

EMQN Best Practice Guidelines for Molecular Genetic Testing of the SCAs

Prepared on behalf of the European Molecular Quality Genetics Network (EMQN) by

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following a EMQN Best Practice Meeting, 17-19 October 2007, Porto, Portugal, as a part of the EU Network of Excellence EuroGentest, and subsequent electronic group discussion in 2008; endorsed by the EMQN board in 2009.

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GENERAL REQUIREMENTS FOR LABORATORIES

Laboratories offering molecular genetic testing for the spinocerebellar ataxias (SCAs) are encouraged to seek accreditation.

Laboratories should also refer to and be acquainted with the OECD Guidelines for Quality Assurance of Molecular Genetic Testing, 2007, as well as the EMQN reporting and internal quality control guidelines.

Both in-house developed and commercial assays should be validated before use.

Annual participation in External Quality Assessment (Proficiency Testing) schemes for SCA analysis is essential.

PANEL OF MOLECULAR GENETIC TESTS

Each laboratory should define an appropriate panel of tests, according to their established priorities, which should be adapted to the prevalence rate or relative frequency of the various SCA types in the population(s) that they serve.

Where not available, specific population studies should be encouraged.

Mechanisms should be in place to keep allelic ranges updated (e.g., a database with key papers, local laboratory data and/or a critical review of up-to-date information).

As a minimum requirement, laboratories offering testing for SCAs must be able to accurately perform molecular genetic testing for SCA1, 2, 3 (MJD), 6 and 7.

Given the partial clinical overlap within the SCAs and of these with other diseases, the offer of testing for other SCA forms (including DRPLA), and for diseases such as FRDA, HD, FXTAS, may be appropriate, particularly in certain populations.

Tests should be excluded from the routine SCA panel until appropriate controls representing alleles in the pathogenic range are available to the laboratory.

If there is a clinical need for a particular test that the laboratory cannot perform, for instance, because it does not have the appropriate positive controls, it should consider transferring the sample to another laboratory which routinely offers that test.

The clinical validity of direct molecular genetic testing for SCA8 by CAG repeat sizing has not yet been established; thus, SCA8 should not currently be offered as a routine diagnostic test, if family history is unavailable or unknown.

Testing for SCA8, however, may be appropriate in large pedigrees in which the expansion has proven to segregate with the disease; nevertheless, the lab should be aware and state in their report that finding an expansion for SCA8 in a patient does not exclude the presence of another causative mutation.

PRE-TEST REQUIREMENTS AND CRITERIA FOR TESTING

- **Criteria for acceptance of samples**

The most appropriate route for the request of a diagnostic test for SCAs is through a clinical neurologist, but this practice may vary amongst countries.

Presymptomatic and prenatal testing procedures request that SCAs should be referred by a clinical geneticist.

- **Test referral and essential samples**

The type of SCA test required - diagnostic, presymptomatic (PST), prenatal diagnosis (PND) or preimplantation genetic diagnosis (PGD), and the clinical questions must be clearly defined by the requesting clinician in writing.

Relevant familial samples other than the sample from the subject may be required and should be requested from the clinician by the laboratory before testing; test results and reports for family members other than for the subject should not be issued, unless they have been specifically requested.

Familial samples other than the sample from the subject are essential for PND and PGD, and preferable for PST.

- **Requirements for diagnostic testing**

There should be a written statement from the referring clinician indicating the presence of clinical symptoms in the patient to avoid performing a PST inadvertently without the appropriate genetic counselling protocol.

The age at onset of first symptoms and a brief clinical description, together with the appropriate written informed consent form, should be provided by clinicians along with the referral.

A family history (including mutation information, where known), or its absence, should be disclosed to the laboratory.

Diagnostic testing of minors is only appropriate provided the parents of the child or of the adolescent have been appraised of the implications of confirming a diagnosis and have given informed consent for the test; whenever there is any uncertainty, the laboratory should confirm with the referring clinician that appropriate counselling is available.

Laboratories should modify their SCA testing priorities and consider testing for further genes on a case by case basis on the basis of local population disease frequencies, the geographic and ethnic origin of the patient and the information provided in the referral with regard to clinical presentation and the apparent mode of inheritance.

Further testing may be appropriate when there is a family history, depending on the clinical presentation and the physician's request.

In the absence of a positive family history, the laboratory may want to consider the possibility of testing for FRDA, FXTAS, or other genes as well, depending on the clinical presentation and the physician's request.

- **Requirements for PST**

At present, no PST of minors should be undertaken for SCAs, as there are no advantages for them.

It is essential that the genetic counselling service and the laboratory jointly document and follow a clearly defined protocol for PST.

Any request for a PST must be made through a genetic counselling service or be accompanied by a document stating that pre-test genetic counselling took place.

Evidence of written informed consent for PST, signed by the consultand and the physician, should be provided with the referral, in accordance with local practices.

PST should only be performed after confirming a molecular genetic diagnosis in the family and establishing a positive familial control; a report from an accredited diagnostic laboratory may be sufficient to establish a diagnosis within a family and offer a PST to a relative.

However, testing in a family with strong clinical evidence but without a confirmed molecular diagnosis may have to be considered occasionally and, if that is the case, the limitations of a non-carrier test result should be discussed in detail in the final report.

Each laboratory should decide, after discussing with their partner laboratories, whether to accept DNA extracted elsewhere for PST or to request blood samples.

For PST, one DNA extraction from one sample is not considered to be sufficient; as a minimum, two DNA extractions on separate occasions should be performed for PST, although a separate collection of two blood samples is preferred.

PST for SCA8 should be undertaken with caution, as its clinical relevance has not yet been established with certainty; however, it may be appropriate for members of large pedigrees in which an expansion clearly segregates with the disease and its penetrance can be estimated.

- **Requirements for PND and PGD**

It is essential that there be clearly documented joint protocols for PND and PGD that are followed by the genetic counselling service, the prenatal or assisted reproduction technologies centre and the laboratory.

Any request for a PND or PGD must be made through a genetic counselling service or accompanied by a document stating that pre-test genetic counselling took place.

Evidence of written informed consent for PND or PGD signed by the consultand and the physician should be provided with the referral, in accordance with local practices.

For both PND and PGD, the mutation must have been previously identified in the family by diagnostic testing in a proband and/or by PST in the at-risk parent.

PND for SCA8 should be undertaken with caution, as its clinical relevance has not yet been established; PGD for SCA8 is not currently recommended with the evidence and methods available.

Direct foetal genotyping is strongly recommended for PND, although exclusion testing may be offered if requested, depending on local practices and legislation.

For PND, both parental samples are desirable; a sample from the parent who is affected or carries the mutation is required as a familial positive control, and a sample from the mother, irrespective of whether she is affected or is a mutation carrier, is also required to exclude possible maternal contamination

For PGD, both parental samples are essential. Samples and studies of other affected and non-affected relatives may provide additional valuable information and should be completed in advance of offering the test to a couple.

ANALYTICAL METHODS

- **Approved/preferred methodologies**

The minimum requirements for any method to perform testing for SCAs, are the following: (a) to be able to discriminate between the presence or absence of an allele in the disease range and (b) to size alleles accurately and be able to resolve alleles one triplet apart in size.

Capillary electrophoresis is preferred, although other methods suiting the minimal requirements are acceptable.

It is appropriate for laboratories to exchange validated standard operating procedures among themselves, and they should be encouraged to do so.

- **Primers and assay design**

Assay design should be carefully considered before any routine testing is offered.

This should include consideration of the potential effect of SNPs under primer sites.

Amplicon sizes should be chosen to maximize the resolution of the assay.

There should be a list of published primer sequences that can be used by all laboratories.

- **Appropriate panel of controls**

If no positive control is available for a given trinucleotide repeat expansion, the test cannot be validated and so should not be offered in a diagnostic routine setting.

It is appropriate for laboratories to exchange positive controls, and they should be encouraged to do so.

The use of a PCR blank and a large expansion control is a minimum requirement; the latter is particularly important when testing possible infantile/juvenile onset cases.

The use of other controls, such as a normal heterozygote (preferably with alleles one repeat apart), intermediate alleles or small expanded alleles, is highly recommended.

Control samples including alleles located at or near the boundaries of normal and mutant ranges are extremely useful; this is particularly important when testing cases with a later age-of-onset.

The use of sequenced alleles as internal controls is highly recommended, to enable accurate repeat sizing.

It is essential that validated, standard and certified reference materials be developed and used for SCA assays.

- **Repeat sizing**

The variable portion of a motif is the ideal fragment to measure; however, a clear definition of pure and interrupted repeats may not be possible on the basis of the evidence currently available.

The current practice of referring to the first description in the literature as a basis for sizing repeats should continue as it promotes consistency and continuity.

Laboratories should be aware of the exact sequence of each oligonucleotide repeat causing SCA, including interrupted and imperfect repeats, and should use the published formula to count the repeat number for each SCA locus.

Laboratories should be aware that determination of the number of repeats is potentially compromised by a mosaic template, or as a consequence of errors during PCR amplification.

The allele size should be designated by its highest peak (capillary electrophoresis) or by its strongest band (polyacrylamide gel electrophoresis).

Laboratories should be aware that the conversion of base pairs into repeat units might not be linear and that standard size ladders may not give correct results; all efforts should be made to determine CAG repeat sizes as accurately as possible by means of an allelic ladder.

Laboratories should be able to provide evidence of repeatability and reproducibility in their determination of allele size.

Wherever possible, laboratories should sequence any large normal, intermediate and/or low penetrance alleles identified and use them routinely as internal controls.

Error margins should be determined for each range and each locus; an uncertainty of ± 1 triplet repeat for smaller normal alleles and of ± 3 for expanded alleles is acceptable in most tests and for most loci; however, an uncertainty of only ± 1 is acceptable for SCA6 expansions.

At the boundaries between normal and mutant alleles, however, as well as for large normal, uncertain and reduced penetrance alleles, repeat sizes should be determined as accurately as possible by sequencing or other method(s).

Phenotypic and genotypic evidence should be gathered continuously with a view to better define allele size ranges, particularly in the case of intermediate alleles.

Any alleles in the size range in which interruptions may be relevant for the phenotype or for the stability of the allele (as is the case for SCA1) must be sequenced or have the interruption pattern determined by another validated method.

- **Allele size ranges**

Appropriate and up-to-date knowledge of available scientific literature is essential.

Reviews of up-to-date ranges of repeat sizes are available for the most frequent SCAs.

Laboratories should be aware that these ranges change with new descriptions in the scientific literature, and should make efforts to keep up with them.

Laboratories should gather data to establish the frequency distribution of normal size and expanded SCA alleles in the populations that they serve, and refer to these when interpreting results.

- **Limitations**

Laboratories should be aware that PCR may fail to amplify very large expansions, and this must be borne in mind particularly when testing very early-onset cases.

Laboratories should also be aware that there may be sequence variations that could give rise to non-amplification of normal or expanded alleles.

Alternative or complementary methods should be available at the laboratory to assist in the resolution of these cases or these samples should be referred to other laboratories that have them.

For loci in which disease may be caused by point mutations (e.g., SCA6), laboratories should be aware that the sensitivity of assays based on sizing triplet repeat arrays may be less than 100%.

- **Homoallelism**

Results showing homozygosity for normal alleles of the same size (homoallelism) should be confirmed by an appropriate method (e.g., triplet primed-PCR or Southern blotting), especially in early-onset cases (below the age of 26); if the patient seems to be homoallelic for a rare normal allele, it may be helpful to obtain samples from the parents or from other relatives.

If homoallelism is not confirmed by a complementary technique, then the possibility of the assay failing to detect a large expansion must be considered and discussed in the report.

Knowing and reporting the local population frequency of that particular allele is useful; population studies should be encouraged in cases in which evidence is lacking.

INTERPRETATION AND REPORTING

The HGVS guidelines for nomenclature are not the most appropriate for reporting the results of SCA caused by repeat expansions.

HGVS nomenclature should be used to report point mutations; classical nomenclature may also be included.

Laboratories are routinely expected to measure allele sizes and determine margins of error (uncertainty) for the purposes of internal quality control and comparing their analytical performance through external quality assessment; in addition, these data allow them to contribute to research on the relationship between genotype and phenotype.

Measurement accuracy and precision should be sufficient for laboratories to assign confidently alleles to recognized ranges; a report should assign alleles to ranges that may include normal,

large normal unstable, uncertain pathogenicity, incomplete penetrance or full penetrance; reporting actual alleles' size is subject to local practice.

The methodologies used, as well as their possible limitations, should be indicated in the report.

When reporting a homoallelic result, particularly in infantile/juvenile cases, a clear mention should be made of the possibility of having missed a large expansion, if complementary methods have not been used or have been uninformative; a request for additional familial samples may also be appropriate; this is particularly important for SCA2 and SCA7.

The report of a homoallelic result should also reflect the population frequency of that allele and that particular genotype, wherever this information is available.

A comment on mitotic instability of expanded and intermediate alleles is appropriate for those alleles at loci known to undergo further large expansion on occasion (as is the case of SCA7); it is also appropriate to mention the increased risk relating to the gender of the transmitting parent.

There must be a recommendation of a referral for genetic counselling in the case of a confirmation of a diagnosis, or in cases in which no mutation is detected in a patient with symptoms and a family history of the disease.

The implications of the confirmation of a diagnosis for relatives of the proband and the availability of testing of other family members and PND should be clearly stated.

If a SCA8 expansion is detected, the report needs to discuss the uncertainty of its significance and the possibility that other mutations may be segregating in the family; a referral for genetic counselling must also be recommended.

In cases in which no mutation is detected in a patient with symptoms, the report should recommend a re-evaluation of the clinical diagnosis and further testing (e.g., other SCAs, recessive ataxias, FXTAS, HD), where appropriate.

The report of a 'non-carrier' result in PST should reflect the degree of certainty of the genetic diagnosis in the family; if the correct diagnosis in a proband could not be confirmed or is uncertain, this must be clearly stated in the report.

24 February 2010

BP Guidelines published in: *Sequeiros J, Martindale J, Seneca S: EMQN Best Practice Guidelines for molecular genetic testing of SCAs. Eur J Hum Genet 2010; Feb 24 [Epub ahead of print]*

See also: *Sequeiros J, Seneca S, Martindale J: Consensus and controversies in best practices for molecular genetic testing of spinocerebellar ataxias. Eur J Hum Genet 2010; Feb 24 [Epub ahead of print]*

For additional material see: **SCAbase - an evidence-based online resource in the field of the spinocerebellar ataxias**: <http://www.scabase.eu>