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

Submitting laboratory: Cardiff RGC

1. Disorder/condition – approved name and symbol as published on the OMIM database (alternative names will be listed on the UKGTN website)
If this submission is for a panel test please complete appendix 1 listing all of the conditions included using approved OMIM name, symbol and OMIM number.

HYPEREKPLEXIA, HEREDITARY 1; HKPX1

HYPEREKPLEXIA 3; HKPX3

HYPEREKPLEXIA 2; HKPX2

2. OMIM number for disorder/condition
If a panel test – see 1. above

#149400

#614618

#614619

3a. Disorder/condition – please provide, in laymen's terms, a brief (2-5 sentences) description of how the disorder(s) affect individuals and prognosis.

Hyperekplexia is a rare disorder that is most severe in early life. Affected individuals are pathologically susceptible to environmental triggers such as noise, touching and visual stimuli. People with hyperekplexia have abnormal tone (typically their trunk and limbs are stiff) and when startled with a stimulus this combination creates a prolonged startled attack. Individuals will be as 'stiff as a board' which is very unpleasant for families to see – and unpleasant for the individuals as they retain full awareness. They will fall (if standing) and their abdomen will stiffen (causing cyanosis). In the more severe cases there are recurrent infantile apnoea attacks and a spectrum of learning disability that includes intellectual disability.

Referrals for hyperekplexia will typically come from neonatologists or paediatric neurologists – although some cases are diagnosed later in life due to delays in recognising the condition so referrals can come from general physicians and adult neurologists. Neonatal and infantile startle attacks can happen dozens of time per day and can lead to developmental delay and learning difficulties when older. Medication is partially helpful in most and very helpful in some and the prognosis can be predicted by knowing which gene is affected.

3b. Disorder/condition – if required please expand on the description of the disorder provided in answer to Q3a.

Classical hyperekplexia is a term used to describe the genetic disorder of neonatal stimulus induced rigidity and exaggerated startle response. This is in contrast to the adjectival use of 'hyperekplexia' which is used to denote prominent startle in later life.

Genetic hyperekplexia always presents in the first year of life (commonly noted in the first week or even in the last trimester). There is an increased frequency of recurrent infantile apnoeas. Learning difficulties (particularly speech delay) and intellectual disability are recognised. If ambulant the stimulus induced startle can be vigorous – knocking the individual off their feet and producing repeated injurious falls. (Thomas *et al.* Brain 2013;136(10):3085-95). It is now noted that there is a ten-fold increased risk of epilepsy in these children.

For some hyperekplexia is a life-long condition that is only partly ameliorated with medication. All stimuli can provoke these startle attacks (which in themselves may last three minutes, and be associated with abdominal splinting and cyanosis). Feeding is a particularly potent trigger for this with risks including aspiration and failure to thrive.

Study of autosomal dominant families with hyperekplexia reported an increased risk of sudden infant death; however there has never been a documented case in a case with a proven mutation.

4. Disorder/condition – mode of inheritance
If this submission is for a panel test, please complete the mode of inheritance for each condition in the table in appendix 1.

Both autosomal dominant and recessive inheritance. There are very rare instances of digenic inheritance. Turkish patients have a recurrent large deletion in GLRA1.

5. Gene – approved name(s) and symbol as published on HGNC database (alternative names will be listed on the UKGTN website)
If this submission is for a panel test please complete appendix 1 listing all of the genes included using approved HGNC name, symbol, number and OMIM number.

glycine receptor, alpha 1; GLRA1
solute carrier family 6 (neurotransmitter transporter, glycine), member 5; SLC6A5
glycine receptor, beta **;** GLRB

6a. OMIM number(s) for gene(s)
If a panel test – see 5. above

GLRA1 = *138491
SLC6A5 = *604159
GLRB = *138492

6b. HGNC number(s) for gene(s)
If a panel test – see 5. above

GLRA1 = HGNC:4326
SLC6A5 = HGNC:11051
GLRB = HGNC:4329

7a. Gene – description(s)
If this submission is for a panel test, please provide total number of genes.

GLRA1 – codes for the alpha 1 subunit if the glycine receptor
SLC6A5 (GlyT2) – encodes for glycine transporter 2
GLRB – codes for the beta subunit of the glycine receptor.

7b. Number of amplicons to provide this test (molecular) or type of test (cytogenetic)
(n/a for panel tests)

34

7c. GenU band that this test is assigned to for index case testing.

F

8. Mutational spectrum for which you test including details of known common mutations
(n/a for panel tests)
If this application is for a panel test to be used for different clinical phenotypes and/or various sub panel tests – please contact the team for advice before completing a Gene Dossier

Missense, nonsense, splice site mutations and small and large insertions/deletions.

9a. Technical method(s) – please describe the test.

Sanger sequencing of the coding region and splice sites and MLPA of all 3 genes.

9b. For panel tests, please specify the strategy for dealing with gaps in coverage.

n/a

9c. Does the test include MLPA? **(For panel tests, please provide this information in appendix 1)**
Yes – all 3 genes are covered in one MLPA test.
9d. If NGS is used, does the lab adhere to the Association of Clinical Genetic Science Best 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 and a copy of their ISO certificate or their CPA number.**
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. If this submission is for a panel test, please provide a summary of evidence of: i) instrument and pipeline validation, and ii) panel verification for the test Please submit as appendices to the Gene Dossier (these will be included in the published Gene Dossier available on the website). Please note that the preferred threshold for validation and verification is 95% sensitivity with 95% Confidence Intervals.
The primer sets used have been SNP-checked, validated to ensure that the full coding sequence is covered, and optimised to work robustly under diagnostic conditions. Additionally 8 samples were tested blind and all results were as expected.
12a. Are you providing this test already?
Yes (in addition to those below, 4 further screens are currently ongoing)
12b(i). If yes, how many reports have you produced?
7
12b(ii). Number of reports mutation positive?
4 (2 homozygous missense changes identified on screens – probably pathogenic, 2 not screens but confirmation of research results)
12b(iii). Number of reports mutation negative?
3
12b(iv). Please provide the time period in which these reports have been produced and whether in a research or a full clinical diagnostic setting.
During the past year.
13a. Is there specialised local clinical/research expertise for this disorder?
Yes
13b. If yes, please provide details
This service is being transferred from the College of Medicine, Swansea University which has been running the screening on a research basis for over 10 years, led by Professor Mark Rees. This group are world leaders in the genetics and clinical presentation of hyperekplexia and can provide all the clinical and research expertise needed.

Additionally the Swansea neurology / neuroscience group have the capacity to perform functional analysis on novel variants on a research basis including electrophysiology, cell surface assays and splice site assays.
14. Based on experience what will be the national (UK wide) activity, per annum, for:
Index cases 15
Family members where mutation is known 20
15. If your laboratory does not have capacity to provide the full national need please suggest 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. If you are unable to answer this question please write "unknown".
We would be able to provide the full national need.
16. If using this form as an Additional Provider application, please explain why you wish to provide this test as it is already available from another provider.
n/a

EPIDEMIOLOGY

17a. Estimated prevalence of conditions in the general UK population
Prevalence is total number of persons with the condition(s) in a defined population at a specific time.
Please identify the information on which this is based.
For panel tests, please provide estimates for the conditions grouped by phenotypes being tested.

Exact prevalence unknown. Estimated to be <1 in 1,000,000

17b. Estimated annual incidence of conditions in the general UK population
Incidence is total number of new cases in a year in a defined population.
Please identify the information on which this is based.
For panel tests, please provide for groups of conditions.

Currently unknown

18. Estimated gene frequency (Carrier frequency or allele frequency)
Please identify the information on which this is based.
n/a for panel tests.

Currently unknown

19. Estimated penetrance of the condition. Please identify the information on which this is based
n/a for panel tests

Approximately 95%, this based on the experience of the clinical team in Swansea: very rarely do cases show incomplete penetrance.

20. Estimated prevalence of conditions in the population of people that will be tested.
n/a for panel tests.

From the 236 index patients screened by the Swansea group, mutations in one of the three genes known to cause the disorder have been identified in 43% of cases.

INTENDED USE (Please use the questions in Annex A to inform your answers)

21. 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

22. 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.
Please note that the preferred threshold for validation and verification is 95% sensitivity with 95% Confidence Intervals.

Bi-directional sequence analysis and MLPA analysis have a high (>99%) sensitivity and specificity.

23. 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).
Please provide the best estimate. UKGTN will request actual data after two years service.

Clinical Sensitivity

From the current data available (from the Swansea research group) we would expect the clinical sensitivity to be approximately 45-50% [Chung *et al.* (2013) GLRB is the third major gene of effect in hyperekplexia. *Human Molecular Genetics;* 22(5):927-40.

Clinical specificity

Approximately 100%

24. 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).
Not currently requested for panel tests

These questions have not been directly studied however:

The inheritance data from Swansea and elsewhere suggests that there is a greater than 95% positive predicted value.

Depending on the clinical scenario - the negative predictive value in a community sample would be high. There are many cases with an atypical hyperekplexia and a negative test result, but very many fewer cases with a typical hyperekplexia (Thomas et al. Brain 2013;136(10):3085-95.)

25. Testing pathway for tests where more than one gene is to be tested sequentially

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.
n/a for panel tests

Due to instances of digenic inheritance (yet to be published), all three genes will be screened simultaneously.

CLINICAL UTILITY

26. How will the test change the management of the patient and/or alter clinical outcome? Please describe associated benefits for patients and family members. If there are any cost savings AFTER the diagnosis, please detail them here.

A confirmed diagnosis of hyperekplexia (known pathogenic mutation in one of the three genes) will;

a) Allow correct management of the condition with the appropriate medication (benzodiazapines)
b) Prevent the use of unnecessary and ineffective medications in a patient due to an incorrect diagnosis of a different disorder (startle epilepsy for example)
c) Prevent patient from being subjected to unnecessary clinical tests (Repeated EEG/ MRI under general anaesthetic)
d) Hyperekplexia is associated with repeated and frequent neonatal apnoeas and correct clinical and parental education can be focussed following a positive result.
e) For many families a confirmed diagnosis allows them to access any additional support required for the care of the patient from their local services.
f) It is now clear that knowing the gene that is mutated can help predict prognosis (Thomas et al.

Brain 2013;136(10):3085-95.). The main gene (GLRA1) produces the mildest phenotype when inherited in an autosomal dominant fashion, and a more extended phenotype if recessive. The genes GLRB and *SLC6A5* are associated with the most severe phenotypes and all cases either have developmental delay or apnoeas; most have both. The prognosis of children with no recognised mutation in these genes is more variable. A confirmed genetic diagnosis would allow clinicians to provide a more accurate prognosis for patients and their carers, and in some cases this will be telling families that symptoms have the potential to resolve over time.

g) Molecular confirmation of the diagnosis permits Agree targeted genetic counselling and testing for families.

h) There are further benefits to securing a correct diagnosis in a rare disorder – such as allowing parents to meet and provide peer support using groups such as 'The Hyperekplexia Society'.

27. If this test was not available, what would be the consequences for patients and family members?

Some patients and families can be subjected to a number of clinical tests to try and rule out other potential diagnoses. If no official diagnosis can be made this often leaves families in limbo, unable to access local services necessary to care for the patient and a feeling of isolation.

Secondly, misdiagnosis could lead to prolonged exposure to inappropriate medications.

Further, in cases of misdiagnosis where genetic testing is not available, the incorrect diagnosis can have a significant impact on the patients' lifestyle. For instance, an incorrect diagnosis of epilepsy (which is very common) will have downstream consequences on everyday things that others take for granted such as driving.

28. 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.

Although expert clinical opinion may suggest a diagnosis of hyperekplexia, this condition is often mis-diagnosed as a similar yet different disorder, usually some type of epilepsy. Therefore, only a molecular test can offer a definitive diagnosis of hyperekplexia.

29a. What unexpected findings could this test show? For example, lung cancer susceptibility when testing for congenital cataract because ERCC6 gene (primarily associated with lung cancer) is included in a panel test for congenital cataract.

None of the three genes are recorded as involved in any other disorders in OMIM.

29b. Please list any genes where the main phenotype associated with that gene is unrelated to the phenotype being tested by the panel.

None

30. If testing highlights a condition that is very different from that being tested for, please outline your strategy for dealing with this situation.

n/a

31. If a panel test, is this replacing an existing panel/multi gene test and/or other tests currently carried out through UKGTN using Sanger sequencing? If so, please provide details below.

n/a

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

None

IS IT A REASONABLE COST TO THE PUBLIC?

33. In order to establish the potential costs/savings that could be realised in the diagnostic care pathway, please list the tests/procedures that would be required in the index case to make a diagnosis if this genetic test was not 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)		
Costs and types of physiological tests (e.g. ECG)		
Cost and types of other investigations/procedures (e.g. biopsy)		
Total cost of tests/procedures no longer required (please write n/a if the genetic test does not replace any other tests procedures in the diagnostic care pathway)		**n/a**

34. Based on the expected annual activity of index cases (Q14), please calculate the estimated annual savings/investments based on information provided in Q33.

Number of index cases expected annually	(a) 15
Cost to provide tests for index cases if the genetic test in this Gene Dossier was not available (see Q32)	(b)
Total annual costs pre genetic test	(a) x (b) = (c)
Total annual costs to provide genetic test	(a) 15x cost of genetic testing for index case £620 = (d) £9,300
Additional investment for 100% positive rate for index cases	(d) £9,300 – (c) = (e)
Percentage of index cases estimated to be negative	(f) 50%
Number of index cases estimated to be negative	(f) 50% x number of index cases 15= (g) 8
Costs to provide additional tests for index cases testing negative	(g) x (b) = (h) n/a
Total costs for tests for index patient activity	(e) + (h) = (i) £9300
Total costs for family members	Costs for family member test £160 x number of family members expected to test in a year 20 (j) = £3,200
If there is a genetic test already available and some of the family testing is already being provided, please advise the cost of the family testing already available	Cost for family member testing already available x estimated number of tests for family members already provided (k) n/a
Total costs for family members minus any family member testing costs already provided	(j) – (k) = (l) n/a
Additional costs for all activity expected in a year	(i) + (j) or (i) + (l) 9300 + 3200 = £12,500

35. REAL LIFE CASE STUDY
Please provide a case study that illustrates the benefits of this test

A case study can be seen in Rees et al 2006 (Nature Genetics 38(7): 801-806). This includes several cases where the lengthy and protracted investigation of the older cases as hyperekplexia struggled to gain clinical recognition before 1994. The genetic tests confirming a transportopathy were key to determining causality and within a neurotransmitter system that was important in the startle reflex. It also retrospectively confirmed a dangerous neonatal window for protracted apnoeas in the first year of life.

A second case study can be seen in Gimenez et al 2012 (JBC 287(34): 28986-9002) and represents a post-mortem diagnosis in a young child with hyperekplexia and encephalopathy type-seizures. A novel mutation and mechanism in GlyT2 gave answers to the family and custodian Clinicians.

TESTING CRITERIA

36. Please only complete this question if there is previously approved Testing Criteria.
Please contact the UKGTN office if you are unsure whether testing criteria is available.

36a. Do you agree with the previously approved Testing Criteria? Yes/No

36b. If you do not agree, please provide revised Testing Criteria on the Testing Criteria form and explain below the reasons for the changes.

UKGTN Testing Criteria

Test name: **Hereditary Hyperekplexia**	
Approved name and symbol of disorder/condition(s):	**OMIM number(s):**
Hyperekplexia, Hereditary 1; HKPX1	149400
Hyperekplexia 3; HKPX3	614618
Hyperekplexia 2; HKPX2	614619
Approved name and symbol of gene(s):	**OMIM number(s):**
glycine receptor, alpha1; GLRA1	138491
solute carrier family 6 (neurotransmitter transporter), member 5; SLC6A5	604159
glycine receptor, beta; GLRB	138492

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**
Evidence of Startle to acoustic or tactile stimuli. (also if positive glabellar tap and hypertonia) **AND**	
Symptoms from early in life **AND**	
Normal EEG and MRI	
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.