAG trinucleotide repeat expansion
65	40	F	Yes	AD	1	Huntington's disease	HTT	(19/72 repeats) Abnormal CAG trinucleotide repeat expansion
66	48	F	Yes	AD	9	Huntington's disease	HTT	(16/45 repeats) Abnormal CAG trinucleotide repeat expansion
67	33	М	Yes	AD	3	Huntington's disease	HTT	(13/42 repeats) Abnormal CAG trinucleotide repeat expansion
68	40	М	No	Sporadic	11	Huntington's disease	HTT	(18/50 repeats) Abnormal CAG trinucleotide repeat expansion
69	42	М	Yes	Father affected	1	Huntington's disease ^d	HTT	(16/44 repeats) Abnormal CAG trinucleotide repeat expansion
70	24	F	Yes	AD	Presymtomatic	Huntington's disease	HTT	(20/39 repeats) Abnormal CAG trinucleotide repeat expansion
71	28	F	Yes	AD	Presymtomatic	Huntington's disease	HTT	(8/43 repeats) Abnormal CAG trinucleotide repeat expansion
72	53	М	Yes	Sporadic	2	Huntington's disease	HTT	(18/44 repeats) Abnormal CAG trinucleotide repeat expansion
73	10	М	Yes	AD	7	Hypokalemic periodic paralysis	CACNA1S	(13/44 repeats) NM_000069·2:c.3716G > A; p.Arg1239His

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Table	1.	(Cont.)
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					Evolution of the			
Detiont (n)	1	Sau	Family	Inhanitanaa	diagnosis (years)	Diagnosis	Cana	Mutation
Patient (II)	Age	Sex	mstory	Inneritance	diagnosis (jears)	Diagnosis	Gene	Mutation
74	72	Μ	Yes	X-linked (recessive)	2	Kennedy disease	AR	Abnormal CAG trinucleotide repeat expansion (43 repeats)
75	18	Μ	No	Sporadic	15	Lafora disease	NHLRC1	NM_198586.2:c.122C > T; p.Pro41L
76	15	Μ	No	Sporadic	1	Leber hereditary optic neuropathy	ND1	NC_012920:m.3460G > A
77	20	Μ	No	Sporadic	2	Leber hereditary optic neuropathy	ND4	NC_012920:m.11778G > A
78	36	Μ	Yes	AD or maternal	17	Leber hereditary optic neuropathy	ND4	NC_012920:m.11778G > A
79	18	Μ	No	Sporadic	1	Leber hereditary optic neuropathy	ND4	NC_012920:m.11778G > A
80	31	Μ	No	Sporadic	1	Leber hereditary optic neuropathy	ND4	NC_012920:m.11778G > A
81	28	Μ	No	Sporadic	8	Leber hereditary optic neuropathy	ND1	NC_012920:m.3460G > A
82	16	Μ	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	Μ	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	М	Yes	AD	5	Myotonic dystrophy	DMPK	Abnormal CTG trinucleotide repeat expansion in the non-coding region
89	20	М	Yes	AD	8	Myotonic dystrophy	DMPK	Abnormal CTG trinucleotide repeat expansion in the non-coding region
90	46	М	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
92	44	F	No	Sporadic	11	Myotonic dystrophy	DMPK	Abnormal CTG trinucleotide repeat expansion
93	23	М	Yes	AD	17	Myotonic dystrophy	DMPK	Abnormal CTG trinucleotide repeat expansion
94	30	F	Yes	AD	14	Myotonic dystrophy	DMPK	Abnormal CTG trinucleotide repeat expansion
95	40	М	Yes	AD	5	Myotonic dystrophy	DMPK	Abnormal CTG trinucleotide repeat expansion
96	29	F	No	Sporadic	7	Niemman Pick-C	NPC1	NM_000271·4:c.[2932C > T]; [3410A > C]; p.
97	21	F	No	Sporadic	13	Niemman Pick-C	NPC1	[Mg/00271.4:c. [3019C > G]; [3104C > T]; p. [Pro1007A1a]; [A1a1035Val]
98	25	F	No	Sporadic	8	Niemman Pick-C	NPC1	$NM_{000271-4:c.} [3134 T > A]; [3451G > A]; p.$
99	6	М	No	Sporadic	1	Niemman Pick-C	NPC1	NM_000271·4:c. [3343G > T]; [3532A > G]; p. [Val1115Phe]; [Ser1178Gly]

100	25	Σ	No	Sporadic	7	Niemman Pick-C	NPC1	NM_000271.4:c.[1201C > A]; $[3019C > G]$; p.
101	19	Σ	No	Sporadic	6	Niemman Pick-C	NPC1	$[r_{10}+0.1111]$; $[r_{10}+0.07A13]$ NM_000271+4:c: $[2292G > A]$; $[2872C > T]$; p.
102	23	Ц	No	Sporadic	18	Pol III-Related leukodystrophies	POLR3A	[Aia/04Aia]; $[Aig950A]NM [0070553: [2781G > A]; [3014G > A]; p.$
103	23	Ц	No	Sporadic	13	Spastic paraplegia 11	SPG11	$V_{1} = V_{1} = V_{2} = V_{2$
104	22	Σ	No	Sporadic	1	Spinal muscular atrophy	SMNI	Homozygous deletion of exons 7 and 8
105	24	ĽL,	No	Sporadic	16	Vanishing white matter	EIF2B5	$NM_{003907.2}$; c.[1030C > T]; [1010A > G]; p.
106	Newborn	M	No	Sporadic	1 month	XLAG	ARX	$NM_{139058-2:c.1034G} > C; p.Arg345Pro$
^a Rodrigu ^b Cordobi ^c Cordobi ^d Rodrigu	lez-Quiroga a <i>et al.</i> (201 a <i>et al.</i> (201: ez-Quiroga	et al. 4). 5). et al.	(2013). (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

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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	10.5 (0-60)
evaluation in our neurogenetic Centre in years (range)	
Pedigrees with confirmatory molecular diagnosis	
Pedigrees with autosomal dominant inheritance	33 families 18/33 (58·3%)
Confirmatory molecular diagnosis	
 Pedigrees with autosomal recessive inheritance 	8 families 5/8 (62.5%)
Confirmatory molecular diagnosis	
 Total number of pedigrees with positive family history 	41 families 23/41 (57.5%)
Confirmatory molecular diagnosis	
• Patients with sporadic ataxia	86 patients 8 patients (9.3%)
Confirmatory molecular diagnosis	

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 wellknown 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 nextgeneration 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

M.K. is a researcher in CONICET and Gobierno de la Ciudad de Buenos Aires. S.R.-Q. has a fellowship from Gobierno de la Ciudad de Buenos Aires. The rest of the authors declare that they have no conflict of interest.

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