National Centre for **Medical Genetics**
Ionad Náisiúnta Gineolaíocht Leighis

The National Centre for Medical Genetics
Our Lady's Children's Hospital, Crumlin
Dublin 12

Division of Molecular Genetics

User Guide 2014

Please note this User Manual must be read in conjunction with any service restrictions that may apply; see www.genetics.ie

This version released August 2014

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Introduction

The National Centre for Medical Genetics was established at Our Lady's Children's Hospital in January 1995 to provide a medical genetics service to the Irish population. There are three divisions; Clinical Genetics, Cytogenetics Genetics, and Molecular Genetics, comprising a fully integrated national genetics service that serves a population of approximately 4.5 million in the Republic of Ireland

The Division of Molecular Genetics provides a fully comprehensive molecular genetic analysis service for a range of inherited disorders and a referral service for analysis for rare disorders. Molecular Genetics also has close links with the Division of Cytogenetics, the Division of Clinical Genetics and University College Dublin.

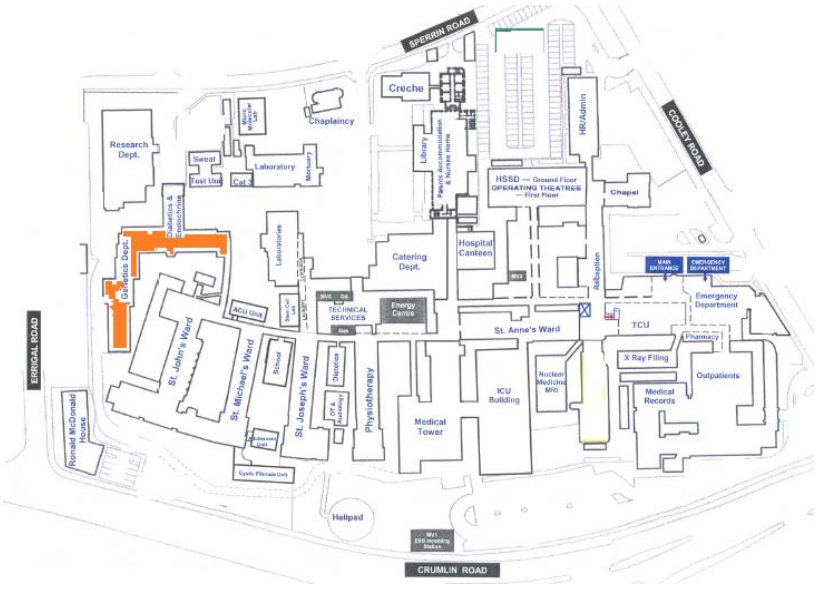
This handbook summarises information on the services provided and should be read in conjunction with any service limitation and restrictions that may be in place (available on www.genetics.ie). The Division aims to return correct results, for the correct patient, to the correct place and person within the correct time frame. The quality of our service is regularly audited and was accredited by CPA (UK) Ltd until end of 2013 (Laboratory Number 3001). The Division is working towards Irish National Accreditation Board (INAB) accreditation to International Standard ISO 15189:2012.

It is important that you contact us if you have any questions, comments or complaints about any aspect of our service. Contact details can be found below.

Division of Molecular Genetics Location

The Division of Molecular Genetics is housed within the National Centre for Medical Genetics, Our Lady's Children's Hospital, Crumlin, Dublin 12.

Please note there is no patient access to the laboratories.



Map of OLCHC site, National Centre for Medical Genetics shown in orange

Postal Address

The address for the Division of Molecular Genetics is:

Division of Molecular Genetics
National Centre for Medical Genetics
Our Lady's Children's Hospital
Crumlin
Dublin 12

Opening Hours

Weekdays 09.30 – 17.00 (excluding public holidays). Please note there is no on-call or out-of-hours service.

Deliveries to the Laboratories

Specimens can be delivered to the laboratories:

- From within Our Lady's Children's Hospital – By delivery to the laboratory or by internal chute system (destination 2770).
- From outside Our Lady's Children's Hospital – By delivery to the specimen reception located at the rear of the National Centre for Medical Genetics. Access is via Gate 5 at the rear of Our Lady's Children's Hospital, Crumlin.

See also page 5, Sending Samples to the Laboratory

Contact Information

General				
	Name	**Telephone**	**Internal**	**email**
Chief Scientist	David Barton	01 409 6738	6749	david.barton@olchc.ie
Quality Manager	Christine Brady	01 428 2705	2705	christine.brady@olchc.ie
Admin/Enquiries	Celine/Audrey	01 409 6733	6733	celine.corcoran@olchc.ie audrey.lewis@olchc.ie
Lab enquires				duty.scientist@olchc.ie

Additional Useful Contacts

Division of Cytogenetics Enquiries
☎ 01 409 6737 (internal 6737) cytolab@olchc.ie
Division of Cytogenetics Quality Manager (Adam Dunlop)
☎ 01 428 2899 (internal 2899) adam.dunlop@olchc.ie
Division of Clinical Genetics Enquiries and Appointments
☎ 01 409 6739 (internal 6739)
National Centre for Medical Genetics General Manager
☎ 01 409 6277 (internal 6277) damien.moyles@olchc.ie
National Centre for Medical Genetics
Website www.genetics.ie

Quality Assurance

As part of its commitment to total quality, the Molecular Genetics Laboratory participates in the external quality assurance schemes run by the United Kingdom National External Quality Assessment Service, UKNEQAS, and by the European Molecular Genetics Quality Network, EMQN. Copies of the results of these external quality assessments are available on request.

The Molecular Genetics laboratory was accredited by CPA(UK) Ltd to the CPA standards incorporating ISO 15189 from 2009-2014 and is currently moving towards being accredited by the Irish National Accreditation Board (INAB). For more information please contact the lab's Quality Manager Christine Brady (christine.brady@olchc.ie).

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Sending Samples to the Laboratory

Samples and information required

Generally 3-5ml of EDTA blood (FBC bottle) is required, except where otherwise indicated. Blood specimens must be appropriately packaged and preferably sent by courier to arrive as soon as possible. Other sample types by arrangement only. Samples for molecular genetic analysis may be refrigerated. Do not freeze prior to or during postage.

Please note that DNA is stored from patients' samples at the National Centre for Medical Genetics, and kept indefinitely unless a written request for its disposal is received from the patient or their parent/guardian. Please note that prior notification is required for all prenatal referrals to the Division of Molecular Genetics. Notification should occur prior to a prenatal specimen being taken, and prenatal testing should be arranged through a Clinical Genetics department if possible. Summary clinical and family history information must accompany every sample, including (when available) names, dates of birth, clinical details and copies of genetic test results from the relevant family member(s).

Genetic tests are usually only carried out once in an individual's life, placing a special emphasis on ensuring that genetic test results go to the correct patient. Accurate patient and sample identification are crucial first steps in achieving this objective. Paperwork must accompany sample(s) and at least 2 identifiers must be present that match sample(s) and any additional paperwork supplied. Any additional identifiers supplied must also match between sample and paperwork.
http://www.genetics.ie/documents/Sample_Identification_Policy.pdf

Transfusions & Transplants

Genetics tests are designed to look at the constitutional genetic makeup of a patient by studying DNA or chromosomes from their lymphocytes. If you send us a blood sample from a patient who has recently had a blood transfusion or has ever had a bone marrow transplant, the result may be compromised because we may be studying the lymphocytes of the donor.

Your patient has recently received a blood transfusion:

- Please indicate the date of any recent transfusion on the request form.
- If possible, wait at least two weeks before taking a blood sample from a patient who has received a transfusion.
- If this is not possible, try to send in the unused portion of the transfusion pack with the patient's sample.
- Alternatively, check with your Pathology Laboratories in case they still have a pre-transfusion blood sample stored from your patient.
- Sometimes we may ask for a different sample type to confirm our results.

Your patient has had a bone marrow transplant:

- Unless the test required is specifically for post-transplant monitoring, we cannot test blood samples from patients who have had a bone marrow transplant.
- Please contact the appropriate laboratory for advice.

Packaging Instruction 650

The following packaging notes apply to all sample types and should be consulted in conjunction with the sample categories listed below.

Diagnostic samples, now classified by the United Nations (UN) as Dangerous Goods, Division 6.2 and assigned to UN 3373, must be packaged for transport in a way that meets the requirements of Packaging Instruction 650. Such packaging may be specially purchased for this purpose or constructed from suitable components.

Packaging should be strong enough to withstand the shocks and loadings normally encountered during transport, including manual and mechanical handling, and should be constructed and closed so as to prevent any loss of contents in the event of leakage or breakage. The packaging consists of:

1. **Primary receptacle**, leakproof and sealed, containing the specimen (e.g. Universal container or blood tube), not exceeding 50ml or 50g, individually wrapped with enough absorbent material to absorb all fluid in the event of leakage or breakage.
2. **Secondary packaging**, durable and leakproof container, to enclose and protect primary receptacle(s). Multiple individually wrapped primary receptacles may be placed in one secondary packaging. Sufficient absorbent material must be used to cushion multiple primary receptacles and absorb the entire contents of the primary receptacles in the event of leakage or breakage.
3. **Outer packaging** to protect the secondary packaging and contents from outside influences, such as physical damage and water while in transit.

In addition, the following **local rules** apply:

- All samples should be in a sealed container accompanied by a fully completed NCMG request form. Packaging instructions are available on the website http://www.genetics.ie/pir/sending_samples.pdf.
  The full sample identification policy is available on the website http://www.genetics.ie/pir/SampleIdentificationPolicyWeb.pdf

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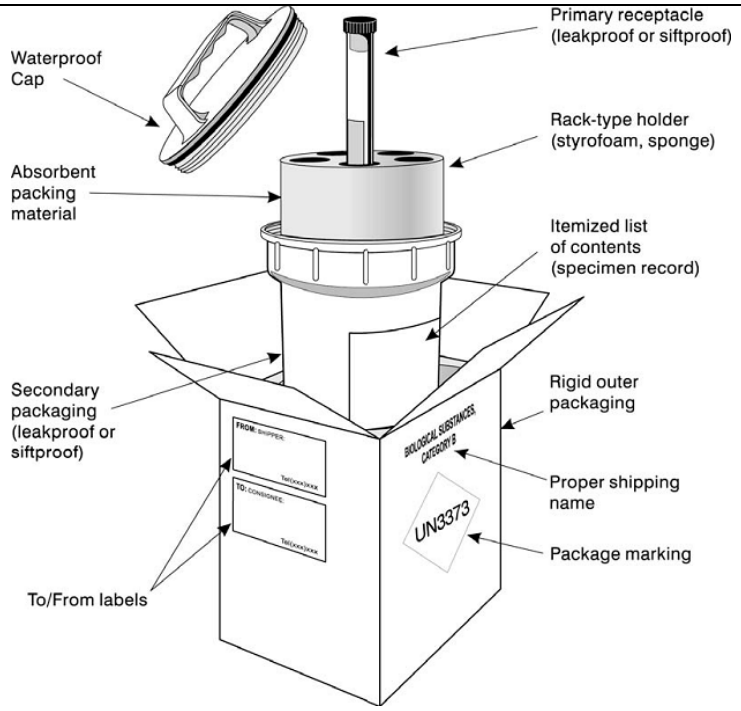

Delivery of Specimens

Specimens should be delivered as soon as possible after sampling. Transport requirements for each sample type are outlined above and on the reverse of the NCMG referral form.

Important note: samples without the above information may be rejected. Samples may also be rejected for other reasons, see individual sample types below.

High Risk Samples

- Please mark **HIGH RISK SAMPLES** appropriately.
- Forms and bottles must be labelled with a red warning sticker.
- The sample must be sealed in a plastic bag. The form must never come into contact with the specimen tube or sample.

Request Forms

If you are sending us a sample please ensure that this form is filled out completely & that you have included your full name & address or the full name of the person to whom the report should be sent, to ensure the report goes to the correct person. Please contact our Molecular Genetics scientist on duty.scientist@olchc.ie if you have any queries regarding Molecular Genetic samples or testing. NCMG Request forms can be downloaded from the website or alternatively local request forms can be used.
http://www.genetics.ie/pir/2006_NCMG_Referral_Form.pdf

Details Required on Request Form

- **Patient details**
  - full name
  - date of birth
  - hospital/medical record number

- **Referral Details**
  - sample type
  - date and time of sampling
  - name of person taking the sample
  - requesting clinician including contact number
  - clinical indication
  - tests required
  - family history and any previous genetic studies on patient or family (including family names & dates of birth)

The Laboratories Acceptance & Rejection Criteria of Samples

Upon receipt, the condition of each sample will be assessed by laboratory staff. Any sample that does not meet the acceptance criteria will be rejected and will not be processed further. A report will be generated to notify the referring health care provider of the reasons for the specimen rejection and request a new sample, if appropriate. Please note that these acceptance and rejection criteria may not apply to irretrievable specimens, such as prenatal specimens or specimens from a deceased individual but will be reviewed on a case by case basis by a Senior Scientist. Specimens are subject to rejection if:

1. Unlabelled or mislabelled samples or Samples/forms received containing insufficient details to identify patients
2. Post Transfusion (see section on Transfusions & Transplants above)
3. Not in EDTA/wrong tube type
4. Compromised sample - the sample appears to have already been used for a pathology test prior to being submitted to us for genetic analysis. Any genetic analysis on this sample would now be compromised and therefore we cannot accept it.
5. Frozen, or grossly contaminated
6. Packaged in improper containers
7. Leaking containers

This list is in no way exhaustive. For any questions regarding these acceptance and rejection criteria, please contact the Laboratory directly at duty.scientist@olchc.ie.

Data Protection and Confidentiality

The National Centre for Medical Genetics adheres to the Data Protection Acts 1998 and 2003; Our Lady's Children's Hospital, Crumlin has a Data Control Officer based in the Information Technology Department.

The National Centre for Medical Genetics is legally bound by the Freedom of Information Acts 1997 and 2003; Our Lady's Children's Hospital, Crumlin has a Freedom of Information

Officer located within the Patient Support Unit.

User Responsibilities

Delivery of our Molecular Genetics service is dependent on the co-operation of the user. The user is responsible for:

- Despatching samples in a timely manner to arrive on a weekday within normal working hours of between 9.30am and 5.00pm.
- Ensuring that all samples are accompanied by a **request form** or covering letter with full details including which specific tests are required.
- Ensure that a copy of the Molecular Genetic report is added to the patient's chart.

Private Hospitals

Samples from private hospitals and clinics must adhere to our acceptance criteria and must have signed an agreement of payment for testing. For further information please contact the General Manager (Damien Moyles) on ☎ 01 409 6277 or Chief Scientist, David Barton on ☎ 01 409 6749.

Enquiries from Patients

Please note that the laboratory cannot deal with direct enquiries from patients. If you have not received results of a genetic test when expected, please contact the doctor who requested the test on your behalf. For general enquiries, where a molecular genetic test has not yet been requested, please contact Clinical Genetics.

Molecular Genetics current In house disease tests - Turnaround times (TAT) for reporting

Disease Name	Synonyms	Current TAT
Angelman Syndrome	**AS**	Urgent = 2 weeks Routine = 8 weeks
Lynch Syndrome	**BRAF**	12 weeks
Hereditary Breast Cancer	**BRCA**	6 weeks
Byler disease	**Byler**	Urgent = 2 weeks Routine = 12 weeks
Cystic Fibrosis	**CF**	Urgent = 2 weeks Routine = 8 weeks
Early-Onset Torsion Dystonia	**DYT1**	Urgent = 4 weeks Routine = 24 weeks
Fragile X Syndrome	**FraX**	24 weeks
Friedreich Ataxia	**FA**	Urgent = 2 weeks Routine = 12 weeks
Huntington Disease	**HD**	Urgent = 4 weeks Predictives = 8 weeks from 2nd sample Routine = 12 weeks
Microsatellite Instability, MSI, analysis	**MSI**	Slides = 12 weeks Blocks = 20 weeks

Osteogenesis Imperfecta Type VIII	**OI-P3H1**	Urgent = 2 weeks Routine = 12 weeks
Prader Willi Syndrome	**PWS**	Urgent = 2wks Routine = 8 wks
Sickle Cell Anaemia	**Sickle**	CVS = 2 weeks
Russell Silver Syndrome	**RSS**	12 weeks
Spinal Muscular Atrophy	**SMA**	Urgent = 2 weeks Routine = 8 weeks
Uniparental disomy	**UPD**	12 weeks

Please note

1. *Urgent samples are patients less than 6 months and those involving pregnancies & Routine samples are all other samples*
2. *For amniotic fluid samples please allow an additional 2 weeks to those indicated above*
3. *TATs may go over during holiday periods at Easter and Christmas*
4. *For TATs for molecular tests that go over the quoted time above, please contact duty.scientist@olchc.ie*
5. *For all other molecular tests (sendouts) please contact duty.scientist@olchc.ie for information regarding TATs.*

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Angelman Syndrome (AS)

Contacts: **Molecular Genetics**
- **Administration** ☎**01 409 6733** **(internal 6733)**
- **Laboratory** **duty.scientist@olchc.ie**

Background

OMIM #105830
Angelman Syndrome (AS) is characterised by severe developmental delay/mental retardation, gait ataxia, seizures, inappropriate laughter and happy disposition. The incidence of AS is estimated to be between 1/10,000 and 1/20,000.
Approximately 80% of AS patients lack expression of the maternal copy of genes in the chromosome 15q11-q13 region. Loss of the maternal allele arises by a de novo deletion of the critical region of the maternal chromosome or by inheritance of two paternal copies of chromosome 15 (paternal uniparental disomy - pUPD). Both of these types of abnormality usually arise de novo and have a very low risk of recurrence. In rare cases, AS may be caused by an imprinting defect – in these cases there can be up to 50% risk of recurrence. Approximately 20% of true AS patients have mutations in the UBE3A gene and other genes. Such cases are associated with a significant risk of recurrence, estimated at between 20% and 50%.

Table 1: Molecular defects and recurrence risks in PWS.

Genetic defect	Proportion of cases	Recurrence risk
De novo deletion of 15q11-q13 on the paternal chromosome	75-80%	<1%
Maternal uniparental disomy (UPD) of chromosome 15	20-25%	<1%
Imprinting defects (with an imprinting centre deletion excluded)	≈1%	<1%
Imprinting centre deletion	≈ 10-15% of patients with an imprinting defect	Up to 50% (if present in father)

(Table taken from "Practice Guidelines for Molecular Analysis of Prader-Willi and Angelman Syndromes". Ramsden et al. BMC Medical Genetics 2010, 11:70)

Essential referral information

In addition to supplying standard patient identification and referral information (see Sample ID Policy), the following should be clearly indicated:
1. Patient's symptoms.
2. Any family history, including names, dates of birth, relationship and genetic test results if available.

It is the responsibility of the referring clinician to ensure consent has been obtained for testing and storage.

Samples required:

3-5ml of blood in an EDTA tube. Sample identification policy is detailed on the NCMG web site.
Blood specimens must be appropriately packaged, and preferably sent by courier to arrive as soon as possible. Do not freeze prior or during postage.
Please note that extracted DNA is stored from patients' samples at the National Centre for Medical Genetics, and kept indefinitely unless a written request for its disposal is received from the patient or their parent/guardian.

Restrictions on testing: *There are no particular restrictions on testing.*

Tests offered:

1. Diagnostic test – Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) is used to detect copy number changes and analyse CpG island methylation patterns within the 15q11-q13 region (Prader-Willi/Angelman critical region). Absence of the maternal methylation pattern confirms a diagnosis of AS.
2. Mechanism of inheritance – when a diagnosis of AS is confirmed, the mechanism of inheritance (i.e. maternal deletion, pUPD or imprinting centre defect) is investigated in order to assess recurrence risks. MS-MLPA analysis can detect deletions of the 15q11-q13 critical region and can determine if the mechanism of inheritance is a maternal deletion. However if a deletion is not detected the mechanism could be either pUPD or an imprinting centre defect. MS-MLPA cannot distinguish between these. Further molecular analysis is necessary in order to investigate UPD. This requires parental samples in EDTA.
3. Where a diagnosis of AS is not excluded by the above test, the laboratory can arrange for detailed mutation screening to be undertaken elsewhere.

Diagnostic sensitivity of tests

1. Approximately 20% of true AS patients have mutations in UBE3A and other genes which will not be detected by this test. Therefore the diagnostic sensitivity of the genetic test is approximately 80%.

Interpretation:

Results are given in the form of a written interpretative report to the referring clinician.

Target reporting times:

As reporting times are constantly evolving, please refer to www.genetics.ie/molecular, or contact the molecular genetics laboratory, to receive up-to-date information on anticipated reporting times for your referral.

- The following are current target reporting times for each category of test offered:
  - Urgent samples (newborns): 2 weeks
  - Routine samples: 8 weeks
  - UPD: 3 months
- Please contact the laboratory if you have not received a report within a week of your patient being due back in clinic.
- Please note it is our policy not to issue verbal results.
- Request for copies of reports on the day that your patient is in clinic cannot normally be accommodated. We usually require 24 hours notice in which to fax a copy of a report.

Further tests

- UBE3A: Bidirectional sequencing of exons 8-16 (functional coding sequence) is available via an external laboratory. TAT = 2 months, cost = £600
- Suspected imprinting centre (IC) defect: When a diagnosis of AS is confirmed using MS-MLPA and both a deletion and UPD have been excluded as the mechanism of inheritance, further analysis can be performed in an external laboratory to confirm/exclude the presence of an IC deletion.

Please contact us to make arrangements for such testing, if required.

Lynch Syndrome

Contacts: **Molecular Genetics**

- **Administration** ☎**01 409 6733** **(internal 6733)**
- **Laboratory** **duty.scientist@olchc.ie**

Background

Lynch Syndrome (previously known as HNPCC - Hereditary Non-Polyposis Colon Cancer) is an autosomal dominant cancer predisposition syndrome characterised by colorectal adenocarcinoma with or without extracolonic cancers (including ovarian, endometrium, small bowel, stomach, hepatobiliary tract, urinary tract, brain, and skin). It is estimated that Lynch Syndrome accounts for 4-6% of colorectal cancer.

Lynch Syndrome is caused by inactivating mutations in genes of the **DNA mismatch repair system** (MMR genes) of which at least five have been identified. MMR gene mutations result in failure to repair errors during DNA replication. Cancer is likely to develop when unrepaired replication errors inactivate genes including tumour suppressors. The MMR genes MLH1 and MSH2 account for approximately 80-90% of Lynch Syndrome cases, MSH6 mutations are found in about 7%-10%; and PMS2 mutations in fewer than 5%.

MMR gene inactivation may be revealed as extra microsatellite alleles in tumour DNA as compared to normal DNA, known as **Microsatellite Instability** (MSI). In addition, loss of expression of the defective MMR protein may be detected by **Immunohistochemistry** of the tumour sample.

REQUESTS FOR DIAGNOSTIC & PREDICTIVE LYNCH SYNDROME TESTING

ARE USUALLY ONLY ACCEPTED VIA A CLINICAL GENETICS SERVICE

For further information regarding Lynch Syndrome referrals to Clinical Genetics at NCMG, please see http://www.genetics.ie/clinical/ or phone 01-4092800.
Once referred, patients are assessed by means of a clinical questionnaire prior to any testing being offered http://www.genetics.ie/services/brca/Family_Cancer_Questionnaire.pdf. Patient consent will also be obtained prior to testing.

Once a patient meets the clinical and family history criteria, genetic testing for Lynch syndrome is usually as follows:

- Evaluation of tumour tissue for **Microsatellite Instability** (MSI) by molecular (DNA) testing and/or **Immunohistochemistry** (IHC) of the four MMR proteins MLH1, MSH2, MSH6, PMS2. IHC testing helps identify the MMR gene that may harbour a germline mutation. The presence of MSI in the tumour alone is not sufficient to diagnosis Lynch syndrome as 10%-15% of sporadic colorectal cancers also exhibit MSI. Further molecular testing of the tumour DNA for the activating somatic **BRAF V600E** mutation may help identify those MSI-H tumours which are more likely to be sporadic rather than hereditary (specifically cases with loss of MLH1 expression).
- For cases where tumours exhibit the molecular characteristics of an MMR gene defect on the basis of the IHC, MSI & BRAF pre-screen (described above), **Sequencing and MLPA analysis** of the patients **MMR genes** (from a blood DNA sample) can then proceed in order to identify the causative germline mutation.
- Once the underlying pathogenic MMR gene mutation has been identified in an index case, **predictive genetic testing** (from a blood DNA sample) can be offered to at risk relatives.

TESTS OFFERED

1. **Tumour Pre-screening**:

NOTE: All cases must be referred via a Clinical genetics service and assessed accordingly prior to submitting any samples for testing (see above).

TEST *(performed in-house at NCMG)*	**SAMPLE TYPE**	**TURNAROUND TIME**
MICROSATELLITE INSTABILITY (MSI)	TUMOUR	3 MONTHS [FOR SLIDES] 5 MONTHS [FOR BLOCKS]
BRAF V600E ANALYSIS	TUMOUR	3 MONTHS

Sample requirements: For MSI and/or BRAF analysis of tumours, we require 8 unstained, uncovered slides (10uM thickness) plus one H&E stained slide (4uM thickness) with the tumour tissue clearly marked on the H&E slide. Each slide should be labelled with the exact block number used and a full pathology report (including IHC results) should accompany each sample submitted. Please contact the laboratory with any further queries regarding submission of tumour samples duty.scientist@olchc.ie

2. **Diagnostic testing:** Mutation screening of the **MLH1, MSH2, MSH6 or PMS2** genes of patients who have clinical signs of Lynch Syndrome, for the purposes of confirming a diagnosis and providing a direct mutation test for at risk relatives (predictive test). *Mutation screening (sequencing and MLPA) is usually only performed after a patient's tumour has been shown to exhibit MSI and/or loss of MMR protein expression by IHC.*
   - Currently, DNA is extracted from patient blood samples (EDTA) and sent to an external laboratory for mutation analysis [Turnaround time = 3-5 months]

3. **Predictive testing**: Testing of asymptomatic individuals who have a family member with Lynch Syndrome in whom a confirmed MMR gene mutation is known and characterised.
   - Currently, DNA is extracted from patient blood samples (EDTA) and sent to an external lab for analysis [Turnaround time = 1 month]

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Familial Breast/Ovarian Cancer

Contacts: **Molecular Genetics**

- **Administration** ☎**01 409 6733** **(internal 6733)**
- **Laboratory** **duty.scientist@olchc.ie**

Background

Pathogenic mutations in the tumour suppressor breast cancer genes *BRCA1** and *BRCA2*** account for approximately 3-5% of all female breast and ovarian cancers. Inheritance follows an autosomal dominant pattern. Females who inherit a pathogenic mutation, in either of these genes, have a 60-85% lifetime risk of developing breast cancer and a lifetime risk of ovarian cancer of between 15 and 40%. Males who carry a mutation in the *BRCA2* gene have a 6.3% risk of developing breast cancer by the age of 70. The risk conferred to males by mutations in the *BRCA1* gene is not well characterised. Female relatives of male carriers of a *BRCA1* or *BRCA2* mutation are at risk of inheriting the *BRCA1* or *BRCA2* mutation and of therefore having a high risk of breast/ovarian cancer. For first degree female relatives, this risk is 50%.

Clinical indicators for hereditary breast cancer/ovarian in a family include the presence of a family history of breast/ovarian cancer, especially in a first degree relative and may also include any of the following: Presence of early onset disease (<40y at diagnosis), bilateral breast cancer, breast and ovarian cancer in the same individual, ovarian cancer before 60yrs of age, male breast cancer.

At the NCMG, testing of hereditary breast/ovarian cancer genes BRCA1 and BRCA2 is available as follows:

- Individuals with breast/ovarian cancer and a family history of breast/ovarian cancer may be offered full mutation screening of the BRCA1 & BRCA2 genes as appropriate, following assessment by a Clinical Genetics service.
- Where a pathogenic mutation in BRCA1 or BRCA2 has been identified in a family, adult family members may be tested for the presence or absence of the mutation as appropriate, following a Clinical Genetics referral.

* OMIM ID-113705
**OMIN ID-600185

Standard service

Essential Referral Information

In addition to supplying standard patient identification and referral information, the following should be clearly indicated:

1. Full mutation screening of BRCA1 and BRCA2: Detailed family history (ideally, in the form of a pedigree), type of cancer (breast/ovarian/prostate), age of diagnosis.
2. Testing for a known family mutation: Whether the patient is affected or unaffected (with any cancer); details of the family mutation, details of the index case (name and date of birth), relationship of the patient to the index case, and family history details (ideally, in the form of a pedigree diagram).

It is the responsibility of the referring clinician to ensure that consent has been obtained for testing & storage.

Samples required:

1. Full mutation screening of BRCA1 and BRCA2: Screening involves sequencing of both of these large genes, therefore approximately 10ml of blood in EDTA anticoagulant is required so that two DNA preps can be obtained.
2. Testing for a known family mutation (predictive and diagnostic testing): Approximately 3-5ml of blood in EDTA anticoagulant

Important note: Samples and associated paperwork must be clearly labelled with at least 2 identifiers (full name AND date of birth/Hospital number) for the individual to be tested. Please refer to NCMG Sample identification policy for further details.

Blood specimens must be appropriately packaged, and preferably sent by courier to arrive as soon as possible. Samples must not be frozen prior to or during posting.

Please note that DNA extracted from samples received are stored at the National Centre of Medical Genetics, and are kept indefinitely unless a written request for disposal is received from the patient.

Restrictions on testing

Molecular genetics analysis of *BRCA1* and *BRCA2* genes is only performed in conjunction with a counselling programme run by the National Centre for Medical Genetics, the Mater Misericordiae University Hospital, or St James's Hospital. Patients should be referred to Professor Andrew Green, Consultant Clinical Geneticist, National Centre for Medical Genetics or Dr David Gallagher at the Mater Misericordiae University Hospital or St James's Hospital.

Predictive testing is only offered to adults (over the age of 16yrs).

Tests offered:

Following DNA extraction by our Centre, *BRCA1* and *BRCA2* full screening is generally carried out externally by the UK West Midlands Regional Genetics Centre in Birmingham (See below for exceptions). Testing for the presence of a know mutation is performed in-house, unless the analysis requires MLPA, or mutation-positive control DNA is unavailable in-house.

1. Full mutation screening of *BRCA1* and *BRCA2*: All coding exons of BRCA1 & BRCA2 are screened using Multi-Plex Ligation dependent Probe Amplification (MLPA) & DNA sequencing. This type of testing is provided for affected individuals with clinical indicators of familial breast/ovarian cancer after assessment by the Clinical Genetics team at the NCMG.
2. Testing for a known family mutation (Predictive and diagnostic testing): Testing is provided both to individuals in the family who do not have cancer (predictive test) and to those who do have cancer (diagnostic test). Testing is offered as appropriate, following assessment by the Clinical Genetics team at the NCMG.

Sensitivity of tests:

Full mutation screening of BRCA1 and BRCA2: This is performed by sequencing which has

an estimated sensitivity of > 99%

Testing for a known mutation in BRCA1 or BRCA2: Estimated sensitivity is 100%

Interpretation:

Following analysis by the West Midlands Regional Genetics Centre in Birmingham, results are provided in the form of a written interpretive report based on whether or not a mutation has been identified and on the type of mutation found. Interpretation of results is based on current best practice guidelines for molecular genetics testing.

Confirmation of the presence of a pathogenic mutation:

- Diagnostic test (Females & males): The result is consistent with a diagnosis of familial breast/ovarian cancer. Predictive/Genotype testing can now be offered to family members.
- Predictive test (Females): The patient has a high lifetime risk of breast/ovarian cancer. Predictive/Genotype testing can now be offered to family members.
- Predictive test (BRCA2, Males): Risk of breast cancer is approximately 6%. Risk of other cancers is unknown. Risk to female relatives. Predictive/Genotype testing can now be offered to family members.
- Predictive test (BRCA1, Males): Risk of cancer in male carriers of BRCA1 mutations is unknown. Risk to female relatives. Predictive/Genotype testing can now be offered to family members.

For all of the above, female relatives are at risk of inheriting the *BRCA1* or *BRCA2* mutation and of therefore having a high risk of breast/ovarian cancer. For first degree female relatives, this risk is 50%.

Confirmation of the absence of a pathogenic mutation:

- Full screening of BRCA1 and BRCA2 – No mutation identified: A mutation in BRCA1 or BRCA2 is unlikely to be the cause of breast/ovarian cancer.
- Testing for a known familial mutation - Predictive test, family mutation is absent: The individual's lifetime risk of breast/ovarian/other cancer has been reduced to that of the general population.
- Testing for a known familial mutation – Diagnostic test, family mutation is absent: This mutation is highly unlikely to be the cause of this patient's cancer

Confirmation of the presence of an Unclassified variant:

- Full mutation screening of BRCA1 and BRCA2 may result in the identification of a sequence variant of unknown clinical significance, with no pathogenic mutation having been identified. The report may state that the clinical significance of the mutation is unknown or that at present there is no evidence of any clinical significance.

In these cases, the UK West Midlands Regional Genetics Centre in Birmingham will present the current information available regarding the pathogenicity status of the sequence variant. It may be appropriate for further studies to be performed. Further tests might include analysis of other members in the family who have cancer in order to ascertain whether the variant is following the disease; or in the case of a putative splice-site mutation, RNA analysis may determine whether the variant has a detrimental affect on RNA. All arrangements for further testing will be made following assessment by the NCMG Clinical Genetics Service.

- In accordance with current best practice guidelines for molecular genetics testing,

predictive testing of family members without cancer is not appropriate for variants of unknown clinical significance.

Target reporting times:

As reporting times are constantly evolving, please refer to www.genetics.ie/molecular, or contact the molecular genetics laboratory, to receive up-to-date information on anticipated reporting times for your referral.

The following are current target reporting times:

- Full mutation screening of BRCA1 and BRCA2: Approx. 3 months
- Testing for a known family mutation (Predictive testing): Approx. 4-6 weeks
- Testing for a known family mutation in a patient with cancer (diagnostic test): Approx 4-6 weeks

Further tests

Where the family mutation has been identified by an external laboratory other than the UK West Midlands Regional Genetics Laboratory, and there is no mutation positive control stored at the NCMG; testing of family members will be performed by the original external laboratory, as appropriate.

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Byler Disease

Contacts **Molecular Genetics**

- **Administration** ☎**01 409 6733** **(internal 6733)**
- **Laboratory** **duty.scientist@olchc.ie**

Background

Byler disease or Progressive Familial Intrahepatic Cholestasis type 1 (PFIC1: OMIM #211600: ATP8B1 gene) is a chronic autosomal recessive disorder causing hepatic fibrosis and end-stage liver disease. Defects in bile secretion and/or absorption, causing hepatic and systemic accumulation of bile acids with reduced enteric bile acid availability underlie PFIC. Clinical symptoms include history of neonatal diarrhoea, sepsis and intermittent jaundice becoming permanent. Intractable pruritus (itch) and growth retardation is also seen (Bourke *et al*, Arch Dis Child 1996 75:223-227). (The formation of bile is a vital function, and its impairment by drugs or infectious, autoimmune, metabolic, or genetic disorders results in the syndrome commonly known as cholestasis).
Benign Recurrent Intrahepatic Cholestasis (BRIC1: OMIM #243300: ATPB81 gene) is an allelic disorder to PFIC and is characterised by intermittent episodes of cholestasis without extra-hepatic bile duct obstruction.

Byler disease causing mutations in the ATP8B1 gene on chromosome 18 (formerly known as FIC1), are heterogeneous. A unique seven base pair deletion c.3622_3628delGCCTACG (p.Ala1208fs) in the ATP8B1 gene has been found in the Irish Traveller population (Klomp *et al.,* Hepatology 2004 40:27-38). Children from the Travelling community with Byler disease are homozygous for this seven base pair deletion and testing for Byler disease (ATP8B1 gene) at the NCMG is only for this specific pathogenic mutation in this population.

PFIC is a genetically heterogeneous disorder and other genes such as ABCB11 (PFIC2) and the ABCB4 gene (PFIC3) are known to be involved and are also caused by defects in the transport of bile acids.

Standard service

Essential referral information

In addition to supplying standard patient identification and referral information, the following should be clearly indicated:

1. Patient's symptoms.
2. Any family history, including names, dates of birth, relationship, and genetic test results of relatives with Byler disease if available.
3. Whether the patient is a member of the Irish Travelling community and whether their parents are from a consanguineous marriage.

It is the responsibility of the referring clinician to ensure consent has been obtained for testing and storage.

Samples required

Generally 5-10ml of EDTA blood (FBC bottle) is required. Sample identification policy is

detailed here.
Blood specimens must be appropriately packaged, and preferably sent by courier to arrive as soon as possible. Do not freeze prior or during postage.
Please note that extracted DNA is stored from patients' samples at the National Centre for Medical Genetics, and kept indefinitely unless a written request for its disposal is received from the patient or their parent/guardian.

Restrictions on testing

Samples for diagnostic testing are generally only accepted from a consultant gastroenterologist/paediatrician or consultant clinical geneticist.

Carrier or prenatal testing is only performed in conjunction with a counselling programme from a clinical genetics service such as offered by the National Centre for Medical Genetics.

Carrier testing is limited to adults over the age of 16 where there is a family history of, or where a family member has been found to be a carrier of the c.3622_3628delGCCTACG ATP8B1 pathogenic mutation.

Tests offered

Diagnostic Test

Diagnostic tests are available for patients with a clinical diagnosis or clinical symptoms suggestive of Byler disease. As the c.3622_3628delGCCTACG ATP8B1 pathogenic mutation is unique to the Irish Travelling population, a family history of Byler disease is highly likely and/or consanguineous marriage.

Carrier Test

Carrier testing is offered to individuals over the age of 16 with a family history of Byler disease and/or a partner with the same.

Prenatal Test

Prenatal testing is available where the c.3622_3628delGCCTACG ATP8B1 pathogenic mutation has been confirmed in both parents. Prenatal testing must be arranged in advance with the laboratory. Prenatal testing is only performed in conjunction with a counselling programme from a clinical genetics service such as offered by the National Centre for Medical Genetics.

Test method

Testing is by bi-directional DNA Sanger sequencing encompassing the c.3622_3628del GCCTACG ATP8B1 pathogenic mutation.

Diagnostic Sensitivity of tests

This test is 100% sensitive for the specific Irish Traveller c.3622_3628delGCCTACG ATP8B1 pathogenic mutation only.
Please note that there >50 distinct Byler disease (PFIC/BRIC) pathogenic mutations (Klomp et al, 2004) in the ATB8B1 gene and that this test does not detect (is not sensitive for) these other mutations.

Interpretation

Results are given in the form of a written interpretative report to the referring clinician. They are based on the clinical indications at referral and whether or not the c.3622_3628delGCCTACG ATP8B1 pathogenic mutation has been detected or not.

Target reporting time

As reporting times are constantly evolving, please refer to www.genetics.ie/molecular, or contact the molecular genetics laboratory, to receive up-to-date information on anticipated reporting times for your referral.

- Please contact the laboratory if you have not received a report within a week of your patient being due back in clinic.
- Please note it is our policy not to issue verbal results.
- Request for copies of reports on the day that your patient is in clinic cannot normally be accommodated. We usually require 24 hours notice in which to fax a copy of a report.

Further tests

As Byler disease (PFIC/BRIC) is genetically heterogeneous and in-house testing is sensitive for only one of >50 pathogenic mutations in the ATP8B1 gene, further mutation testing of the ATP8B1 gene or other PFIC genes (ABCB11) is available from external referral laboratories. Please contact the laboratory to enquire about the availability and cost of these tests.

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Cystic Fibrosis (CF)

Contacts **Molecular Genetics**

- **Administration** ☎**01 409 6733** **(internal 6733)**
- **Laboratory** **duty.scientist@olchc.ie**

Background

Cystic fibrosis (CF) is an autosomal recessive disease, caused by mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The incidence of cystic fibrosis is approximately 1 in 1,461 in Ireland and approximately 1 in 19 Irish people carry a CF mutation. If partners each carry a CF mutation, they have a 1 in 4 chance for each pregnancy of having a child with CF.
Mutations result in a wide phenotypic spectrum, from severe classical CF, characterised by pancreatic insufficiency and chronic endobronchial infection, through to milder forms. Other pathologies linked to mutations in the CFTR gene include Congenital Bilateral Absence of Vas Deferens (CBAVD), liver disease, recurrent pancreatitis and disseminated bronchiectasis. More than 1800 CF mutations have been identified to date.

Standard service

Essential referral information

In addition to supplying patient identification and referral information, the following should be clearly indicated:

- Patient's symptoms – so we can determine whether it is a diagnostic or a carrier test referral
- Sweat test levels, if performed, and the test centre location
- Any family history, including names, date of birth, relationship, genetic test results or the test centre location
- If testing is required as a pre-requisite to assisted reproduction.
- Ethnic origin of patient, if not of Irish decent

Cystic Fibrosis patient information request (PIR) forms to help service users to give us the information we require are available here.
It is the responsibility of the referring clinician to ensure consent has been obtained for testing and storage.

Samples required

Generally 5-10ml of EDTA blood (FBC bottle) is required. Sample identification policy is detailed here.
Blood specimens must be appropriately packaged, and preferably sent by courier to arrive as soon as possible. Do not freeze prior or during postage.

Please note that extracted DNA is stored from patients' samples at the National Centre for Medical Genetics, and kept indefinitely unless a written request for its disposal is received

from the patient or their parent/guardian.

Restrictions on testing

- Carrier testing is limited to adults over the age of 16 where there is a family history of CF, or where a family member has been found to be a carrier of a CF mutation (i.e. NOT a population screen).
- Predictive testing for newborns is available only when both CF mutations in the family have been identified by NCMG, or if a copy of the report on the index case (or parents of the index) from another test centre is provided.
- Prenatal testing must be arranged in advance with the laboratory, through our clinical genetics department if possible.
- Self-referrals are not accepted.

Tests offered

Diagnostic Test

Diagnostic tests for patients with a clinical diagnosis of CF, or clinical symptoms strongly suggestive of CF.
For patients (especially newborns) where there is a strong family history of CF, and the likelihood of being an (unaffected) carrier is a strong possibility, we will ask to test the parents first to assess the likelihood of the couple having a child with CF. This avoids unintentionally revealing carrier status in a minor and increasing parental anxiety.

Carrier Test

Carrier detection for individuals over the age of 16 with a family history of CF and/or a partner with the same.
Carrier detection is also offered where it is a pre-requisite for a couple awaiting/undergoing assisted reproduction.

Prenatal Diagnosis

Prenatal diagnosis – in cases where the mutations in both parents have been characterised. Prenatal testing must be arranged in advance with the laboratory, through a Clinical Genetics department if possible.

Predictive Test

Predictive testing for newborn babies when both CF mutations have been identified in the parents and/or full siblings with CF. Predictive testing for half siblings is not carried out. Testing of the new partner is recommended instead to assess the likelihood of the new couple having a child with CF.

Tests available include:

- Screen of the 39 mutations by allele-specific oligonucleotide hybridisation using the Luminex xTAG® Cystic Fibrosis 39 kit v2 and Luminex™ Liquid Bead Array Platform.
- Mutations analysed: p.Ala455Glu (A455E), p.Ala559Thr (A559T), p.Phe508del (ΔF508), p.Ile507del (ΔI507), p.Gly85Glu (G85E), p.Gly551Asp (G551D), p.Gly542X (G542X), p.Met1101lLys (M1101K), p.Asn1303Lys (N1303K), p.[Arg117His] (R117H), p.Arg334Trp (R334W), p.Arg347His (R347H), p.Arg347Pro

(R347P), p.Arg553X (R553X), p.Arg560Thr (R560T), p.Arg1162X (R1162X), p.Ser549Asn (S549N), p.Ser549Arg (S549R), p.Ser1255X (S1255X), p.Val520Phe (V520F), p.Trp1282X (W1282X), p.Tyr122X (Y122X), p.Tyr1092X (Y1092X), p.Leu88fs (394delTT), c.489+1G>T (621+1G→T), c.579+1G>T (711+1G>T), p.Phe316fs (1078delT), c.1585-1G>A (1717-1G>A), c.1766+1G>A (1898+1G>A), c.1766+5G>T (1898+5G>T), c.2051_2052delAAinsG (2183AA>G), p.Lys684fs (2184delA), p.Glu726fs (2307insA), c.2657+5G>A (2789+5G>A), c.2988+1G>A (3120+1G>A), p.Lys1177fs (3659delC), c.3717+10kbC>T (3849+10kbC→T), p.Lys1250fs (3876delA), p.Leu1258fs (3905insT).

- Intron 8 poly T tract variant – Screened in CBAVD cases, when the R117H mutation has been identified in association with another CF mutation in query affected cases/diagnostic cases, and when 1 mutation has been identified in a patient presenting with pancreatitis/bronchiectasis.

Diagnostic Sensitivity of tests

The test used is estimated to identify 93.5% of the CF alleles in the Irish population. This means that fewer than 1 person with CF in 200 would have no mutations detected by this test.
Risks for couple to have a child with CF in different scenarios are set out below.

Please note: coverage may be reduced or unknown for other populations. Please always provide information on the ethnic origin of your patient.

Interpretation:

Results are given in the form of a written interpretative report to the referring clinician.

Query Affected/Genotype Report

- The presence of two mutations confirms a diagnosis of CF.
- Less than 1% of patients with classical CF in the Irish population would not have their mutations detected by the above 39 mutation screen. In these cases (and in cases where only one mutation has been identified), where CF is clinically indicated (e.g. positive sweat test; Cl^- & Na^+ > 60mmol/L, or combined NaCl > 90mmol/L. However, published guidelines recommend that a sweat test that measures sodium and chloride separately should be performed), samples will be screened for the presence of rare mutations (see H Further Tests)

Carrier Status Reports

- The presence of one mutation confirms that the individual is a carrier of a CF mutation.
- The absence of all 39 Irish mutations in referrals for carrier status determination (in the absence of a family history of CF) reduces the risk of being a carrier from ~1/19 (Irish population risk) to approximately 1 in 277.
- Where there is a family history of CF, and the familial mutations are known and excluded for the sibs of a CF patient, the risk of being a carrier is negligible.
- Carrier results for a couple are reported as a joint report where they have both been tested at this Centre, and their risk of having a child with CF for each pregnancy is stated. Where a member of the couple has been tested at another test centre, this is referred to in the interpretation of the report and their risk of having a child with

CF is stated, based on the information provided.

Couples' risk of having a child with CF

Father / Mother	Affected	Carrier	39 tested mutations absent
Affected	1	1/2	~ 1/500
Carrier	1/2	1/4	~ 1/1000
39 tested mutations absent	~ 1/500	~ 1/1000	~ 1/250,000

CBAVD Reports

- All 39 Irish mutations absent: Presence of rare CF mutations is assumed in cases where CBAVD has been diagnosed by a consultant urologist.
- One mutation identified: As above
- 5T/5T (interpretation is difficult, as this allele is present in 5% of the normal population). The 5T variant has been shown to be associated with CBAVD. Some studies suggest that the 5T variant may be a CF mutation in its own right, causing disease with partial penetrance.

R117H/5T

- R117H is a mild mutation when inherited alongside a classical CF mutation, disease severity may be increased when 5T is also present.

Target reporting times:

As reporting times are constantly evolving, please refer to www.genetics.ie/molecular, or contact the molecular genetics laboratory, to receive up-to-date information on anticipated reporting times for your referral.

The following are current target reporting times for each category of test offered;

Urgent Diagnostic and Carrier Tests

Reports on babies < 6months of age, CVS samples and pregnant couples are reported within 2 weeks.
Prenatal diagnosis on amniocentesis samples (which require 2 weeks of culture) are reported within 4 weeks.

Routine Reports

Currently dispatched within 8 weeks from receipt of sample and relevant clinical details/family history information.

- Request for copies of reports on the day that your patient is in clinic cannot normally

be accommodated. We usually require 24 hours notice in which to fax a copy of a report.

- All requests for copies of reports, when not from the original referring clinician or referring centre, must be made in writing via email (to duty.scientist@olchc.ie), fax (01 409 6971) or by letter.
- Please contact the laboratory if you have not received a report within a week of your patient being due back in clinic.
- Please note that it our policy not to issue verbal results.

Further tests

In cases where there is a diagnosis of CF, or strong clinical suspicion of CF or a positive sweat test, and where both CF mutations have not been identified by the 39-mutation screen; samples can be added to a panel for analysis by full sequencing of the CFTR gene & MLPA analysis for the detection of large deletions/rearrangements.

Further CFTR gene screening is now routinely sent to the Manchester Genetics Laboratory and are reported within 10 weeks. Previously further screening was carried out as a research collaboration with Professor Claude Ferec's Laboratory, in Brest, France and the results took many months.

Web Links to Related Documents

Cystic Fibrosis Patient Information Request (PIR) form: http://www.genetics.ie/pir/CF_pir.pdf

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Torsion Dystonia (DYT1)

Contacts **Molecular Genetics**

- **Administration** ☎**01 409 6733** **(internal 6733)**
- **Laboratory** **duty.scientist@olchc.ie**

Background

Torsion Dystonia, DYT1 - OMIM number 128100

Early onset torsion dystonia is an autosomal dominant disorder due to a deletion mutation in the TOR1A gene on chromosome 9q34.
Penetrance is low: around 30% of individuals with a mutation express the disease. However penetrance varies between families. A 3-bp GAG deletion (c.904_906delGAG) in the Exon 5 region of the TOR1A gene is the only mutation so far detected in a large number of patients from different ethnic backgrounds. DYT1 represents only one of a clinically and genetically heterogeneous group of idiopathic torsion dystonias. Most patients with atypical presentation for DYT1 do not have the GAG deletion.
Primary torsion dystonia usually begins in childhood or adolescence with involuntary posturing of the trunk, neck, or limbs. Early-onset primary dystonia (DYT1) is considered a primary dystonia because it is not associated with other neurological abnormalities.

Standard service

Essential referral information

In addition to supplying standard patient identification and referral information, the following should be clearly indicated:

1. Patient's symptoms
2. Any family history, including names, dates of birth, relationship, and genetic test results if available.

It is the responsibility of the referring clinician to ensure consent has been obtained for testing and storage.

Samples required

Generally 5-10ml of EDTA blood (FBC bottle) is required. Sample identification policy is detailed here.

Blood specimens must be appropriately packaged and preferably sent by courier to arrive as soon as possible. Do not freeze prior or during postage.

Please note that extracted DNA is stored from patients' samples at the National Centre for Medical Genetics, and kept indefinitely unless a written request for its disposal is received from the patient or their parent/guardian.

Restrictions on testing

Testing would not normally be considered for asymptomatic children under the age of 16.

This policy is consistent with international guidelines for genetic testing of children.

Tests offered

Standard analysis is to test for a 3-bp GAG deletion (c.904_906delGAG) in the Exon 5 region of the TOR1A gene. Testing performed includes the following:

- Diagnostic tests for patients with clinical symptoms suggestive of DYT1.
- Predictive tests / presymptomatic diagnosis may be possible in families, but only where an index case has previously been identified. A referral to Clinical Genetics is recommended prior to presymptomatic testing

Prenatal testing must be arranged in advance with the laboratory, through a Clinical Genetics department if possible.
Analysis methodology based on Ozelius et al. 1997, Nature Genet. 17, 40-8

Diagnostic Sensitivity of tests

Diagnostic testing of DYT1 is carried out to reveal the presence or absence of a 3 bp deletion, c.904_906delGAG, in affected individuals. Other forms of torsion dystonia are not excluded by this analysis.
Please contact the laboratory if it is appropriate to perform other tests.

Interpretation:

Results are given in the form of a written interpretative report to the referring clinician.

Target reporting times:

As reporting times are constantly evolving, please refer to www.genetics.ie/molecular, or contact the molecular genetics laboratory, to receive up-to-date information on anticipated reporting times for your referral.
The following are current target reporting times for each category of test offered:
Target reporting time for DYT1 is up to 6 months. Analysis is performed in batches due to low supply of positive control.

- Please contact the laboratory if you have not received a report within a week of your patient being due back in clinic.
- Please note it is our policy not to issue verbal results.
- Request for copies of reports on the day that your patient is in clinic cannot normally be accommodated. We usually require 24 hours notice in which to fax a copy of a report.

Further tests

Please contact the laboratory to discuss any other tests, e.g. possible linkage studies for large families with no 3bp GAG deletion in DYT1.

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Fragile X Syndrome

Contacts: **Molecular Genetics**
- **Administration** ☎**01 409 6733** **(internal 6733)**
- **Laboratory** **duty.scientist@olchc.ie**

Background

Fragile X Syndrome (FRAX) is an X-linked syndrome of mild to severe mental Retardation [OMIM #300624]. Clinical features include macroorchidism in post-pubertal males, long face, coarse features, large everted ears, behavioural disturbances.
Clinical presentation is very variable and can be subtle in younger prepubescent children, making diagnosis difficult.

The Fragile X Mental Retardation (FMR1) gene, is located on the X chromosome at Xq27.3. Approximately 99% of Fragile X cases are caused by an increase in the number of CGG triplet repeats near exon 1 of the gene.
FMR1 alleles are classed according to the number of CGG repeats as shown in the table below, following the EMQN best practice guidelines.

Status	Number of CGG repeats
Normal/Unaffected	<50 approx.
Intermediate	50 - 58 approx.
Premutation	58 - 200 approx.
Affected/Full mutation	More than 200 (and methylated)

The genetic test detects increases in the number of CGG triplet repeats above the normal range.
The prevalence of a Fragile X full mutation is 1 in 4000 - 9000 [males] and 7000 - 15000 [females]. The prevalence of a Fragile X premutation is 1 in 810 - 1100 [males] and 1 in 246 - 468 [females].

Two distinct adult onset disorders are described for certain individuals with a Fragile X premutation:
Fragile X-associated tremor/ataxia syndrome (**FXTAS**) is progressive neurodegenerative disorder characterized by late-onset progressive cerebellar ataxia and intention tremor in both middle aged & elderly males and females. Other neurological findings include short-term memory loss, executive function deficits, cognitive decline, dementia, parkinsonism, peripheral neuropathy, lower-limb proximal muscle weakness, and autonomic dysfunction. The prevalence of FXTAS is estimated at 40% overall for males (> 50 yrs) with premutations and the penetrance is aged related. The prevalence of FXTAS in females is less than that for males.
Premature ovarian failure (**POF**) or **primary ovarian insufficiency** (**POI**) is defined as cessation of menses before the age of 40 in a woman with an FMR1 premutation. POF/ POI occurs with a penetrance of approximately 20% in female with a FMR1 premutation.

Standard service

Essential referral information

In addition to supplying standard patient identification and referral information, the following should be clearly indicated:

1. Patient's symptoms
2. Any family history, including names, dates of birth, relationship, and genetic test results if available.

Note: It is the responsibility of the referring clinician to ensure consent has been obtained for testing and storage.

Samples required

Generally 3 to 5ml of EDTA blood (FBC bottle) is required. Sample identification policy is detailed here.
Blood specimens must be appropriately packaged, and preferably sent by courier to arrive as soon as possible. Do not freeze prior or during postage.
Please note that extracted DNA is stored from patients' samples at the National Centre for Medical Genetics, and kept indefinitely unless a written request for its disposal is received from the patient or their parent/guardian.

Restrictions on testing

Testing would not usually be considered for asymptomatic children under 16 years of age.

Children under 4 to 6 months are not routinely tested for Fragile X; please contact the NCMG for further information.

Tests offered

Prenatal testing must be arranged in advance with the laboratory, through a Clinical Genetics department if possible.

Diagnostic test:

To determine whether a patient is affected with:
Fragile X Syndrome- full mutation)
Fragile X-associated tremor/ataxia syndrome (FXTAS) – premutation
Premature ovarian failure (POF) or primary ovarian insufficiency (POI) - premutation

Carrier test:

To determine whether a person without symptoms in a known Fragile X syndrome family carries a premutation (i.e. approx 58-200 CGG repeats). Such tests are only performed in conjunction with a counselling programme run by the National Centre for Medical Genetics. Patients should be referred to the Director, Prof. A. Green.
In a female carrying a premutation the number of CGG repeats can increase to a full mutation when passed on to offspring.
In a male carrying a premutation the number of CGG repeats does not increase significantly upon transmission, so a premutation is passed on to offspring.

Prenatal diagnosis:

To determine whether a foetus has inherited the full mutation; once a premutation or full mutation has been identified in the mother. Prenatal diagnosis can only be performed in conjunction with a counselling programme run by the National Centre for Medical Genetics. Patients should be referred to the Director, Prof. A. Green.

Diagnostic Sensitivity of tests

The sensitivity of the diagnostic test is approximately 99%.

In very rare cases, Fragile X Syndrome may result from:
Point mutations or deletions in the FMR1 (FRAXA) gene.
Mosaicism (the presence of both normal and expanded alleles).
These rare events cannot be excluded by the test.

Rare families have been described in which CGG expansions in the nearby FMR2 (FRAXE) locus appear to be responsible for developmental delay/mental retardation (OMIM #309548). Expansions at the FMR2 locus are not detected by the test.

Interpretation:

Results are given in the form of a written interpretative report to the referring clinician.

The results of the genetic test indicate whether a person has the normal number of CGG repeats, is carrying a premutation, or has a full mutation.
A diagnostic test for a male showing a full mutation confirms a diagnosis of Fragile X.
Approximately 30-50% of females with a full mutation on one of their X chromosomes will suffer from mental retardation (less severe than seen in affected males).

Target reporting times:

As reporting times are constantly evolving, please refer to www.genetics.ie/molecular, or contact the molecular genetics laboratory, to receive up-to-date information on anticipated reporting times for your referral.
The following are current target reporting times for each category of test offered:

Query affected males: up to 6 months (most reported approx. 2 months)
Query affected females: up to 6 months
Prenatal diagnosis (CVS): 4 weeks
Prenatal diagnosis (Amniocentesis): 6 weeks
Query carrier status females & males (confirmed familial mutation): up to 6 months
FXTAS [males]: up to 6 months (most reported approx. 2 months)
FXTAS [females]: up to 6 months
POF/ POI: up to 6 months

- Please contact the laboratory if you have not received a report within a week of your patient being due back in clinic.
- Please note it is our policy not to issue verbal results.
- Request for copies of reports on the day that your patient is in clinic cannot normally be accommodated. We usually require 24 hours notice in which to fax a copy of a report.

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Friedreich Ataxia (FRDA)

Contacts: **Molecular Genetics**
- **Administration** ☎**01 409 6733** **(internal 6733)**
- **Laboratory** **duty.scientist@olchc.ie**

Background

Friedreich Ataxia (FRDA, OMIM 229300) is the one of the most common hereditary ataxias with an estimated prevalence of 1 in 50,000 in Caucasian populations. The carrier frequency for FRDA in the European population is estimated to be 1 in 90.
FRDA is characterised by progressive ataxia, areflexia of the legs, pyramidal weakness and impaired sense of vibration. Cardiomyopathy and diabetes are also seen with variable penetrance. FRDA is an autosomal recessive disorder and the majority of patients (98%) have a homozygous expansion mutation of a (GAA)n repeat within intron 1 of the Frataxin gene, FXN). The normal range is 9-33 repeats and the size range associated with disease is 66 to 1,700 repeats, but the majority of pathogenic alleles contain 600 to 1,200 repeats. A number of point mutations have been reported in patients who are heterozygous for the expansion. To date, no FRDA patients without at least one expansion have been reported.

Standard service

Essential referral information:

In addition to supplying standard patient identification and referral information, the following should be clearly indicated:
- Patient's symptoms, if any, so that we can determine if a diagnostic or carrier test is required
- Any family history, including names, dates of birth and genetics test results if available.

It is the responsibility of the referring clinician to ensure consent has been obtained for testing and storage

Samples required:

Generally 5-10ml of EDTA blood (FBC bottle) is required. Sample identification policy is detailed here.
Blood specimens must be appropriately packaged, and preferably sent by courier to arrive as soon as possible. Do not freeze prior or during postage.
Please note that extracted DNA is stored from patients' samples at the National Centre for Medical Genetics, and kept indefinitely unless a written request for its disposal is received from the patient or their parent/guardian.

Restrictions on testing

Carrier or presymptomatic testing is not offered for minors (children under the age of 16) except in cases of early onset FRDA, but only where an index case has previously been identified. A referral to Clinical Genetics is recommended prior to presymptomatic testing. This policy is consistent with international guidelines for genetic testing of children.

Tests offered:

Standard analysis is to test for GAA expansion mutations in the FXN gene. Three types of test may be performed:

- Diagnostic tests for patients with clinical symptoms suggestive of FRDA
- Carrier tests for asymptomatic individuals who have a family history of FRDA or whose partner may be a carrier
- Predictive tests / presymptomatic diagnosis may be possible in families, especially in cases of early onset FRDA, but only where an index case has previously been identified. A referral to Clinical Genetics is recommended prior to presymptomatic testing

Analysis methodology uses Long PCR based on Filla *et al.* (1996) Am. J. Hum. Genet. **59(3):** 554-560 and Triplet Primed PCR based on Warner *et al.* (1996) J. Med. Genet. **33:** 1022-1026

Diagnostic Sensitivity of tests:

The PCR-based tests used allow for the detection and relative sizing of the GAA repeat in both the normal and pathogenic size ranges. Approximately 98% of patients with Friedreich Ataxia have no normal sized alleles and have 2 alleles with a GAA expansion in the pathogenic size range (66 – 1,700 repeats). Approximately 2% of individuals with FRDA have an expanded GAA repeat mutation in one allele and an intragenic inactivating *FXN* mutation (e.g., point mutation or deletion outside of the GAA repeat region) in the other allele.

Interpretation:

Results are given in the form of a written interpretative report to the referring clinician.
Reports will normally not state the number of GAA repeats; alleles are simply classified as normal, intermediate or pathogenic. Individual reports with estimated repeat numbers are available on request when required (e.g. for participation in clinical trials), but experience tells us that GAA repeat number estimates vary markedly between testing laboratories.

Target reporting times:

As reporting times are constantly evolving, please refer to www.genetics.ie/molecular, or contact the molecular genetics laboratory, to receive up-to-date information on anticipated reporting times for your referral.
The following are current target reporting times for each category of test offered:

Urgent = 2 weeks, Routine = 12 weeks

- Please contact the laboratory if you have not received a report within a week of your patient being due back in clinic.
- Please note it is our policy not to issue verbal results.
- Request for copies of reports on the day that your patient is in clinic cannot normally be accommodated. We usually require 24 hours notice in which to fax a copy of a report.

Further tests

Where appropriate, point mutation analysis (DNA sequencing) may be performed in patients who have clinical symptoms but who only have one GAA expansion mutation. This further screening must be specifically requested by the clinician following receipt of the initial report on the GAA expansion analysis. Please contact the laboratory if it is appropriate to perform other tests, for example into spinocerebellar ataxia (SCA) genes or mitochondrial disorders.

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Huntington disease (HD)

Contacts: **Molecular Genetics**
- **Administration** **☎01 409 6733** **(internal 6733)**
- **Laboratory** duty.scientist@olchc.ie

Background

OMIM#143100
Huntington disease (HD) is an autosomal dominant progressive neurodegenerative disease, caused by mutation in the HTT gene on chromosome 4p. Typical clinical features include motor symptoms, cognitive deterioration, and psychiatric symptoms.
The nature of the mutation is an increase in the number of CAG repeats within exon 1 of this gene. Patients with HD have an allele with 36 or more repeats. Alleles are classified according to CAG repeat number as follows: (Am J Hum Genet 62, 1243-7, 1998):

CAG repeat no.	Allele description
≤ 26	Normal allele
27 – 35	Mutable normal allele – may occasionally mutate to HD alleles in subsequent generations
36 – 39	HD allele with reduced penetrance
≥ 40	HD allele

Laboratory tests determine the number of CAG repeats in patient samples.

Standard service

Essential referral information

In addition to supplying standard patient identification and referral information, the following should be clearly indicated:
- Patient's symptoms - so that we can be sure this is a diagnostic test
- Any family history, including names, dates of birth and genetics test results if available.
- An indication of informed patient consent for diagnostic testing, which must be on an NCMG consent form.

Samples required

Generally 5-10ml of EDTA blood (FBC bottle) is required. Sample identification policy is detailed here.
Blood specimens must be appropriately packaged, and preferably sent by courier to arrive as soon as possible. Do not freeze prior or during postage. Please note that extracted DNA is stored from patients' samples at the National Centre for Medical Genetics, and kept indefinitely unless a written request for its disposal is received from the patient or their parent/guardian.

Restrictions on testing

- We would normally only accept diagnostic requests from a Consultant Neurologist, Psychiatrist, Geriatrician or Clinical Geneticist, where full informed consent of the patient has been obtained.

- **Consent is only accepted on NCMG consent forms**.
- Predictive testing is only performed in conjunction with a counselling programme run by the Clinical Genetics Division of the National Centre for Medical Genetics. For further information regarding referrals, please see http://www.genetics.ie/clinical/ or phone 01-4096739.
- Predictive testing is not normally considered for children under age 16.

Tests offered

A number of tests are performed on a routine basis. Molecular confirmation of Huntington Disease (HD) is possible using a PCR based assay to detect the number of CAG repeats within exon 1 of the HTT gene. See the table above for the classification of these alleles.

1. Diagnostic tests for patients with clinical symptoms suggestive of Huntington disease (HD). Due to the implications of a positive test result, we recommend that such tests should be performed only with full informed consent of the patient.
2. Predictive tests for asymptomatic individuals who have a family history of HD. Such tests are only performed in conjunction with a counselling programme run by the National Centre for Medical Genetics. Patients should be referred to the Director, Prof. A. Green.
3. Prenatal testing must be arranged in advance with the laboratory through our own Clinical Genetics team.

Diagnostic Sensitivity of tests

The test is highly sensitive and highly specific. An HD allele is detected in ~100% of affected individuals; HD alleles are detected in ~0% of normal individuals; individuals with a family history of HD and who have an HD allele are ~100% likely to develop the disease within the average lifespan.

Interpretation:

Following laboratory analysis, a report is prepared which gives results based on the CAG repeat classification system above. This is sent to the referring clinician. Results are given in the form of a written interpretative report to the referring clinician.

Target reporting times:

As reporting times are constantly evolving, please refer to www.genetics.ie/molecular, or contact the molecular genetics laboratory, to receive up-to-date information on anticipated reporting times for your referral.

- The following are current target reporting times for each category of test offered:

Urgent = 2 weeks

Predictive = 8 weeks from 2nd sample
Routine = 12 weeks

- Please contact the laboratory if you have not received a report within a week of your patient being due back in clinic.
- Please note it is our policy not to issue verbal results.
- Request for copies of reports on the day that your patient is in clinic cannot normally be accommodated. We usually require 24 hours notice in which to fax a copy of a report.

Further tests

Please contact the laboratory to arrange any other related tests, e.g. prenatal diagnosis, prenatal exclusion testing, or testing for Dentatorubral-Pallidoluysian atrophy (DRPLA) or Spinal Cerebellar Ataxia (SCA) – which may be advised for a query affected patient who has been

shown to not have Huntington Disease.

Web Links to Related Documents

NCMG Huntington Disease Consent form http://www.genetics.ie/pir/HD_consent.pdf

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Osteogenesis Imperfecta (OI)

Contacts: **Molecular Genetics**

- **Administration** ☎**01 409 6733** **(internal 6733)**
- **Laboratory** **duty.scientist@olchc.ie**

Background

Osteogenesis imperfecta (OI) is a group of heterogeneous conditions characterised by a varying degree of bone fragility, susceptibility to fracture, short stature, bowing of the long bones and other clinical features. OI is usually caused by a heterozygous mutation in either of the type I collagen genes and it is inherited in an autosomal dominant fashion. The collagen genes COL1A1 and COL1A2 encode the α1 and the α2 chain of type 1 collagen. Type 1 (pro) collagen is the major extracellular matrix protein of bone and skin.

The existence of recessive forms of lethal/severe OI has long been suspected since the original 1979 Sillence classification. However, a recent study has identified a number of genes that cause recessive OI including the genes CRTAP (encodes the protein cartilage-associated protein, CRTAP) and LEPRE1 (which encodes prolyl-3-hydroxylase-1 (PH31)) which are involved in type 1 collagen formation. A unique loss of function mutation c.232delC in the LEPRE1/P3H1 gene (OMIM #601905) was identified in the Irish Traveller population (Cabral *et al,* Nature Genetics 2007 39(3) 359-365).

Type VIII OI caused by pathogenic mutations the LEPRE1/P3H1 gene has a variable phenotype from perinatal lethal to surviving phenotypes. It is clinically and radiographically comparable to OI Type II/III. In the perinatal period, it is very difficult to distinguish radiographically severe OI due to LEPRE1 mutations from severe OI caused by COL1A1 or COL1A*2*. However with increasing age there are some distinctive clinical and radiological characteristics.

Children from the Travelling community with OI Type VIII are homozygous for this c.232delC LEPRE1/P3H1 deletion and it is testing for this specific pathogenic mutation that forms the basis of the service offered.

Standard service

Essential referral information

In addition to supplying standard patient identification and referral information, the following should be clearly indicated:

1. Patient's symptoms.
2. Any family history, including names, dates of birth, relationship, and genetic test results of relatives with OI Type VIII if available.
3. Whether the patient is a member of the Irish Travelling community and whether their parents are from a consanguineous marriage.

It is the responsibility of the referring clinician to ensure consent has been obtained for testing and storage.

Samples required

Generally 5-10ml of EDTA blood (FBC bottle) is required. Sample identification policy is detailed here.

Blood specimens must be appropriately packaged, and preferably sent by courier to arrive as soon as possible. Do not freeze prior or during postage.

Please note that extracted DNA is stored from patients' samples at the National Centre for Medical Genetics, and kept indefinitely unless a written request for its disposal is received from the patient or their parent/guardian.

Restrictions on testing

Samples for diagnostic testing are generally only accepted from a consultant paediatrician or consultant clinical geneticist.

Carrier or prenatal testing is only performed in conjunction with a counselling programme from a clinical genetics service such as offered by the National Centre for Medical Genetics.

Carrier testing is limited to adults over the age of 16 where there is a family history of, or where a family member has been found to be a carrier of the c.232delC LEPRE1/P3H1 pathogenic mutation.

Tests offered

Diagnostic Test

Diagnostic tests are available for patients with a clinical diagnosis or clinical symptoms suggestive of OI Type VIII. As the c.232delC LEPRE1/P3H1 pathogenic mutation is unique to the Irish Travelling population, a family history of OI Type VIII is highly likely and/or consanguineous marriage.

Carrier Test

Carrier testing is offered to individuals over the age of 16 with a family history of OI Type VIII and/or a partner with the same.

Prenatal Test

Prenatal testing is available where the c.232delC LEPRE1/P3H1 pathogenic mutation has been confirmed in both parents. Prenatal testing must be arranged in advance with the laboratory. Prenatal testing is only performed in conjunction with a counselling programme from a clinical genetics service such as offered by the National Centre for Medical Genetics.

Test method

Testing is by bi-directional DNA Sanger sequencing encompassing the c.232delC LEPRE1/P3H1 pathogenic mutation

Diagnostic Sensitivity of tests

This test is 100% sensitive for the specific Irish Traveller c.232delC LEPRE1/P3H1 pathogenic mutation only.
Please note that there numerous distinct OI Type VIII pathogenic mutations (Baldridge *et al*, Human Mutation 2008 0, 1-8) in the LEPRE1/P3H1 gene and that this test does not detect (is not sensitive for) these other mutations.

Interpretation

Results are given in the form of a written interpretative report to the referring clinician. They are based on the clinical indications at referral and whether or not the c.232delC LEPRE1/P3H1 pathogenic mutation has been detected or not.

Target reporting time

As reporting times are constantly evolving, please refer to www.genetics.ie/molecular, or contact the molecular genetics laboratory, to receive up-to-date information on anticipated reporting times for your referral.

- Please contact the laboratory if you have not received a report within a week of your patient being due back in clinic.
- Please note it is our policy not to issue verbal results.
- Request for copies of reports on the day that your patient is in clinic cannot normally be accommodated. We usually require 24 hours notice in which to fax a copy of a report.

Further tests

As Osteogenesis Imperfecta is extremely genetically heterogeneous (autosomal dominant and recessive forms) and in-house testing is sensitive for only one of several pathogenic mutations in the LEPRE1/P3H1 gene (recessive form), further mutation testing for (many) other genes involved in OI may be available from external referral laboratories. Please contact the laboratory to enquire about the clinical suitability, availability and cost of these tests.

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Prader-Willi Syndrome (PWS)

Contacts: **Molecular Genetics**
- **Administration** ☎**01 409 6733** **(internal 6733)**
- **Laboratory** **duty.scientist@olchc.ie**

Background

OMIM# 176270
Prader-Willi Syndrome is a neurological disorder characterised by neonatal hypotonia, hyperphagia with obesity, hypogonadism, mental and psychomotor retardation. The estimated prevalence is 1/15,000 to 1/30,000.

Several genes are believed to be involved in PWS and are located on chromosome 15
(15q11-q13). Normally these genes are only active (unmethylated) on the chromosome inherited from the father. In PWS, expression of these paternally active genes is lost resulting in abnormal methylation patterns.
Loss of the paternal allele arises by a de novo deletion of the critical region of the paternal chromosome in approximately 75% of PWS cases, or by inheritance of two maternal copies of chromosome 15 (maternal uniparental disomy - mUPD) in approximately 25% of cases.
Both of these types of abnormality usually arise de novo and have a very low risk of recurrence.
In very rare cases, PWS may be caused by an imprinting defect – in these cases there can be up to 50% risk of recurrence.

Genetic defect	Proportion of cases	Recurrence risk
De novo deletion of 15q11-q13 on the paternal chromosome	75-80%	<1%
Maternal uniparental disomy (UPD) of chromosome 15	20-25%	<1%
Imprinting defects (with an imprinting centre deletion excluded)	≈1%	<1%
Imprinting centre deletion	≈ 10-15% of patients with an imprinting defect	Up to 50% (if present in father)

(Table taken from "*Practice Guidelines for Molecular Analysis of Prader-Willi and Angelman Syndromes*". CMGS/EMQN 2008)

Standard service

Essential referral information

In addition to supplying standard patient identification and referral information, the following should be clearly indicated:

- Patient's symptoms.
- Any family history, including names, dates of birth, relationship and genetics test

results if available.

It is the responsibility of the referring clinician to ensure consent has been obtained for testing and storage.

Samples required:

Generally 3-5ml of EDTA blood (FBC bottle) is required. Sample identification policy is detailed here.

Blood specimens must be appropriately packaged, and preferably sent by courier to arrive as soon as possible. Do not freeze prior or during postage.

Please note that extracted DNA is stored from patients' samples at the National Centre for Medical Genetics, and kept indefinitely unless a written request for its disposal is received from the patient or their parent/guardian.

Restrictions on testing:

Referrals on patients where obesity is the only clinical indication are not processed.
There are no other particular restrictions on testing.

Tests offered:

- Diagnostic test – Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) is used to detect copy number changes and analyse CpG island methylation patterns within the 15q11-q13 region (Prader-Willi/Angelman critical region). Absence of the paternal methylation pattern confirms a diagnosis of PWS.

- Mechanism of inheritance – when a diagnosis of PWS is confirmed, the mechanism of inheritance (i.e. paternal deletion, mUPD or imprinting centre defect) is investigated in order to assess recurrence risks. MS-MLPA analysis can detect deletions of the 15q11-q13 critical region and can determine if the mechanism of inheritance is a paternal deletion. However if a deletion is not detected the mechanism could be either mUPD or an imprinting centre defect. MS-MLPA cannot distinguish between these. Further molecular analysis is necessary in order to investigate UPD. This requires parental samples in EDTA.

Diagnostic sensitivity of tests

The diagnostic sensitivity of the genetic test is greater than 99%.

Interpretation:

Results are given in the form of a written interpretative report to the referring clinician.

Target reporting times:

As reporting times are constantly evolving, please refer to www.genetics.ie/molecular, or contact the molecular genetics laboratory, to receive up-to-date information on anticipated reporting times for your referral.

- The following are current target reporting times for each category of test

offered;

Urgent samples (newborns): 2 weeks
Routine samples: 8 weeks
UPD: 3 months

- Please contact the laboratory if you have not received a report within a week of your patient being due back in clinic.
- Please note it is our policy not to issue verbal results.
- Request for copies of reports on the day that your patient is in clinic cannot normally be accommodated. We usually require 24 hours notice in which to fax a copy of a report.

Further tests

- ? Imprinting centre (IC) defect: When a diagnosis of PWS is confirmed using MS-MLPA and both a deletion and UPD have been excluded as the mechanism of inheritance, further analysis can be performed in an external laboratory to confirm/exclude the presence of an IC deletion.

Please contact us to make arrangements for such testing, if required.

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Russell Silver Syndrome

Contact **Molecular Genetics**
- **Administration** **☎01 409 6733** **(internal 6733)**
- **Laboratory** duty.scientist@olchc.ie

Background

Russell Silver Syndrome (RSS) is a malformation syndrome characterised by pre- and post-natal growth retardation. Genetic alterations can be identified in approximately 50% of RSS patients: 7–10% carry a maternal uniparental disomy of chromosome 7 [mUPD(7)] that is, the patient has inherited both copies of chromosome 7 from the mother and none from the father, a further 38–63% show a hypomethylation of the H19/IGF2 DMR mapping to chromosome 11p15. If present, mUPD(7) contributes to diagnosis and indicates a low recurrence risk. Specific clinical features of RSS include; pre and post natal growth retardation, cerebral haemorrhage, feeding difficulties (at 16 months) triangular face, downturned mouth, micrognathia (unusually small jaw), broad high forehead, pointed chin, low prominent dysplastic ears, clinodactyly (inward bending) of little fingers and toes and mild psychomotor developmental delay. There is some evidence to suggest that mUPD(7) patients are less likely to have the triangular face, downturned mouth and micrognathia. Referrals are usually made by a clinical geneticist.

Standard service

Essential referral information

In addition to supplying standard patient identification and referral information, the following should be clearly indicated:

- Patient's symptoms
- Any family history, including names / dobs, relationship, and genetic test results if available.

It is the responsibility of the referring clinician to ensure consent has been obtained for testing and storage.

Samples required

Generally 3-5ml of EDTA blood (FBC bottle) is required. Sample identification policy is detailed here.
Blood specimens must be appropriately packaged, and preferably sent by courier to arrive as soon as possible. Do not freeze prior or during postage.
Please note that extracted DNA is stored from patients' samples at the National Centre for Medical Genetics, and kept indefinitely unless a written request for its disposal is received from the patient or their parent/guardian.

Restrictions on testing

Testing would not normally be considered for asymptomatic children under age 16 and where the patient has not been seen by a clinical geneticist. Referrals are only

accepted from Clinical Geneticists.

Tests offered

Diagnostic tests are performed for patients where clinical symptoms are suggestive of RSS and where the patient has been seen by a clinical geneticist. Microsatellite analysis of markers distributed along the length of chromosome 7 is performed on DNA extracted from both parents and the proband. Prenatal testing must be arranged in advance with the laboratory, through a Clinical Genetics department if possible.

Diagnostic Sensitivity of tests

The sensitivity of such analysis is dependent on factors which are unique to each family assessed. As each polymorphic marker only tests a single point on the chromosome, it is never possible to exclude the presence of UPD at other points along the chromosome.

Interpretation:

Results are given in the form of a written interpretative report to the referring clinician. Following laboratory analysis, a report is prepared indicating the presence or absence of maternal uniparental disomy of chromosome 7 [mUPD(7)] and an interpretation of the result. Absence of mUPD(7) neither supports nor refutes a diagnosis of Russell Silver Syndrome (RSS), as less than 10% of sporadic RSS result from mUPD of chromosome 7. The analysis does not exclude the possibility of segmental mUPD(7).

Target reporting times:

As reporting times are constantly evolving, please refer to www.genetics.ie/molecular, or contact the molecular genetics laboratory, to receive up-to-date information on anticipated reporting times for your referral. The following are current target reporting times for each category of test offered:

1. Urgent samples (newborns and PNDs): 2 weeks.
2. RSS - UPD7: 3 months.
    - Please contact the laboratory if you have not received a report within a week of your patient being due back in clinic.
    - Please note it is our policy not to issue verbal results.
    - Request for copies of reports on the day that your patient is in clinic cannot normally be accommodated. We usually require 24 hours notice in which to fax a copy of a report.

Further tests

If a clinical diagnosis of Russell Silver syndrome is still considered, DNA samples can be sent to an external laboratory (Birmingham) for MS-MLPA analysis to determine methylation of the H19 gene.

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Uniparental Disomy (UPD)

Contacts **Molecular Genetics**

- **Administration** ☎**01 409 6733** **(internal 6733)**
- **Laboratory** **duty.scientist@olchc.ie**

Background

UPD is defined as the inheritance of both homologues of a pair of chromosomes from only one parent. The presence of both homologues is termed heterodisomy while the presence of two copies of one homologue is termed isodisomy. A mixture of both types is possible. UPD may arise through the following mechanisms:

- Chromosome loss in trisomy (trisomic rescue) to generate a disomic foetus. This is the most common mechanism for UPD
- Gamete complementation in which both gametes are coincidentally abnormal, one disomic and the other nullisomic, where one parent has contributed both members of a homologous pair to the zygote and the other parent has contributed none.
- Duplication in monosomy in which one chromosome from a normal gamete from one parent has nothing to pair with from a nullisomic gamete from the other parent - monosomic rescue describes duplication of the chromosome in the foetus and restoration of euploidy (complete set of chromosomes)
- Post fertilisation error in which there is mitotic loss of 1 homologue of a chromosome pair and reduplication of the remaining one

UPD does not necessarily involve a whole chromosome; segmental UPD occurs due to somatic recombination between parental chromosomes before one of the events above.

The phenotypic consequences of UPD for several chromosomes are still unknown or poorly understood. UPD for some chromosomes e.g. chromosomes 13, 21, 22, 16, seems to have no effect or no constant effect on phenotype. Placental/foetal mosaicism is usually due to trisomy rescue and can cause severe growth retardation and possible developmental delay. UPD can cause reduction to homozygosity of autosomal recessively inherited mutations e.g. CF in UPD 7. However, most diseases associated with UPD are due to loss of the active homologue of an imprinted gene e.g. Prader Willi and Angelman syndromes, Beckwith Wiedemann syndrome. UPD in many cases is correlated with advanced maternal age (35 years and over), evidence that this is the result of meiotic non-disjunction.
Referrals are usually as a result of cytogenetic findings in a patient e.g. chromosomal missegregations including confined placental mosaicism (CPM) and apparently balanced Robertsonian translocations or where unexpected homozygosity for a recessive allele is found. Referrals are usually made by a clinical geneticist.

Standard service

Essential referral information

In addition to supplying standard patient identification and referral information, the following should be clearly indicated:

- Patient's symptoms
- Any family history, including names / dobs, relationship, and genetic test results if available.
- Copy of cytogenetic report where relevant

It is the responsibility of the referring clinician to ensure consent has been obtained for testing and storage.

Samples required

Generally 3-5ml of EDTA blood (FBC bottle) is required. Sample identification policy is detailed here.
Blood specimens must be appropriately packaged, and preferably sent by courier to arrive as soon as possible. Do not freeze prior or during postage.

Please note that extracted DNA is stored from patients' samples at the National Centre for Medical Genetics, and kept indefinitely unless a written request for its disposal is received from the patient or their parent/guardian.

Restrictions on testing

Testing would not normally be considered for asymptomatic children under age 16. A referral to a clinical geneticist may be suggested prior to testing.

Tests offered

Diagnostic tests are performed for patients where clinical symptoms and / or cytogenetic findings indicate the possibility of UPD. Analysis of polymorphic DNA markers distributed along the length of the chromosome under investigation is performed on DNA extracted from the proband and both parents.
Prenatal testing must be arranged in advance with the laboratory, through a Clinical Genetics department if possible.

Diagnostic Sensitivity of tests

The sensitivity of such analysis is dependent on factors which are unique to each family assessed. As each polymorphic marker only tests a single point on the chromosome, it is never possible to exclude the presence of UPD at other points along the chromosome.

Interpretation:

Following laboratory analysis, a report is prepared indicating the presence or absence of uniparental disomy and an interpretation of the result. Results are given in the form of a written interpretative report to the referring clinician.

Target reporting times:

As reporting times are constantly evolving, please refer to www.genetics.ie/molecular, or contact the molecular genetics laboratory, to receive up-to-date information on anticipated reporting times for your referral. The following are current target reporting times for each category of test offered:

- Urgent samples (newborns and prenatal): 2 weeks.
- UPD: 3 months.

- Please contact the laboratory if you have not received a report within a week of your patient being due back in clinic.
- Please note it is our policy not to issue verbal results.
- Request for copies of reports on the day that your patient is in clinic cannot normally be accommodated. We usually require 24 hours notice in which to fax a copy of a report.

Further tests

Samples may be sent to an external lab (Essen, Germany) for further analysis of the 14q32 imprinted region.

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Spinal Muscular Atrophy (SMA)

Contacts **Molecular Genetics**

- **Administration** **☎01 409 6733** **(internal 6733)**
- **Laboratory** duty.scientist@olchc.ie

Background

Spinal Muscular Atrophy (SMA) OMIM#253300
SMA is autosomal recessive, with a frequency of 1 in 10,000 (carrier frequency of approximately 1 in 38). Clinical features include: proximal muscle weakness, floppy baby, poor feeding, absent reflexes, arthrogyphosis, and fasciculation of tongue. SMA results from the degeneration of the anterior horn cells of the spinal cord. Approximately 95% of SMA patients have homozygous absence of exons 7 and 8 (or exon 7 only) of the Survival Motor Neuron 1 (*SMN1*) gene (i.e. they have no functional copies of the *SMN1* gene). The remainder of patients are compound heterozygotes for *SMN1* mutations, with a subtle mutation on one chromosome and a deletion or gene conversion on the other. The copy number of the adjacent *SMN2* gene has been shown to correlate with disease severity, however prediction of disease severity on this basis may not be accurate. SMA is clinically heterogeneous, classified into 4 types based on clinical severity:

SMA Types	Age of Onset	Prognosis
Type I (Werdnig-Hoffmann)	0 - 6 months	most severe, never sit, death in early infa
Type II	< 2 years	never stand, death in early twenties
Type III (Kugelberg-Welander)	> 2 years	muscle wasting, survive into adulthood
Type IV	30-50 years	Least severe

Standard service

Essential referral information

In addition to supplying standard patient identification and referral information, the following should be clearly indicated:

- Patient's symptoms.
- Any family history, including names, dates of birth, relationship, and genetic test results if available.
- It is the responsibility of the referring clinician to ensure consent has been obtained for testing and storage.

Samples required

Generally 5-10ml of EDTA blood (FBC bottle) is required. Sample identification policy is detailed here.
Blood specimens must be appropriately packaged, and preferably sent by courier to arrive as soon as possible. Do not freeze prior or during postage.
Please note that extracted DNA is stored from patients' samples at the National Centre for Medical Genetics, and kept indefinitely unless a written request for its disposal is received from the patient or their parent/guardian.

Restrictions on testing

Carrier testing is only available via a Clinical Geneticist. Please refer your patient to Clinical Genetics if carrier testing for SMA is required.
Carrier testing is not offered for minors. This policy is consistent with international guidelines for genetic testing of children.
Prenatal or presymptomatic diagnosis is offered for families, only where an index case has previously been identified as either 1) homozygously deleted for the *SMN1* gene or 2) hemizygously deleted for the *SMN1* gene and with either a 2nd causative mutation identified OR a firm diagnosis of SMA, including a characteristic muscle biopsy.

Tests offered

The SMA P021 MLPA assay is available in the National Centre for Medical Genetics, and is a quantitative test for *SMN1* gene copy number, http://www.mrc-holland.com.

- **Diagnostic:** Molecular confirmation of a suggested clinical diagnosis.
- **Carrier testing:** A direct test, to confirm carrier status or estimate the risk of being a carrier of the common *SMN1* mutation, is available at the NCMG. Referrals are accepted from individuals with a family history of SMA, and partners of such individuals. Carrier testing is only available via a Clinical Geneticist, and is not offered for minors.
- **Prenatal & Presymptomatic:** Prenatal and presymptomatic diagnosis/exclusion (using MLPA, & additional linkage analysis if required) may be possible in families, but only where an index case has previously been identified as either 1) homozygously deleted for the *SMN1* gene or 2) hemizygously deleted for the *SMN1* gene, and with a 2nd causative mutation characterised OR a firm diagnosis of SMA, including a characteristic muscle biopsy.
- Prenatal testing must be arranged in advance with the laboratory, through a Clinical Genetics department if possible.

Diagnostic Sensitivity of tests

- The SMA MLPA assay is a quantitative test for *SMN1* gene copy number, and will not pick up subtle deletions, inversions or point mutations in *SMN1* – screening for such mutations can be arranged via external laboratories, where relevant. Diagnostic sensitivity of the MLPA assay is additionally influenced by the fact that approximately 4% of the *SMN1* alleles in the general population have two *SMN1* copies on a single chromosome.
- **Diagnostic:** Homozygous deletion of the *SMN1* gene will be evident in approximately 95% of SMA Type I patients.
- **Carrier testing:** Carrier status will be confirmed in approximately 96% of *SMN1* deletion carriers.
- **Prenatal & Presymptomatic:** Providing linkage analysis is informative, prenatal & presymptomatic diagnosis should be possible, with an error rate due to recombination of less than 1%.

Interpretation:

Results are given in the form of a written interpretative report to the referring clinician.

- **Diagnostic:** Diagnosis is confirmed where a homozygous deletion of exons 7 and 8 (or exon 7 alone) of the *SMN1* gene is indicated. Hemizygous deletion of *SMN1* (i.e. 1 copy) reduces the likelihood that a patient is affected with SMA, but does not rule out a diagnosis of SMA. Over 99% of 5q13-linked SMA cases are excluded by an

MLPA result which indicates 2 copies of *SMN1*.

- **Carrier testing:** Detection of only one copy of the *SMN1* gene confirms deletion carrier status. In the absence of a family history, detection of two or three copies of the *SMN1* gene indicates a very low risk of carrying a deletion (<1%). Where there is a family history of SMA, detection of two copies of the *SMN1* gene indicates an intermediate risk of carrying a deletion (an estimate of this risk will be provided with individual reports), while detection of three copies of the *SMN1* gene indicates a very low risk of carrying a deletion (<1%).
- **Prenatal & Presymptomatic**: For families in which an index case has been identified as homozygously deleted for the *SMN1* gene, prenatal/presymptomatic diagnosis is confirmed where a homozygous deletion of exons 7 and 8 (or exon 7 alone) of the *SMN1* gene is indicated. The absence of homozygous deletion of *SMN1* indicates a low risk of developing SMA (<1%). The clinical severity of SMA cannot be accurately predicted.

Target reporting times:

As reporting times are constantly evolving, please refer to www.genetics.ie/molecular, or contact the molecular genetics laboratory, to receive up-to-date information on anticipated reporting times for your referral.
The following are current target reporting times for each category of test offered:

Diagnostic:	6-8 weeks Routine / 2 weeks Urgent (i.e. a neonate)
Carrier testing:	6-8 weeks Routine / 2 weeks Urgent (i.e. pregnancy)
Prenatal:	2 weeks CVS (4 weeks for amniotic fluid specimens)

- Please contact the laboratory if you have not received a report within a week of your patient being due back in clinic.
- Please note it is our policy not to issue verbal results.
- Request for copies of reports on the day that your patient is in clinic cannot normally be accommodated. We usually require 24 hours notice in which to fax a copy of a report.

Further tests

The SMA MLPA assay is a quantitative test for *SMN1* gene copy number, and will not pick up subtle deletions, inversions or point mutations in *SMN1*. However, screening for such mutations can be arranged by us in external laboratories, where relevant (e.g. in the case of a suspected clinical diagnosis in the presence of a hemizygous deletion of *SMN1*). Please contact the laboratory for further information.

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External referral laboratories

Contacts Molecular Genetics

- **Administration** ☎**01 409 6733** **(internal 6733)**
- **Laboratory** **duty.scientist@olchc.ie**

We also maintain a register of hundreds of genetic disorders for which tests are available abroad, and for which we provide a referral service, which includes DNA preparation. Turnaround times for DNA preparation + despatch are as follows

Sendouts	Current sendout dispatch TAT
Very urgent	To be sent within 1 working week from activation
Urgent	To be sent within 3 working weeks from activation
Routine	To be sent no more than 8 working weeks from activation

These turnaround times for DNA preparation and despatch indicate the time taken to dispatch the sample(s) from our lab and not the TAT for reporting the result (which is determined by the external lab). Due to the high number of samples we receive we have a queue system and process all requests by urgency and in the order received.

Costs for tests carried out at external referral laboratories will be invoiced directly to the referring clinician/institution by the testing laboratory.

Please contact us at duty.scientist@olchc.ie for all queries regarding molecular genetic tests.

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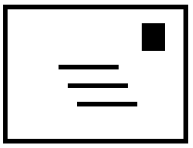
Complaints and Feedback

Your feedback is important to us. Any complaints, compliments or feedback may be directed to either the Quality Manager or Chief Scientist.

Christine Brady	Quality Manager & Senior Clinical Scientist	☎ 01 428 2705 ☎ 01 428 2899	christine.brady@olchc.ie
David Barton	Chief Scientist	☎ 01 409 6749	david.barton@olchc.ie

Or by mail to:



Christine Brady
Quality Manager
Division of Molecular Genetics
National Centre for Medical Genetics
Our Lady's Children's Hospital
Crumlin
Dublin 12

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