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

TEST – DISORDER/CONDITION – POPULATION TRIAD			
Submitting laboratory:	**Oxford RGC**	**Approved:**	**Sept 2013**
1. Disorder/condition – approved name and symbol as published on the OMIM database (alternative names will be listed on the UKGTN website)	MYASTHENIC SYNDROME, CONGENITAL, WITH TUBULAR AGGREGATES 2; CMSTA2 CONGENITAL DISORDER OF GLYCOSYLATION, TYPE Ij; CDG1J.		
2. OMIM number for disorder/condition	614750, 608093		
3a. Disorder/condition – please provide, in laymen's terms, a brief (2-5 sentences) description of how the disorder(s) affect individuals and prognosis.	Limb-girdle myasthenia with tubular aggregates is a progressive neuromuscular disorder characterized by onset of proximal muscle weakness, usually in the first decade and often accompanied by the presence of tubular aggregates on muscle biopsy. EMG classically shows a decremental response to repeated nerve stimulation. Affected individuals showed a favorable response to acetylcholinesterase (AChE) inhibitors. There are at least two types – type 1 has been shown to be caused by mutations in the GFPT1 gene. The different types are indistinguishable at the clinical level. Mutations in DPAGT1 have also been identified in a congenital disorder of glycosylation, type Ij; CDG1j. Patients with this disorder have severe multisystem problems. Several cases have very recently been reported.		
3b Disorder/condition – if required please expand on the description of the disorder provided in answer to Q3a.			
4. Disorder/condition – mode of inheritance	Autosomal recessive		
5. Gene – approved name(s) and symbol as published on HGNC database (alternative names will be listed on the UKGTN website)	dolichyl-phosphate (UDP-N-acetylglucosamine) N-acetylglucosaminephosphotransferase 1 (GlcNAc-1-P transferase); DPAGT1		
6a. OMIM number for gene(s) **6b HGNC number for gene(s)**	191350 HGNC:2995		
7a. Gene – description(s)	The DPAGT1 gene is located at 11q23.3 and consists of 11 exons, 9 of which encode the enzyme dolichyl-phosphate N-acetylglucosamine phosphotransferase (the first two exons encode 5'UTR). N-linked glycosylation requires participation of a special lipid called dolichol phosphate, and DPAGT1 catalyzes the first step in the dolichol cycle: the synthesis of N-acetylglucosaminyl- pyrophosphoryldolichol (GlcNAc-PP-dolichol) from dolichol phosphate and UDP-GlcNAc.		
7b. Number of amplicons to provide this test (molecular) or type of test (cytogenetic)	9		

7c. GenU band that this test is assigned to for index case testing	Band E
8. Mutational spectrum for which you test including details of known common mutations	Nonsense, missense, small insertions, deletions and splicing mutations. As glycosylation is essential for cell survival it is expected that *DPAGT1* mutations create hypomorphic alleles, and no CMSTA2 or CDG1J patient has been found to date that carries two null mutations in the constitutive exons of the gene (Belaya *et al* 2012, Am J Hum Genet 91:193-201).
9a. Technical method(s)	Bidirectional fluorescent sequencing
9b If a panel test using NGS please state if it is a conventional panel or a targeted exome test.	N/A
9c. Panel/targeted exome Tests **i) Do the genes have 100% coverage? If not what is the strategy for dealing with the gaps in coverage?**	N/A
ii) Does the test include MLPA?	No
iii) Does this use sanger sequencing or Next Generation Sequencing (NGS)?	Sanger sequencing
iv) If NGS is used, does the lab adhere to the Practice Guidelines for NGS?	N/A
10 Is the assay to be provided by the lab or is it to be outsourced to another provider? **If to be outsourced, please provide the name of the laboratory.**	To be provided by the lab
11. Validation process Please explain how this test has been validated for use in your laboratory or submit your internal validation documentation	Bidirectional fluorescent sequencing is used by our laboratory for mutation scanning of several genes including the congenital myasthenia genes DOK7, RAPSN, CHRNA1, CHRNB1, CHRND, CHRNE, CHAT, CHRNG, COLQ and GFPT1. Prior to use all primers will be checked for SNPs and 2 normal controls sequenced to confirm specific amplification. Confirmation of known mutations using controls from Prof Beeson's research laboratory will also be carried out.
12a. Are you providing this test already?	✓☐ **No** ☐ **Yes** This service is currently offered on a research basis by Professor David Beeson's laboratory and will transfer to our laboratory in 2013.
12b. If yes, how many reports have you produced? **Please provide the time period in which these reports have been produced and whether in a research or a full clinical diagnostic setting.**	Professor Beeson has analysed 33 CMS samples in a research setting over the last 2 years.

12c. Number of reports mutation positive	Professor Beeson found 5 positive samples (15%).
12d. Number of reports mutation negative	Professor Beeson found 28 negative samples.
13. For how long have you been providing this service?	Professor Beeson has been offering this service for 2 years.
14a. Is there specialised local clinical/research expertise for this disorder?	☐ **No** ✓ ☐ **Yes**
14b. If yes, please provide details	Professor David Beeson's research group at the Weatherall Institute of Molecular Medicine has worked on many of the CMS genes and is able to provide functional studies for unclassified variants, which if pathogenic may require different treatments depending upon the effect of the mutation on neuromuscular junction function. An HSS funded referral centre for CMS is based at the John Radcliffe, Oxford, with weekly clinics for patients. The lead clinician is Dr Jackie Palace.
15. Are you testing for other genes/disorders/conditions closely allied to this one? Please give details	Yes – CHRNA1, CHRNB1, CHRND, CHRNE, RAPSN, DOK7, CHAT, COLQ, CHRNG, GFPT1. DOK7 and GFPT1 mutations give a similar limb-girdle pattern of weakness.
16. Based on experience what will be the national (UK wide) activity, per annum, for:	
16a. Index cases	Based on research experience to date – 16 per annum (<2 from Wales or Northern Ireland), of which approx 2 will be positive
16b. Family members where mutation is known	At least 4, <2 will be from Wales or Northern Ireland
17a. Does the laboratory have capacity to provide the expected national activity?	Our laboratory should be able to provide the full national need. We are not aware of any other laboratories in the UK that are offering this test.
17b. If your laboratory does not have capacity 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".	N/A
18. Please justify the requirement for another laboratory to provide this test e.g. insufficient national capacity.	N/A

EPIDEMIOLOGY	
19a. Estimated prevalence of condition in the general UK population	The prevalence is estimated to be no higher than 1 in 500,000 (estimated prevalence for DOK7). However, as this is a newly identified disorder this may change as knowledge of the phenotype becomes more widely known.
19b. Estimated incidence of condition in the general UK population Please identify the information on which this is based	Based on limited data this is a fully penetrant disorder that is present by age 10. Based on a UK population of 63 million, a birth rate of 12.3 per 1000 population and a gene frequency of 1/700, the UK birth incidence is predicted to be 1:490,000.
20. Estimated gene frequency (Carrier frequency or allele frequency) Please identify the information on which this is based	As this is an autosomal recessive disorder the gene frequency is estimated to be 1/700 based on the prevalence figure given above.
21. Estimated penetrance Please identify the information on which this is based	Based on reported families penetrance is 100% (Belaya et al 2012 Am J Hum Genet 91:193-201)
22. Estimated prevalence of condition in the population of people that meet the Testing Criteria.	As other genes have overlapping clinical features, DPAGT1 mutations would be expected to be identified in less than 25% of patients that meet all of the above criteria.

INTENDED USE

23. Please tick either yes or no for each clinical purpose listed.

Panel Tests: a panel test would not be used for pre symptomatic testing, carrier testing and pre natal testing as the familial mutation would already be known in this case and the full panel would not be required.

Diagnosis	✓☐ **Yes**	☐ **No**
Treatment	✓☐ **Yes**	☐ **No**
Prognosis & management	✓☐ **Yes**	☐ **No**
Presymptomatic testing (n/a for panel tests)	✓☐ **Yes**	☐ **No**
Carrier testing for family members (n/a for panel tests)	✓☐ **Yes**	☐ **No**
Prenatal testing (n/a for panel tests)	✓☐ **Yes**	☐ **No**

TEST CHARACTERISTICS

24. 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.

Bidirectional sequencing is expected to have an analytical sensitivity >90%, as no whole exon deletions or duplications have been reported to date. As mutations are believed to retain some residual protein function, homozygous deletions would not be expected, nor a hemizygous deletion with another null allele. The specificity is also expected to be high, as polymorphisms in the gene are well characterised, and facilities exist in Professor Beeson's research laboratory to undertake functional studies to determine pathogenicity of missense variants.

25. Clinical sensitivity and specificity of test in target population The *clinical sensitivity* of a test is the probability of a positive test result when condition is known to be present; the *clinical specificity* is the probability of a negative test result when disorder is known to be absent. The denominator in this case is the number with the disorder (for sensitivity) or the number without condition (for specificity).

For individuals who meet the diagnostic criteria (given below) of limb- girdle CMS, effective response to AchE inhibitors or tubular aggregates in muscle biopsies, clinical sensitivity is likely to be fairly low due to genetic heterogeneity. At present approximately 15% of referrals for *DPAGT1* testing are positive, and a definitive diagnosis is made following a positive genetic result. The clinical specificity would be approaching 100%.

26. 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 condition or predisposition. It is measured by its *positive predictive value* (the probability of getting the condition given a positive test) and *negative predictive value* (the probability of not getting the condition given a negative test).

Positive predictive value approaches 100% in probands and affected relatives. Due to genetic heterogeneity *DPAGT1* mutations are expected to be identified in less than 25% of individuals in the target population (based on very limited data from the service to date).

27. Testing pathway for tests where more than one gene is to be tested 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 will be added to the published Testing Criteria.

Cases which fully meet the diagnostic criteria will be tested for *GFPT1* and *DPAGT1* initially, whereas all other limb-girdle CMS patients will be screened first for *DOK7*. Other cases may be tested for other relevant CMS genes initially depending on the diagnostic criteria supplied. All referrals will be assessed for appropriateness by the Oxford CMS service. Testing will not be carried out until appropriate clinical details have been received.

CLINICAL UTILITY

28. How will the test change the management of the patient and/or alter clinical outcome?

No further molecular or neurological investigations required if mutation identified. A series of different treatments are available for the congenital myasthenic syndromes, depending on the underlying disease mechanism. A beneficial treatment for one disorder may be contraindicated in another. Thus definitive diagnosis is important for appropriate therapy. Patients with *DPAGT1* mutations respond well to anticholinesterase medication, but in the past these cases were commonly mistaken for 'seronegative' myasthenia gravis. Finding a mutation also confirms the mode of inheritance and allows carrier testing in family members and appropriate counselling. Prenatal diagnosis would be available to couples where both were identified carriers.

29. Benefits of the test for the patient & other family members Please provide a summary of the overall benefits of this test.

Finding a mutation confirms the mode of inheritance as autosomal recessive and allows carrier testing in family members and appropriate counselling. Prenatal diagnosis would be available to couples where both were identified carriers.
Patients with mutations should respond to treatment with AChE inhibitors and 3.4-diaminopyridine significantly improving their quality of life.

30. What will be the consequences for patients and family members if this test is not approved?

The disorder is slowly progressive, and may lead to loss of ambulation and wheel chair dependency. Therapy with anticholinesterases may prevent this. Moreover defining a cohort of patients with these mutations will allow the best therapeutic options to be determined. The disorder may be mistaken for 'seronegative' myasthenia gravis and inappropriate therapies such as immunosuppression and thymectomy instigated. We have one case who died due to sudden respiratory insufficiency aged 26, and it may have been possible to avoid this were a definitive genetic diagnosis known.

31. 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.

Diagnosis is currently on a clinical basis only. EMG's are not definitive.

32. Please describe any specific ethical, legal or social issues with this particular test.

Variability in clinical phenotype may cause a problem in interpreting prenatal results, but generally mutations within a family result in a similar disease severity. Predictive testing has not been requested to date, but could theoretically occur. Carrier testing in minors may be an issue.

33. Only complete this question if there is previously approved Testing Criteria and you do not agree with it. Please provide revised Testing Criteria on the Testing Criteria form and explain here the changes and the reasons for the changes.

N/A

34. List the diagnostic tests/procedures that an index case no longer needs if this genetic test is available.

	Type of test	Cost (£)
Costs and type of imaging procedures		
Costs and types of laboratory pathology tests (other than molecular/cyto genetic test proposed in this gene dossier) DOK7 gene screen CHAT gene screen COLQ gene screen CHRNE gene screen CHRNA1 gene screen CHRNB1 gene screen CHRND gene screen RAPSN gene screen GFPT1 gene screen aCGH DM1 Various limb girdle muscular dystrophy genes	 510 (HSS) 540 (HSS) 540 (HSS) 510 (HSS) 490 (HSS) 510 (HSS) 510 (HSS) 490 (HSS) 540 (HSS) 500 221 HSS	
Costs and types of physiological tests (e.g. ECG)	~£1377 (EMG) ~£1000 (SFEMG)	
Cost and types of other investigations/procedures (e.g. biopsy)	>£2805 muscle biopsy with histology: intercostal muscle biopsy requires hospital admission (cost unknown) £1000+ Inpatient stays to monitor treatment	
Total cost tests/procedures no longer required		**£8800**

Each case will be different and therefore it is difficult to give a precise figure – a case fulfilling all criteria for DPAGT1 testing may have this done initially and all other tests would not be required if positive – however other cases may have DOK7 or other genes done initially. If DPAGT1 testing is negative, some or all of the other genes may be tested, depending on the phenotype. We have therefore assumed that, on average, half of the cost of the molecular tests would be saved if DPAGT1 testing is positive.

35. Based on the expected annual activity of index cases (Q15a), please calculate the estimated annual savings/investments based on information provided in Q33.

Number of index cases expected annually	16
Cost to provide tests for index cases if the genetic test in this gene dossier was not available (see Q34)	(b)-Approx £8800
Total annual costs pre genetic test	16x8800 = £140,800
Total annual costs to provide genetic test	Financial section states cost per test is £540 and therefore 16 multiply by £540 is £8640.
Total savings	£140,800 currently spending for 16 £8640 to provide genetic test for 16 Only 25% expected to be positive. Therefore 75% of £140800 is £105,600 and still needs to be spent. Consequently spend for 16 with 75% still requiring other tests is £8640 + £105,600 = £114,240. £140,800 - £114,240 = **£25,560 saving**.

36. REAL LIFE CASE STUDY
In collaboration with the clinical lead, describe TWO real case examples:

1. **prior to availability of genetic test**
2. **post availability of genetic test**

to illustrate how the test improves patient experience and the costs involved.

Case example one – pre genetic test

Case 1 (III-14) was a female who was symptom-free up to school age, when she presented with mild and fluctuating proximal weakness of lower extremities. Diagnosis of myasthenia gravis (MG) was proposed when she was 7 years old and administration of pyridostigmine, prednisone and physical and occupational therapy resulted in partial relief. She was able to perform almost normally in daily activities for the next 4-5 years. Aged 15 she was hospitalized with respiratory failure requiring ITU. Increase in pyridostigmine and prednisolone led to partial recovery with no history of hospitalisation for the subsequent few years. The patient again required hospital intervention aged 24, when her symptoms worsened following a viral illness. At this time azathioprine was added to her treatment regimen.
On examination, aged 25, she had severe weakness of her proximal muscles. Deep tendon reflexes were reduced throughout. Antibodies to the acetylcholine receptors were not present. Electrophysiological testing revealed abnormal decrement (up to 32%) on repetitive nerve stimulation in keeping with a myasthenic disorder. Myopathic potentials were seen on standard needle EMG examination of all muscles tested and were most prominent proximally. Fibrillations and positive sharp waves were also detected on EMG. Nerve conduction studies were normal. CT of the thymus was normal. A muscle biopsy from the right vastus lateralis showed a reduced number of muscle fibres and replacement with fat and connective tissue. The remaining muscle fiber size was variable and some demonstrated centralization of nuclei. The withdrawal of prednisone and azathioprine and symptomatic treatment with pyridostigmine resulted in the patient regaining much of her normal strength. However, the following year the patient died during a further respiratory crisis.

PRE GENETIC TEST COSTS

	Type of test	Cost
Costs and type of imaging procedures CT scan of thymus	 400?	
Costs and type of laboratory pathology tests DOK7 gene screen CHRNE gene screen CHRNA1 gene screen CHRNB1 gene screen CHRND gene screen RAPSN gene screen Anti-AChR Antibodies	 510 510 490 510 510 490 15	
Costs and type of physiological tests (e.g. ECG)	~£1377 x 2 (EMG)	
Cost and type of other investigations/procedures (e.g. biopsy) muscle biopsy with histology Two ITU admissions	 >£2805 >£20000	
Cost outpatient consultations (genetics and non genetics) 4 Out-patient appointments to discuss diagnosis and treatments (~£300 each)	 £1200	
Total cost pre genetic test		**>£30194**

Case example two – post genetic test

The patient had problems weight bearing with a tendency to fall which initially fluctuated, beginning at 2.5 years of age. Distal weakness was worse than proximal. There were mild swallowing difficulties and slurring and nasal speech and shortness of breath. He was poor at sports during childhood although was able to run short distances.

During childhood his walking deteriorated and he started to use a wheelchair at around 9 years. At that time he had a muscle biopsy and was told he had Werdnig-Hoffman spinal muscular atrophy. It became apparent over subsequent years that this diagnosis was not correct and CMS was suspected. In adult life he has been stable but with long-term fluctuations lasting weeks to months not associated with exacerbating factors such as infection.

The patient at this time started anticholinesterase medication with benefit. Since genetic diagnosis he takes oral salbutamol with good effect, which has prevented his major fluctuations, improved his grip and has enabled him to go from wheelchair bound to walking within his home. He has noted cramps secondary to this treatment.

POST GENETIC TEST COSTS

	Type of test	Cost
Costs and type of imaging procedures		
Costs and types laboratory pathology tests (other than molecular/cyto genetic proposed in this gene dosier) SMA test DOK7 gene screen CHRNE gene screen CHRNA1 gene screen CHRNB1 gene screen CHRND gene screen RAPSN gene screen	 221 510 510 490 510 510 490	
Cost of genetic test proposing in this gene dossier	490	
Costs and type of physiological tests (e.g. ECG)	~£1377 x 2 (EMG)	
Cost and type of other investigations/procedures (e.g. biopsy) Two muscle biopsies with histolog	 >£2805 x2	

Cost outpatient consultations (genetics and non genetics) 5 yearly appointments to discuss test results, diagnosis and treatment	£3000		
Total cost post genetic test	[redacted]	**£15095**	

37. Estimated savings between two case examples described >£15099

UKGTN Testing Criteria

Test name: **Myasthenia, Limb-Girdle, With Tubular Aggregates Type 2 And Congenital Disorder Of Glycosylation, Type Ij**	
Approved name and symbol of disorder/condition(s): Myasthenic Syndrome, Congenital, with Tubular Aggregates 2; CMSTA2 Congenital Disorder of Glycosylation, Type Ij; CDG1J. **Approved name and symbol of gene(s):** dolichyl-phosphate (UDP-N-acetylglucosamine) N-acetylglucosaminephosphotransferase 1 (GlcNAc-1-P transferase); DPAGT1	**OMIM number(s):** 614750 608093 **OMIM number(s):** 191350

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	
Consultant paediatric neurologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Congenital Myasthenic Syndrome with a limb-girdle pattern of weakness **AND** at least one of the following: • anti-cholinesterase responsive • tubular aggregates on muscle biopsy	
OR at risk family members where familial mutation is known	

Additional Information:
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.