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

Test – Disease – Population Triad

Disease – name	Hereditary Leiomyomatosis and renal cell cancer (HLRCC)
OMIM number for disease	150800, 605839
Disease – alternative names please provide any alternative names you wish listed	Multiple Cutaneous and Uterine Leiomyomatosis (MCUL) It is often referred to as HLRCC/MCUL
Disease – please provide a brief description of the disease characteristics	Hereditary leiomyomatosis and renal cell cancer (HLRCC) is a disorder in which affected individuals tend to develop benign tumors containing smooth muscle tissue (leiomyomas) in the skin and, in females, the uterus. This condition also increases the risk of kidney cancer. Uterine leiomyomata are present in almost all females with HLRCC and tend to be numerous and large; age at diagnosis ranges from 18 to 52 years, with most women experiencing irregular or heavy menstruation and pelvic pain. Renal tumors causing hematuria, lower back pain, and a palpable mass are usually unilateral, solitary, and aggressive and range from type 2 papillary to tubullo-papillary to collecting-duct carcinomas. They occur in about 2-6% of individuals with HLRCC; the median age of detection is 44 years (Toro et al 2005 Br J Dermatology). It is often referred to as multiple cutaneous and uterine leiomyomatosis (MCUL) especially in families with no renal cell cancer. There are rare reports of malignant uterine leiomyosarcomas in females with HLRCC/MCUL (Lehtonen et al 2006 J Med Genet & Ylisaukkoja et al 2006 Int J Cancer). Germline fumarate hydratase mutations have also been detected in 2 out of 29 (6.9%) adult Leydig Cell tumours of the Testis (Carvajal-Carmona et al 2006 J Clin Endocrin & Metab).
Disease - mode of inheritance	Autosomal dominant
Gene – name(s)	Fumarate Hydratase (FH) 1q42.1
OMIM number for gene(s)	136850
Gene – alternative names please provide any alternative names you wish listed	Fumarase
Gene – description(s) (including number of amplicons).	FH is the only gene associated with HLRCC or MCUL. It has 10 coding exons over 22,152 base pairs which can be amplified in 10 fragments. It encodes fumarase, an enzyme of the Kreb's TCA cycle which catalyses the conversion of fumarate to malate. Fumarase is a homotetrameric enzyme, composed of 510 amino acids (molecular weight 54,637 Da) with four identical subunits (50 kDa each); three active DNA binding sites and one lower affinity substrate site.

Mutational spectrum for which you test including details of known common mutations.	DNA sequencing of all 10 coding exons including 25-50bp of intronic sequence at intron/exon boundaries to detect all missense, nonsense, splice site mutations, small deletions and insertions. In addition MLPA (MRC Holland kit P198) is available for large deletions/duplications (~6% frequency) (Bayley et al 2008, BMC Med Genet). The mutations are scattered throughout the gene. The most common mutation reported is p.Arg233His (previously reported as R190H) in exon 4 (17% frequency) (Alam et al 2005 Br J Dermatology).
Technical Method (s)	Direct DNA sequencing and MLPA
Validation Process Note: please explain how this test has been validated for use in your laboratory	DNA sequencing has been extensively validated within the laboratory against scanning techniques (SSCP, CSGE) and is the main route for mutation detection within the lab. MLPA analysis has been validated in the lab for many disorders using known deletion positive and normal controls with no false negatives or positives. We also participate in EQA (UKNEQAS and EMQN) including the sequencing scheme and schemes involving the use of MLPA as a technique.
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	Yes, we have received 12 referrals since June 2008 (3 from abroad). 8 were positive for a mutation; 4 were negative from DNA sequencing.
For how long have you been providing this service?	Since May 2008
Is there specialised local clinical/research expertise for this disease?	Yes: X No: Please provide details Dr G Sobey, Consultant Dermatologist specialising in Genetics. Professor Albert Ong, Consultant Nephrologist. Dr Simon Olpin, Consultant Biochemist providing a national biochemical service for FH. Dr Olpin has a research record with 8 publications as co-author for both MCUL/HLRCC and FH deficiency.
Are you testing for other genes/diseases closely allied to this one? Please give details	We are also testing for the autosomal recessive condition fumarate hydratase deficiency caused by bi-allelic mutations in the FH gene (gene dossier submitted and approved in 2008-2009). We have an interest in other kidney disorders, particularly Wilms tumour due to mutations in the WT1 gene. We are also planning to set up a service for autosomal dominant polycystic kidney disease. We have a strong interest in other skin and connective tissue disorders such as Ehlers Danlos Syndrome (EDS), Pseudo-xanthoma elasticum (PXE) and Osteogenesis Imperfecta (OI). We have strong links with Nephrology and Clinical Genetics.

Your Activity If applicable - How many tests do you currently provide annually in your laboratory?	Index cases: multiplying up from 12 referrals in 10 months we would expect to test at least 14 individuals per year but this is likely to increase as the service becomes more established. Family members where mutation is known: None so far.
Your Activity 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: We are set up for very high through-put sequencing and can easily process the anticipated number of national requests. Family members where mutation is known: Unlimited.
Based on experience how many tests will be required nationally (UK wide)? Please identify the information on which this is based	Index cases: Unknown but likely to be between 10-20 Family members where mutation is known: Unknown
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”.	Not applicable – we are able to provide the full national need.

Epidemiology

Estimated prevalence of disease in the general UK population Please identify the information on which this is based	Not known but 37 MCUL probands with FH germline mutations were identified in UK (Alam et al 2003 Hum Mol Genet 12 and Alam et al 2005 Br J Dermatology).
Estimated gene frequency (Carrier frequency or allele frequency) Please identify the information on which this is based	See above
Estimated penetrance Please identify the information on which this is based	Penetrance of Leiomyomatosis is very high. Penetrance of Renal cell cancer is ~2-6% without selection bias (Alam et al 2005 Br J Dermatology) but is widely reported as between 10-16%. There is also a rare risk of leiomyosarcomas (Lehtonen et al 2006 J Med Genet & Ylisaukkoja et al 2006 Int J Cancer) and Leydig Cell tumours of the Testis (Carvajal-Carmona et al 2006 J Clin Endocrin & Metab).
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 disease and appropriate family members. Gene mutations have been detected in individuals from different ethnic backgrounds and the testing will therefore not be limited to a particular ethnic group. The target population will consist of: A) Individuals with multiple cutaneous leiomyomas and a family history of HLRCC/MCUL **OR** B) Individuals with multiple cutaneous **and** uterine leiomyomas **OR** C) Tubullo-papillary or Type 2 papillary or Collecting duct renal cell cancer **AND** multiple cutaneous or uterine leiomyomas **OR** D) Two or more Tubullo-papillary or Type 2 papillary or Collecting duct renal cell tumours **OR** E) Tubullo-papillary or Type 2 papillary or Collecting duct renal cell cancer with documented history of similar renal tumour or of multiple cutaneous or uterine leiomyomas in a 1st degree relative OR F) Proven *FH* mutation detected in relative.
Estimated prevalence of disease in the target population	In a review of MCUL/HLRCC cases reported worldwide, germline FH mutations have been found in 76 out of 89 (85%) Alam et al 2005 Br J Dermatology.

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. If more than one gene will be tested, please include your testing strategy and data on the expected proportions of positive results for each part of the process. Please illustrate this with a flow diagram.	Sensitivity of DNA sequencing is over 95%. Since all mutations are checked in two separate amplicons, if possible by two independent methods, specificity is 100% where the mutation or type of mutation has been previously reported. Where the change is novel or an unclassified variant is detected, it may be necessary to carry out family studies and it still may not be possible to reach a conclusion. For mutation negative cases MLPA will be available if required.
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)	The clinical sensitivity and specificity of FH gene analysis is high in patients with clinically/histologically diagnosed HLRCC/MCUL. Published literature suggests that ~80% of patients with HLRCC/MCUL carry FH point mutations or small deletions which are detectable by direct DNA sequencing. Deletions/duplications detectable by MLPA are present in ~6% of affected individuals (Bayley et al 2008 BMC Med Genet).
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 published literature, this test has a positive predictive value of 95-100%. The clinical validity may be reduced if an unclassified variant is detected (for example, novel missense or atypical splice mutations). In the absence of functional studies and/or segregation analysis it is not always possible to determine the significance of these variants in terms of pathogenicity. In families with a known FH mutation, the negative predictive value when testing asymptomatic family members is close to 100%, since the new mutation rate is expected to be low.

Clinical utility of test in target population (Please refer to Appendix A) Please provide a description of the clinical care pathway.	Clinical Care Pathway: Diagnostic referrals: • Referral for testing from relevant specialists, including results from histological investigations from skin (cutaneous leiomyomas), kidney or uterus. • Decision whether testing is appropriate from referral information • Pre-test counselling by clinical geneticist due to association with malignancy. • Mutation analysis • Result to referring clinician for post test counselling • Management plan Family studies: • Referral for testing from Consultant Geneticist (after appropriate pre-test counselling). • Check that appropriate information is included i.e. the mutation has been identified in affected family members; positive control sample available for testing etc. • Mutation analysis • Result to Consultant Geneticist for post-test counselling • Referral to Consultant Dermatologist, Nephrologist or Gynaecologist (if appropriate)
How will the test add to the management of the patient or alter clinical outcome?	*FH* is the only gene known to be associated with HLRCC/MCUL. The molecular test allows confirmation of diagnosis. Approximately 85% of individuals with HLRCC/MCUL have identifiable sequence variants in *FH*. Differential diagnoses eg. familial renal cancer syndromes including: Von-Hippel-Lindau syndrome, Hereditary papillary renal cancer and Birt-Hogg Dube syndrome, can be excluded if a mutation is found. If no mutation is found the differential diagnoses should be further investigated. Once a diagnosis is confirmed specific evaluations are recommended including renal U/S and abdominal CT with contrast; detailed dermatological and pelvic examinations to detect the presence of any early malignant leiomyosarcomas. Manifestations of the condition require specific treatment. Confirmation of diagnosis in an affected family member will allow predictive testing of at-risk relatives. Those found to have inherited the mutation can then have appropriate regular screening and treatments.
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?	The negative predictive value is near to 100% and familial cases who do not inherit the familial mutation will no longer require regular dermatological, pelvic and renal screening thus improving their quality of life.

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 there is a biochemical test available for the autosomal recessive fumarate hydratase deficiency, Fumarate hydratase enzyme activity can be difficult to interpret for patients with a heterozygous FH gene mutation even following extensive family analysis. It is therefore not recommended for individuals with HLRCC/MCUL (personal communications, Simon Olpin)
Please describe any specific ethical, legal or social issues with this particular test?	None

Please complete the testing criteria form.

UKGTN Testing criteria

Name of Disease(s):
LEIOMYOMA, HEREDITARY MULTIPLE, OF SKIN (150800)
LEIOMYOMATOSIS AND RENAL CELL CANCER, HEREDITARY (605839)
Name of gene(s): Fumarate Hydratase; FH (136850)

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	**Lab ID:**
Title/Position:	

Referrals will only be accepted from one of the following:

Referrer	Tick if this refers to you.
Consultant Clinical Geneticist	

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

Criteria	Tick if this patient meets criteria
Multiple cutaneous leiomyomas with family history of HLRCC/MCUL **OR**	
Multiple cutaneous **and** uterine leiomyomas **OR**	
Tubullo-papillary, collecting duct or papillary type 2 renal tumour **and** multiple cutaneous or uterine leiomyomas **OR**	
Two or more tubullo-papillary, collecting duct or papillary type 2 renal tumours **OR**	
Tubullo-papillary, collecting duct or papillary type 2 renal tumour with documented history of similar renal tumour or of multiple cutaneous or uterine leiomyomas in a 1st degree relative **OR**	
Relative in family with proven fumarate hydratase gene 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.