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 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
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 – SCA3a | ATXN3| Abnormal CAG trinucleotide repeat expansion (23/64 repeats) |
| 25          | 54  | M   | Yes            | AD          | 3                                                   | Ataxia – SCA3a | ATXN3| Abnormal CAG trinucleotide repeat expansion (19/63 repeats) |
| 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<sup>a</sup> | 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<sup>b</sup> | 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 | 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<sup>c</sup> | GRIK2 | NM_021956-4:c.592C > T; p.Arg198X |
| 41         | 34  | F   | Yes            | AR          | 34                                                | Developmental delay/dystonia<sup>c</sup> | GRIK2 | NM_021956-4:c.592C > 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_Gly156InsCtfsX2 |
| 45         | 59  | F   | Yes            | AR          | 10                                                | Familial amyloid neuropathy | TTR | NM_000371-3.c.148G > 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_001242896-3.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) |
| ID | Age | Sex | Onset | Diagnosis | HTT | CAG Repeat | Mutation |
|----|-----|-----|-------|-----------|-----|------------|----------|
| 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 | HTT | Abnormal CAG trinucleotide repeat expansion (20/39 repeats) |
| 70 | 24  | F   | Yes   | Presymptomatic | Huntington’s disease | HTT | Abnormal CAG trinucleotide repeat expansion (8/43 repeats) |
| 71 | 28  | F   | Yes   | Presymptomatic | 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 |
| Patient (n) | Age | Sex | Family history | Inheritance | Evolution of the disease until the diagnosis (years) | Diagnosis | Gene | Mutation |
|------------|-----|-----|----------------|-------------|---------------------------------------------------|-----------|------|----------|
| 74         | 72  | M   | Yes            | X-linked     | 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     | 8                                                 | Leber hereditary optic neuropathy | ND1   | NC_012920:m.3460G > A |
| 81         | 28  | M   | No             | Sporadic     | 1                                                 | Leber hereditary optic neuropathy | ND1   | NC_012920:m.3460G > A |
| 82         | 16  | M   | No             | Sporadic     | 1                                                 | MELAS | MT-TL1 | NC_012920:m.3243A > G |
| 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                                                 | Niemann Pick-C | NPC1  | NM_000271-4:c.[2932C > T]; [3410A > C]; p. [Arg978Cys]; [Asn1137Ser] |
| 97         | 21  | F   | No             | Sporadic     | 13                                                | Niemann Pick-C | NPC1  | NM_000271-4:c. [3019C > G]; [3104C > T]; p. [Pro1007Ala]; [Ala1035Val] |
| 98         | 25  | F   | No             | Sporadic     | 8                                                 | Niemann Pick-C | NPC1  | NM_000271-4:c. [3134 T > A]; [3451G > A]; p. [Leu1045Pro]; [Ala1151Thr] |
| 99         | 6   | M   | No             | Sporadic     | 1                                                 | Niemann Pick-C | NPC1  | NM_000271-4:c. [3343G > T]; [3532A > G]; p. [Val1115Phe]; [Ser1178Gly] |
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: 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-e mia
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

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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