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ARTICLE

Consensus and controversies in best practices for molecular genetic testing of spinocerebellar ataxias

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Many laboratories worldwide are offering molecular genetic testing for spinocerebellar ataxias (SCAs). This is essential for differential diagnosis and adequate genetic counselling. The European Molecular Genetics Quality Network (EMQN) started an SCA external quality assessment scheme in 2004. There was a clear need for updated laboratory guidelines. EMQN and EuroGentest organized a Best Practice (BP) meeting to discuss current practices and achieve consensus. A pre-meeting survey showed that 36 laboratories (20 countries) conducted nearly 18 000 SCA tests the year before, and identified issues to discuss. Draft guidelines were produced immediately after the meeting and discussed online for several months. The final version was endorsed by EMQN, and harmonized with guidelines from other oligonucleotide repeat disorders. We present the procedures taken to organize the survey, BP meeting, as well as drafting and approval of BP guidelines. We emphasize the most important recommendations on (1) pre-test requirements, (2) appropriate methodologies and (3) interpretation and reporting, and focus on the discussion of controversial issues not included in the final document. In addition, after an extensive review of scientific literature, and responding to recommendations made, we now produce information that we hope will facilitate the activities of diagnostic laboratories and foster quality SCA testing. For the main loci, this includes (1) a list of repeat sequences, as originally published; (2) primers in use; and (3) an evidence-based description of the normal and pathogenic repeat-size ranges, including those of reduced penetrance and those in which there is still some uncertainty. This information will be maintained and updated in http://www.scabase.eu.
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INTRODUCTION

Spinocerebellar ataxias (SCAs) are a clinically and genetically heterogeneous group of autosomal dominant, neurodegenerative disorders. At present, the genetic defect is chromosomally mapped or molecularly characterized for about half of them. The causative mutation involves a triplet repeat expansion for all of the more common forms, but other repeat configurations, deletions and point mutations are also known.[1–3] The different types of SCA have a considerable geographical and ethnic variation in prevalence.[4]

Some clinical features, when present, may help in their differential diagnosis, for example, ophthalmoparesis, bulging eyes and face and tongue fasciculations in MJD/SCA3; very slow saccadic eye movements in SCA2; retinopathy in SCA7; or myoclonic epilepsy and dementia in DRPLA. A clinical diagnosis of a specific SCA can often be made, or at least suspected, mainly in large families, with many affected patients, showing all the characteristics of the disease. The phenotypic overlap among SCAs is, however, still considerable, and there is a striking variation within each form and even in single families. Patients with short disease duration, some end-stage cases, the absence of family history, atypical presentation and age at onset, as well as families with few members affected, are more difficult to diagnose. A clinical diagnosis is often further challenged by anticipation of age at onset. A detailed genetic and molecular analysis is thus required for resolution of genetic heterogeneity among SCAs, to provide for a definitive diagnosis, as well as being essential for adequate genetic counselling.

Sizing of CAG trinucleotide repeats for several SCAs is now a widespread service, routinely offered by medical genetic laboratories around the world, for molecular confirmation or exclusion of a clinical diagnosis, presymptomatic testing (PST) and prenatal diagnosis (PND). Nevertheless, little information is available on current practices; besides the UK Clinical Molecular Genetics Society guidelines prepared in 1998,[5] and retired in 2007, no other recommendations existed to assist molecular genetics laboratories with technical aspects and in the interpretation and reporting of results. The UK NEQAS held a best practice (BP) meeting on 20 September 2004, but no final document was produced.

The need for laboratory BP guidelines was further highlighted by the results of our external quality assessment (EQA) SCA scheme organized by the European Molecular Genetics Quality Network (EMQN) since 2004. The experience of the first 3 years showed that, although there was some improvement in the quality of SCA testing, the rate of gross genotype errors and the inability to correctly interpret and report the results by some laboratories were still a cause for great concern.[6]

Thus, the need was felt for widespread information on appropriate methodologies, for standardization and improvement of accurate

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repeat sizing, and to update reference ranges for normal and pathogenic alleles. The EMQN and EuroGentest addressed this by organizing an SCA BP meeting that was aimed at obtaining international consensus and developing updated BP guidelines.

We hereby discuss the methods for organizing the SCA BP meeting and producing the BP guidelines, the consensus reached and, mainly, the polemic issues and discussion in which consensus was not obtained and, thus, were not included in the guidelines.

METHODS

Pre-meeting survey

A pre-meeting survey was sent to all laboratories registered with EMQN, including all non-European ones participating in our EQA scheme. We inquired about general issues such as (1) the set of SCA types that should be tested as a minimum; (2) the SCA loci tested and number of tests performed in 2006; (3) availability of relative frequencies among SCAs for that country; (4) existence of population studies regarding allele frequencies in the country; (5) regular participation in SCA EQA schemes; and (6) the most relevant topics to be discussed at a BP meeting.

BP meeting

A call for a BP meeting was then issued to respondents and to all other laboratories registered with EMQN and offering SCA testing in Europe or beyond. EMQN national representatives were asked to nominate one laboratory to ensure a wide representation of European countries. The main aims were to discuss current practices and achieve consensus to formulate BP guidelines for molecular genetic testing of SCAs. The EMQN BP meeting was supported by EuroGentest, and took place in Porto between 17 through 19 October 2007 (http://www.eurogentest.org/web/db/event/128/index.xhtml).

A total of 33 persons from 17 European countries, as well as from Australia and Brazil, participated in the 2-day meeting. Participants were mostly molecular geneticists, but also included some clinicians and patient representatives, the EMQN EQA SCA scheme organizer and assessors, a delegate from the EMQN administration, as well as a UK NEQAS representative.

The initial plenary sessions covered the clinical aspects and epidemiology of the most common SCAs, a view of ongoing research and patients' perspectives (by Ataxia UK). The next sessions included a discussion of the results from the pre-meeting survey, pre-test requirements and criteria, analytical methods, as well as interpretation and reporting. Small group discussions followed on (1) pre-test requirements and acceptance criteria for samples and documentation (in a diagnostic, PST or PND context) and (2) appropriate methodologies for genotyping and repeat sizing (availability and use of controls and reference materials; infrequent findings and how to deal with them; how far to test in a negative diagnostic case). At the end of the first day, participants were asked for their five most important issues, from which a set of 12 questions were selected for the next day's discussion.

The second day was mostly devoted to an in-depth discussion of the 12 selected issues. Three related to pre-test requirements: (1) definition of ranges of normal, pathogenic and 'intermediate' repeats; (2) required and acceptable samples for diagnostic, PST and PND; and (3) acceptable referrals and information requirements (patient identifiers, copies of family results, DNA sample from an index case, testing isolated cases or known SCA families). The six analytical issues discussed included (4) striving for accurate repeat sizing and available size standards; (5) standard positive and negative samples for internal controls, and proper procedure in the event that no positive control is available; (6) testing strategy and protocol, acceptable methodologies, additional testing and protocols in case of homoallelism (ie, two normal alleles with the same repeat size) and the need to discriminate alleles that are just one repeat apart; (7) limitations of methodologies and detection of large expansions (TP-PCR, Southern blotting); (8) role of exclusion testing in PND; and (9) should SCA8 be offered as a diagnostic test and, if so, in which conditions? Finally, the last three issues related to the post-analytical phase: (10) reporting normal and pathogenic repeat sizes, dealing with the limits of the methods used and further testing recommended; (11) formulation of 'positive' or 'negative' test results in diagnostic, PST or PND, and essential recommendations, including counselling (and mention of recurrence risks for family members and offer of PND), if an expansion is found, or other loci to be tested, in the event that no expansion is detected; and (12) addressing and disclosing results to the referring and other physicians. Each of six groups discussed two of these questions, and each question was thus covered by two different groups. The conclusions were then presented by the rapporteurs and discussed at an open forum. At the end of the day, participants were asked to write the five major conclusions they would like to see included in the guidelines, which were then considered in the drafting process.

BP guidelines

The draft guidelines, reflecting the discussion and the final consensus reached, were then composed by the organizers immediately after the meeting. For more than 3 months, an electronic discussion group followed. After this online discussion, which included two reminders for participation and three rounds of updating, redrafting and recirculation, a consensus document was finalized in 2008. This was submitted to the EMQN board and then subjected to further revision. An effort was made to exclude more general recommendations, not specific to SCA testing. Finally, the guidelines were endorsed by the EMQN board, but still went through a process of harmonization with other BP guidelines existing or being developed for triplet repeat diseases, including Friedreich ataxia (FRDA), myotonic dystrophy, fragile-X syndrome and, mainly, Huntington disease.

RESULTS

Premeeting survey

Results from this survey (36 laboratories from 20 countries) showed that more than three-quarters believed SCA1, SCA2, MJD/SCA3, SCA6 and SCA7 should be offered as a minimum; more than half also included SCA17 and DRPLA. The menu of SCA tests that the laboratories actually offered was not much different from this, except that half also test routinely for SCA12 and 86% did offer DRPLA. Searching point mutations in selected loci (SCA4-5, 11, 13–14, 27) was not considered essential for the vast majority. The number of SCA tests performed by 33 laboratories in 2006 added up to nearly 18 000, but ranged from 17 to 2770 (10 laboratories performed less than 200, and only five conducted over 1000 SCA tests that year). Only seven of the respondents (mostly EMQN registered) did not perform any EQA for SCAs. Over 40% of laboratories referred no evidence for relative frequency of SCAs in their respective country or region, and 56% reported the absence of any population studies for SCA allele frequencies.

BP guidelines

The final product, the BP guidelines for molecular genetic testing in SCAs, is now published separately in this issue of the journal,[7] and will also be available from the websites of EMQN (http://www.emqn.org) and EuroGentest (http://www.eurogentest.org). They reflect the consensus obtained at the BP meeting and the electronic discussion group, and the process of endorsement by EMQN and of harmonization with other guidelines. Publishing the guidelines alone would, however, deprive all those interested of the rich discussion that was had during and after the meeting, and of the many lessons derived from it, the main reason for this paper.

SCAbase – an evidence-based online resource

The repeat definition, as originally published, is indicated for all the main SCA loci in Table 1, with respective references. The sequences of primers described originally, for the most common SCA loci, and the respective references, are included in Table 2. We are thus fulfilling those two recommendations from the BP guidelines. Another major recommendation of the meeting and of the guidelines was the need to review scientific literature and update the reference ranges for normal

Table 1 Definition of the repeat, as originally published for the main SCA loci

ATAXIA	*Gene name*	*Repeat*	*References*[a]
SCA1	*ATXN1*	$(CAG)_n(CAT)_n(CAG)_n$[b]	Orr *et al*[15]; Chung *et al*[16]; Sobczak *et al*[17]
DRPLA	*ATN1*	$(CAG)_n$	Koide *et al* (1994); Nagafuchi *et al* (1994)
SCA2	*ATXN2*	$[(CAG)_n$ CAA $(CAG)_n]_n$[c]	Sobczak *et al*[17]; Pulst *et al*[18]
MJD/SCA3	*ATXN3*	$(CAG)_2$ CAA AAG CAG CAA $(CAG)_n$	Kawaguchi *et al*[19]; Maciel *et al*[20]
SCA6	*CACNA1A*	$(CAG)_n$	Zhuchenko *et al*[13]
SCA7	*ATXN7*	$(CAG)_n$	David *et al*[14]
SCA8	*ATXN8*	$(CAG/TAG)_n$	Koob *et al*[28]
SCA10	*ATXN10*	$(ATTCT)_n$[d]	Matsuura *et al* (2000)
SCA12	*PPP2R2B*	$(CAG)_n$	Holmes *et al* (1999)
SCA17	*TBP*	$[(CAG)_n$ $(CAA)_n$ $(CAG)_n]_n$	Koide *et al*[21]

[a]Full references are available at: http://www.scabase.eu.
[b]May be interrupted by 1 to 3, or exceptionally 4, CATs.
[c]The CAG repeat may be pure or have 1–4 CAA interruptions.
[d]Interruptions with multiple pentarepeats (ATGCT, ATTGT or TTTCT) or septarepeats (ATTCTAT, ATTTTCT and ATATTCT) or complex ATTGT-TTTCT repeats may be found.

and pathogenic alleles, including those with reduced or uncertain penetrance for the major loci, on the basis of evidence available. Two or more reports were required to define 'normal', 'reduced penetrance' or 'full penetrance' ranges for each allele size; whenever there was only one, or two or more contradictory reports, the pathogenicity of that repeat size was classified as 'uncertain.' Those ranges are provided in Table 3.

All this information on (1) repeat definitions and formulae, (2) primers in use for repeat sizing and (3) reference size ranges for each SCA locus will now be kept at a curated database (http://www.scabase.eu), together with the respective, annotated, bibliographic references. This will be updated continuously with the information received from those willing to submit their own data and experience, and should become an important resource for diagnostic laboratories.

DISCUSSION

Pre-test requirements and criteria

The appropriate *panel of tests* to be offered by any given laboratory is difficult to establish, given the paucity of information on relative frequencies of each SCA in specific populations, and the fact that it is absolutely not possible to rely on the overall frequencies worldwide:[4] the most common dominant ataxias in Portugal are MJD/SCA3 and DRPLA, whereas in the United Kingdom, SCA2 and SCA6 predominate; in Germany, SCA6 and MJD/SCA3 are the most frequent, whereas SCA2 and SCA1 are the most prevalent in Italy. It is important to avoid comparison with single populations; each laboratory should gather their own data to gain insight into the local prevalence of the different SCAs and tailor its test panel accordingly. The consensus was, however, that all laboratories should offer testing for SCA1, SCA2, MJD/SCA3, SCA6 and SCA7, as a minimum, other forms depending on local prevalence. Most laboratories consider it essential also to offer testing for SCA17 and DRPLA. However, detection rates will vary considerably among countries, and also between different regions within the same country. A list of known SCA genes and mutations can be found elsewhere[4] and will be regularly updated in SCAbase (http://www.scabase.eu). The diagnostic tests available, laboratories offering them and research projects on SCAs can all be found in Orphanet (http://www.orpha.net).

It is highly desirable to have complete *clinical information*, with emphasis on age of onset, main symptoms, family history, as well as origins and ethnicity; this aids in prioritizing certain loci on a general request for 'SCA screening', or when recommending further testing, if appropriate. Very importantly, lack of clinical information also gives rise to the danger of inadvertently performing a PST outside the proper counselling context and procedures.

Particular care should be taken with *diagnosing minors*, if there is any doubt that the parents are aware of the implications to themselves of a confirmation of a diagnosis in their child. *PST of minors* should follow the recommendations for diseases for which there is currently no treatment and, hence, no benefit from testing a child.[8,9]

To carry out a *PST*, in the context of the appropriate counselling of unaffected family members, there should have been a molecular diagnosis in a proband. The ideal is to have a 'mutation-positive' familial control; failing this, a mutation report from another trusted laboratory may be sufficient. Some feel that PST should not be offered in the absence of this confirmation, but, in some situations, it cannot be avoided; in that case, the appropriate disclaimers should be included in the report, to make it clear that there is still a residual risk in the event of a 'negative' result, as a different diagnosis could not be ruled out in that family.

There are currently a variety of approaches to *sample requirements* for PST. It was felt that either a blood sample, or extracted DNA from another trusted laboratory, can be used; however, some still prefer to extract their own DNA samples to increase quality control. The consensus, however, was that one DNA extraction from one blood sample is *not* sufficient. Although the BP is to have two DNA samples extracted from two separately drawn blood samples, this may not always be practicable, depending on local procedures for PST and counselling. An acceptable compromise may be to take two tubes of blood on the same occasion or split one tube into two aliquots, followed by DNA extraction on separate occasions.

For *PND* or *preimplantation genetic diagnosis* (PGD), a molecular diagnosis in a proband is essential. For PND, a sample from the mother is required, irrespective of whether she is the affected parent, for exclusion of maternal contamination of foetal DNA. Some argue about inadvertent detection of non-paternity, stating that a sample from the father may not be required if he is unaffected; however, laboratories are used to dealing with this situation, and most feel it is a small price to pay for the improved quality with both parental samples available. For PGD, samples of both parents, and also of other affected and unaffected relatives, are essential, because the gold standard for monogenic diseases is the use of multiplex PCR with linked markers to be able to detect allele dropout and contamination; family samples

Table 2 List of primers published for the main SCA loci

ATAXIA	*Gene*	*Primers sequences*	*References*[a]
SCA1	*ATXN1*	Rep-1: AACTGGAAATGTGGACGTAC	Orr *et al*[15]
		Rep-2: CAACATGGGCAGTCTGAG	
		CAG-a: CCGGAGCCCTGCTGAGGT	Orr *et al*[15]
		CAG-b: CCAGACGCCGGGACAC	
DRPLA	*ATN1*	CTGB37.5 F: CACCAGTCTCAACACATCACCATC	Li *et al* (1993)
		CTGB37.5 R: CCTCCAGTGGGTGGGGAAATGCTC	
		DRPLA F: CCCAGTCCACCGCCCACCCACCA	Majounie *et al* (2007)
		DRPLA R: TGCTCCAGGAGGAGGGGGCCCAGA	
SCA2	*ATXN2*	SCA2-A: GGGCCCCTCACCATGTCG	Pulst *et al*[18]
		SCA2-B: CGGGCTTGCGGACATTGG	
		UH13: CGTGCGAGCCGGTGTATGGG	Imbert *et al* (1996)
		UH10: GGCGACGCTAGAAGGCCGCT	
MJD/SCA3	*ATXN3*	MJD52: CCAGTGACTACTTTGATTCG	Kawaguchi *et al*[19]
		MJD25: TGGCCTTTCACATGGATGTGAA	
		SCA3 F: TTTTTAAATATACTTCACTTTTGAATG	Juvonen *et al* (2005)
		SCA3 R: TGTGAACTCTGTCCTGATAGGT	
		SCA3 Fanch: GAATGTTTCAGACAGCAGCAAAAGCAG	(Martindale, unpublished)
		used with the MJD25 reverse primer[19]	
SCA6	*CACNA1A*	S-5-F1: CACGTGTCCTATTCCCCTGTGATCC	Zhuchenko *et al*[13]
		S-5-R1: TGGGTACCTCCGAGGGCCGCTGGTG	
SCA7	*ATXN7*	4U1024: TGTTACATTGTAGGAGCGGAA[b]	David *et al*[14]
		4U716: CACGACTGTCCCAGCATCACTT	
		H2: GTAGGAGCGGAAAGAATG	Del-Favero *et al* (1998)
		H1: TTCAGGACTGGGCAGAGG	
		SCA7 F: CGGCGGCGGGCGGAGCAGCG	Juvonen *et al* (2005)
		SCA7 R: TGCGCCGTGGCGGCGGTGGCG	
		SCA7 F: ATGTCGGAGCGGGCCGCGGATGACGTCAGG	Martindale, unpublished)
		SCA7 R: GGAGGCGGCGGCTGCTGCTGCTGCTGCTGC	
SCA8	*ATXN8*	SCA8-F3: TTTGAGAAAGGCTTGTGAGGACTGAGAATG	Koob *et al*[28]
		SCA8-R4: GGTCCTTCATGTTAGAAAACCTGGCT	
		SCA8 F: GCAGTATGAGGAAGTGTGGAAA	Majounie *et al* (2007)
		SCA8 R: GGTCCTTCATGTTAGAAAACCT	
SCA10	*ATXN10*	attct-L: AGAAAACAGATGGCAGAATGA	Matsuura *et al* (2000)
		attct-R: GCCTGGGCAACATAGAGAGA	
		SCA10 F: CTCCAGTGCAACCACTTTTAGA	Majounie *et al* (2007)
		SCA10 R: AGGCAGGAGAATTGCTTGAA	
SCA12	*PPP2R2B*	A: TGCTGGGAAAGAGTCGTG	Holmes *et al* (1999)
		B: GCCAGCGCACTCACCCTC	
		SCA12 F: TGCTGGGAAAGAGTCGTG	Majounie *et al* (2007)
		SCA12 R: CAGCGCACTCACCCTCAC	
SCA17	*TBP*	TBP-F: CCTTATGGCACTGGACTGAC	Koide *et al*[21]
		TBP-R: GTTCCCTGTGTTGCCTGCTG	
		SCA17 F: AACACCAATAGTCTGTCTATTTTG	Juvonen *et al* (2005)
		SCA17 R: TGCTGGGACGTTGACTGCTG	
		SCA17 F: GACCCCACAGCCTATTCAGA	Majounie *et al* (2007)
		SCA17 R: GGGACGTTGACTGCTGAAC	

[a]Full references are available at: http://www.scabase.eu.
[b]A SNP at the underlined nucleotide has been reported to EMQN (by Ottie O'Brien, Newcastle, UK).

must be sufficient to determine segregation patterns and identify the high-risk haplotype.[10,11]

Before offering any SCA test, assays should be validated with relevant positive and negative controls. The consensus was that a test should not be offered unless a 'mutation-positive' control is available. Exchange of controls between laboratories is to be encouraged, taking advantage of regional or ethnic differences, to allow them to offer a wider range of tests.

Methodologies

The group discussion regarding appropriate *analytical methods* led to agreement that any one was suitable, as long as it had the capacity to detect alleles in the normal and expanded ranges and resolve alleles one repeat apart. Agarose gels are thus clearly not appropriate, and direct sequencing will not be needed in most instances[6] (see exceptions below). Most methods are PCR based; however, access to additional testing protocols, which can include TP-PCR or Southern

Table 3 Reference ranges for oligonucleotide repeat sizes at the main SCA loci[a]

ATAXIA	*Repeat*	*Normal*	*Uncertain*[b]	*Reduced penetrance*	*Full penetrance*	*References*[a]
SCA1	$(CAG)_n$ $(CAT)_n$ $(CAG)_n$[c]	6–38; 39–44 CAT interrupted	—[d]	—	39–44 CAGs uninterrupted; 45–91	Orr *et al*[15]; Quan *et al*[22]; Goldfarb *et al*, 1996; Zühlke *et al*, 2002)
DRPLA	$(CAG)_n$	6–35	—	—	49–93	(Koide *et al*, 1994; Nagafuchi *et al*, 1994; Shimojo *et al*, 2001)
SCA2	$[(CAG)_n$ CAA $(CAG)_n]_n$[e]	14–31	32–34[f]	—	35–500	(Imbert *et al*, 1996; Pulst *et al*[18]; Sanpei *et al*, 1996; Cancel *et al*, 1997; Leggo *et al*, 1997; Costanzi-Porrini *et al*, 2000; Fernandez *et al*, 2000; Choudhry *et al*, 2001; Silveira *et al*, 2002; Mao *et al*[34]; Kim *et al*[25])
MJD/SCA3	$(CAG)_2$ CAA AAG CAG CAA $(CAG)_n$	11–44	45–59[g]	—	61–87	(Maciel *et al*[20]; Takiyama *et al*, 1997; van Schaik *et al*, 1997; Egan *et al*, 2000; Maciel *et al*, 2001; van Alfen *et al*, 2001; Padiath *et al*, 2005; Gu *et al*, 2004)
SCA6	$(CAG)_n$	4–18	—	19	20–33	(Ishikawa *et al*, 1997; Zhuchenko *et al*[13]; Stevanin *et al*, 1997; Shizuka *et al*,1998, Yabe *et al*, 1998; Katayama *et al*, 2000; Mariotti *et al*, 2001; Takahashi *et al*, 2004)
SCA7	$(CAG)_n$	4–19	28–33	34–35	36–460	(David *et al*, 1998; Stevanin *et al*, 1998; Koob *et al*, 1998; Benton *et al*, 1998; Johannson *et al*, 1998; Giunti *et al*, 1999; Nardacchione *et al*, 1999; Van de Warrenburg *et al*, 2001; Ansorge *et al*, 2004; Whitney *et al*[35])
SCA8	$(CAG/TAG)_n$	14–42	—[h]	$\geqslant$74–1000[h]	—[h]	Koob *et al*[28]; Silveira *et al*[29]; Vincent *et al*, 2000; Cellini *et al*, 2001; Ikeda *et al*[31]; Sulek *et al*, 2004)
SCA10	$(ATTCT)_n$[i]	8–32	280	>280–850	850–4500	(Matsuura *et al*, 2000; Alonso *et al*, 2006; Matsuura *et al*, 2006; Wang *et al*, 2008; Raskin *et al*, 2007)
SCA12	$(CAG)_n$	4–32[j]	40–45[j]	—	51–78[j]	(Holmes *et al*, 1999; Fujigasaki *et al*, 2001; Holmes *et al*, 2003; Hellenbroich *et al*, 2004; Bahl *et al*, 2005)
SCA17	$[(CAG)_n$ $(CAA)_n$ $(CAG)_n]_n$	25–42	—	43–48	49–66	(Zühlke *et al*, 2003; Oda *et al*, 2004, Mariotti *et al*, 2007; Zühlke and Bürk, 2007; Stevanin and Brice, 2008)

[a]Full references are available at: http://www.scabase.eu.
[b]'Uncertain' range was defined whenever there was only one, or two or more contradictory reports.
[c]May be interrupted by 1 to 3, or exceptionally 4, CATs.
[d]One report of non-penetrance with a 44 repeat allele, but not described as pure or interrupted (Goldfarb *et al*, 1996).
[e]The CAG repeat may be pure or have 1 to 4 CAA interruptions.
[f]An interrupted 32 repeat allele found in a patient (Silveira *et al*, 2002); an uninterrupted 32 CAGs allele in a (young) asymptomatic person (Cancel *et al*, 1997); a 33 pure CAG repeat in one patient (Fernandez *et al*, 2000); a 34 interrupted repeat in one patient (Constanzi-Porrini *et al*, 2000); 32, 34 and 35 interrupted repeats found in patients with Parkinsonism.[25]
[g]A 45 CAG allele in one patient (Padiath *et al*, 2005); one family segregating a 51 CAG allele, apparently not associated with the disease (Maciel *et al*, 2001); a 51 allele in one patient from a MJD family (Gu *et al*, 2004); one family segregating 53 and 54 alleles associated with an 'abnormal phenotype' (van Alfen *et al*, 2001); a 54 CAGs allele in a patient from a MJD family (van Schaik *et al*, 1997); a 55 allele described in one patient (Egan *et al*, 2000); a 56 allele described in one patient (Takiyama *et al*, 1997).
[h]Pathogenic ranges and incomplete penetrance are very uncertain in SCA8; there is a large overlap of repeat sizes in patients and in persons with no symptoms and no family history of ataxia, but it may depend on the family; however, expansions were also present in 0.4% of controls;[31] Silveira *et al*[29] had reported before different ranges, seeing no overlap in controls (15–91) and pathogenic (100–152 repeat) alleles; however, they found a very high instability in sperm (contractions and expansions), both for expanded and normal alleles; patients were found with schizophrenia or bipolar disorder, depression, or borderline personality disorder with 1140 and 1300 repeats (Vincent *et al*, 2000).
[i]Interruptions with multiple penta or septarepeats or complex ATTGT-TTTCT repeats are found both in normal alleles with $\geqslant$17 repeats, and in pathogenic alleles (Matsuura *et al*, 2006; Hagerman *et al*, 2009).
[j]Alleles with 40 and 41 repeats, in two patients, were included in a review by Hellenbroich *et al* (2004); a 45 repeat allele was seen in an Indian individual without neurological or psychiatric signs or symptoms and with no known family history (Fujigasaki *et al*, 2001); an asymptomatic homozygote for expanded alleles (52 and 59 repeats) was reported by Bahl *et al* (2005); an Iranian woman with unipolar depression and her MZ twin sons with schizophrenia all had a 53-CAG repeat (Holmes *et al*, 2002).

blotting (either in-house or elsewhere), is essential for the detection of very large expansions. This is particularly important in SCA2 and SCA7, in which juvenile-onset cases are known to exist and homoallelism is frequent. Exchange of validated protocols between laboratories is to be encouraged. Sequencing of alleles at the boundary or at the interval between normal and pathogenic ranges is essential; this is particularly so in SCA1, for example, in which normal and disease ranges overlap and in which whether the allele is pathogenic depends on its interspersed or pure structure.

Laboratories should be aware of the *limitations of the assays* used and include the appropriate disclaimers in their reports. In some diseases in which expansions may not account for all mutations, sensitivity will be less than 100%. Assay design is extremely important; keeping amplicons as small as possible maximizes resolution and the chance of amplifying large alleles. There is a potential to fail to amplify alleles if the primer binding sites contain SNPs (Table 2); an ongoing review of primer sequences against regularly updated SNP databases, such as dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/), is vital. It is important to remember that SNPs and their frequencies are likely to vary among populations; hence, although protocol sharing is to be encouraged, it may not be possible to standardize assays. The impact of primer site SNPs has been discussed for the diagnosis of Huntington disease.[12]

It is necessary to know exactly what is being measured and *how the repeat is defined* for any given SCA, as interspersions within the array may have a bearing on whether the repeat is pathogenic. One

definition of 'the repeat' is the stretch of sequence containing the repeated motif(s), bounded by unique flanking sequences. Whereas the variable portion of a motif seems the logical thing to measure, a clear definition of what constitutes a pure or interrupted repeat may not be so easy. It may be necessary to define a 'repeat' differently for different SCAs, depending on the structure of the array in each gene. The significance of any interspersions should be well understood. Moreover, appropriate and current knowledge of scientific literature is essential, and the formulae from the original publications for each SCA should be used to ensure consistency. Unlike *CACNA1A*[13]and *ATXN7*,[14] in which the repeat motif is pure (uninterrupted), several $(CAG)_n$ tracts contain variant sequences, such as CAT, CAA and/or AAG in *ATXN1*,[15–17] *ATXN2*,[17,18] *ATXN3*[19,20] and *TBP*[21] genes. Conventionally, these variant triplets are included when determining the total repeat number (Table 1).

Accurate *repeat sizing* is obviously important in determining whether an allele is in the pathogenic or normal range, particularly when it lies outside or at the boundary between defined ranges. For any SCA, there is a theoretical formula relating the amplicon size in base pairs to the number of repeats, by removing non-repetitive flanking sequences and dividing by three; this gives the repeat size, *including* any existing interruptions (Table 1). Sizing by an internal size marker may not be accurate, as this is not necessarily a linear conversion. Inaccuracies may also be generated by a mosaic template or by errors during amplification. The presence of CAT interruptions (not coding for glutamines) may hold some pitfalls. Special caution is needed when assessing the $(CAG)_n$ near the border of normal and expanded repeats in the *ATXN1* gene. Normal SCA1 repeats contain usually one to three (exceptionally four) CAT interruptions, resulting in shorter polyglutamine tracts when translated. In contrast, expanded SCA1 alleles are characterized by a pure CAG stretch; however, in rare cases, SCA1 expansions also have stabilizing CAT interruptions and may be wrongly considered pathogenic if only sized:[22] the precise size and exact repeat configuration of these expanded CAG/CAT alleles need to be determined carefully, for example, by sequencing. In SCA2, expansions over 36 repeats are generally composed of pure CAG stretches; nevertheless, there are reports of intermediate size SCA2 alleles with CAG repeats interspersed by CAA in patients manifesting with Parkinson disease.[23–26]

Although there were no absolute conclusions about the exact *acceptable margins of error*, the consensus was that this could be different for each SCA, as well as for normal alleles (± 1) and most larger expansions (± 3); outside or close to the boundary of normal and pathogenic alleles, precision should be to the repeat unit, as this may have clinical consequences (together with the exact sequence, if interruptions are possible).

The use of *accurate size controls* is strongly recommended, in which the size of alleles has been determined by sequencing (or any other appropriate method). This allows laboratories to construct their own allelic ladder for each SCA being tested, and confers a high level of repeatability and reproducibility. A sufficient number of alleles should be sized in this manner to allow correlation between base pairs and repeats, including some at the upper border of the normal range. For larger alleles, sizing only on the basis of a panel of normal alleles is likely to be underestimated, and this may have clinical relevance. It is desirable to accurately determine the largest abnormal allele available and include it in any allelic ladder.

Validated, standard and *certified reference materials* (CRMs) are not yet available for SCAs; there is a definite need for development of such materials and to consider the issues of possible sources of CRMs, obtaining sufficient quantities of DNA, organizing its distribution and quality control, and responsibility for its production, validation and certification for diagnostic use. If lymphoblastoid cell lines are to be the source of CRMs, there will be the need to validate each new culture batch.

The extent of *multiple testing* for SCA loci should depend on the physician's request, and the clinical information and family history provided. Prioritizing loci for testing is an important issue for laboratories and clinicians, both in terms of costs and time saved to achieve a correct diagnosis and enable genetic counselling; as more powerful and cheaper technologies become available, this may tend to change. It should not be forgotten, however, that multiple testing may augment the error rate (false positives) and increase the chance of disclosing unrequested and unwanted information; it was consensual that this is not appropriate for PST. Testing for other disorders, such as Huntington disease or *FMR1* premutation testing for fragile-X tremor-ataxia syndrome, may be clinically indicated, but should also require a specific request from the physician. In cases in which there is no clear family history, testing for FRDA may also need to be considered; indeed, the detection rate for FRDA seems to be higher than that for any of the SCAs among isolated cases of ataxia, even if of late-onset.

Consideration was given to the issue of *clinical utility of SCA8* testing and whether it should be offered on a routine basis. In spite of recent advances, the pathogenesis of this disease is still poorly understood, and it has shown reduced penetrance on multiple occasions, even in familial cases.[27] There is a danger of assigning too much relevance to an expansion, given its high prevalence and when it is still disputed by some in which size range SCA8 expansions are pathogenic.[28–31] Families in which affected individuals have another SCA, in addition to an SCA8 expansion, are known.[30,32] Ceasing to test other loci, if a SCA8 expansion is found, could lead to missing the causative mutation. There is a thought that PST or PND should not be offered for SCA8; however, some consider that genetic linkage testing could be offered, if the expansion segregates with the disease and penetrance can be confirmed in the family.

It was strongly felt that a direct prenatal test is far preferable to prenatal *exclusion testing* (PNE); however, some couples insist on PNE, and the at-risk partners have a right not to know their status. A precedent has been set in HD;[33] although it may be difficult to justify refusal of a PNE test for a SCA, this will depend on local practices and legislation. It is essential, however, that samples from sufficient affected and unaffected relatives are available to establish the phase, before offering any such test.

Interpretation and reporting

Definition of *normal and pathogenic ranges* can present a problem, as the data available in literature are not consistent, being based on studies by different groups and in different populations, and with variable accuracy and methodologies (see, eg, GeneReviews in http://www.genetests.org). It is very important for laboratories to have a mechanism to regularly review the literature and update the range definitions used. Normal and pathogenic size ranges, however, are not definitive, and, ideally, a curated resource of repeat definitions and updated ranges, formulae and primers (Tables 1–3; to be updated in SCAbase, http://www.scabase.eu) was an aspiration.

The issue of whether allele sizes should be reported was presented at the BP meeting and in the electronic discussion group, but was mostly debated before endorsement by EMQN and the harmonization process with other repeat diseases. Arguments *in favour of reporting allele sizes* include the following: (1) laboratories should not hide data

that they must obtain anyway; (2) a recommendation to use accurately sized controls has been made, and there is no reason why alleles cannot be sized accurately; (3) allele sizes provide precise data, allowing better interpretation; (4) a precise measure is important for quality control; (5) it allows other laboratories to test family members more readily (particularly important for PST and PND); (6) the evidence needed for precise ranges will be more readily available in different populations; (7) size is critical for interpretation of 'intermediate' alleles and, more importantly, of alleles at or near the boundaries of normal and expanded ranges, as (8) definitions of these ranges are likely to change over time.

Arguments *against reporting allele sizes*, raised mainly by some UK laboratories, include the following: (1) the laboratory would spend time sizing normal alleles, generating unnecessary work with no useful benefit; (2) unless allele sizes can be accurately determined, they should not be reported; (3) too much emphasis can be put on the exact size of expanded alleles both by clinicians and patients; (4) it causes too much complication for clinicians; (5) nonpaternity might be revealed; (6) a genotype–phenotype correlation is not always clear; (7) quoting sizes introduces a potential for error; and (8) allele size may differ between laboratories in any case.

Debate ensued particularly around the issue of *reporting normal allele sizes*; however, the consensus at the BP meeting was that (1) both alleles should always be mentioned in reports and (2) their sizes should be provided. Laboratories also need to include in the report (3) the methods used to size repeats and their limitations; (4) the error limits of their measurement; (5) the normal, uncertain and pathogenic reference size ranges; and (6) a clear interpretation. (7) In the event of homoallelism, particularly if no additional methods could be used, the report should definitely mention the repeat size and the population frequency of that normal allele and/or of that particular genotype: the clinical implications of reporting two alleles of a common size are clearly different from homoallelism for a rarer variant.

Experience and updated knowledge of relevant scientific literature is essential in the field of SCA testing, including for the *particularities of each SCA*. The existence or absence of CAT stabilizing interruptions is very important in SCA1. The low polymorphism of normal SCA2 alleles is of great help, particularly in cases of homoallelism. Overlap of low penetrance and complete penetrance alleles in SCA6 must be considered. The possibility of very large expansions in SCA7, and to a lesser extent in SCA2, should always be taken into account.[14,34,35]

The *gender of the patient* or presymptomatic carrier is also crucial in that regard: instability and potential for very large expansions in SCAs caused by a $(CAG)_n$ within its coding region are mainly observed in paternal transmissions; in SCA8, however, this may occur mainly on maternal transmission.

Finally, it was agreed that the *use of HGVS-approved nomenclature* is potentially confusing and, thus, not appropriate for reporting repeat expansions, although it should always be used to report point mutations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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