Lynch syndrome/Hereditary Non-Polyposis Colorectal Cancer (HNPCC) – OMIM 120435/609310/614350

Background

Lynch syndrome is a rare condition and is the most common cause of hereditary bowel cancer. The condition increases the likelihood of specific types of cancer developing so regular screening is important. Lynch syndrome cancers typically develop at a younger age than sporadic cancers. It is inherited in an autosomal dominant manner so there is a 50% chance of a parent passing the condition on to each child. There are various analysis steps in the diagnosis process depending upon referral criteria and family history: microsatellite instability (MSI) in the tumour tissue, immunohistochemistry (IHC) testing for loss of DNA mismatch repair gene expression in tumour tissue, *BRAF* (OMIM 164757) Val600Glu mutation analysis, *MLH1* promoter methylation testing and screening of a number of DNA mismatch repair genes including *MLH1* (OMIM 120436), *MSH2* (OMIM 609309) and *MSH6* (OMIM 600678). An overview of the Lynch syndrome diagnosis pathway is shown in figure 1.

Recommended Clinical Referral Criteria

Germline testing of the Lynch syndrome gene panel

Patients are referred to clinical genetics for germline testing of the Lynch syndrome gene panel if they fulfil the following criteria:

- The patient's tumour sample has previously shown microsatellite instability or loss of mismatch repair protein and no detection of the BRAF V600E variant and MLH1 promoter methylation.
- The affected proband comes from a modified Amsterdam criteria positive family irrespective of the dMMR status of the tumour.

Germline MLH1 promoter methylation analysis

Patients are referred to clinical genetics for germline MLH1 promoter methylation for a diagnosis of Lynch syndrome if they fulfil the following criteria:

- MLH1 promoter methylation has previously been detected in a Lynch-related tumour specimen.
- The patient is less than 50 years of age
- The patient has a first or second degree relative with bowel or endometrial cancer at any age.

MMR gene sequencing on FFPE tissue

Tumour samples referred for a somatic MMR gene screen are sent out for testing at the Manchester Centre for Genomic Medicine.

Tumour samples are referred for a somatic MMR gene screen if:

- Microsatellite instability or loss of mismatch repair proteins previously shown in the tumour sample **AND**
- BRAF V600E variant and MLH1 promoter methylation both absent in the tumour sample **AND**
- Subsequent germline testing did not identify a pathogenic variant in the Lynch syndrome gene panel.

Archive tissue samples (preferably normal, otherwise tumour) may also be referred by clinical genetics when a germline blood gene screen would normally be performed but there is no living affected individual on whom to perform a germline test.

Family follow-up testing

Relatives of individuals with an identified Lynch syndrome variant can access predictive testing through the clinical genetics team.

Molecular Analysis

- **Pre-screen:** MSI analysis of the selected tumour tissue to check mismatch repair deficiency; IHC testing of the tumour to look at expression of the mismatch repair genes.
  - MSI-positive samples: pyrosequencing analysis for c.1799T>A (p.Val600Glu) *BRAF* gene mutation and/or bisulphite conversion then pyrosequencing analysis of *MLH1* promoter methylation on the tumour tissue. Both of these tests are to check for sporadic (non-Lynch) tumours with microsatellite instability.
- **Variant screen:** Next generation sequence (NGS) analysis of the *MSH2, MSH6* and *MLH1* genes (any gaps will be filled by Sanger sequencing) using the TruSight Cancer sequencing panel. MLPA is also used to perform dosage analysis of *EPCAM* exon 9 to detect the recurrent *MSH2* disrupting 10Mb paracentric inversion.
- **MLH1 promoter methylation analysis:** Germline MLH1 promoter methylation analysis using pyrosequencing is performed on a blood sample.

Family follow-up: Testing for known familial variant in *MLH1, MSH2* and *MSH6* genes

Turnaround Times (TAT)

Prices available on request, please contact the laboratory using details below.

Test	TAT (calendar days)
Testing for known familial variant in *MLH1*, *MSH2* and *MSH6*	28
MSI ,*BRAF* (p.Val600Glu) and MLH1 promoter methylation analysis	28
MLH1 promoter methylation analysis	14
Dosage analysis only (*MLH1, MSH2* or *MSH6)* gene	42
MSH2 inversion breakpoint analysis	42
Variant screening and dosage analysis (*MLH1*, *MSH2* (plus common inversion), *MSH6* and dosage analysis of *EPCAM* exon 9	42

Analysis of a subset of genes is available if required.
The turnaround time for an FFPE MMR gene screen is determined by the performing laboratory (Manchester)

Contact Details

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Sample Requirements

Paraffin-embedded tumour tissue: should contain the maximum quantity of viable tumour and be accompanied by a histology report.
Blood: 5ml in EDTA (for control purposes and screening).
Please label samples with three identifiers and date of collection.
All samples must be accompanied by request form.
Consent for testing & DNA **storage is assumed when a test request and samples are received.**

Links

Orphanet
http://www.orpha.net/
OMIM
http://www.omim.org/
Genetic Test Registry
http://www.ncbi.nlm.nih.gov/gtr/
Support
http://www.bowelcanceruk.org.uk

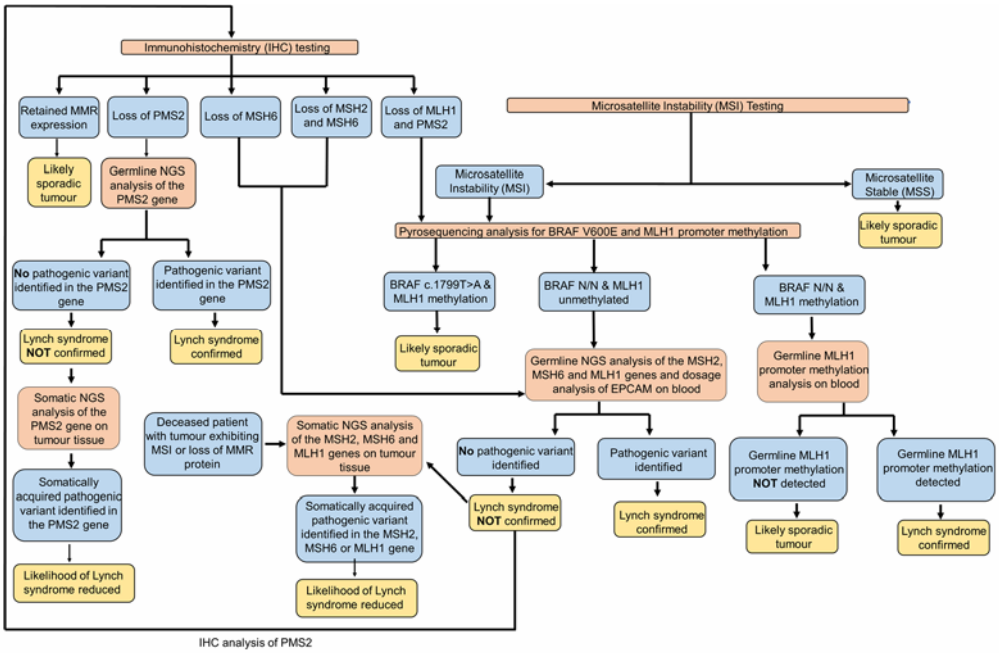

Figure 1: The Lynch syndrome diagnostic pathway.