Proposal form for the evaluation of a genetic test for NHS Service Gene Dossier

Test – Disease – Population Triad

Disease – name	Amyotrophic Lateral Sclerosis 10 (ALS10) and Amyotrophic Lateral Sclerosis 6 (ALS6)
OMIM number for disease	612069, 608030
Disease – alternative names please provide any alternative names you wish listed	Motor Neurone Disease (MND) Familial Amyotrophic Lateral Sclerosis (FALS)
Disease – please provide a brief description of the disease characteristics	MND/ALS is an adult-onset, relentlessly progressive neurodegenerative disease caused by progressive cell death of the upper and lower motor neurones in the brain, brainstem and spinal cord. This results in muscular atrophy, cramps and fasciculations. Onset can occur in the limb or bulbar muscles, but generally spreads quickly to other muscle groups, leading to death, usually as a result of respiratory failure, 2-3yrs after disease onset. MND/ALS is genetically heterogeneous. Mutations in both *TARDBP* and *FUS* are inherited in an autosomal dominant manner.
Disease - mode of inheritance	It is usually sporadic, although 5-10% of cases are familial, predominantly inherited in an autosomal dominant manner.
Gene – name(s)	TAR DNA binding protein (*TARDBP*) Fused in liposarcoma (*FUS*)
OMIM number for gene(s)	605078, 137070
Gene – alternative names please provide any alternative names you wish listed	*TDP43* Translocated in liposarcoma (*TLS*)
Gene – description(s) (including number of amplicons).	The *TARDBP* gene is comprised of 6 exons and can be alternatively spliced - 11 mRNA transcripts have been detected. This gene encodes TDP43, originally identified as a protein that binds the transactive response (TAR) region of HIV-1 DNA. However, it also acts as a transcriptional repressor, an activator of exon skipping (in the *CFTR* gene) and also as a scaffold protein for nuclear bodies. The *FUS* gene is comprised of 15 exons and is located on chromosome 16p11.2. It forms oncogenic fusion proteins associated with liposarcomas and leukaemia. This gene encodes a protein which is localised in both the nucleus and cytoplasm and is involved in transcriptional activation, alternative splicing, transportation of RNA and is also present in heterogeneous ribonuclear proteins.

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Mutational spectrum for which you test including details of known common mutations.	Mutation screening can be performed on all 6 exons of *TARDBP* to include intron/exon boundaries and flanking 5' and 3' UTRs. However, to date, all bar one mutation have been located in exon 6 of the *TARDBP* gene, in which recurrent mutations have been identified. The other mutation was found in exon 3. *FUS* mutations have been identified most frequently in exons 14 and 15, although non-synonymous changes, some of which have also been identified in control populations, have also been found in exons 5 and 6. However, since the *FUS* gene was only identified in 2009, there are currently insufficient publications to definitively state which exons harbour the most common mutations.
Technical Method (s)	Bidirectional sequencing of genomic DNA and analysis on capillary sequencer (ABI3730).
Validation Process Note: please explain how this test has been validated for use in your laboratory	The *TARDBP* test was validated by blinded sequencing of a panel of anonymised control DNAs tested in a research laboratory; some with a mutation identified and some without. All results obtained were congruent with the results obtained on a research basis. The *FUS* test has not yet been validated. However, a panel of anonymised control DNAs tested in a research laboratory is available for blinded testing; some of which will have had a mutation identified and some which will not. The results obtained will be compared with the results obtained on a research basis.
Are you providing this test already? **If yes, how many reports have you produced?** **Please give the number of mutation positive/negative samples you have reported**	***TARDBP*: Yes** **Number of reports issued: 9** **Number of reports mutation positive: 3** **Number of reports mutation negative: 6** ***FUS*: No**
For how long have you been providing this service?	***TARDBP*: 10 months**
Is there specialised local clinical/research expertise for this disease?	**Yes** | **Please provide details** Professor Pamela Shaw and Dr Christopher McDermott run a motor neurone disorders clinic in which they see referrals with MND/ALS from around the UK. They also have an active research programme on various aspects of MND/ALS and have published widely on the subject
Are you testing for other genes/diseases closely allied to this one? Please give details	**Yes** Familial cases are referred and the superoxide dismutase-1 (*SOD1*, OMIM 147450) gene is screened first, as this represents the most common genetic cause of familial MND/ALS (up to 20% of cases).
Your Current Activity If applicable - How many tests do you currently provide annually in your laboratory?	*TARDBP* index cases: Only 8 have been tested to date in this laboratory. Family members where mutation is known: 1 has been tested

Your Capacity if Gene Dossier approved How many tests will you be able to provide annually in your laboratory if this gene dossier is approved and recommended for NHS funding?	Index cases: up to 50 samples, to be tested for both genes Family members where mutation is known: up to 100 (although there is not likely to be a need for this many tests).
Based on experience how many tests will be required nationally (UK wide)? Please identify the information on which this is based	Up to approximately 50 for each gene. There are approximately 1000 new cases of MND a year in the UK, which equates to 50 familial cases assuming that 5% of MND is familial. If we assume 10% of these will be due to *SOD1* mutation, it leaves approximately 45 cases that could potentially be due to *TARDBP* or *FUS*.
National Activity **(England, Scotland, Wales & Northern Ireland)** **If your laboratory is unable to provide the full national need please could you provide information on how the national requirement may be met.** For example, are you aware of any other labs (UKGTN members or otherwise) offering this test to NHS patients on a local area basis only? This question has been included In order to gauge if there could be any issues in equity of access for NHS patients. It is appreciated that some laboratories may not be able to answer this question. If this is the case please write "unknown".	The full national need could be met.

Epidemiology

Estimated prevalence of disease in the general UK population Please identify the information on which this is based	MND/ALS has a prevalence of 5-7 per 100,000 (McDermott & Shaw, *BMJ* 2008; **336:** 658 – 662). *TARDBP* mutations are estimated to account for approximately 5% of familial and 0.4% of sporadic MND/ALS cases (Kirby *et al* 2010, *Neurogenetics* **11:** 217 – 225). *FUS* mutations are also estimated to account for approximately 5% of familial and 0.4% of sporadic MND cases (Hewitt *et al* 2010, *Arch Neurol* **67(4):** 455 – 461; Kwiatkowski *et al* 2009, *Science* **323:** 1205 – 1208).
Estimated gene frequency (Carrier frequency or allele frequency) Please identify the information on which this is based	The overall prevalence of MND/ALS is 7 per 100,000, of which 10% (7 per million) are familial cases. *TARDBP* is estimated to account for 5% i.e. 3.5 cases per 10 million cases and this corresponds to a disease allele frequency of approximately 1.75×10^{-7}. *FUS* also accounts for 5% i.e. 3.5 cases per 10 million cases and this corresponds to a disease allele frequency of approximately 1.75×10^{-7}.

Estimated penetrance Please identify the information on which this is based	There is very little information in the published literature as yet, since both genes were only linked with MND/ALS in 2008. The penetrance for both might be expected to be similar to that observed for other autosomal dominant MND/ALS genes, such as *SOD1* i.e. 80 – 100%.
Target Population Description of the population to which this test will apply (i.e. description of the population as defined by the minimum criteria listed in the testing criteria)	The target population will be limited to patients with manifest progressive disease and a family history of similar, with the presence of upper and lower motor neuron degeneration and the absence of evidence of other disease processes that could be causative. This will be after exclusion of a mutation in the *SOD1* gene and will be regardless of ethnicity or age. Family members will also be tested for known mutations where appropriate.
Estimated prevalence of disease in the target population	5% of the familial cases, which have a prevalence of 7 per million.

Intended Use (Please use the questions in Annex A to inform your answers)

Please tick the relevant clinical purpose of testing	YES	NO
Diagnosis	√	
Treatment		√
Prognosis & Management		√
Presymptomatic testing	√	
Risk Assessment for family members	√	
Risk Assessment – prenatal testing	√	

Test Characteristics

Analytical sensitivity and specificity This should be based on your own laboratory data for the specific test being applied for or the analytical sensitivity and specificity of the method/technique to be used in the case of a test yet to be set up.	Sensitivity of DNA sequencing is over 95%. Since all mutations are checked in two separate amplicons, specificity is 100% where the mutation or type of mutation has been previously reported. Where the change is novel, it may be necessary to carry out family / RNA / population studies and it still may not be possible to reach a definitive conclusion.
Clinical sensitivity and specificity of test in target population The *clinical sensitivity* of a test is the probability of a positive test result when disease is known to be present; the *clinical specificity* is the probability of a negative test result when disease is known to be absent. The denominator in this case is the number with the disease (for sensitivity) or the number without disease (for specificity)	Published literature suggests that approximately 5% of all patients with familial ALS carry *TARDBP* point mutations and a further 5% carry *FUS* point mutations, all of which are detectable by direct DNA sequencing (Kirby *et al* 2010, *Neurogenetics* **11:** 217 – 225, Hewitt *et al* 2010, *Arch Neurol* **67(4):** 455 – 461; Kwiatkowski *et al* 2009, *Science* **323:** 1205 – 1208). DNA sequencing has a high clinical specificity. Although some polymorphisms in the coding sequence of the *TARDBP* gene have been identified, the nucleotide changes have no effect on the protein (Kirby *et al* 2010, *Neurogenetics* **11:** 217 – 225). Some polymorphisms in the coding sequence of the *FUS* gene have been identified; however, the nucleotide changes have also been detected in unaffected control individuals (Hewitt *et al* 2010, *Arch Neurol* **67(4):** 455 – 461). In this laboratory the clinical sensitivity of the *TARDBP* point mutation testing in the cohort tested thus far equates to 3/8 or 37.5%.
Clinical validity (positive and negative predictive value in the target population) The *clinical validity* of a genetic test is a measure of how well the test predicts the presence or absence of the phenotype, clinical disease or predisposition. It is measured by its *positive predictive value* (the probability of getting the disease given a positive test) and *negative predictive value* (the probability of not getting the disease given a negative test).	According to the (limited) published literature, this test is expected to have a positive predictive value of 90 – 100%. In families with a known *TARDBP* or *FUS* mutation, the negative predictive value when testing asymptomatic family members is close to 100%, since the new mutation rate is expected to be low.
Testing pathway Please include your testing strategy if more than one gene will be tested and data on the expected proportions of positive results for each part of the process. Please illustrate this with a flow diagram. This can be added to the document as a separate sheet if necessary.	See next page

Referral Pathway Template –

TARGET POPULATION

Individuals with motor neurone disease and a family history of similar symptoms, or with no family history but a classical motor neurone disease phenotype, where other differential diagnoses and mutations in the *SOD1* gene have been excluded.

WHAT TYPE AND LEVEL OF PROFESSIONAL OR REFERRER DO YOU ACCEPT SAMPLES FROM?

We envisage that initially patients with symptoms of motor neurone disease will present to their GP, who will then refer to secondary care. This is likely to involve neurology services and/or clinical genetics

From here, the patient will be referred to a specialised motor neurone disease clinic / neurology services. If such a clinic is not available the request may be initiated by the referring consultant neurologist / geneticist / paediatrician. If the patient is suspected of having familial MND, genetic testing is requested after counselling and consent.

PLEASE PROVIDE DETAILS OF HOW REFERRALS WILL BE ASSESSED FOR APPROPRIATENESS?

SOD1 mutation to be excluded first (accounting for 10-20% of familial cases), referrals then assessed using *TARDBP* and *FUS* test criteria forms. If cognition is affected or there is a family history of dementia, *TARDBP* testing would be performed first.

HOW MANY TESTS DO YOU EXPECT TO PERFORM ANNUALLY?

Up to 50 diagnostic samples to be tested for both genes.
Testing will be carried out in parallel until such time as there is sufficient evidence to demonstrate whether either gene is a more common cause of FALS; the testing strategy will then be reviewed.
Familial tests for known mutations will be done as needed.

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Clinical utility of test in target population (Please refer to Appendix A) Please provide a description of the clinical care pathway.	History and examination together with imaging and neurophysiology will make a definite diagnosis of MND/ALS. A detailed family history will be taken and in those with a confirmed diagnosis and a family history of MND/ALS, counselling with regard to diagnostic genetic testing for familial MND/ALS will be undertaken.
How will the test add to the management of the patient or alter clinical outcome?	Once familial ALS is suspected (more than one individual in a family), this adds an enormous anxiety and distress to both affected and well members of the family. Patients will undergo genetic counselling and will be offered screening for mutations in the *TARDBP* and *FUS* genes. The patient will be given the results, which if positive for either gene will provide an explanation for the ALS. Counselling regarding predictive testing, prenatal testing and preimplantation screening can then be given to at risk family members, who can also be reassured by a negative predictive test result.
What impact will this test have on the NHS i.e. by removing the need for alternative management and/or investigations for this clinical population? Please provide evidence from your own service.	The test will not have any major impact on the NHS. It is unlikely to alter other investigations a patient undergoes. The numbers refered for screening are likely to be low, as a family history is only present in 5% of the MND/ALS population. As there are approximately 1000 new cases in England and Wales each year, this would translate to approximately 50 index cases with familial MND/ALS per year. In the Sheffield region alone we receive up to 5 new familial cases per year.
What are the consequences of not doing this genetic test. Commissioners have asked for specific information to support introduction of tests.	Families in which there is more than one individual affected by MND/ALS would not be given the opportunity to have appropriate counselling regarding predictive testing and planning a family (prenatal testing, pre-implantation screening etc).
Utility of test in the NHS In a couple of sentences explain the utility of this test for the disease(s)	Providing a definite diagnosis of familial ALS, defining the mode of inheritance and allowing appropriate counselling of an individual and at risk family members, who may also have their risk defined.
Is there an alternative means of diagnosis or prediction that does not involve molecular diagnosis? If so (and in particular if there is a biochemical test) please state the added advantage of the molecular test	There is no definitive test for familial ALS, which is diagnosed following the exclusion of other diseases which mimic symptoms. The advantage of the molecular diagnosis is that it provides a definite diagnosis, which may have an impact on other family members.
Please describe any specific ethical, legal or social issues with this particular test?	The ethical issues relate to the principles of predictive testing for a disease with no current treatment. The department has experience in these matters.

UKGTN Testing criteria

Name of Disease(s):
AMYOTROPHIC LATERAL SCLEROSIS 10, WITH OR WITHOUT FRONTOTEMPORAL DEMENTIA WITH TDP43 INCLUSIONS; ALS10 (612069)
AMYOTROPHIC LATERAL SCLEROSIS 6 WITH OR WITHOUT FRONTOTEMPORAL DEMENTIA; ALS6 (608030)

Name of gene(s): TAR DNA binding protein (605078), fused in sarcoma; FUS (137070)

Patient name: **Date of birth:**

Patient postcode: **NHS number:**

Name of referrer:

Title/Position:
Lab ID:

Referrals will only be accepted from one of the following:

Referrer	Tick if this refers to you.
Consultant Clinical Geneticist	
Consultant Neurologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:

Criteria	Tick if this patient meets criteria
The **presence** of: • Evidence of lower motor neuron (LMN) degeneration by clinical, electrophysiologic, or neuropathologic examination **AND** • Evidence of upper motor neuron (UMN) degeneration by clinical examination **AND** • Progressive spread of symptoms or signs within a region or to other regions, as determined by history or examination **TOGETHER WITH**	
The **absence** of: • Electrophysiologic and pathologic evidence of other disease processes that might explain the signs of LMN and/or UMN degeneration **AND** • Neuroimaging evidence of other disease processes that might explain the observed clinical and electrophysiologic signs.	
AND Relentless progression of symptoms and signs during follow-up period	
AND Additional family history with affected first degree relative or two second degree relatives	
AND Exclusion of *SOD1* mutation	

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed, please contact the laboratory to discuss testing of the sample.

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