The North East Thames Regional Genetics Service is based at Great Ormond Street Hospital. It is comprised of the Molecular and Cytogenetics laboratories along with Clinical Genetics. Having merged with the North West Thames Regional Genetics laboratory in October 2018, we now serve a population of approximately 9 million across North London, Hertfordshire, Essex, including referrals from the North West Thames clinical genetics team.

The laboratory service has a staff of approximately 100 including consultant and HCPC registered clinical scientists, genetic technologists and administrative support staff. The staff work closely with clinical colleagues and other healthcare scientists in pathology as well as with research staff at the UCL Great Ormond Street Institute for Child Health.

The laboratory provides an extensive range of diagnostic testing services and is a UKAS accredited medical laboratory No. 7883. The laboratory accreditation schedule and list of accredited services can be viewed via the UKAS website: https://www.ukas.com/wp-content/uploads/schedule_ascend_uploads/00007/7883%20Medical%20Single.pdf

The service is a member of the South East of England Genetics Network (SEEGEN) and the United Kingdom Genetics Testing Network (UKGTN).

The laboratory service processes over 30,000 samples per year and issues approximately 18,500 analytical reports. The service repertoire is regularly updated, but includes a regional service for postnatal and prenatal microarray, karyotyping and a number of single gene and multi-gene panel tests. Patients with developmental delay and/or dysmorphism, or patients with an anomalous ultrasound scan during pregnancy are offered a 750K microarray analysis. Karyotyping is performed for a smaller number of patients, whilst samples from pregnancy losses are analysed using molecular methods.

The Unit receives nationally commissioned funding for a number of specialised services including Bardet-Biedl syndrome, craniosynostoses, lysosomal storage disorders and severe combined immunodeficiencies.

The laboratory also provides both a national and international service for non-invasive prenatal diagnosis and a range of specialist Next Generation Sequencing panels. Referrals are received for diagnostic, predictive, carrier and prenatal testing.

We undertake externally funded research projects and accept private referrals. Please contact the laboratory for further information.

Research and development is a key objective of the Unit, a number of staff having joint academic appointments with University College London. The service has a strong commitment to education and training and public and patient engagement and participates in clinician, scientist and technologist training programmes. A number of staff are also actively involved at a regional and national level in policy development, training and examination.

The laboratory provides a DNA banking service and can forward samples to other centres for approved requests provided funding is available. A complete list of in-house services and corresponding information sheets can be found in this service pack. Further details regarding tests which may be available from other laboratories can be found on page 3.

**Laboratory Opening Hours**

The laboratory is staffed Monday - Friday, 9.00am – 5.30pm excluding bank holidays.

Sample reception is open from 9.00am to 5.30pm. Specimens arriving outside these hours are refrigerated / frozen prior to processing. There is no out-of-hours service.
Sample Requirements

For sample requirements, please refer to our request cards which can be found at: http://www.labs.gosh.nhs.uk/laboratory-services/genetics/sending-a-sample

It is the responsibility of the patient’s clinician to ensure that all requests meet testing criteria, that samples are correctly labelled and request forms are completed to a minimum standard.

In submitting samples the clinician confirms that consent for testing and possible storage has been obtained.

Samples

5 mls venous blood in plastic EDTA bottles (>1 ml from neonates)

For DNA samples, it is requested that the referral laboratory provides sufficient DNA for the analysis being requested.

Samples must be labelled with:

- Patient’s full name (surname/family name and first/given name)
- Date of birth and unique hospital/NHS number
- It is also desirable to have the date and time sample was taken and/or location

Prenatal Samples

Please contact the laboratory in advance of arranging a prenatal sample.

Cytogenetic analysis of prenatal samples is routinely available. The type of test offered is dependent on criteria such as the patient having a serum screen risk only, or having abnormalities detected on ultrasound scan or individual service level agreements.

Prenatal diagnosis for molecular genetic disorders can only be offered by prior arrangement where diagnosis has been confirmed by molecular means and parental samples are fully informative. It is standard practice for the laboratory to exclude maternal cell contamination of all fetal samples; a maternal blood sample is required for this analysis.

CVS / Amniocentesis: Tissue type and date of biopsy should be clearly documented on the referral information. In the case of twins, special attention must be given to the identity of each sample.

Cell Free Fetal DNA Analysis: 20ml of maternal blood in EDTA is required. If the sample is expected to take over 24 hours to arrive, blood should be taken into a STRECK tube. Sample date & gestation as confirmed by ultrasound scan must be provided along with a valid clinical indication for early gender determination or single gene testing. This test does not apply to twin pregnancies.

Minimum sample labelling criteria:

- Patient’s full name and date of birth
- Unique hospital/NHS number
Request form

The Regional Genetics Laboratory has its own referral card; an electronic version is available on our website.

Alternative referral cards / letters are acceptable; it is preferable that any referral card is fully completed. However, referral documents must provide the minimum criteria of:

- Patient’s full name and date of birth
- NHS number (essential) and hospital number
- Full name and address of referring clinician/consultant
- Patient’s postcode
- Patient’s GP name and address
- Clearly mark if referral is for a non-NHS patient
- Analysis can only be carried out if a specific disease / gene test(s) is requested
- For family / targeted mutation tests, a mutation report or GOS genetics family ID is required along with the relationship of your patient to family members previously tested.

Sending samples

Samples sent by Royal Mail or courier must comply with PI 650 for category B substances.

- This is a triple layer system which comprises a primary leak-proof receptacle within a secondary leak-proof receptacle contained in a rigid outer package. The packaging should be strong enough to withstand a 95 kPA pressure differential and a drop of 1.2 m.
- There should be sufficient absorbent material between the primary and secondary packaging to absorb any spillage. The primary container and absorbent material must be placed into a single bag with the request form in the pouch.
- The package should be clearly labelled ‘diagnostic specimen UN3373’.

Pricing

NHS Provider to Provider and Private / Overseas test prices

Please contact the laboratory for an up-to-date price list. Prices are also available on our UKGTN listing and website.


Tests carried out by other laboratories

UK Genetic Testing Network www.ukgtn.nhs.uk

The United Kingdom Genetic Testing Network (UKGTN) is a collaborative group of UK laboratories and their clinicians, commissioners and patient representatives. The network, which is overseen by the Department of Health, aims to ensure that the UKGTN services provided by the member laboratories are of high quality, that new services are evaluated for effectiveness and that the NHS commissioning mechanisms are appropriately informed in order to promote equity of access.

Subject to meeting recognised referral criteria and available funding for specific tests, DNA may be extracted and forwarded to the relevant UKGTN laboratory for tests not available in-house. The UKGTN website lists evaluated tests (including a
search function and alphabetical list of tests), however this has not been updated since April 2018. From October 2018 the National Genomic Test Directory will specify which genomic tests are commissioned by the NHS in England, the technology by which they are available and the patients who will be eligible to access a test. The final draft 2018/2019 National Genomic Test Directory for rare and inherited disorders and cancer can be accessed at https://www.england.nhs.uk/publication/national-genomic-test-directories.

The NE Thames Regional Genetics Laboratory will continue to receive samples, perform genomic tests and dispatch DNA samples for specialist testing to the appropriate laboratory. Further information about future genomic testing arrangements will be issued as it becomes available.

The current accreditation status of UK laboratories registered with UKAS can be checked at http://www.ukas.com/

Testing carried out by laboratories outside the UKGTN

Molecular genetic screening for some disorders may not currently be available from UKGTN laboratories. These tests may be available at other diagnostic laboratories within and outside the UK and in some cases samples can be forwarded provided funding is available. Please contact the laboratory for further information.
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<th>Gene</th>
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<th>OMIM Gene</th>
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### Core disorders

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### Cell-free fetal DNA testing

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### Cardiovascular Disorders

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### Cardiovascular Disorders

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### Skeletal Dysplasias

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### Craniosynostosis

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### Craniosynostosis

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<td>13q11-q12</td>
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<td>*121011</td>
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<td>EAST syndrome</td>
<td>KCNJ10</td>
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<td>Non-Syndromic Hearing Loss</td>
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<td>Pendred syndrome</td>
<td>SLC26A4</td>
<td>7q31</td>
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<td>Usher Syndrome</td>
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<td>Waardenburg syndrome Types 1 - 4</td>
<td>Various – see NGS panel section</td>
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<td>X-Linked Deafness (DFN3)</td>
<td>POU3F4</td>
<td>Xq21.1</td>
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### Metabolic Disorders

<table>
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<tr>
<th>Disorder</th>
<th>Gene</th>
<th>Locus</th>
<th>OMIM Disease</th>
<th>OMIM Gene</th>
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<tr>
<td>Carbamoylphosphate synthetase 1 deficiency</td>
<td>CPS1</td>
<td>2q35</td>
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<td>Fabry disease</td>
<td>GLA</td>
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<td>Gaucher disease</td>
<td>GBA</td>
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<td>#230800 (Type1)</td>
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<td>#230900 (Type2)</td>
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<td>#231000 (Type 3)</td>
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<td>Glycogen Storage disease type 1a</td>
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<td>Disorder</td>
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<td>Glycogen storage disease type 2 (Pompe disease)</td>
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<td>Krabbe disease GALC</td>
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<td>Long-chain, deficiency of Acyl-CoA dehydrogenase</td>
<td>HADHA</td>
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<td>HADHB</td>
<td>2q23</td>
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<tr>
<td>Medium-chain, deficiency of Acyl-CoA dehydrogenase</td>
<td>ACADM</td>
<td>1p31</td>
<td>#201450</td>
<td>*607008</td>
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<td>Metachromatic Leukodystrophy (incl. pseudodeficiency of arylsulphatase A)</td>
<td>ARSA</td>
<td>22q13.31-qter</td>
<td>#250100</td>
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<td>Mucopolysaccharidosis type 1 (Hurler / Scheie)</td>
<td>IDUA</td>
<td>4p16.3</td>
<td>#607014 (Hurler)</td>
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<td>#607015 (H/S)</td>
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<td>#607016 (Scheie)</td>
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<tr>
<td>Mucopolysaccharidosis type 2 (Hunter)</td>
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<tr>
<td>Mucopolysaccharidosis type 3 (Sanfilippo)</td>
<td>SGSH</td>
<td>17q25.3</td>
<td>#252900 (MPS3A)</td>
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<tr>
<td></td>
<td>NAGLU</td>
<td></td>
<td>#252920 (MPS3B)</td>
<td>*609701</td>
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<tr>
<td>Neuronal Ceroid Lipofuscinosis type 1 (incl. infantile Batten disease)</td>
<td>PPT1</td>
<td>1p32</td>
<td>#256730</td>
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<tr>
<td>Neuronal Ceroid Lipofuscinosis type 2 (late-infantile Batten)</td>
<td>TPP1</td>
<td>11p15.5</td>
<td>#204500</td>
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<td>Neuronal Ceroid Lipofuscinosis type 3 (juvenile Batten)</td>
<td>CLN3</td>
<td>16p12.1</td>
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<td>Lipofuscinosis type 5 (variant late-infantile Batten)</td>
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<td>Neuronal Ceroid Lipofuscinosis type 6 (variant late-infantile Batten)</td>
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<tr>
<td>Neuronal Ceroid Lipofuscinosis type 7 (variant late-infantile Batten)</td>
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<td>4q28.2</td>
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<tr>
<td>Neuronal Ceroid Lipofuscinosis type 8 (variant late-infantile Batten)</td>
<td>CLN8</td>
<td>8pter-p22</td>
<td>#600143</td>
<td>*607837</td>
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<tr>
<td>Neuronal Ceroid Lipofuscinosis – multiplex</td>
<td>Various – see NGS panel section</td>
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<tr>
<td>Ornithine transcarbamylase deficiency</td>
<td>OTC</td>
<td>Xp21.1</td>
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<tr>
<td>Osteopetrosis, autosomal recessive</td>
<td>TCIRG1</td>
<td>11q13.4-q13.5</td>
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<tr>
<td>Pyridoxine Dependent Epilepsy</td>
<td>ALDH7A1</td>
<td>5p23.2</td>
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<td>Schindler disease (Kanzaki disease)</td>
<td>NAGA</td>
<td>22q13.2</td>
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## Renal Disorders

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<tr>
<td>CFHRS Nephropathy</td>
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<td>Cystinosis (Adult)</td>
<td>CTNS</td>
<td>17p13</td>
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<td>Cystinosis (Juvenile)</td>
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<td>#219750</td>
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<td></td>
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<td>#219900</td>
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<tr>
<td>Juvenile Nephronphthisis</td>
<td>NPHP1</td>
<td>2q13</td>
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<td>Steroid-resistant nephrotic syndrome</td>
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<td>1q25-q31</td>
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<tr>
<td>Renal tubulopathies</td>
<td>Various</td>
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## Immunodeficiencies

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<th>OMIM Gene</th>
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<tbody>
<tr>
<td>Autoimmune lymphoproliferative syndrome (ALPS)</td>
<td>TNFRSF6</td>
<td>10q24.1</td>
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<td>Activated PI3 Kinase Delta syndrome (Immunodeficiency 14)</td>
<td>PIK3CD</td>
<td>1p36.22</td>
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<td>Cartilage Hair Hypoplasia</td>
<td>RMRP</td>
<td>9p21-p12</td>
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<td>Familial hemophagocytic lymphohistiocytosis</td>
<td>PRF1</td>
<td>10q22</td>
<td>#603553</td>
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<td>Interleukin 7 receptor alpha severe combined immunodeficiency</td>
<td>IL7Ra</td>
<td>5p13</td>
<td>#600802</td>
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<tr>
<td>JAK3-deficient severe combined immunodeficiency</td>
<td>JAK3</td>
<td>19p13.1</td>
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<td>Lymphoproliferative syndrome 1 (ITK deficiency)</td>
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<td>Netherton Syndrome</td>
<td>SPINK5</td>
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<td>Primary immunodeficiency</td>
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<td>Radiation-sensitive SCID</td>
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<td>RAG-deficient severe combined immunodeficiency</td>
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<td>RAG2</td>
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<td>Wiskott-Aldrich syndrome</td>
<td>WAS</td>
<td>Xp11.23-p11.22</td>
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<td>X-linked agammaglobulinaemia</td>
<td>BTK</td>
<td>Xq21.3-q22</td>
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## Immunodeficiencies

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<td>X-linked Hyper IgM syndrome (HIGM)</td>
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<td>X-linked Lymphoproliferative syndrome</td>
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<td>XIAP</td>
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<td>X-linked Severe combined immunodeficiency</td>
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<td>Xq13</td>
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## Next generation sequencing panels

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<td>Bardet-Biedl syndrome</td>
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<td>Ciliopathies (various sub-panels available)</td>
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<td>Clinical exome (targeted panels - GOSHHome)</td>
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<td>Coffin-Ciril &amp; Nicolaides Baraitser syndromes</td>
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<td>Colorectal polyposis</td>
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<td>Dermatology (various sub-panels available)</td>
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<td>Early Infantile Epileptic Encephalopathy</td>
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<td>Familial breast/ovarian cancer</td>
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<td>Familial Hypercholesterolaemia</td>
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<td>Inherited hearing loss panel</td>
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<td>Lynch syndrome</td>
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<td>Neuronal Ceroid lipofuscinoses (NCL)</td>
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<td>Oculome (Congenital eye disorders – various sub-panels available)</td>
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<td>Primary immunodeficiency</td>
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<td>106</td>
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<td>Very early onset inflammatory bowel disease</td>
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<td>Surfactant deficiency</td>
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<td>Pulmonary fibrosis</td>
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<td>Waardenburg syndrome</td>
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<td>Postnatal tests</td>
<td>Major referral category</td>
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<td>FISH</td>
<td>Rapid neonatal testing</td>
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<td>For trisomy 13, 18 &amp; 21 and chromosomal sex assignment</td>
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<tr>
<td>Microarray (750K SNP)</td>
<td>Developmental delay / learning difficulties / multiple congenital abnormalities / epilepsy</td>
<td>109</td>
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<tr>
<td>Karyotyping</td>
<td>Sexual development / infertility</td>
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<tr>
<td></td>
<td>Breakage syndromes. Specific tests apply to each syndrome.</td>
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<tr>
<td></td>
<td>Tests are referred out to other accredited providers</td>
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<tr>
<td>Targeted (partial)</td>
<td>Query sex chromosome abnormality</td>
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<td>Follow up of known, documented familial chromosome rearrangements</td>
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<td>Mosaicism screening</td>
<td>As requested or indicated e.g. for selected query sex chromosome abnormalities</td>
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<tr>
<td>QF-PCR and MLPA †</td>
<td>Pregnancy loss investigation</td>
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<td>Products of conception (3rd or subsequent miscarriage in 1st trimester; any thereafter)</td>
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<td>Parental cytogenetic testing</td>
<td>Parental cytogenetic testing</td>
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<td>Follow-up to exclude carrier status for positional rearrangements (e.g. Robertsonian translocations) that may be associated with copy number changes identified in products of conception</td>
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<td>Cell culture</td>
<td>Culturing of fibroblasts, chorionic villi and amniocytes for DNA extraction and other tests</td>
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<tr>
<td>QF-PCR</td>
<td>Rapid (invasive) prenatal testing for common aneuploidies</td>
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<tr>
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<td>e.g. increased risk of Down syndrome as judged by maternal screening</td>
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<tr>
<td>Microarray (750K SNP) in conjunction with QF-PCR</td>
<td>Structural fetal abnormalities identified by ultrasound scan (including raised NT &gt; 3.5mm at 12-14 weeks)</td>
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<tr>
<td>Karyotyping</td>
<td>As required, following identification of trisomy 13 or 21 by QF-PCR or confirmation of other abnormal copy number findings as required</td>
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</tbody>
</table>

† Note that this service is offered in place of routine parental karyotyping of couples with a history of recurrent (3 or more) miscarriages in line with RCOG Green Top Guidelines (2010).
OMIM notes

An asterisk (*) before an entry number indicates a gene of known sequence.

A number symbol (#) before an entry number indicates that it is a descriptive entry, usually of a phenotype, and does not represent a unique locus. Discussion of any gene(s) related to the phenotype resides in another entry (entries) as described in the first paragraph.

A plus sign (+) before an entry number indicates that the entry contains the description of a gene of known sequence and a phenotype.

A percent sign (%) before an entry number indicates that the entry describes a confirmed Mendelian phenotype or phenotypic locus for which the underlying molecular basis is not known.
**Introduction**

Alexander disease (AD) (MIM 203450) is characterized clinically by the development of megalencephaly in infancy, accompanied by progressive spasticity and dementia. Both infantile and adult onset cases have been reported. Imaging studies of the brain typically show cerebral white matter abnormalities, preferentially affecting the frontal region. Pathologically, AD is characterized by astrocytic inclusion bodies, termed Rosenthal fibres, found adjacent to areas of demyelination. AD results from dominant gain of function mutations in the GFAP gene that frequently arise de novo. Pathogenic variants in the GFAP gene are the only known cause of AD.

**Referrals**

- Confirmation of diagnosis in individuals clinically suspected of having AD
- Testing of individuals with a relative with genetically confirmed AD

**Service offered**

Analysis of the GFAP gene by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is not validated to detect large deletions / duplications. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported.

Testing for previously identified pathogenic variants is available to other family members.

**Target reporting time**

8 weeks for a full screen in an index case (next generation sequencing and MLPA). 4 weeks for familial testing.

Please note this service does not currently have UKAS accreditation.
Angelman Syndrome

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
Angelman syndrome (MIM 105830) occurs in 1/15000 - 1/20000 individuals. It is characterised by severe motor and intellectual retardation, seizures associated with characteristic EEG traces, microcephaly, ataxia, frequent jerky limb movements and flapping of the arms and hands, hypotonia, hyperactivity, hypopigmentation (39%), absence of speech, characteristic face shape, and episodes of paroxysmal laughter. The AS phenotype results from the lack of a maternal contribution at chromosome 15q11-q13. This can be caused by deletion (~75%), paternal uniparental disomy (UPD) (~2%) or pathogenic variants in the imprinting centre (IC) (~5%) that cause abnormal methylation at exon alpha of the SNRPN gene at 15q11-13. These are all detected by disrupted methylation. About 20% of AS patients have a normal methylation pattern and are believed to have a pathogenic variant in a putative Angelman gene (UBE3A). Deletions and UPD are usually de novo events, associated with low recurrence risks, although it is important to determine whether either parent of an affected child has a predisposing chromosomal translocation. There is a recurrence risk of up to 50% in families with confirmed AS who do not show maternal deletion or UPD.

Referrals
- Confirmation of clinically suspected AS in children/adults.
- Investigation of the molecular defect in genetically confirmed AS cases (parental samples required).
- Carrier testing in adult relatives of confirmed (genetic) AS patients who are suspected of having an IC mutation (samples from appropriate family members are required).

Prenatal testing
Prenatal diagnosis is available to couples where AS has been confirmed in the family and to couples at risk of having a child affected with AS due to a balanced chromosomal rearrangement involving chromosome 15 in one of the parents. Please contact the laboratory to discuss, prior to sending prenatal samples.

Service offered
Confirmation of AS by methylation analysis and microsatellite analysis to determine the underlying cause in confirmed cases and carrier testing for adults (requires samples from appropriate family members). The UBE3A gene is on the EIEE panel.

Technical
For diagnostic referrals, the initial test is to determine the methylation status of exon alpha of the SNRPN gene. Methylation analysis is performed by methylation-specific PCR following bisulphite modification of genomic DNA. Normal individuals yield a 313bp maternally-derived fragment and a 221bp paternally-derived fragment. Patients with AS show a single 221bp paternal fragment only. Positive results are confirmed by either MS-MLPA or aCGH analysis. Chromosome 15 microsatellite markers from within and flanking the commonly deleted region can also be used to characterise the mechanism in patients shown to have abnormal methylation. Cytogenetic analysis is also helpful in identifying deletions and predisposing parental translocations. NB: A similar testing process is undertaken for Prader-Willi syndrome.

Target reporting time
Routine analysis - the initial methylation test takes up to 4 weeks. Microsatellite marker analysis takes 4 weeks from receipt of parental samples. Please contact the laboratory for urgent cases.
Familial breast/ovarian cancer

Introduction

Hereditary breast and ovarian cancer due to pathogenic variants in BRCA1 and BRCA2 genes is the most common cause of hereditary forms of both breast and ovarian cancer. The prevalence of BRCA1/2 pathogenic variants is ~1/400 to 1/800; however this varies depending on ethnicity. Notably, in Ashkenazi Jewish populations there are three well-described founder pathogenic variants and their combined frequency in this population is 1/40. Pathogenic variants in the BRCA1 and BRCA2 genes are also associated with other forms of cancer including fallopian tube carcinoma and primary papillary serous carcinoma of the peritoneum. The risk of prostate cancer in male BRCA2 carriers is increased with a relative risk of 4.6 and with evidence of earlier age of onset, while some studies suggest that male BRCA1 carriers may have a very slightly increased risk of prostate cancer. Pathogenic variants causing breast/ovarian cancer have also been identified in the PALB2, RAD51C, RAD51D and BRIP1 genes in a small proportion of cases.

Li-Fraumeni is an inherited cancer syndrome characterised by the early onset of tumours within an individual and multiple tumours including breast cancer (MIM151623). Pathogenic variants in the TP53 gene account for ~70% and ~40% individuals with Li-Fraumeni syndrome or Li-Fraumeni like syndrome respectively.

Referrals

Referrals are accepted via Clinical Genetics and approved mainstreaming referrers.

Service Offered

- Next generation sequencing of the BRCA1 and BRCA2 genes is offered to individuals who meet the criteria defined by the NICE and Pan Thames guidelines. Testing for large gene deletions and duplications in the BRCA1, BRCA2 (using NGS data or MLPA) and TP53 (by MLPA) genes.
- Common variant screening is offered to individuals of populations where there is a high frequency of BRCA1/2 pathogenic variants:

<table>
<thead>
<tr>
<th>Ashkenazi Jewish panel:</th>
<th>BRCA1 c.68_69del</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BRCA1 c.5266dup</td>
</tr>
<tr>
<td></td>
<td>BRCA2 c.5946del</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Polish panel:</th>
<th>BRCA1 c.181T&gt;G</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BRCA1 c.4035del</td>
</tr>
<tr>
<td></td>
<td>BRCA1 c.5266dup</td>
</tr>
</tbody>
</table>

- Predictive testing is offered to individuals who have a known familial pathogenic variant in the BRCA1, BRCA2, TP53, PALB2, BRIP1 RAD51C or RAD51D genes. A familial control is required for analysis.

Technical

Testing is carried out by next generation sequencing with library preparation using an Agilent SureSelect kit followed by sequencing on the Illumina NextSeq or MiSeq. Data is analysed using an in-house pipeline with all pathogenic variants confirmed by Sanger sequencing. This assay is validated to detect large deletions/duplications in the BRCA1 and BRCA2 genes. Confirmation of large BRCA1/BRCA2 deletions/duplications and deletion/duplication analysis of the TP53 gene is carried out using MLPA analysis. Common variant screens are carried out by Sanger sequencing of the relevant exons. Predictive testing is carried out by Sanger sequencing or MLPA as appropriate.

Target reporting time

8 weeks for a full screen in an index case. 4 weeks for a common variant screen. 2 weeks for a predictive test. Please contact the laboratory for urgent cases.
Canavan disease

Introduction

Canavan disease (CD) (MIM 271900) is an autosomal recessive leukodystrophy, characterised clinically by hypotonia, macrocephaly and developmental delay. Pathogenic variants in the \textit{ASPA} gene affect the function of the aspartoacylase protein, demonstrated biochemically by an increase of N-acetyl-L-aspartic acid (NAA) in the urine. CD has an incidence of around 1/6500 – 1/13500 in the Ashkenazi Jewish population (approximately 1/40 individuals are carriers), and around 1/100000 in individuals of non-Ashkenazi Jewish ancestry. Pathogenic variants in the \textit{ASPA} gene are the only known cause of CD. \textit{ASPA} has 6 coding exons and family specific variants are found throughout the gene. Three common variants account for 98% of pathogenic variants in the Ashkenazi Jewish population, and a single common variant accounts for approximately 30-60% of pathogenic variants in the non-Ashkenazi Jewish population.

Referrals

- Confirmation of diagnosis in individuals clinically suspected of having CD.
- Investigation of the molecular defect in individuals with an increased NAA concentration
- Carrier testing in adult relatives of confirmed (genetic) CD patients
- Carrier testing in individuals at increased risk (above the population risk) of having an affected pregnancy, for example a family history of CD, a partner shown to be a carrier or first cousin partnerships within the Ashkenazi Jewish population.

Prenatal testing

Prenatal testing is available for couples in whom pathogenic variants have been identified.

Service offered

Analysis of of the \textit{ASPA} gene by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is not validated to detect large deletions / duplications. Detection of large deletions/duplications in the \textit{ASPA} gene is carried out using MLPA. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported. Testing for previously identified pathogenic variants is available to other family members.

Target reporting time

8 weeks for a full screen in an index case (next generation sequencing and MLPA). 4 weeks for familial testing.

Please note this service does not currently have UKAS accreditation.
Nicolaides-Baraitser/Coffin-Siris Syndromes

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
De novo germline pathogenic variants in several components of the SWI/SNF-like BAF complex can cause Coffin Siris Syndrome (CSS) and Nicolaides Baraitser syndrome (NBS).

CSS (MIM 614609) is a rare congenital malformation syndrome characterised by developmental delay/intellectual disability, coarse facial appearance, feeding difficulties, frequent infections and hypoplastic to absent fifth fingernails and fifth distal phalanges. CSS can be caused by pathogenic variants in SMARC\textsubscript{B}1, SMARCA\textsubscript{4}, ARID\textsubscript{1}A, ARID\textsubscript{1}B, SMARCE\textsubscript{1} and SOX\textsubscript{1}1.

NBS (MIM 601358) is an autosomal dominant condition manifesting primarily as severe developmental delay with short stature, microcephaly, seizures, and a characteristic facial dysmorphism. Notable features include sparse hair on the scalp, and progressive skin wrinkling; additionally, the hands tend to show brachydactyly, inter-phalangeal joint swellings and broad distal phalanges. It is generally caused by \textit{de novo} heterozygous pathogenic variants within the SMARCA\textsubscript{2} gene.

Although CSS and NBS are different clinical entities there is some overlap as both syndromes feature intellectual disability, hair abnormalities (hypertrichosis and sparse scalp hair) and digital abnormalities (short 5\textsuperscript{th} fingers, prominent interphalangeal joints and prominent distal phalanges).

Referrals
We offer testing for confirmation of diagnosis of either CSS or NBS in affected individuals and family members.

Prenatal testing
Prenatal testing may be available for families in whom specific pathogenic variants have been identified. Please contact the laboratory to discuss.

Service offered
Next generation sequencing of the SMARCA\textsubscript{2}, SMARCA\textsubscript{4}, SMARC\textsubscript{B}1, ARID\textsubscript{1}A, ARID\textsubscript{1}B, SMARCE\textsubscript{1} and SOX\textsubscript{1}1 genes with variants confirmation by sanger sequence analysis.

Testing for previously identified familial pathogenic variants is available in family members.

Technical
Analysis is by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5\% (with 95\% confidence) for regions covered >30x. This assay is not currently validated to detect large deletions / duplications. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported.

Target reporting time
Panel test: 8 weeks.
Familial variant test: 4 weeks.
For urgent samples please contact the laboratory.
Colorectal polyposis

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- Tumour testing: FFPE tissue. 4x5um unstained slides with indication of tumour content and a H&E stained slide with region of tumour highlighted
- Germline testing: 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
Colorectal polyposis is associated with an increased risk of colorectal cancer and number of different inherited cancer syndromes:
Familial adenomatous polyposis (FAP) is associated with 100-1000s of adenomatous polyps and is caused by pathogenic variants in the APC gene. A milder form, attenuated FAP (aFAP) is associated with fewer polyps (<100) and lower cancer risk. aFAP has a phenotypic overlap with MUTYH-associated polyposis (MAP), caused by pathogenic variants in the MUTYH gene. There are three well described MUTYH pathogenic founder variants, two in the Caucasian population which account for ~85% of pathogenic variants in this population and a third frequently seen in the Indian Gujarati population.
The much rarer polymerase proofreading associated polyposis (PPAP) and NTHL1 associated polyposis (NAP) due to pathogenic variants in the POLD1, POLE and NTHL1 genes are associated with variable phenotypes and other cancers including endometrial, pancreatic, breast and ovarian have been reported.
Hereditary mixed polyposis syndrome (HMPS) is associated with mixed polyps and a duplication located upstream of the GREM1 gene (Ashkenazi Jewish founder variant). Juvenile Polyposis Syndrome (JPS) is characterised by juvenile polyps and a pathogenic variant in the SMAD4 or BMPR1A gene. Pathogenic SMAD4 variants are also associated with a high risk of hereditary haemorrhagic telangiectasia (HHT). Peutz-Jeghers syndrome (PJS) is characterised by hamartoma polyps in the GI tract, distinctive mucocutaneous pigmentation and pathogenic variants in the STK11 gene. PTEN-Hamartoma Tumour syndrome (PHTS) is a spectrum of disorders caused by pathogenic variants in the PTEN gene. These disorders include Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, Proteus and Proteus like syndromes. Common indications for referral include macrocephaly, developmental delay and cancer. JPS, PJS and PHTS are all also associated with an increased risk of other cancers including gastric, pancreatic, breast and ovarian.

Referrals
Referrals are accepted from Clinical Genetics, family cancer clinics and approved gastroenterologists.

Service Offered
- Germline DNA testing: Next generation sequencing of the above genes. MLPA analysis to detect large gene deletions and duplications in the APC, MUTYH, GREM1, SMAD4, BMPR1A, STK11, and PTEN genes.
- Common variant screening is offered to individuals of populations where there is a high frequency of MUTYH pathogenic variants:
<table>
<thead>
<tr>
<th>Caucasian variants:</th>
<th>Indian Gujarati variant:</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.536A&gt;G p.Tyr179Cys</td>
<td>c.1438G&gt;T p.Glu480*</td>
</tr>
</tbody>
</table>
- Predictive testing is offered to individuals who have a known familial pathogenic variant in the above genes. A familial control is required for analysis.

Technical
Germline testing is carried out by next generation sequencing with library preparation using an Agilent SureSelect kit followed by sequencing on the Illumina NextSeq or MiSeq. Data is analysed using an in-house pipeline with all pathogenic variants confirmed by Sanger sequencing. Detection of large deletions/duplications is carried out using MLPA analysis (APC, MUTYH, GREM1, SMAD4, BMPR1A, STK11, and PTEN).
Predictive testing is carried out by Sanger sequencing or MLPA as appropriate.

Target reporting time
8 weeks for a full screen in an index case. 4 weeks for a common variant screen. 2 weeks for a predictive test. Please contact the laboratory for urgent cases.
Introduction

Congenital Tufting Enteropathy (CTE; OMIM #613217) is a rare, neonatal-onset autosomal recessive condition characterised by chronic intractable diarrhoea leading to severe malabsorption and significant morbidity and mortality. The severity of intestinal malabsorption in most cases results in complete dependence on long-term parenteral nutrition.

Histologically, affected individuals display focal epithelial tufts in the duodenum and jejunum composed of closely packed enterocytes.

The prevalence of CTE is estimated between 1/50'000 to 1/100'000 in Western Europe but is higher in consanguineous populations and patients of Arabic ethnic origin.

Congenital Tufting Enteropathy is caused by pathogenic variants in the EPCAM gene at 2p21.

The EPCAM gene (NM_002354.2, ENST00000263735) consists of 9 coding exons and encodes an epithelial cell adhesion molecule.

Referrals

Referrals are accepted from Consultant Clinical Geneticists and Consultant Paediatric Gastroenterologists in the following:

- Patients with clinically suspected Congenital Tufting Enteropathy
- Familial testing in family members for known familial pathogenic variants

For clinical enquiries, please contact Dr Neil Shah, Gastroenterology, GOSH
Tel: +44 (0) 20 7405 9200 ext 5949, email: Neil.Shah@gosh.nhs.uk

Prenatal testing

Prenatal testing is available for families in whom specific clearly pathogenic variants have been identified or in whom appropriate family studies have been undertaken - please contact the laboratory to discuss.

Service offered

Analysis for substitutions and small insertions / deletions is by direct Sanger sequencing of the coding region in 9 amplicons.

Technical

Molecular screening is carried out by direct sequencing analysis.

Target reporting time

The target reporting time is 8 weeks for an EPCAM gene full screen and 4 weeks for familial testing. Please contact the laboratory for urgent cases.
Cystic Fibrosis

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Introduction
Cystic fibrosis (MIM 219700) is an autosomal recessive condition caused by pathogenic variants in the cystic fibrosis transmembrane regulator (CFTR) gene. To date, over 2000 pathogenic variants with varying frequency have been identified in this gene. The ethnic origin of the patient influences the incidence of CF in the population and the pathogenic variants most commonly identified.

Referrals
- Confirmation of diagnosis in individuals clinically suspected of having CF. A sweat test should be undertaken prior to molecular genetic analysis wherever possible.
- Testing in individuals who may have a mild variant form of CF (CFTR-related disease), e.g. congenital bilateral absence of the vas deferens (CBVAD), bronchiectasis and pancreatitis.
- Carrier testing in pregnant couples with fetal echogenic bowel.
- Carrier testing in individuals at increased risk (above the population risk) of having an affected pregnancy, for example a family history of CF, a partner shown to be a carrier or first cousin partnerships. Accurate carrier testing in CF families ideally requires either a sample from an affected family member or information regarding the pathogenic variants carried in the family. Without this information, the extent to which we can reduce an individual’s carrier risk is less than if information on familial pathogenic variants is available.
- In accordance with UK genetic testing guidelines carrier testing is only exceptionally undertaken in minors.

Prenatal testing
Prenatal testing is available for couples in whom pathogenic variants have been identified. Non-invasive prenatal diagnosis may be available - please contact the laboratory to discuss.

Service offered
Common variant analysis is carried out using the CFEU2v1 kit from Elucigene which tests for 50 common pathogenic variants and the partially penetrant intronic polyT variant in cases referred for CFTR-related disease where the p.Arg117His variant has been detected.

Full gene analysis using Agilent SureSelect and Illumina NextSeq and MLPA testing using the MLPA P091 kit from MRC-Holland.

Technical
- The detection system in use in this laboratory is the CFEU2v1 kit from Elucigene. As only 50 of the most commonly identified pathogenic variants are covered by this analysis failure to identify a pathogenic variant cannot exclude affected/carryer status, a residual risk to the individual is therefore calculated and reported wherever possible. In the European population, this system detects approximately 86% of cystic fibrosis pathogenic variants. Information regarding the ethnic origin of the patient is important for calculation of residual risk as the pathogenic variant spectrum, and hence the detection rate of the assay used, varies in different populations.
- Analysis of the CFTR gene by next generation sequencing. A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported. Large deletions and duplications are currently tested for using the MLPA kit (P091) from MRC-Holland.
- Detection of known pathogenic variants in relatives of patients with confirmed pathogenic variants by Sanger sequencing and/or MLPA analysis, as appropriate.

Target reporting time
2 weeks for routine analysis or routine testing of known familial pathogenic variants. 8 weeks for NGS analysis. Please contact the laboratory if urgent or prenatal testing is required.
Cystic Fibrosis Newborn Screening Programme

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
• 1 x dried blood spot
• A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring laboratory

Introduction
Cystic fibrosis (MIM 219700) is an autosomal recessive condition caused by pathogenic variants in the cystic fibrosis transmembrane regulator (CFTR) gene. To date over 2000 pathogenic variants with varying frequency have been identified in this gene. The ethnic origin of the patient influences the incidence of CF in the population and the pathogenic variants most commonly identified.

Referrals
• Testing is performed for all newborns with a raised IRT following referral from the newborn screening laboratory.
• This laboratory accepts referrals from three newborn screening laboratories:
  • North Thames – based at Great Ormond Street Hospital for Children
  • South East Thames – based at St Thomas’ Hospital
  • South West Thames – based in St Helier Hospital

Service offered
Initial testing involves screening for the four most common pathogenic variants using the CF4v2 kit from Elucigene: c.489+1G>T, c.1521_1523del p.(Phe508del), c.1624G>T p.(Gly542*) and c.1652G>A p.(Gly551Asp)

Any cases where one of more pathogenic variants are detected using this kit are tested with the CFEU2v1 kit (also from Elucigene), which tests for 50 most common pathogenic variants. The kit also detects the presence of the partially penetrant intronic polyT variant, which is only analysed when the p.(Arg117His) variant is detected.

Technical
The detection system in use in this laboratory is the CF4v2 kit and the CFEU2v1 kit. As only 4 or 50 of the most commonly identified pathogenic variants are covered by this analysis, respectively, failure to identify a pathogenic variant cannot exclude affected status. The results are reported back to the screening laboratories, who produce a final report including all biochemical and genetic analyses.

Target reporting time
The nationally agreed turnaround time is 6 days. The screening laboratories are notified if this will be exceeded, and are also immediately notified of any failed samples or unusual results, as repeat samples will be required to complete analysis.
Early Infantile Epileptic Encephalopathy (EIEE)

**Contact details**
Molecular Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

**Samples required**
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- A completed DNA request card should accompany all samples

**Patient details**
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

**Introduction**
The early infantile epileptic encephalopathies (EIEE) are a group of disorders characterized by early onset seizures and developmental delay. Many are associated with intractable seizures, severe developmental delay and require lifelong care. Early mortality is common amongst severely affected individuals due to seizures and/or respiratory tract infections.

An increasing number of individual genetic disorders are now recognised to cause EIEE. In only a small subset of the disorders is the clinical phenotype sufficiently recognisable or distinctive to allow targeted testing of specific genes.

**Referrals**
- Patients with EIEE and insufficient clinical features to target gene testing to a single gene. **Clinical details must be provided with all referrals.**
- Targeted parental testing to clarify inheritance and potential significance of relevant variants detected in the proband.
- Mutation testing can be offered to the relatives of EIEE patients once a disease causing mutation has been identified.

**Service offered**
Next generation sequencing of 82 genes (see list below) with mutation confirmation by Sanger sequencing.

**Updated List of genes included in version 10 panel**
ADSL, ALG13, ARHGEF9, ARX, ATP1A3, ATRX, BRAT1, CDKL5, CHD2, CHRNA2, CHRNA4, CHRN2B, CLN5, CLN6, CLN8, CNTNAP2, DNM1, DOCK7, DYRK1A, EHMT1, FOXG1, GABRA1, GABRB3, GATA2B, GNAO1, GRIN1, GRIN2A, GRIN2B, HCN1, IOSSEC2, KCNA2, KCNB1, KCNC1, KCONQ2, KCNT1, KIAA1279 (KIF1B1P), KIAA2022, LG11, MAGI2, MBDS, MECP2, MEF2C, MFSD8, NACC1, NRXN1, PCDH19, PIGA, PIGN, PLCB1, PNKP, POLG, PRRT2, PURA, QARS, SCN1A, SCN1B, SCN2A, SCN8A, SETD5, SIK1, SLC12A5, SLC13A5, SLC16A2, SLC2A2, SLC2A3, SLC6A1, SLC9A6, SMC1A, SPTA1, STX1B, STXBP1, SYNGAP1, TBC1D24, TCF4, TBP1, UBE2A, UBE3A, UNC80, WDR45, WWOX, ZEB2

**List of genes removed from panel**
CBL, CSNK1G1, FASN, GABBR2, PIGQ, RYR3

**Technical**
Mutation screening is carried out by next generation sequencing with library preparation using a Sure Select XT custom kit followed by sequencing on the Illumina MiSeq. Data is analysed using an in-house pipeline with all mutations confirmed by Sanger sequencing.

**Target reporting time**
4 months for a full mutation screen in an index case (next generation sequencing).
4 weeks for family follow-up testing.
Please contact the laboratory for urgent cases.
**Fragile X Syndrome**

**Contact details**
Regional Genetics Service  
Levels 4-6, Barclay House  
37 Queen Square  
London, WC1N 3BH  
T +44 (0) 20 7762 6888  
F +44 (0) 20 7813 8578

**Samples required**
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

**Patient details**
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

**Introduction**
Fragile X syndrome (MIM 309550) is an X-linked mental retardation syndrome associated with dysmorphic features (large everted ears, coarse facies, elongated face, macro-orchidism) in a proportion of cases. Around 1 in 5000 of the population is affected with fragile X, they are predominantly male but females can also be affected. The majority of fragile X cases are caused by expansion of the (CGG)n repeat in the promoter region of the FMR1 gene on chromosome Xq27.3 (FRAX A cases). Expansion of the (CGG)n repeat sequence to >200 repeats accompanied by methylation of the adjacent CpG island extinguishes the FMR1 gene expression (full mutation expansion). Premutation alleles with 59-200 (CGG)n repeats are unstable at meiosis and can lead to full expansion mutations in subsequent generations. Intermediate alleles (46-58 repeats) are not believed to be associated with fragile X syndrome, but may display size instability in future generations. FMR1 point mutations and deletions are rare causes of the syndrome. Premutation allele carriers can display additional phenotypes such as premature ovarian failure (POF) and a neurodegenerative disorder of older adults, fragile X associated tremor/ataxia syndrome (FXTAS).

**Referrals**
Children/adults (male or female) in whom a diagnosis of fragile X syndrome is suspected. Adults with a suspected clinical diagnosis of POF and FXTAS. Carrier testing for adults with a confirmed or suspected family history of fragile X syndrome. Prenatal samples (see below).

**Prenatal testing**
Prenatal testing is available for confirmed fragile X carriers - analysis can be carried out on prenatal samples by direct analysis of the FMR1 (CGG)n repeat and/or by linked marker analysis if samples from the relevant family members are available.

**Service offered**
Direct analysis of the FMR1 (CGG)n repeat to identify intermediate alleles, premutations and full mutations. Linked marker analysis is available in families where we are unable to identify a mutation in a clinically affected individual: this relies on the clinical diagnosis being correct and sample availability from the affected individual and appropriate family members.

**Technical**
DNA is analysed by PCR of the (CGG)n repeat within the 5’ untranslated region of the FMR1 gene. The AmplideX™ FMR1 PCR kit is used to detect large premutations and full mutations which cannot be detected using the routine PCR assay. Neither of these assays is able to detect point mutations or deletions within the FMR1 gene, and they are also unable to exclude mosaicism. Please note that the PCR/ AmplideX™ assay are not methylation sensitive and provide no information on the methylation status of the FMR1 promoter.

**Target reporting time**
Routine analysis - 4 weeks for the initial PCR-based mutation screen (and AmplideX™ if required). For urgent samples please contact the laboratory.
**Albrights Hereditary Osteodystrophy / Pseudohypoparathyroidism type 1a / Pseudopseudohypoparathyroidism (AHO/PHP1a/PPHP) & Acrodysostosis**

**Introduction**

Albright’s hereditary osteodystrophy (AHO) is an autosomal dominant disorder characterised by short stature, obesity, brachydactyly, subcutaneous ossifications and mental defects. There is a 2:1 ratio of affected females to males. AHO can present in one of two ways: with the somatic features of AHO alone (pseudopseudohypoparathyroidism, PPHP); or with AHO plus resistance to multiple hormones which increase cAMP in their target organs (pseudohypoparathyroidism type 1a, PHP 1a). Both PHP1a and PPHP are caused by inactivating pathogenic variants in the GNAS1 gene. PHP1a is usually caused by pathogenic variants in maternal GNAS1, PPHP in paternal allele.

GNAS1 encodes the α subunit of the G protein Gs. The G proteins are a family of guanine nucleotide binding proteins involved in transmembrane signalling. They form heterotrimeric of α, β and γ.

GNAS1 (located on 20q13.3) has 13 exons, 6 polyadenylation sites 3’ and 4 isofoms (due to differential splicing of exons 3 and 4). There are two alternatively spliced transcripts using exons upstream of GNAS1 (termed XLαs and NESP55) spliced to GNAS1 ex2-13 (+/- exon 3) expressed in most fetal tissue. Although the gene is biallelically expressed in most fetal tissue, XLαs is only expressed from the paternal chromosome and NESP55 only expressed from the maternal chromosome.

Pathogenic variants in the PRKAR1A and PDE4D genes are associated with Acrodysostosis type 1 and 2 respectively. Acrodysostosis is a type of skeletal dysplasia and is a differential diagnosis of AHO. Clinical symptoms include facial dysostosis, short stature, nasal hypoplasia and severe brachydactyly. Patients may also have advanced bone age, obesity and hormone resistance. PDE4D (5q11.2-q12.1) encodes a cAMP-specific phosphodiesterase (PDE). PRKAR1A (17q24.2) encodes a cAMP-dependent regulatory subunit of protein kinase A.

We also offer testing for GNAS1 somatic mutations for two codons associated with McCune Albright syndrome. For further information, please refer to the McCune Albright syndrome pack sheet.

**Referrals**

- Patients with clinical symptoms as above.
- Familial mutation testing for family members.

**Prenatal testing**

Prenatal testing is available for families in whom specific pathogenic variants have been identified or in whom appropriate family studies have been undertaken - please contact the laboratory to discuss.

**Service offered**

Analysis of GNAS1, PDE4D & PRKAR1A genes by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is not currently validated to detect large deletions / duplications. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported.

**Target reporting time**

The target reporting time is 8 weeks for a diagnostic screen and 4 weeks for familial testing. Please contact the laboratory for urgent cases.
IL10-related Infantile Inflammatory Bowel Disease

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
Inflammatory bowel disease (OMIM # 266600) is a heterogeneous group of disorders commonly presenting in adolescence or adulthood, but may present in infancy and be inherited as an autosomal recessive condition.

Interleukin-10 (IL10) is an anti-inflammatory cytokine secreted by several cell types that is essential for the regulation of immune homeostasis in the gastro-intestinal tract. IL10 activates downstream signalling pathways by binding to the IL10 receptor, comprised of 2 α subunits (encoded by IL10RA) and 2 β subunits (encoded by IL10RB).

Loss-of-function pathogenic variants in IL10 at 1q32.1 (NM_000572.2), IL10RA at 11q23.3 (NM_001558.3) and IL10RB (NM_000628.3) are associated with hyper-inflammatory immune responses in the intestine resulting in severe, infantile-onset inflammatory bowel disease.

Referrals
Referrals are accepted from Consultant Clinical Geneticists and Consultant Paediatric Gastroenterologists in the following:
- Patients with clinically suspected IL10-related inflammatory bowel disease
- Carrier testing in family members for known clearly pathogenic variants
- For clinical enquiries, please contact Dr Neil Shah, Gastroenterology, GOSH
Tel: +44 (0) 20 7405 9200 ext 5949, email: Neil.Shah@gosh.nhs.uk

Prenatal testing
Prenatal testing is available for families in whom clearly pathogenic variants have been identified- please contact the laboratory to discuss.

Service offered
Analysis for substitutions and small insertions / deletions is by direct Sanger sequencing analysis of the coding regions of IL10, IL10RA and IL10RB in 21 amplicons.

Technical
Molecular screening is carried out by direct sequencing analysis.

Target reporting time
The target reporting time is 8 weeks for an IL10 / IL10RA / IL10RB full screen and 4 weeks for familial testing. Please contact the laboratory for urgent cases.
Lynch syndrome

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7752 6888
F +44 (0) 20 7813 8578

Samples required
- Tumour testing: FFPE tissue. 4x5um unstained slides with indication of tumour content and a H&E stained slide with region of tumour highlighted
- Germline testing: 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
Lynch syndrome is an inherited cancer syndrome which accounts for 5-10% of cases of inherited colon cancer. It is characterised by a family history of colon cancer at an early age, but can also present with other tumours including endometrial, small bowel, pancreatic, biliary tract, stomach, ovarian, urinary tract and brain.

It is caused by defective DNA mismatch repair as a result of pathogenic variants in one of the mismatch repair genes: MLH1, MSH2, MSH6, EPCAM or PMS2. Lynch syndrome may be indicated in patients with a tumour showing loss of expression of one or more of the MLH1, MSH2, MSH6 or PMS2 proteins and/or microsatellite instability. Loss of MLH1/PMS2 protein expression may also arise sporadically due to somatic hypermethylation of the MLH1 gene.

Referrals
Referrals are accepted from Clinical Genetics, family cancer clinics and approved gastroenterologists.

Service Offered
- Tumour testing: Microsatellite instability testing (MSI). MLPA analysis to detect hyper-methylation of MLH1 in patients with loss of MLH1/PMS2 protein expression
- Germline DNA testing: Next generation sequencing of the MLH1, MSH2 and MSH6 genes is offered to individuals who meet the criteria defined by the revised Bethesda and Pan Thames guidelines. MLPA analysis to detect large gene deletions and duplications in the MLH1, MSH2, MSH6 and EPCAM genes.
- Predictive testing is offered to individuals who have a known familial pathogenic variant in the MLH1, MSH2, MSH6 or EPCAM gene. A familial control is required for analysis.
- PMS2 gene testing is not currently offered

Technical
Tumour testing: MSI testing is carried out using the Promega Microsatellite kit which contains 5 mononucleotide markers Bat25, Bat26, M mono27, NR1, NR24). MLH1 hypermethylation testing is carried out using methylation specific MLPA analysis.
Germline testing is carried out by next generation sequencing with library preparation using an Agilent SureSelect kit followed by sequencing on the Illumina NextSeq or MiSeq. Data is analysed using an in-house pipeline with all pathogenic variants confirmed by Sanger sequencing. Detection of large deletions/duplications in MLH1, MSH2, MSH6 and EPCAM is carried out using MLPA analysis.
Predictive testing is carried out by Sanger sequencing or MLPA as appropriate.

Target reporting time
6 weeks for tumour testing. 8 weeks for a full screen in an index case. 2 weeks for a predictive test. Please contact the laboratory for urgent cases.

Please note that tumour testing (MSI and MLH1 hypermethylation testing) for Lynch syndrome is not currently included in our scope for UKAS accreditation.
**McCune Albright Syndrome (Somatic GNAS1 testing)**

**Contact details**
Regional Genetics Service  
Levels 4-6, Barclay House  
37 Queen Square  
London, WC1N 3BH  
T +44 (0) 20 7762 6888  
F +44 (0) 20 7813 8578

**Samples required**
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

**Patient details**
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

**Introduction**
McCune Albright syndrome (MAS) is characterised by precocious puberty, café au lait spots and polyostic fibrous dysplasia of bone where the normal interior of bone is replaced by fibro-osseous connective tissue. McCune Albright syndrome is caused by somatic activating pathogenic variants in exons 8 and 9 of GNAS1 (codons p.Arg201 and p.Gln227 respectively, NM_00516.5). All MAS patients are mosaics.

GNAS1 encodes the α subunit of the G protein Gs. The G proteins are a family of guanine nucleotide binding proteins involved in transmembrane signalling. They form heterotrimers of α, β and γ.

We also offer a full diagnostic screen of GNAS1 for Albright’s Hereditary dystrophy (AHO) and related disorders. Please refer to the AHO & Acrodysostosis service sheet.

**Referrals**
Patients with clinical symptoms as above.
As we offer other types of GNAS1 testing, to prevent any delay to testing, please clearly state on the referral form that McCune Albright syndrome testing is required.

**Service offered**
Targeted next generation sequencing (NGS; Illumina MiSeq) for the p.Arg201 and p.Gln227 pathogenic variants. This has shown increased sensitivity for low level mosaicism compared to Sanger sequencing. DNA from an affected tissue such as bone has given more successful results than DNA extracted from lymphocytes.

**Target reporting time**
The target reporting time is 4 weeks for MAS mutation screening. Please contact the laboratory for urgent cases.
Pulmonary Surfactant Metabolism Dysfunction / Pulmonary Fibrosis

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7613 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
Pulmonary surfactant metabolism dysfunction / pulmonary fibrosis comprise a genetically heterogeneous group of disorders that result in severe respiratory insufficiency or failure in full-term neonates, or infants. These disorders are associated with various pathologic entities, including pulmonary alveolar proteinosis (PAP), desquamative interstitial pneumonitis (DIP), cellular nonspecific interstitial pneumonitis (NSIP), or pulmonary fibrosis. A number of genes are associated with surfactant metabolism dysfunction, pulmonary (SMDP), including SFTPB (SMDP1 – autosomal recessive), SFTPC (SMDP2 – autosomal dominant), ABCA3 (SMDP3 – autosomal recessive), and CSF2RB (SMDP5 – autosomal recessive). The NKX2-1/TITF1 gene is associated with a neonatal respiratory distress syndrome and is autosomal dominant. The SFTPA2, TERC, and TERT genes are associated with pulmonary fibrosis.

Referrals
- Full term neonates with severe respiratory distress of unknown aetiology and older children with respiratory distress of unknown cause.
- Carrier testing can be offered to the adult relatives of patients once a pathogenic variant has been identified.

Prenatal testing
Prenatal testing is available for families in whom pathogenic variants have been identified or in whom appropriate family studies have been undertaken; please contact the laboratory to discuss.

Service offered
- Analysis of the ABCA3, CSF2RB, NKX2-1/TITF1, SFTPA2, SFTPB, SFTPC, TERC, and TERT genes by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is not currently validated to detect large deletions / duplications. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported.
- Detection of known pathogenic variants in relatives of patients with confirmed pathogenic variants by Sanger sequencing.

Target reporting time
8 weeks for NGS screening. 4 weeks for routine testing of familial pathogenic variants. Please contact the laboratory for urgent cases.
Interferon Regulatory Factor 6 (IRF6) gene disorders
(Popliteal Pterygium syndrome and Van der Woude syndrome)

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
• 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
• Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
• Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
• A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
Van der Woude syndrome (VWS; MIM) and Popliteal Pterygium syndrome (PPS) are allelic autosomal dominant disorders caused by pathogenic variants in the interferon regulatory factor 6 gene (IRF6; MIM *607199). VWS is the most common form of cleft lip and/or palate accounting for 1-2% of cases. Lip pits and/or sinuses are cardinal features of the syndrome present in 70-80% of patients. PPS combines the symptoms of VWS with popliteal webs, unusual nails, syndactyly, ankyloblepharon and genital abnormalities.

It has been proposed that orofacial development is affected in VWS as a result of haploinsufficiency with protein truncating pathogenic variants commonly identified throughout the IRF6 gene. The features of PPS are thought to result from dominant negative pathogenic variants (generally missense) in the DNA binding domain of the protein. Confirmation of diagnosis enables prenatal testing for PPS and clarification of recurrence risk for VWS (50% as opposed to 3-5% for isolated cleft/lip palate families).

The IRF6 gene (1q32-q41) has 9 exons (exons 1 and 2 are non-coding). c.250C>T p.(Arg84Cys) and c.251G>A p.(Arg84His) are recurrent pathogenic variants identified in PPS patients. A variety of point variants and small deletions have been identified in VWS located throughout the IRF6 gene.

Referrals
• Referrals are only accepted via a Clinical Geneticist or cleft surgeon.
• Testing of other family members will be possible upon identification of a pathogenic variant in the index case.

Prenatal testing for Popliteal Pterygium syndrome
Prenatal testing is available for families in whom pathogenic variants causing PPS have been identified or in whom appropriate family studies have been undertaken. Please contact the laboratory to discuss.

Service offered
Analysis is by next generation sequencing of the entire coding region of IRF6 (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is not currently validated to detect large deletions / duplications. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported.

Testing for known pathogenic variants in relatives of patients with confirmed PPS / VWS mutations by sanger sequencing.

Contact details for Consultant Cleft Geneticist at Great Ormond Street
Dr M Lees, Clinical Genetics, Great Ormond Street Hospital, Level 4 Barclay House, 37 Queen Square, London WC1N 3BH

Target reporting time
8 weeks for routine screen in an index case. 4 weeks for familial testing. Please contact the laboratory for urgent cases.
Prader-Willi Syndrome

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7613 8578

Introduction
Prader-Willi syndrome (PWS) (MIM 176270), occurring in 1/15000 - 1/20000 individuals, is characterised by diminished fetal activity, obesity, muscular hypotonia, developmental delay, short stature, hypogonadotropic hypogonadism, and small hands and feet. The PWS phenotype results from the lack of a paternal contribution at 15q11-q13. This can be caused by a deletion (~70%), maternal uniparental disomy (UPD) (25-30%) and rarely due to mutations in the imprinting centre (IC) that cause abnormal methylation at exon alpha of the SNRPN locus. These are all detected by disrupted methylation. Deletions and UPD are usually de novo events, associated with low recurrence risks, although it is important to determine whether either parent of an affected child has a predisposing chromosomal translocation. There is a recurrence risk of up to 50% in families with confirmed PWS who do not have a deletion or UPD and are therefore likely to have an IC mutation.

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Referrals
- Confirmation of clinically suspected PWS in children/adults.
- Investigation of the molecular defect in confirmed PWS cases, distinguishing between UPD, deletion and IC mutations (parental samples required).
- Carrier testing in adult relatives of confirmed PWS patients who are suspected of having an IC mutation (samples from appropriate family members are required).

Prenatal testing
Prenatal diagnosis is available to couples where PWS has been confirmed in the family and to couples at risk of having a child affected with PWS due to a balanced chromosomal rearrangement involving chromosome 15 in one of the parents. Please contact the laboratory to discuss each case prior to sending prenatal samples to the laboratory.

Service offered
Confirmation of a PWS diagnosis by methylation analysis and microsatellite analysis to determine the molecular defect in confirmed cases (requires samples from appropriate family members).

Technical
For diagnostic referrals, the initial test is to determine the methylation status of exon alpha of the SNRPN gene. Methylation analysis is undertaken by methylation specific PCR following bisulphite modification of genomic DNA. Normal individuals yield a 313bp maternally derived fragment and a 221bp paternally derived fragment. Patients with Prader-Willi syndrome show a single 313bp maternal fragment only.
Positive results are confirmed by either MS-MLPA or aCGH analysis. Chromosome 15 microsatellite markers from within and flanking the commonly deleted region can also be used to characterise the mechanism in patients shown to have abnormal methylation. Cytogenetic analysis is also helpful in identifying deletions and predisposing parental translocations. NB: A similar testing process is undertaken for Angelman syndrome.

Target reporting time
Routine analysis - the initial methylation test takes up to 4 weeks. Microsatellite marker analysis takes 4 weeks from receipt of parental samples. Please contact the laboratory for urgent cases.
Primary Congenital Glaucoma (PCG)

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
Primary congenital glaucoma (PCG; MIM: #231300) is a congenital or infantile onset condition, characterised by raised intraocular pressure. Signs of this may include enlargement of the globe (buphthalmos), cloudy/hazy corneas, lacrimation and optic disc changes. The condition causes irreversible optic nerve damage and blindness if untreated.

This autosomal recessive condition has an incidence of 1/10,000 and is caused by pathogenic variants in the CYP1B1 gene (MIM:*601771) located at 2p22.2.

Patients with primary congenital glaucoma (PCG) are defined by elevated intraocular pressure (IOP) >21 mm Hg and/or signs consistent with elevated IOP, including
- disc cupping >0.3 or disc asymmetry ≥0.2
- progressive disc cupping
- buphthalmos (prominent, enlarged eye)
- enlarged corneal diameter (>11 mm in newborn, >12 mm in a child <1 year, or >13 mm in a child >1 year)
- corneal edema
- Descemet's membrane splits (Haab's striae)
- visual field defects
- progressive myopia in a child < 2 years of age.

Sensitivity: 50% of patients with the symptoms described above will be expected to have pathogenic variants in CYP1B1. No other genes have yet been described.

Later-onset disease (e.g. in middle childhood/adolescence) caused by CYP1B1 pathogenic variants is rarely reported.

Referrals
Referrals are accepted from Consultant Clinical Geneticists and Consultant Paediatric Ophthalmologists in the following:
- Patients with the symptoms detailed above
- Carrier testing in family members for familial pathogenic variants

Prenatal testing
Prenatal testing is available for families in whom specific pathogenic variants have been identified or in whom appropriate family studies have been undertaken - please contact the laboratory to discuss.

Service offered
Next generation sequencing of CYP1B1 gene with variant confirmation by Sanger sequencing. Testing for previously identified pathogenic variants is available to other family members.

Technical
Analysis is by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is not currently validated to detect large deletions / duplications. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported.

Target reporting time
The target reporting time is 8 weeks for a CYP1B1 screen and 4 weeks for carrier testing. For urgent samples please contact the laboratory.
### SMARCB1 – Rhabdoid Tumour

**Contact details**
Regional Genetics Service  
Levels 4-6, Barclay House  
37 Queen Square  
London, WC1N 3BH  
T +44 (0) 20 7762 6888  
F +44 (0) 20 7813 8578

**Samples required**
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)  
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.  
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis  
- A completed DNA request card should accompany all samples

**Patient details**
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

**Introduction**
Rhabdoid tumour predisposition syndrome (MIM# 609322) is a rare syndrome occurring in infancy and early childhood. The incidence is not currently known but is thought to occur in ~ 0.1-0.05 per million children per year. Rhabdoid tumours are highly malignant neoplasms that develop in the brain and spinal cord (atypical teratoid rhabdoid tumour), kidney and/or soft tissue (malignant rhabdoid tumour or extra-renal rhabdoid tumour). Patients may present with apparently sporadic tumours in one site or with multiple tumours arising in the brain, kidney and/or soft tissues. Approximately 1/3 of rhabdoid tumours are due to germline pathogenic variants in the **SMARCB1** gene. The **SMARCB1** gene encodes the INI-1/hSNF5 protein which is a member of the SW1/SNF chromatin re-modelling complex and functions in controlling gene transcription.

Rhabdoid tumours are highly resistant to conventional chemotherapies and radiotherapy; patients frequently succumb rapidly to disease.

Loss of protein expression of SMARCB1 can be detected by immunohistochemistry, however a small number of tumours show expression.

**Referrals**
Referrals are accepted only via a Consultant Clinical Geneticist.

**Prenatal testing**
Prenatal testing is available for families in whom pathogenic variants have been identified on referral from a Clinical Genetics unit only.

**Service offered**
Next generation sequencing of the **SMARCB1** gene with clinically relevant variants confirmed by Sanger sequencing. MLPA to detect large deletions and duplications.

**Technical**
Testing is carried out by next generation sequencing with library preparation using an Agilent SureSelect kit followed by sequencing on the Illumina NextSeq or MiSeq. Data is analysed using an in-house pipeline with all mutations confirmed by Sanger sequencing. Dosage analysis is by Multiplex Ligation-dependent Probe Amplification (MLPA) analysis.

**Target reporting time**
8 weeks for screen in index case (next generation sequencing and MLPA). 4 weeks for familial testing. For urgent samples please contact the laboratory.
Rett Syndrome

Introduction

Rett syndrome (MIM 312750) is an X-linked dominant neurodevelopmental disorder that occurs mainly in females. It is characterized by arrested development between 6 and 18 months of age, regression of acquired skills, loss of speech, stereotypical movements (classically of the hands), microcephaly, seizures and mental retardation. Approximately 90% of cases with classical RTT are caused by mutations in the methyl-CpG-binding-protein 2 (MECP2) gene on Xq28 (MIM 300005). MECP2 mutations have also been reported in individuals with Angelman-like syndrome, non-specific mental retardation and neonatal severe encephalopathy.

Samples required

- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Referrals

- Confirmation of diagnosis in individuals clinically suspected of having a MECP2-related disorder.
- Maternal carrier testing once a mutation has been identified in the proband (a mother who has a MECP2 mutation may have favourably skewed X-chromosome inactivation that results in her being unaffected or mildly affected).

Prenatal testing

Prenatal testing is available for couples 1) when the mother has been found to be a carrier 2) to rule out recurrence due to germ-line mosaicism in either parent when a mutation has been identified in their affected child - please contact the laboratory to discuss.

Service offered

- **MECP2**
  - MLPA analysis of the MECP2 gene to identify large deletions and duplications
  - Mutation screening analysis of MECP2 gene (all 4 exons and intron-exon boundaries) by next generation sequencing (Agilent SureSelect and Illumina NextSeq).

Technical

Dosage analysis is by Multiplex Ligation-dependent Probe Amplification (MLPA). MECP2 uses kit P015-F2 from MRC-Holland where 17 MECP2 probes cover all 4 exons. Mutation screening is carried out by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported.

Nomenclature

Nomenclature for the MECP2 gene is based on reference sequence NM_001110792.1 - this encodes MECP2-e1, is the longest isoform of the MECP2 protein and encompasses exon 1 but not exon 2 of the MECP2 gene. It has been shown that this is the predominant isoform expressed in the brain and central nervous system. NM_004992 encodes MECP2_e2 which encompasses exons 2, 3 and 4 and was the originally identified transcript. It was later shown that expression of this transcript is mainly confined to fibroblast and lymphoblast cells.

Target reporting time

8 weeks for routine sequence and dosage analysis of MECP2. Please contact the laboratory if urgent or prenatal testing is required.
X-Inactivation

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
The X-inactivation status of females may be determined using X-linked methylation sensitive polymorphic markers. This information may be useful to explain the manifestation of X-linked recessive conditions in females or to indicate carrier status for certain X-linked disorders.

In females, random X-inactivation/lyonisation occurs where one of the two X chromosomes is randomly inactivated in every somatic cell. Hence the expression levels of most genes on the X chromosome are similar in males and females. However, 5-20% of the normal female population appear to have non-random or skewed X-inactivation. Non-random X-inactivation is also thought to increase with age. In certain conditions, if a female has a mutation in a given gene on one X chromosome then non-random X-inactivation can occur, but this can be tissue dependent and therefore care must be taken to ensure the most appropriate tissue is analysed.

The technique can be applied to any appropriate condition, however in this laboratory X-inactivation studies are most commonly used to indicate carrier status for the immunodeficiency conditions Wiskott Aldrich syndrome (WAS), X-linked severe combined immunodeficiency (XSCID) and X-linked agammaglobulinaemia (XLA). In these conditions carrier females have unilateral X-inactivation patterns in their whole blood, T cells only, or B cells only, respectively (separated cells will be required for this analysis, please see information below).

Referrals
- To indicate carrier status of females with a suspected family history of the immunodeficiency disorders, WAS, XSCID and XLA, where no sample is available from the affected male or where no mutation has been identified.
- For studies in other X-linked recessive conditions, please contact the laboratory to discuss.

Service offered
X-inactivation status at the androgen receptor (AR) gene, Xq11-q12 (MIM 313700).

Technical
A methylation sensitive restriction enzyme is used to detect differential methylation patterns between the inactive and active X chromosomes. The methylation sensitive sites are in close proximity to the polymorphic site allowing the two X chromosomes to be distinguished. The androgen receptor is very informative and has a heterozygosity of 90%.

Target reporting time
Routine analysis (immunodeficiency disorders) - 4 weeks. For urgent samples please contact the laboratory.
For studies in other diseases please contact the laboratory to discuss the utility of the analysis, the samples required and the expected reporting time.
Zygosity testing

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
Molecular analysis of polymorphic markers can be used for ‘DNA fingerprinting’ to determine the zygosity of twins. Monozygotic (identical) twins will inherit the same alleles from their parents for all of the markers tested, whereas dizygotic twins are likely to inherit different alleles (but they may inherit the same alleles by chance). The likelihood of monozygosity can be determined by testing both parents and twins and calculating the likelihood of the same alleles being inherited by chance. If parents are not available then samples from the twins alone may be used and allele frequencies used to calculate the likelihood that the same alleles have been inherited by chance, however there is limited data available for allele frequencies in different ethnic groups.

Referrals
Zygosity studies are often requested when one twin has developed a clinical phenotype which is thought to be genetic in origin. These studies may help to establish the recurrence risk.

Service offered
9 highly polymorphic markers plus the amelogenin locus (a segment of the X-Y pseudoautosomal region) are analysed.

Technical
Zygosity analysis makes use of an AmpfISTR Profiler Plus PCR amplification kit (manufactured by Applied Biosystems). This contains primers to amplify by PCR 9 polymorphic markers on 9 different chromosomes, plus the amelogenin locus. Different alleles are detected by size differentiation and analysed on a genetic analyser. Allele frequencies for the US Caucasian and Afro-American population have been determined and can be used to calculate the likelihood of monozygosity to greater than 99% probability in these ethnic groups, when parents are not available.

Target reporting time
Routine analysis 4 weeks.
Please contact the laboratory for urgent cases.
**Introduction**

Free fetal DNA may be detected in maternal plasma from early in gestation and used for determination of fetal gender. The sex of the fetus is determined by the presence of Y-specific sequence for a male fetus and the absence of Y-specific material in the cell-free DNA extract in the case of a female fetus.

The analytical sensitivity and specificity of the Real Time PCR assay was measured in 189 pregnancies (394 tests) over a period of 2 years from April 2007 to March 2009. When audited against pregnancy outcome there were 145 cases with a known outcome and in these cases the test demonstrated 100% (95% CI 97.5-99.9) concordance with no false positives or false negatives.

This is achieved by testing two separate maternal samples for the presence of SRY and by stipulating that the fetus is at least 7 weeks gestation at the time of sampling.

**Referrals**

All referrals should be made via a Clinical Genetics Department or Fetal Medicine Unit, please contact the laboratory in advance of sending a sample. Samples are accepted from patients from 7 weeks gestation (confirmed by scan) at which time there should be a sufficient concentration of free fetal DNA in the circulation.

*From 7 to 9 weeks gestation, 2 x 10ml samples are required, taken one week apart. At 9+ weeks gestation 2 x 10ml samples may be taken at the same time. Samples should be sent to arrive in the laboratory within 24 hours of sampling (24-48 hours for Streck tubes) if possible. Advance notice is required so samples can be processed rapidly upon receipt. Information on the outcomes of the pregnancy will be requested as part of a national ethically approved audit. Information sheets for parents and the audit are available on our laboratory website.

**Service offered**

We offer this service to pregnancies at risk of X-linked disorders or congenital adrenal hyperplasia. It is not available for non-medical indications. This test may not be applicable in multiple pregnancies including those with a possible vanishing twin.

A male fetus is detected by the presence of SRY-specific sequence. The assay cannot distinguish between a lack of SRY indicative of a female fetus and a failure to extract sufficient free fetal DNA for analysis. A second sample ideally at later date but dependent on the gestation age is therefore required to repeat the analysis. Consistent absence of SRY in the presence of the control marker is taken as evidence that the fetus is female.

**Technical**

Maternal EDTA blood is separated as rapidly as possible after collection. Cell-free DNA is then extracted from the plasma. Molecular analysis is performed using real time PCR and Taqman assays for the SRY marker and a CCR5 control marker. Results of the duplicate analysis will be released following analysis of the second sample.

**Target reporting time**

The results of the Y-specific probe analysis should be available within 3 days of the laboratory receiving the second sample.
NIPD for FGFR3-related skeletal dysplasias

Introduction

Achondroplasia (ACH) (MIM 100800) is an autosomal dominant skeletal disorder due to mutations in the FGFR3 gene on chromosome 4p16.3. Around 80-90% of cases are sporadic. Thanatophoric Dysplasia (TD), a sporadic neonatal lethal skeletal dysplasia, is divided into two subsets based upon radiological findings. TD type I (MIM 187600) is associated with curved femora and variable and milder craniosynostosis and TD type II (MIM 187601) with straight femora and often cloverleaf skull. Mutations in the FGFR3 gene have been identified in almost 100% of confirmed cases of TD. A single mutation, p.(Lys650Glu), accounts for all TD type II patients reported to date. Several recurrent mutations have been identified in TD type I. Hypochondroplasia (HCH) (MIM 146000) has very similar skeletal features to those seen in ACH but tends to be milder. About 70% of affected individuals are heterozygous for a mutation in FGFR3. Non-invasive prenatal genetic diagnosis (NIPD) by next generation sequencing (NGS) is possible using cell free fetal DNA (cffDNA) in pregnancies at risk of FGFR3-related skeletal dysplasias.

Referrals

All referrals should be made via a Clinical Genetics Department or Fetal Medicine Unit and will be accepted in either of the categories given below. If you wish to refer a case which does not fulfil these criteria please contact Professor Lyn Chitty (l.chitty@ucl.ac.uk) (Clinical) or the laboratory (Genetics.Labs@gosh.nhs.uk).

1. At risk pregnancy
   - Paternal FGFR3-related skeletal dysplasia OR a previous pregnancy has been confirmed to have FGFR3-related skeletal dysplasia, thus there is a very small risk of recurrence due to germline mosaicism

2. Abnormal ultrasound findings

Achondroplasia
- Femoral length on or above the 3rd percentile (i.e. within normal range) at routine 18-20 week scan AND femur length and all long bones below 3rd percentile after 25 weeks gestation AND head circumference and abdominal circumference within or above the normal range for gestation at diagnosis. Fetal and maternal dopplers should be normal

Thanatophoric dysplasia
- The following features must be present: All long bones below the 3rd percentile AND small chest with short ribs
- Additional features include polyhydramnios, bowed femora, relative macrocephaly, cloverleaf skull, short fingers

Service offered

NGS for FGFR3 mutations associated with skeletal dysplasia.

Technique

Maternal EDTA blood is spun as soon as possible after collection, cffDNA is extracted from plasma. Molecular analysis is performed by PCR, followed by NGS (Illumina MiSeq). Amplification of fetal DNA will be confirmed using genetic analysis in males or by the presence of paternal HLA sequences. When this is uninformative a separate NGS assay is used containing 50 heterogeneous SNPs to identify paternally inherited alleles.

Target reporting time

Results are normally available within 5 days of sample receipt.
Disease

NIPD for Apert syndrome

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- Pregnant Women
  2x 10mls venous blood in plastic EDTA bottles or glass Streck tubes, this should ideally reach the laboratory within 24-48 hours of sampling
- The minimum gestation (by scan) is 9wks for accepting a sample. If earlier than 18wks then 2 blood samples a week apart may be required
- Testing must be arranged in advance, through your Local Clinical Genetics Department or Fetal Medicine Unit
- A completed DNA request card and ultrasound report should accompany all samples with an appropriate telephone number and a secure fax number.
- Pregnancy outcome
  Details of pregnancy outcome will be required for confirmation of laboratory results as part of the ongoing validation of new tests

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address), details of any relevant family history and full contact details for the referring clinician

Introduction
Apert syndrome (MIM 101200) is a congenital disorder characterised primarily by craniosynostosis, midface hypoplasia, and syndactyly of the hands and feet with a tendency to fusion of bony structures. Most cases are sporadic, but autosomal dominant inheritance has been reported. Apert syndrome can be very severe and is easily distinguishable from other craniosynostosis syndromes. Two mutations in FGFR2 exon 8, c.755C>G p.(Ser252Trp) and c.758C>G p.(Pro253Arg), account for over 98% of reported cases.

Non-invasive prenatal genetic diagnosis (NIPD) is now possible using cell free fetal DNA (cffDNA) in pregnancies at risk of Apert syndrome.

Referrals
All referrals should be made via a Clinical Genetics Department or Fetal Medicine Unit and will be accepted in either of the categories given below. If you wish to refer a case which does not fulfil these criteria please contact Professor Lyn Chitty (l.chitty@ucl.ac.uk) (Clinical) or the laboratory (Genetics.Labs@gosh.nhs.uk)
1. At risk pregnancy
   - Paternal Apert syndrome OR
   - a previous pregnancy has been confirmed to have Apert syndrome, thus there is a very small risk of recurrence due to germline mosaicism
2. Abnormal ultrasound findings
   - Acrocephaly AND
   - Symmetrical syndactyly

Service offered
Targeted next generation sequencing (NGS) for c.755C>G p.(Ser252Trp), c.758C>G p.(Pro253Arg) and c.755_756delinsTT p.(Ser252Phe) mutations in FGFR2.

Technical
Maternal EDTA blood is spun as soon as possible after collection, cffDNA is extracted from plasma. Molecular analysis is performed by PCR, followed by NGS (Illumina MiSeq). Amplification of fetal DNA will be confirmed using gender analysis in males or by the presence of paternal HLA sequences. When this is uninformative a separate NGS assay is used containing 50 heterogeneous SNPs to identify paternally inherited alleles.

We cannot rule out the possibility of false negative results due to the complex architecture of fetal cell free DNA confounding this analysis. To date, we have carried out over 500 tests on 93 different amplicons and identified one true false negative case (1.1%) that may be attributable to non-random shearing of the fetal cell free DNA.

We continue to collect outcomes and request a cord blood sample from the baby at birth.

Target reporting time
Results are normally available within 5 days of sample receipt.
Introduction

Crouzon syndrome is an autosomal dominant disorder characterised by craniosynostosis causing secondary alterations of the facial bones and facial structure. Crouzon syndrome with acanthosis nigricans (CAN) (MIM 612247) presents with congenital craniofacial abnormalities consistent with classic Crouzon syndrome plus velvety hyperpigmentation of the skin and is caused by a single missense mutation in FGFR3 (c.1172C>A p.(Ala391Glu)). Choanal atresia or stenosis is often present (41%), and is considered highly suggestive of CAN. Other commonly reported signs include hydrocephalus (43%), oral abnormalities such as cleft palate, dental malocclusion, and cementomas of the jaw (34%), and melanocytic nevi (25%). Some of these specific features are rare in patients with classic Crouzon syndrome.

Non-invasive prenatal genetic diagnosis (NIPD) is now possible using cell free fetal DNA (cffDNA) in pregnancies at risk of Crouzon syndrome with acanthosis nigricans (CAN).

Referrals

All referrals should be made via a Clinical Genetics Department or Fetal Medicine Unit and will be accepted in either of the categories given below. If you wish to refer a case which does not fulfil these criteria please contact Professor Lyn Chitty (l.chitty@ucl.ac.uk) (Clinical) or the laboratory (Genetics.Labs@gosh.nhs.uk).

- Paternal Crouzon syndrome with acanthosis nigricans (CAN) syndrome
- a previous pregnancy has been confirmed to have Crouzon syndrome with acanthosis nigricans (CAN), thus there is a very small risk of recurrence due to germline mosaicism

Targeted next generation sequencing (NGS) for FGFR3 c.1172C>A p.(Ala391Glu).

Technical

Maternal EDTA blood is spun as soon as possible after collection, cffDNA is extracted from plasma. Molecular analysis is performed by PCR, followed by NGS (Illumina MiSeq). Amplification of fetal DNA will be confirmed using gender analysis in males or by the presence of paternal HLA sequences. When this is uninformative a separate NGS assay is used containing 50 heterogeneous SNPs to identify paternally inherited alleles.

We cannot rule out the possibility of false negative results due to the complex architecture of fetal cell free DNA confounding this analysis. To date, we have carried out over 500 tests on 93 different amplicons and identified one true false negative case (1.1%) that may be attributable to non-random shearing of the fetal cell free DNA.

We continue to collect outcomes and request a cord blood sample from the baby at birth.

Target reporting time

Results are normally available within 5 days of sample receipt.
## Disease

### Contact details

Regional Genetics Service  
Levels 4-6, Barclay House  
37 Queen Square  
London, WC1N 3BH  
T +44 (0) 20 7762 6800  
F +44 (0) 20 7813 8578

### Samples required

- **Pregnant Women**  
  2x 10mls venous blood in plastic EDTA bottles or glass Streck tubes, this should ideally reach the laboratory within 24-48 hours of sampling

- **The minimum gestation (by scan)** is 9wks for accepting a sample. If earlier than 18wks then 2 blood samples a week apart may be required

- **Testing must be arranged in advance**, through your Local Clinical Genetics Department or Fetal Medicine Unit and will be accepted in either of the categories given below. If you wish to refer a case which does not fulfil the criteria please contact Professor Lyn Chitty (l.chitty@ucl.ac.uk) (Clinical) or the laboratory (Genetics.Labs@gosh.nhs.uk).

- **A completed DNA request card** should accompany all samples with an appropriate telephone number and a secure fax number.

- **Pregnancy outcome**  
  Details of pregnancy outcome will be required for confirmation of laboratory results as part of the ongoing validation of new tests

### Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

### NIPD for FGFR2-related craniosynostosis

#### Introduction

The FGFR2-related craniosynostosis syndromes are characterised by skull deformity such as cloverleaf skull, distinctive facial features, and variable hand and foot findings. A wide range of presentations have been described among individuals with identical mutations in FGFR2 who have Crouzon (MIM 123500), Pfeiffer (101600) or Jackson-Weiss (123150) syndromes. This suggests that other factors play a role in determining symptoms. Within a family the same mutation is usually reported to result in the same clinical syndrome. These conditions are autosomal dominant or de novo with complete penetrance and variable expression. Antley-Bixler syndrome (ABS) (MIM 207410) is an exceptionally rare craniosynostosis syndrome characterised by radiohumeral synostosis present from the perinatal period. FGFR2-related isolated coronal synostosis is characterised by uni- or bicornal craniosynostosis only. Non-invasive prenatal genetic diagnosis (NIPD) by next generation sequencing (NGS) is possible using cell free fetal DNA (cffDNA) in pregnancies at risk of FGFR2-related craniosynostosis syndromes.

#### Referrals

- All referrals should be made via a Clinical Genetics Department or Fetal Medicine Unit and will be accepted in either of the categories given below. If you wish to refer a case which does not fulfil these criteria please contact Professor Lyn Chitty (l.chitty@ucl.ac.uk) (Clinical) or the laboratory (Genetics.Labs@gosh.nhs.uk).

- **Paternal FGFR2-related craniosynostosis syndrome** due to one of the mutations listed below OR

- **a previous pregnancy has been confirmed to have FGFR2-related craniosynostosis syndrome due to one of the mutations listed below, thus there is a very small risk of recurrence due to germline mosaicism**

  - **c.755C>T**, p.(Ser252Leu)
  - **c.760C>T**, p.(His254Tyr)
  - **c.863T>G**, p.(Ile288Ser)
  - **c.866A>C**, p.(Gln289Pro)
  - **c.867G>C**, p.(Trp290Arg)
  - **c.868T>C**, p.(Trp290Cys)
  - **c.870G>C**, p.(Trp290Ser)
  - **c.1018T>A**, p.(Tyr340His)
  - **c.1019A>C**, p.(Thr341Pro)
  - **c.1021A>C**, p.(Thr341Pro)
  - **c.1023,1025del**, p.(Cys342del)
  - **c.1024T>A**, p.(Cys342Ser)
  - **c.1025G>A**, p.(Cys342Ser)
  - **c.1025G>T**, p.(Cys342Phe)
  - **c.1030G>C**, p.(Ala344Pro)
  - **c.1032G>A**, p.(=)

  - **Paternally inherited alleles.**

#### Target reporting time

Results are normally available within 5 days of sample receipt

#### Service offered

NGS for 28 FGFR2-related craniosynostosis syndrome mutations

#### Technical

Maternal EDTA blood is spun as soon as possible after collection, cffDNA is extracted from plasma. Molecular analysis is performed by PCR, followed by NGS (Illumina MiSeq). Amplification of fetal DNA will be confirmed using gender analysis in males or by the presence of paternal HLA sequences. When this is uninformative a separate NGS assay is used containing 50 heterogeneous SNPs to identify paternally inherited alleles.

#### Results reporting time

Results are normally available within 5 days of sample receipt
Disease  

NIPD for Cystic Fibrosis

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- Pregnant Women
  2x 10mls venous blood in plastic EDTA bottles or glass Streck tubes, this should ideally reach the laboratory within 24-48 hours of sampling
- Paternal
  blood (5ml EDTA) or DNA
- Proband (or confirmed unaffected child)
  blood (1ml EDTA) or DNA
- The minimum gestation (by scan) is 9wks for accepting a sample. If earlier than 16wks then 2 blood samples a week apart may be required
- Testing must be arranged in advance, through your Local Clinical Genetics Department or Fetal Medicine Unit
- A completed DNA request card and ultrasound report should accompany all samples with an appropriate telephone number and a secure fax number.
- Pregnancy outcome
  Details of pregnancy outcome will be required for confirmation of laboratory results as part of the ongoing validation of new tests

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address), details of any relevant family history and full contact details for the referring clinician

Introduction
Cystic fibrosis (MIM 219700) is an autosomal recessive condition caused by mutations in the cystic fibrosis conductance regulator (CFTR) gene. CF affects epithelia of the respiratory tract, exocrine pancreas, intestine, male genital tract, hepatobiliary system, and exocrine sweat glands, resulting in complex multisystem disease. Pulmonary disease is the major cause of morbidity and mortality in CF. Definitive non-invasive prenatal diagnosis (NIPD) by relative haplotype dosage analysis (RHDO) is now possible using cell free fetal DNA (cffDNA) in pregnancies at risk of cystic fibrosis for confirmed carrier couples. This test replaces the previous paternal mutation exclusion panel test and is also applicable to couples who are carriers of the same CF mutation.

Referrals
All referrals should be made via a Clinical Genetics Department or Fetal Medicine Unit. Both parental mutations must have been confirmed by molecular genetic testing. This test is only applicable to couples
  1) who are known carriers of CF mutations AND
  2) DNA is available from the affected proband or a confirmed unaffected child
Relative haplotype dosage analysis will be used to determine if the fetus has inherited the high risk allele from both parents. If you wish to refer a case which does not fulfil these criteria please contact Professor Lyn Chitty (l.chitty@ucl.ac.uk) (Clinical) or the laboratory (Genetics.Labs@gosh.nhs.uk).

Service offered
Relative haplotype dosage analysis by targeted next generation sequencing (NGS).

Technical
Maternal EDTA blood is spun as soon as possible after collection, cffDNA is extracted from plasma. Molecular analysis is performed by Agilent SureSelect target enrichment, followed by NGS (Illumina). Amplification of fetal DNA will be confirmed using paternal-specific sequences.

Target reporting time
Results are normally available within 5 days of sample receipt.
Disease

NIPD for Cystic Fibrosis – paternal mutation exclusion

Introduction

Cystic fibrosis (MIM 219700) is an autosomal recessive condition caused by mutations in the cystic fibrosis conductance regulator (CFTR) gene. CF affects epithelia of the respiratory tract, exocrine pancreas, intestine, male genital tract, hepatobiliary system, and exocrine sweat glands, resulting in complex multisystem disease. Pulmonary disease is the major cause of morbidity and mortality in CF. Paternal mutation exclusion by non-invasive prenatal genetic diagnosis (NIPD) is now possible using cell free fetal DNA (cffDNA) in pregnancies at risk of cystic fibrosis for carrier couples where the parental mutations differ and the paternal mutation is one of 10 mutations covered by the panel.

Referrals

All referrals should be made via a Clinical Genetics Department or Fetal Medicine Unit. Both parental mutations must have been confirmed by molecular genetic testing. This test is only applicable to couples

1) who are known carriers of different CF mutations AND
2) the paternal mutation is one of the 10 mutations listed below

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Clinical *</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.489+1G&gt;T</td>
<td>c.1519_1521delATC p.(Ile507del)</td>
</tr>
<tr>
<td>c.1521_1523delCTT p.(Phe508del)</td>
<td>c.1624G&gt;T p.(Gly542*)</td>
</tr>
<tr>
<td>c.1646G&gt;A p.(Ser549Asn)</td>
<td>c.1647T&gt;G p.(Ser549Arg)</td>
</tr>
<tr>
<td>c.1652G&gt;A p.(Gly551Asp)</td>
<td>c.1657C&gt;T p.(Arg553*)</td>
</tr>
<tr>
<td>c.1679G&gt;C p.(Arg560Thr)</td>
<td>c.3846G&gt;A p.(Trp1282*)</td>
</tr>
</tbody>
</table>

The absence of a paternal mutation indicates that the fetus is at most only a carrier for CF and is therefore predicted to be unaffected. This test is not applicable to couples who carry the same CF mutation, or where the CF mutations have not been characterised. If you wish to refer a case which does not fulfil these criteria please contact Professor Lyn Chitty (l.chitty@ucl.ac.uk) (Clinical) or the laboratory (Genetics.Labs@gosh.nhs.uk).

Service offered

Targeted next generation sequencing (NGS) for 10 mutations in CFTR.

Technical

Maternal EDTA blood is spun as soon as possible after collection, cffDNA is extracted from plasma. Molecular analysis is performed by PCR, followed by NGS (Illumina MiSeq). Amplification of fetal DNA will be confirmed using gender analysis in males or by testing for the presence of paternal HLA sequences. When this is uninformative a separate NGS assay is used containing 50 heterogeneous SNPs to identify paternally inherited alleles.

We cannot rule out the possibility of false negative results due to the complex architecture of fetal cell free DNA confounding this analysis. To date, we have carried out over 500 tests on 93 different amplicons and identified one true false negative case (1.1%) that may be attributable to non-random shearing of the fetal cell free DNA. We continue to collect outcomes and request a cord blood sample from the baby at birth.

Target reporting time

Results are normally available within 5 days of sample receipt.
Familial Hypercholesterolaemia

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
• 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
• A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
Familial hypercholesterolaemia (FH) (MIM 143890) is a relatively frequent autosomal dominant condition, characterised clinically by elevations in low-density lipoprotein cholesterol (LDL-C), tendon xanthomata (TX) and premature coronary heart disease (CHD). Heterozygous FH has an incidence of around 1/500 individuals in the UK, and severe homozygous FH affects 1/1000 000 individuals. FH is genetically heterogeneous; however the primary genetic defect in FH is a pathogenic variant in the gene encoding the LDL-receptor (LDLR). LDLR has 18 exons and family specific pathogenic variants are found throughout the gene, although some recurrent pathogenic variants are reported. Large deletions or duplications encompassing one or more exons accounts for 5% of pathogenic variants in LDLR. A clinically indistinguishable disorder, familial defective apolipoprotein B100 (FDB), is due to a pathogenic variant in the gene encoding apolipoprotein B (APOB), which is one of the ligands of the LDL-receptor. The majority of FDB cases (2-5% of hypercholesterolaemic individuals) have a single pathogenic variant: p.(Arg3527Gln).

Pathogenic variants causing FH have also been identified in the PCSK9 and LDLRAP1 genes that account for a small proportion of cases.

Referrals
Referral criteria for testing are as determined by the Simon Broome Steering Committee:

a) Total cholesterol >7.5mmol/l or LDL-C >4.9mmol/l if >16yrs. If <16yrs total cholesterol >6.7mmol/l or LDL-C >4.0mmol/l
b) TX in patient or in first or second degree relative
c) Family history of myocardial infarction (MI) <60yrs in first degree relative or family history of MI <50yrs in second degree relative
d) Family history of total cholesterol >7.5mmol/l in first or second degree relative

Patients are separated into two groups, ‘definite FH’ and ‘possible FH’. For a diagnosis of ‘definite FH’ both a) & b) must be present, but for ‘possible’ FH both a) & c) or a) & d) must be observed. Both groups are appropriate for genetic testing. Testing can be offered to the relatives of FH patients once a pathogenic variant has been identified.

Service offered
Analysis of the LDLR, APOB, PCSK9 and LDLRAP1 genes by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is validated to detect large deletions / duplications in the LDLR gene is carried out using MLPA. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported.

Testing for previously identified pathogenic variants is available to other family members.

Target reporting time
8 weeks for a full screen in an index case (next generation sequencing and MLPA). 4 weeks for familial testing.
Hypertrophic cardiomyopathy (MYBPC3, MYH7, TNNT2, TNNI3)

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
Hypertrophic cardiomyopathy (HCM) is an autosomal dominant disease characterised by unexplained hypertrophy of the left ventricle and is one of the leading causes of premature sudden death in young adults (especially between 10 and 30 years). The disease is mainly caused by pathogenic variants in genes encoding protein components of the cardiac sarcomere. There is a wide heterogeneity with at least fourteen genes responsible for HCM. There is also a variation in expressivity and penetrance.

Referrals
- Predictive testing is available for family members in whom the causative pathogenic variant has been confirmed in a UKAS accredited laboratory.
- Confirmatory testing can be carried out for patients in whom a pathogenic variant has been detected in a research laboratory.

Prenatal testing
Prenatal testing is available for families in whom specific pathogenic variants have been identified or in whom appropriate family studies have been undertaken; please contact the laboratory to discuss.

Service offered
Predictive and confirmatory testing can be offered to families where a pathogenic variant has been identified in the MYBPC3, MYH7, TNNT2 or TNNI3 genes.

Technical
Testing is carried out by direct sequencing analysis.

Target reporting time
The turn around time for familial testing (including predictive tests) is 4 weeks. Please contact the laboratory for urgent cases.
Introduction

Loeys-Dietz syndrome (LDS) is an aortic aneurysm syndrome characterised by widely spaced eyes (hypertelorism), bifid uvula and/or cleft palate and generalised arterial tortuosity with ascending aortic aneurysm and dissection. Affected patients have a high risk of aortic dissection or rupture at an early age and at aortic diameters that ordinarily would not be predictive of these events. LDS shows autosomal dominant inheritance and variable clinical expression. Approximately 25% of individuals diagnosed with LDS have an affected parent, with the remaining 75% due to de novo pathogenic variants. LDS is caused by pathogenic variants in TGFBR1 and TGFBR2. Both proteins are transmembrane serine/threonine receptor kinases. TGFBR1 contains 9 exons and spans approximately 53 kb. TGFBR2 contains 8 exons in the longest transcript, including the alternatively spliced exon 1A, and spans approximately 91 kb. Pathogenic variants throughout the coding region of both genes have been reported including frame shift, nonsense, missense and splice site pathogenic variants as well as small insertions or deletions. Most pathogenic variants are missense variants in or immediately flanking the serine-threonine kinase domains of either receptor. Analysis is by sequencing of all coding exons of both genes and will detect >95% of pathogenic variants in individuals with typical findings of LDS.

Referrals

• New referrals should fulfill the UKGTN testing criteria.
• Mutation testing can be offered to the relatives of LDS patients once a pathogenic variant has been identified.

Prenatal testing

Prenatal testing is available for families in whom pathogenic variants have been identified or in whom appropriate family studies have been undertaken; please contact the laboratory to discuss.

Service offered

Screening of the entire coding regions and intron-exon boundaries of TGFBR1 & 2 for diagnostic tests.

Testing for previously identified familial pathogenic variants is available to other family members.

Technical

Analysis is by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is not currently validated to detect large deletions / duplications. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported.

Familial testing is carried out by direct sequencing analysis.

Target reporting time

The target reporting time for both genes is 8 weeks. The target turnaround time for familial variants (incl. predictive tests) is 4 weeks. Please contact the laboratory for urgent cases.
Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples
- For NIPD analysis: 2x 10mls venous blood in plastic EDTA bottles or glass Streck tubes, this should ideally reach the laboratory within 24-48 hours of sampling
- The minimum gestation (by scan) is 9wks for accepting a sample. If earlier than 18wks then 2 blood samples a week apart may be required

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
Achondroplasia (MIM 100800), Hypochondroplasia (MIM 146000) and Thanatophoric dysplasia (MIM 187600 and MIM 187601) are autosomal dominant skeletal disorders caused by mutations in the FGFR3 gene on chromosome 4p16.3. Achondroplasia (ACH) has a birth incidence of between 1/15,000 and 1/77,000. Around 80-90% of cases are sporadic and there is an association with increased paternal age at the time of conception, suggesting that new mutations are generally of paternal origin. There are rare familial forms, as well as reported cases of germline and somatic mosaicism. Hypochondroplasia (HCH) has very similar skeletal features to those seen in ACH but tends to be milder. Because of its mild nature, HCH can be difficult to diagnose and may be genetically heterogeneous. Approximately 70% of HCH patients have one of two mutations in the FGFR3 gene. Of the remaining 30%, some families are reported that do not link to chromosome 4p16.3 Thanatophoric Dysplasia (TD), a sporadic neonatal lethal skeletal dysplasia, is divided into two subsets based upon radiological findings. TD type I is associated with curved femora and variable but milder craniosynostosis and TD type II with straight femora and often cloverleaf skull. A single mutation, p.(Lys650Glu), accounts for all TD type II patients reported to date. Several recurrent mutations have been identified in TD type I. Mutations in the FGFR3 gene on chromosome 4 have been identified in almost 100% of confirmed cases of TD.

Referrals
We offer testing for confirmation of diagnosis in affected individuals and family members.

Prenatal testing
Prenatal testing is available to families in whom specific mutations have been identified and also to confirm a diagnosis of skeletal dysplasia suspected on antenatal ultrasound scan. This can be carried out by Non Invasive Prenatal Diagnosis (NIPD) – please contact the laboratory to discuss.

Please note that NIPD analysis is not applicable in cases where the mother has the mutation.

Service offered
Next generation sequencing of the FGFR3 gene with variant confirmation by Sanger sequencing. Testing for previously identified pathogenic variants is available to other family members. For prenatal samples targeted Sanger sequence analysis of the commonly mutated exons of FGFR3 gene.

Technical
Analysis is by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported. Targeted Sanger sequence analysis of exons 7, 10, 13, 15 and 19 of FGFR3.

Target reporting time
8 weeks for mutation screen (NGS) and 4 weeks for familial variant testing. For urgent samples please contact the laboratory.
**Craniosynostosis**

**Contact details**
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

**Samples required**
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples
- For NIPD analysis:
  - 2x 10mls venous blood in plastic EDTA bottles or glass Streck tubes, this should ideally reach the laboratory within 24-48 hours of sampling
  - The minimum gestation (by scan) is 9wks for accepting a sample. If earlier than 18wks then 2 blood samples a week apart may be required

**Patient details**
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

**Introduction**
Craniosynostosis is the premature fusion of one or more of the cranial sutures, resulting in abnormal skull growth, and affects approximately 1 in 2500 individuals. Craniosynostosis represents a heterogeneous group of disorders arising from both genetic and environmental factors. The craniosynostosis syndromes are usually sporadic, autosomal dominant disorders that have significant clinical overlap.

**Referrals**
We offer testing for confirmation of diagnosis in affected individuals and family members. Supra regional funding covers referrals for the following:
- Muenke Syndrome / Non-syndromic Craniosynostosis (MIM 602849)
- Saethre-Chotzen Syndrome (MIM 101400)
- Pfeiffer Syndrome (MIM 101600)
- Crouzon Syndrome (MIM 123500)
- Apert Syndrome (MIM 101200)

**Prenatal testing**
Prenatal testing is available for families in whom specific mutations have been identified - please contact the laboratory to discuss.
Non Invasive Prenatal Diagnosis (NIPD) is currently available for Aperts syndrome, FGFR2-related craniosynostosis and Crouzon syndrome with acanthosis nigricans – please contact the laboratory to discuss. Please note that NIPD analysis is not applicable in cases where the mother has the mutation.

**Service offered**
Next generation sequencing of the FGFR1, FGFR2, FGFR3, ERF, TCF12, ILLRA1, EFNB1 and TWIST1 genes with variant confirmation by Sanger sequencing for all syndromic craniosynostosis referrals. MLPA to detect large deletions and duplications in the TWIST1 and EFNB1 genes. Testing for previously identified pathogenic variants is available to other family members.
Apert syndrome: targeted analysis for common pathogenic variants c.755C>G p.(Ser252Trp) and c.758C>G p.(Pro253Arg).
Muenke syndrome/Non-syndromic: test for c.749C>G p.(Pro250Arg) in FGFR3

**Technical**
Analysis is by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is not currently validated to detect large deletions / duplications. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported.
Dosage analysis for TWIST and EFNB1 is by Multiplex Ligation-dependent Probe Amplification (MLPA) analysis using the craniofacial MLPA kit from MRC-Holland.

**Target reporting time**
Routine analysis: 8 weeks for mutation screen (NGS) and MLPA analysis and 4 weeks for familial variant testing. For urgent samples please contact the laboratory.
Mitochondrial testing m.1555A>G

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
Some mitochondrial variants have been associated with deafness, the most commonly reported being m.1555A>G.


This variant has been detected in families with maternally transmitted deafness and seems to have an age dependent penetrance for deafness, which is enhanced by treatment with aminoglycosides.

Referrals
- Patients with hearing loss for m.1555A>G analysis.
- Patients who may require aminoglycosides.
- Maternal relatives of patients with the m.1555A>G variant.

Service offered
Analysis for the m.1555A>G variant.

Technical
Restriction enzyme assay is performed to detect the m.1555A>G variant. All positive results are confirmed by sequence analysis.

Target reporting time
Two weeks for routine testing of m.1555A>G variant in index case.
Two weeks for maternal relatives of patients with the m.1555A>G variant.
Please contact the laboratory for urgent cases.
Branchio-oto-renal syndrome (BOR)

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
Branchio-oto-renal syndrome (BOR) (MIM 113650) is an autosomal dominant condition that manifests with the following phenotypes:
- Hearing loss
- Preauricular pits (“ear pits”)
- Pinnae abnormalities
- Branchial fistulae (lateral fistula of the neck)
- Renal anomalies

BOR has an incidence of approximately 1/40,000, accounts for about 2% of profoundly deaf children, and can be caused by pathogenic variants in the EYA1, SIX1 or SIX5 genes. Approximately 20% of cases have a large deletion of the EYA1 gene.

Referrals
Prospective patients should have at least three of the four following major features:
- hearing loss, branchial defects, ear pits and renal anomalies.
Testing can be offered to relatives of affected patients once a disease causing variant has been identified.

Prenatal testing
Prenatal testing is available for families in whom specific variants have been identified - please contact the laboratory to discuss.

Service offered
- Variant screening by next generation sequencing of the EYA1, SIX1 and SIX5 genes with variant confirmation by Sanger sequencing. MLPA analysis of the EYA1 gene to identify larger deletions and duplications.
- Detection of the familial variant in relatives of patients with a confirmed EYA1, SIX1 or SIX5 pathogenic variant

Technical
Variant screening is by next generation sequencing with library preparation using an Agilent SureSelect kit for the inherited deafness panel followed by sequencing on the Illumina NextSeq or MiSeq. Data from the EYA1, SIX1 and SIX5 genes only are analysed using an in-house pipeline with variants confirmed by Sanger sequencing. Dosage analysis is by Multiplex Ligation-dependent Probe Amplification (MLPA) analysis. Family tests are by targeted Sanger sequencing or MLPA, as appropriate.

Target reporting time
8 weeks for EYA1, SIX1 and SIX5 variant screening including EYA1 MLPA analysis
4 weeks for familial variant testing
Please contact the laboratory for urgent cases.
Introduction

Pre-lingual non-syndromic sensorineural hearing loss (NSSNHL) is predominantly due to recessive pathogenic variants. DFNB1 was the first locus described for autosomal recessive NSSNHL and accounts for a high proportion of cases.

The GJB2 gene (located at 13q11-q12) encodes the gap junction protein, beta 2 - also known as connexin 26. GJB2 pathogenic variants may account for 10-30% of sporadic non-syndromic deafness. The c.35del pathogenic variant is the most common GJB2 pathogenic variant described so far and is found in the majority of families linked to DFNB1. Other common pathogenic variants have been detected in specific ethnic groups.

A small proportion of individuals with DFNB1 have one identifiable GJB2 pathogenic variant and one of two large deletions (del(GJB6-D13S1830) – 309kb, del(GJB6-D13S1854) – 232kb) that include a part of GJB6 (encoding connexin 30) inherited on the opposite chromosome (del Castillo et al, J Med Genet (2005) 42:588-594).

Specific heterozygous GJB2 pathogenic variants have also been described in patients with idiopathic autosomal dominant hearing loss and rare cases of hearing loss associated with skin phenotypes: (Keratoderma ichthyosis and deafness syndrome (KID), Vohwinkel syndrome, and palmoplantar keratoderma (PPK) and deafness.

Referrals

- Patients with hearing loss for screening of connexin 26
- Patients with hearing loss and a relevant skin phenotype for screening of connexin 26.
- Adult relatives of patients with connexin 26 pathogenic variants for carrier status.

Service offered

Screening of connexin 26 gene, GJB2, coding region and intron boundaries (including the intron 1 splice site pathogenic variant c.-23+1G>A). Analysis for the 309kb deletion (GJB6-D13S1830) and 232kb deletion (GJB6-D13S1854). Detection of known pathogenic variants in relatives of patients with confirmed connexin 26 mutations.

Technical

Direct sequencing analysis of the coding region of connexin 26 and intron boundaries (including the intron 1 splice site pathogenic variant c.-23+1G>A). Size separation assay for the 309kb and 232kb deletions.

Target reporting time

Routine mutation screen in index case takes 8 weeks.
Routine testing of specific mutations takes 4 weeks.
Please contact the laboratory for urgent cases.
EAST Syndrome

Introduction

EAST syndrome (OMIM #612780) is an autosomal recessive condition that manifests with the following phenotypes:

- epilepsy
- ataxia
- sensorineural hearing loss
- tubulopathy (electrolyte imbalance)

Also known as SESAME syndrome, EAST syndrome is a rare disorder caused by homozygous or compound heterozygous pathogenic variants in the KCNJ10 gene (OMIM *602208).

Referrals

- Prospective patients should have a clear clinical phenotype, preferably referred by clinical genetics or specialist renal departments
- Asymptomatic (carrier) testing can be offered to relatives of affected patients once the pathogenic variant(s) have been identified.

Prenatal testing

Prenatal testing is available for families in whom specific pathogenic variants have been identified - please contact the laboratory to discuss.

Service offered

- Variant screening by next generation sequencing of the KCNJ10 gene with variant confirmation by Sanger sequencing
- Detection of the familial variant in relatives of patients with confirmed KCNJ10 pathogenic variant(s).

Technical

Variant screening is by next generation sequencing with library preparation using an Agilent SureSelect kit for the inherited deafness panel followed by sequencing on the Illumina NextSeq or MiSeq. Data from the KCNJ10 gene only are analysed using an in-house pipeline with variants confirmed by Sanger sequencing. Family tests are by targeted Sanger sequencing.

Target reporting time

8 weeks for KCNJ10 variant screening of an index case.
4 weeks for familial variant testing
For urgent samples please contact the laboratory.

Contact details

Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required

- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician.
Pendred Syndrome

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis.
- A completed DNA request card should accompany all samples.

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician.

Introduction
Pendred syndrome is an autosomal recessive form of hearing loss due to pathogenic variants in the SLC26A4 gene on chromosome 7q31 that presents with other features including goitre, enlarged vestibular aqueducts (EVA) and Mondini malformation. Pendred syndrome is the most common form of syndromic hearing loss with estimates of incidence ranging from 1 in 10,000 to 1 in 15,000 and a carrier frequency of 1 in 50 to 1 in 60.

The polypeptide pendrin is expressed in non-sensory epithelia of the inner ear and in thyroid folliculocytes. Pathogenic variants in SLC26A4 disrupt ion exchange activity of pendrin and have been reported across the gene, although there are a small number that appear to be recurrent: p.Leu236Pro, p.Gly209Val, c.1001+1G>A, p.Glu384Gly, p.Thr410Met and p.Thr416Pro have been reported amongst Western patients (Coyle et al., Hum Mol Genet 1998, 7:1105-1112). c.919-2A>G, p.His723Arg, p.Ser90Leu and p.Leu676Gln have been reported to be recurrent in particular Asian populations (Park et al., J Med Genet, 2003; 40:242-248).

Referrals
- Patients with a clinical diagnosis / a strong likelihood of Pendred syndrome.
- Adult relatives of patients with SLC26A4 pathogenic variants for carrier status.

Prenatal testing
Prenatal testing may be available for families in whom specific pathogenic variants have been identified or in whom appropriate family studies have been undertaken—please contact the laboratory to discuss.

Service offered
- Next generation sequencing of the SLC26A4 gene with variant confirmation by Sanger sequencing.
- In cases where variant screening has not confirmed a diagnosis but Pendred syndrome is still suspected, MLPA analysis can be performed upon request to detect large deletions and duplications in the SLC26A4 gene.
- Detection of the familial variant in relatives of patients with confirmed SLC26A4 pathogenic variant(s).

Technical
Variant screening is carried out by next generation sequencing with library preparation using an Agilent SureSelect kit for the inherited deafness panel followed by sequencing on the Illumina NextSeq or MiSeq. Data from the SLC26A4 gene only are analysed using an in-house pipeline with clinically relevant variants confirmed by Sanger sequencing. Dosage analysis is by Multiplex Ligation-dependent Probe Amplification (MLPA) analysis. Family tests are by targeted Sanger sequencing.

Target reporting time
8 weeks for variant screening in an index case by next generation sequencing
4 weeks for familial variant testing (carrier testing) or MLPA analysis
For urgent samples please contact the laboratory.
### Waardenburg Syndrome types 1-4

#### Contact details
Regional Genetics Service  
Levels 4-6, Barclay House  
37 Queen Square  
London, WC1N 3BH  
T +44 (0) 20 7762 6888  
F +44 (0) 20 7813 8578

#### Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

#### Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

#### Introduction
Waardenburg syndrome (WS) is an auditory-pigmentary disorder consisting of four clinical subtypes with an annual incidence of 1/212 000 births and accounts for approximately 3% of congenitally deaf children. WS1 (MIM #193500) and WS3 (MIM #148820) are defined by deafness, depigmentation features and dysmorphology. WS3 individuals also have musculoskeletal abnormalities of the upper limbs.

The Paired Box Gene 3 (PAX3) on chromosome 2q35 is the only gene known to be associated with WS1 and WS3 with pathogenic sequence variants identified in more than 90% of affected individuals. No common variants are known. Partial and total gene deletions have also been described and may represent 10% of cases without identified pathogenic sequence variants. WS1 is autosomal dominant but may arise de novo and demonstrates variable expressivity. In WS3 homozygous and compound heterozygous (severe phenotype) or heterozygous (moderate phenotype) pathogenic variants are seen.

Waardenburg syndrome Type 2 (WS2) and Type 4 (WS4) are genetically heterogeneous disorders. WS2 is associated with autosomal dominant pathogenic variants or large deletions in the MITF or SOX10 genes, and also with autosomal recessive pathogenic variants in the SNAI2 gene. MITF can also cause Tietz syndrome, a severe WS2 phenotype characterized by uniform hypopigmentation. WS4 is associated with pathogenic changes in the SOX10, EDNRB or EDN3 genes.

#### Referrals
- Patients with a clinical suspicion of WS
- Testing can be offered to relatives of affected patients once a disease-causing variant has been identified

#### Service offered
- Variant screening by next generation sequencing of a panel of 6 WS-associated genes: PAX3, MITF, SOX10, EDNRB, EDN3 and SNAI2. Dosage analysis by MLPA for large deletions in PAX3, MITF and SOX10
- Detection of the familial variant in relatives of patients with a confirmed pathogenic variant in one of the 6 WS-associated genes

#### Technical
Variant screening is by next generation sequencing with library preparation using an Agilent SureSelect kit for the inherited deafness panel followed by sequencing on the Illumina NextSeq or MiSeq. Data from the PAX3, MITF, SOX10, EDNRB, EDN3 and SNAI2 genes only are analysed using an in-house pipeline with clinically relevant variants confirmed by Sanger sequencing. Dosage analysis is by Multiplex Ligation-dependent Probe Amplification (MLPA) analysis. Family tests are by targeted Sanger sequencing or MLPA, as appropriate.

#### Target reporting time
- 8 weeks for variant screening of 6-gene panel including MLPA analysis
- 4 weeks for familial variant testing
- Please contact the laboratory for urgent cases
X-linked deafness (POU3F4)

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
Hearing loss affects 1 to 3 in 1000 newborns with 50% of these cases due to genetic causes. The majority of these are nonsyndromic (70%) and 1-2% are X-linked. The POU3F4 gene, DFN3 locus, (located at Xq21.1) encodes a transcription factor of the POU-domain family, comprised of two domains: POU-specific domain and a homeodomain. X-linked hearing loss can also exhibit as part of a syndrome; e.g. Norrie disease. Clinically DFN3 is characterised by congenital sensorineural or mixed hearing loss with an abnormally wide opening in the bone separating the basal turn of the cochlea and the inner auditory meatus, a fixation of the stapes with a perilymphatic gusher occurring during stapes surgery. The condition produces characteristic radiological findings. There are no common pathogenic variants but micro and large deletions 5' of the POU3F4 gene account for ~50% of cases.

Referrals
- Patients with hearing loss for variant screening of POU3F4.
- Adult relatives (females) of patients with POU3F4 pathogenic variants for carrier status.

Service offered
- Variant screening by next generation sequencing of the POU3F4 gene with variant confirmation by Sanger sequencing. MLPA analysis to identify larger deletions
- Detection of the familial variant in relatives of patients with a confirmed POU3F4 pathogenic variant

Technical
Variant screening is by next generation sequencing with library preparation using an Agilent SureSelect kit for the inherited deafness panel followed by sequencing on the Illumina NextSeq or MiSeq. Data from the POU3F4 gene only are analysed using an in-house pipeline with clinically relevant variants confirmed by Sanger sequencing. Dosage analysis is by Multiplex Ligation-dependent Probe Amplification (MLPA) analysis. The MLPA kit covers both coding sequence and 5' putative enhancer regions. Family tests are by targeted Sanger sequencing or MLPA, as appropriate.

Target reporting time
8 weeks for POU3F4 variant screening of an index case including MLPA analysis
4 weeks for familial variant testing
For urgent samples please contact the laboratory
Carbamoylphosphate synthetase 1 (CPS1) deficiency

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
Carbamoylphosphate synthetase 1 deficiency (MIM 237300) is a rare autosomal recessive metabolic disorder. CPS1 deficiency affects the first enzymatic step in the urea cycle and results in hyperammonemia that can lead to lethargy, vomiting, coma and premature death. The clinical presentation is varied from neonatal onset, where patients have severe hyperammonemia which is fatal in the first few days of life, to a case reported where a woman in her third decade of life collapsed and died after a normal pregnancy and delivery. The onset of CPS1 deficiency may also be exacerbated by infection, metabolic stress or excessive protein intake.

The CPS1 gene (2q35) consists of 38 exons.

Referrals
Prior to genetic analysis, clinically affected patients should wherever possible be confirmed as having CPS1 deficiency by enzyme analysis on a liver biopsy. Linkage analysis may be requested in the affected proband of a family, please supply details of biochemical testing undertaken, clinical details and any relevant pedigree.

Linkage analysis can be offered to the siblings for diagnostic testing and to adult relatives for carrier testing of CPS1 patients once an informative haplotype has been identified.

Prenatal testing
Prenatal testing is available for confirmed CPS1 families in whom linkage analysis has been shown to be informative - please contact the laboratory to discuss.

Service offered
Linkage analysis of the CPS1 gene region is undertaken in the affected patient and their parents.

Technical
There are eight microsatellite markers available spanning the CPS1 region which may be useful for family studies, please contact the laboratory to discuss.

Target reporting time
8 weeks for linkage analysis in the index case and parents. Please contact the laboratory for urgent cases.
Fabry disease (α-galactosidase A deficiency)

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7613 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
Fabry disease (MIM 301500) is an X-linked lysosomal storage disorder affecting ~1/40,000 males. It is due to a deficiency of the lysosomal hydrolase, α-galactosidase A. Males with classical Fabry disease have no residual enzyme activity, whereas atypical patients, usually with symptoms confined to the heart (cardiac variant), have varying degrees of residual activity. These enzyme activity levels allow the clinical diagnosis to be confirmed. The symptoms of Fabry disease begin during childhood or teenage years and include angiokeratoma, acroparesthesia and ocular features. Cerebrovascular, cardiovascular and renal malfunction may develop later. Clinical manifestation in carrier females can range from being asymptomatic to being as severely affected as affected males. Enzyme replacement therapy for Fabry disease is now well established and in wide use.

The gene encoding α-galactosidase A (GLA) (Xq22.1) consists of 7 exons and family specific pathogenic variants are found throughout the gene, although some recurrent pathogenic variants are reported and one pathogenic variant, p.(Asn215Ser), is commonly found in patients with the cardiac variant.

Referrals
- Clinically affected male patients should have their diagnosis confirmed by biochemical analysis; this should be arranged either locally or with the Enzyme Unit, Chemical Pathology, Great Ormond Street Hospital (tel: 0207 4059200 ext 1785/6751). Biochemically confirmed patients can be referred for genetic analysis.
- Clinically affected female patients can be referred directly for genetic analysis (due to unreliability of heterozygote detection by biochemical testing).
- Genetic testing can be offered to relatives of affected patients once a pathogenic variant has been identified.

Prenatal testing
Prenatal testing is available, if required, for families where specific pathogenic variants have been identified - please contact the laboratory to discuss.

Service offered
Analysis of the GLA gene by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is not currently validated to detect large deletions / duplications. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported.

MLPA dosage analysis to detect large deletions/duplications in the GLA gene.

Pathogenic variant testing for previously identified pathogenic variants is also available in family members.

Target reporting time
8 weeks for routine genetic screen (including MLPA) in index case. 4 weeks for family pathogenic variant specific tests. Please contact the laboratory for urgent cases.
Gaucher disease (β-glucocerebrosidase deficiency)

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
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F +44 (0) 20 7813 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
Gaucher disease (MIM 230800) is an autosomal recessive condition caused by a deficiency of the lysosomal enzyme β-glucocerebrosidase (GBA) and the resultant accumulation of its undegraded substrate, glucosylceramide, in the lysosomes. Biochemical enzyme analysis confirms a clinical diagnosis in affected individuals. The disease can be broadly divided into three clinical forms on the basis of the absence (type 1) or presence (types 2 and 3) of primary CNS involvement although there is actually likely to be a clinical continuum. Type 2 is considered to be the most severe form and type 1, the least severe. All forms are characterised by hepatosplenomegaly and anaemia with bone involvement common in types 1 and 3. Treatment involves bone marrow transplantation or enzyme replacement therapy. Type 1 is the most prevalent form and is particularly common in the Ashkenazi Jewish population with an incidence of ~1/855 individuals. Type 1 shows a broad spectrum of severity ranging from severely affected individuals to asymptomatic, presenting in childhood or adulthood. Types 2 and 3 are rarer.

All three subtypes are caused by pathogenic variants in the GBA gene; the phenotypic heterogeneity correlates to some extent with the different nature of the pathogenic variants identified. The GBA gene (1q21) comprises 12 exons. Although many novel pathogenic variants are known, there are ‘common’ pathogenic variants within the gene, particularly in the Ashkenazi Jewish population.

Referrals
- Clinically affected patients should have their diagnosis confirmed by biochemical analysis; this should be arranged either locally, or with the Enzyme Unit, Chemical Pathology, Great Ormond Street Hospital (tel: 0207 4059200 ext 1785/6751). Biochemically confirmed patients can be referred for genetic analysis.
- Carrier testing can be offered to adult relatives of affected patients once a pathogenic variant has been identified.

Prenatal testing
Prenatal testing is available for families in whom specific pathogenic variants have been identified or in whom appropriate family studies have been undertaken - please contact the laboratory to discuss.

Service offered
Testing is offered for the following recurrent GBA pathogenic variants which account for ~86% pathogenic variants in the Ashkenazi population and 70% of pathogenic variants in the non-Jewish UK population. Analysis is carried out by PCR & restriction enzyme digest, ARMS PCR and nested PCR analysis: p.(Asn409Ser), p.(Leu483Pro), p.(Arg502Cys), p.(Asp448His), c.84dupG, c.(1263_1319)del55 and c.115+1G>A.

Target reporting time
4 weeks for routine genetic screen. 4 weeks for carrier testing using pathogenic specific tests. Please contact the laboratory for urgent cases.
Glycogen storage disease type 1a (GSD1a)

**Contact details**
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

**Samples required**
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

**Patient details**
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

**Introduction**
Glycogen storage disease type 1a (GSD1a, MIM 232200), also known as Von Gierke disease, is an autosomal recessive inborn error of glycogen metabolism, occurring in ~1/100,000 live births worldwide. The condition usually manifests during the first year of life with severe hypoglycemia, growth retardation, hepatomegaly, bleeding diathesis, lactic acidemia, hyperlipidemia and hyperuricemia. Long-term complications include gout, hepatic adenomas, osteoporosis and renal disease.

GSD1a is caused by a deficiency of the enzyme glucose-6-phosphatase (G6Pase), which has an important role in glycogen metabolism and blood glucose homeostasis. G6Pase is normally expressed in the liver, kidney and intestinal mucosa and absence of G6Pase activity is associated with the excessive accumulation of glycogen in these organs. A clinical diagnosis of GSD1a can be confirmed by enzyme analysis on a liver biopsy. The G6PC gene consists of 5 exons and family specific pathogenic variants are found throughout the gene, however, ethnic specific pathogenic variants are recognised and information regarding ethnic origin is a useful indicator. In the North European Caucasian population two pathogenic variants, namely p.(Gln347*) and p.(Arg83Cys) account for approximately 62% of all pathogenic variants.

**Referrals**
- Clinically affected patients can have their diagnosis confirmed by biochemical demonstration of a deficiency of G6Pase activity on liver biopsy. This should be arranged either locally, or with the Enzyme Unit, Chemical Pathology, Great Ormond Street Hospital (tel: 0207 4059200 ext 1785/6751).
- Affected patients can be referred for genetic analysis. If the necessary patient samples are unavailable genetic testing can be undertaken in the parents of the affected child. Information regarding ethnic origin is useful.
- Carrier testing can be offered to the adult relatives of affected patients once a pathogenic variant has been identified.

**Prenatal testing**
Prenatal testing is available for families in whom specific pathogenic variants have been identified or in whom appropriate family studies have been undertaken - please contact the laboratory to discuss.

**Service offered**
Analysis of the G6PC gene by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is not currently validated to detect large deletions / duplications. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported.

**Target reporting time**
8 weeks for genetic screen in index case. 4 weeks for routine testing of specific pathogenic variants. Please contact the laboratory for urgent cases.
**Contact details**

Regional Genetics Service  
Levels 4-6, Barclay House  
37 Queen Square  
London, WC1N 3BH  
T +44 (0) 20 7762 6888  
F +44 (0) 20 7813 8578

**Samples required**

- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

**Patient details**

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

**Introduction**

Glycogen storage disease type 2 (GSD2, MIM 232300) is an autosomal recessive inborn error of glycogen metabolism caused by deficiency of acid α-glucosidase (GAA), which is required for the degradation of lysosomal glycogen. More commonly used names for this disorder include Pompe disease, acid maltase deficiency and glycogenosis type 2. GSD2 is characterised by lysosomal accumulation of glycogen in many body tissues as opposed to the exclusive cytoplasmic accumulation of glycogen that occurs in most other glycogen storage disorders.

Clinical presentation varies from a rapidly fatal infantile disease to a slowly progressive late-onset myopathy frequently associated with respiratory insufficiency. Generally there is a correlation between the severity of the disorder and the amount of residual GAA activity. Incidence varies by ethnicity; in the Caucasian population the frequency of infantile disease is between 1:100,000 and 1:200,000 and late-onset disease possibly as high as 1:60,000. Enzyme replacement therapy is available which may slow or reverse symptoms of the disease.

The GAA gene consists of 20 exons (exon 1 is non-coding) and family specific pathogenic variants are found throughout the gene. A mild splicing pathogenic variant in intron 1 (c. -32–13T>G) in combination with a more severe pathogenic variant is commonly associated with the late-onset phenotype in Caucasians.

**Referrals**

- Clinically affected patients should have their diagnosis confirmed by enzyme analysis; this should be arranged either locally, or with the Enzyme Unit, Chemical Pathology, Great Ormond Street Hospital (tel: 0207 4059200 ext 1785/6751).
- Biochemically confirmed patients can be referred for genetic analysis. If the necessary patient samples are unavailable genetic testing can be undertaken in the parents of the affected child. Information regarding ethnic origin is useful.
- Carrier testing can be offered to adult relatives of affected patients once a pathogenic variant has been identified.

**Prenatal testing**

Prenatal testing is available, if required, for families where specific pathogenic variants have been identified - please contact the laboratory to discuss.

**Service offered**

Analysis of the GAA gene by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is not currently validated to detect large deletions / duplications. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported.

Pathogenic variant specific testing for previously identified pathogenic variants is also available in family members.

**Target reporting time**

8 weeks for routine genetic screen in index case. 4 weeks for routine testing of specific pathogenic variants. Please contact the laboratory for urgent cases.
Krabbe disease (Globoid Cell Leukodystrophy)

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7613 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
Krabbe disease (MIM #245200) is an autosomal recessive inborn error of metabolism caused by deficiency of the enzyme galactosylceramidase (galactocerebrosidase). Galactosylceramidase (EC 3.2.1.46) is a lysosomal enzyme involved in the catabolism of galactosylceramide, a major lipid in myelin, kidney, and epithelial cells of the small intestine and colon. Enzyme deficiency results in the build-up of undigested fats affecting growth of the nerve’s protective myelin sheath and causes severe degeneration of mental and motor skills. The disease may be diagnosed by its characteristic grouping of certain cells (multinucleated globoid cells), nerve demyelination and degeneration, and destruction of brain cells. Special stains for myelin (e.g.; luxol fast blue) may be used to aid diagnosis. Definitive testing is by direct enzyme analysis.
Infants with Krabbe disease are normal at birth. Symptoms begin between the ages of 3 and 6 months with irritability, inexplicable crying, fevers, limb stiffness, seizures, feeding difficulties, vomiting, and slowing of mental and motor development. In infants, the disease is generally fatal before age 2. There are also juvenile- and adult-onset cases of Krabbe disease, which have similar symptoms but slower progression and significantly longer lifespan. Although there is no cure for Krabbe disease, bone marrow transplantation has been shown to benefit mild cases early in the course of the disease. The incidence of Krabbe disease is around 1 in 100,000 – 200,000 births.

The GALC gene is situated at 14q31 and consists of 17 exons. A recurrent 30kb deletion has been described which extends from intron 10 to intron 17 of the GALC gene and in the homozygous state is associated with infantile onset disease. The allele frequency of this deletion in Krabbe patients is reported to be approximately 50% in Dutch patients and 35% in non-Dutch European patients (Kleijer, WJ et al. (1997) J Inher Metab Dis 20:587-594).

Referrals
- Clinically affected patients should have their diagnosis confirmed by enzyme analysis; this should be arranged either locally or with the Enzyme Unit, Chemical Pathology, Great Ormond Street Hospital (tel: 0207 4059200 ext 1785/6751). Biochemically confirmed patients can be referred for genetic testing. If the necessary patient samples are unavailable, genetic testing can be undertaken in the parents of the affected child. Information regarding ethnic origin is useful.
- Carrier testing can be offered to adult relatives of affected patients once a pathogenic variant has been identified.

Prenatal testing
Prenatal testing is available for families in whom the 30kb deletion has been identified or in whom appropriate family studies have been undertaken - please contact the laboratory to discuss.

Service offered
Testing for the common 30kb deletion by three-primer PCR analysis. Other pathogenic variants are heterogeneous and testing is not currently offered as part of this diagnostic service.

Target reporting time
4 weeks for routine deletion test in the index case and family member carrier testing. Please contact the laboratory for urgent cases.
### Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

### Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis.
- A completed DNA request card should accompany all samples

### Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician.

### Introduction
Long chain acyl-CoA dehydrogenase (LCHAD) deficiency / mitochondrial trifunctional protein (MTF) deficiency is an autosomal recessive disorder of mitochondrial beta-oxidation of fatty acids. The mitochondrial trifunctional protein is composed of 4 alpha and 4 beta subunits, which are encoded by the HADHA and HADHB genes, respectively. It is characterized by early-onset cardiomyopathy, hypoglycemia, neuropathy, and pigmentary retinopathy, and sudden death. There is also an infantile onset form with a hepatic Reye-like syndrome, and a late-adolescent onset form with primarily a skeletal myopathy. Tandem mass spectrometry of organic acids in urine, and carnitines in blood spots, allows the diagnosis to be unequivocally determined. An additional clinical complication can occur in the pregnant mothers of affected fetuses; they may experience maternal acute fatty liver of pregnancy (AFLP) syndrome or hypertension/haemolysis, elevated liver enzymes and low platelets (HELLP) syndrome. The genes encoding the HADHA and HADHB subunits are located on chromosome 2p23.3. The pathogenic variant c.1528G>C, p.(Glu510Gln) on the HADHA gene accounts for approximately 87% of LCHAD alleles in affected patients.

### Referrals
- Clinically affected patients should have their diagnosis confirmed by biochemical analysis; this should be arranged either locally or with Chemical Pathology, Great Ormond Street Hospital (tel: 0207 4059200 ext 5009). Affected patients can then be referred for genetic testing, if the necessary patient samples are available, genetic testing can be undertaken in the parents of an affected child.
- Pregnant patients who have AFLP or HELLP can be referred for carrier testing for the c.1528G>C pathogenic variant, along with their partners.
- Carrier testing can be offered to the adult relatives of affected patients once a pathogenic variant has been identified.

### Prenatal testing
Prenatal testing is available, if required, for families where pathogenic variants have been identified - please contact the laboratory to discuss.

### Service offered
- Level 1 analysis: testing for the c.1528G>C pathogenic variant in the HADHA gene by Sanger sequencing analysis.
- Level 2 analysis: Analysis of the HADHA and HADHB genes by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is not currently validated to detect large deletions / duplications. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported.
- Detection of known pathogenic variants in relatives of patients with HADHA and HADHB pathogenic variants by Sanger sequencing.

### Target reporting time
4 weeks for routine level 1 screen in index case, 8 weeks for level 2 screen. 4 weeks for carrier testing for known pathogenic variants. Please contact the laboratory for urgent cases.
Medium chain acyl-CoA dehydrogenase (MCAD) deficiency

Introduction

Medium chain acyl-CoA dehydrogenase (MCAD) deficiency is an autosomal recessive disorder of fatty acid metabolism (MIM 201450), caused by a deficiency of the MCAD enzyme. Tandem mass spectrometry of organic acids in urine, and carnitines in blood spots, allows the diagnosis to be unequivocally determined.

MCAD deficiency has an incidence in the UK of approximately 1/10,000 live births. It is clinically heterogeneous but often presents as an episodic disease resembling Reye syndrome, with vomiting, lethargy and coma after metabolic stress, prolonged fasting or infection. Patients may also have cardiomyopathy and/or skeletal myopathy, and some patients present as sudden infant death cases. Between episodes, patients can appear normal and biochemical abnormalities can be absent. The gene encoding the MCAD enzyme (ACADM) is located at 1p31 and the pathogenic variant c.985A>G causes the replacement of the amino acid lysine with glutamic acid at codon 329, p.(Lys329Glu). This causes loss of MCAD activity and accounts for approximately 90% of pathogenic variant alleles in clinically affected North European patients, and 71% of alleles in newborn screening.

Referrals

- Clinically affected patients should have their diagnosis confirmed by biochemical analysis; this should be arranged either locally or with Chemical Pathology, Great Ormond Street Hospital (tel: 0207 4059200 ext 5009). Affected patients can then be referred for genetic testing. If the necessary patient samples are unavailable, genetic testing can be undertaken in the parents of an affected child.
- Carrier testing may be offered to the adult relatives of affected patients once a pathogenic variant has been identified and partner testing for the common c.985A>G pathogenic variant can be offered if appropriate.

Prenatal testing

Prenatal testing, by genetic analysis, is available to couples that have both been shown to be carriers of pathogenic variants. Please contact the laboratory to discuss.

Service offered

Level 1 analysis - testing for the common c.985A>G pathogenic variant by Sanger sequence analysis.

Level 2 analysis - Analysis of the ACADM gene by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is not currently validated to detect large deletions / duplications. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported.

Target reporting time

4 weeks for routine level 1 screen in index case, 8 weeks for level 2 screen, 4 weeks for carrier testing for known pathogenic variants. Please contact the laboratory for urgent cases.
Metachromatic Leukodystrophy & Pseudodeficiency of arylsulphatase A

Introduction

Metachromatic leukodystrophy (MLD) is an autosomal recessive lysosomal storage disorder caused by deficiency of the enzyme arylsulphatase A which catalyses the first step in the degradation of the sphingolipid 3-O-sulphoglucosyl ceramide (sulphatide). Accumulation of sulphatide in the brain leads to progressive demyelination of the central and peripheral nervous systems causing a variety of neurological symptoms including gait disturbances, ataxias, optical atrophy, dementia, seizures and spastic tetraparesis. Disease severity can range from mild to severe and can be broadly grouped into 3 subtypes (late-infantile, juvenile and adult). The majority of patients with arylsulphatase A deficiency and signs of MLD will have pathogenic variants in the ARSA gene however there is a much less common form of MLD caused by deficiency of saposin B, a non-enzymatic sphingolipid activator protein. Arylsulphatase A is also defective in multiple sulphatide deficiency due to pathogenic variants in SUMF1. The ARSA gene (22q13.31-qter) comprises 8 exons. Although many novel pathogenic variants are known, there are 'common' pathogenic variants within the gene, particularly the c.459+1G>A and c.1277C>T, p.(Pro426Leu) pathogenic variants, which account for around 50% of disease alleles in the Northern European population.

Pseudodeficiency of arylsulphatase A (PDASA)

Pseudodeficiency of arylsulphatase A is a condition of reduced arylsulphatase A activity (<15% normal) without clinical consequence, which can complicate the biochemical diagnosis of MLD. PDASA is caused by two sequence variants in the ARSA gene, namely PD2 (Poly A / c.*96A>G) and PD1 (NGly / p.(Asn350Ser)). PD2 is almost invariably seen on a background with PD1 but PD1 can occur independently of PD2 and its effect on causing PDASA is controversial.

Referrals

PDASA testing is used to assist the interpretation of arylsulphatase A activity results. Referrals are generally via the Enzyme Unit, Great Ormond Street Hospital however referrals may be accepted from other centers who carry out biochemical testing for arylsulphatase A. Biochemical confirmation of arylsulphatase A deficiency can only be confirmed after PDASA testing. In families with PDASA, prenatal testing by enzyme analysis can be complicated and in many cases impossible. For these families genetic testing is particularly useful but this can also mean that in some cases testing for MLD may have to be performed without biochemical confirmation. In these cases a very strong clinical picture of MLD must be present.

Prenatal testing

Prenatal testing is available for families in whom pathogenic variants have been identified or in whom appropriate family studies have been undertaken. Prenatal testing for PDASA may also be requested by the Enzyme Unit, Great Ormond Street Hospital.

Service offered

- PDASA: Testing for the presence of PD1 and PD2 by Sanger sequencing.
- MLD Level 1 analysis: testing for the common pathogenic variants c.459+1G>A and c.1277C>T, p.(Pro426Leu) by Sanger sequencing.
- MLD Level 2 analysis: Analysis of the ARSA gene by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is not currently validated to detect large deletions / duplications. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported.

Target reporting time

4 weeks for PDASA testing and MLD routine level 1 screen in index case, 8 weeks for level 2 screen. 4 weeks for routine testing of specific pathogenic variants. Please contact the laboratory for urgent cases.

Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician.
Mucopolysaccharidosis 1 (MPS1) (Hurler / Scheie syndrome)

Introduction

MPS1 (MIM 252800) is an autosomal recessive lysosomal storage disorder, also known as Hurler syndrome (severe) or Scheie syndrome (milder variant). The condition is caused by a deficiency of the enzyme alpha-L-iduronidase (IDUA), which is required for lysosomal degradation of the glycosaminoglycans, heparin sulphate and dermatan sulphate. Affected individuals have a characteristic pattern of urine metabolites and a deficiency in the IDUA enzyme activity; biochemical enzyme analysis utilises these features to confirm a clinical diagnosis.

Hurler patients are usually diagnosed by the age of 2 years and characteristically have short stature, coarse facial features, developmental delay, heart defects and hepatosplenomegaly. Scheie patients can present at a later age, and have a milder course of symptoms, including joint stiffness, corneal clouding and aortic valve disease. Other patients have an intermediate phenotype. The phenotypic heterogeneity correlates to some extent with the different nature of the pathogenic variants identified in the IDUA gene, although many novel pathogenic variants are known, there are ‘common’ pathogenic variants within the gene.

The IDUA gene (4p16.3) has 14 exons and pathogenic variants have been found throughout the gene. The recurrent pathogenic variants p.(Gln70*), p.(Ala327Pro) and p.(Trp402*) account for approx. 70% of disease alleles in the Northern European population. The p.(Trp402*) and p.(Gln70*) are the most common pathogenic variants seen in Hurler patients. p.(Arg89Gln) and c.590-7G>A are generally associated with Scheie syndrome.

Referrals

- Clinically affected patients should have their diagnosis confirmed by biochemical analysis; this should be arranged either locally or with the Enzyme Unit, Great Ormond Street Hospital (tel: 0207 4059200 ext 1785/6751). Biochemically confirmed patients can be referred for genetic analysis. If the necessary patient samples are unavailable genetic testing can be undertaken in the parents of a child.
- Carrier testing can be offered to the adult relatives of affected patients once a pathogenic variant has been identified.

Prenatal testing

Prenatal testing is available for families in whom pathogenic variants have been identified or in whom appropriate family studies have been undertaken. This service is also offered by biochemical analysis. Please contact the laboratory to discuss.

Service offered

- Level 1 analysis: detection of the ‘common’ pathogenic variants p.(Gln70*), p.(Ala327Pro) and p.(Trp402*) by Sanger sequence analysis.
- Level 2 analysis: Analysis of the GLA gene by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is not currently validated to detect large deletions / duplications. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported.
- Detection of known pathogenic variants in relatives of patients with confirmed MPS1 pathogenic variants by Sanger sequencing.

Target reporting time

4 weeks for routine level 1 screen in index case, 8 weeks for level 2 screen. 4 weeks for carrier testing using pathogenic variant specific tests. Please contact the laboratory for urgent cases.
Mucopolysaccharidosis type 2 (MPS2) (Hunter syndrome)

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
Hunter syndrome (MIM 309900) is an X-linked recessive lysosomal storage disorder. The condition is caused by a deficiency of the enzyme iduronate-2-sulphatase (IDS), which is required for the lysosomal degradation of the glycosaminoglycans, heparan sulphate and dermatan sulphate. Affected males have a characteristic pattern of urine metabolites and a deficiency of the IDS enzyme; biochemical enzyme analysis utilises these features to confirm a clinical diagnosis.

Hunter syndrome is clinically heterogeneous, but the predominant clinical features include coarse facial features, stiff joints, hepatosplenomegaly, cardiovascular and respiratory disorders, developmental delay and mental retardation. The IDS gene consists of 9 exons and family specific pathogenic variants are found throughout the gene. Homologous recombination between the IDS gene and the unexpressed IDS pseudogene, located 20kb telomeric of IDS, leads to inversions and deletions, a common inversion accounts for ~10% of Hunter cases.

Referrals
- Clinically affected patients should have their diagnosis confirmed by biochemical analysis; this should be arranged either locally or with the Enzyme Unit, Great Ormond Street Hospital (tel: 0207 4059200 ext 1785/6751). Such patients may then be referred for genetic analysis. If the necessary patient samples are unavailable genetic testing can be undertaken in the mother of the affected child.
- Carrier testing can be offered to female relatives of affected patients once a pathogenic variant has been identified.

Prenatal testing
Prenatal testing is available for families in whom specific pathogenic variants have been identified. This service is also offered by biochemistry. Please contact the laboratory to discuss.

Service offered
All confirmed Hunter patients (or their mothers if no sample is available from the affected male) are first tested for the presence of the common inversion, which has been shown to occur in ~10% of Hunter patients. Analysis of the IDS gene by next generation sequencing (Agilent SureSelect and Illumina NextSeq) is then carried out. A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is not currently validated to detect large deletions / duplications. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported. MLPA analysis is also carried out to detect large deletions and duplications. Pathogenic variant specific testing for previously identified pathogenic variants is also available in family members.

Target reporting time
4 weeks for inversion test. 8 weeks for routine genetic screen and MLPA in index case. 4 weeks for carrier testing of specific pathogenic variants. Please contact the laboratory for urgent cases.
Mucopolysaccharidosis type 3 (MPS3) (Sanfilippo syndrome)

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
Mucopolysaccharidosis type 3 (MPS3 / Sanfilippo syndrome MIM #252900) is an autosomal recessive lysosomal storage disorder caused by impaired degradation of heparan sulphate (found in the urine of affected patients). The syndrome is characterised by severe central nervous system degeneration, but only mild somatic disease (moderately severe claw hand and visceromegaly, little or no corneal clouding or skeletal change). Onset of clinical features usually occurs between 2 and 6 years; severe neurologic degeneration occurs in most patients between 6 and 10 years of age leading to a vegetative state, and death occurs typically during the second or third decade of life. Type A is reported to be the most severe of the 4 subtypes of Sanfilippo syndrome with earlier onset and rapid progression of symptoms and shorter survival (typically during the teens). Affected patients have a characteristic pattern of urine metabolites and a deficiency in one of the enzymes involved in heparan sulphate degradation; biochemical enzyme analysis utilises these features to confirm a clinical diagnosis.

Testing is currently available for types A and B which are due to deficiencies of the enzymes N-sulfoglucosamine sulfohydrolase (SGSH) and alpha-N-acetylglucosaminidase (NAGLU), respectively. There have been several recurrent pathogenic variants identified in both the SGSH and NAGLU genes although these are generally population specific.

Referrals
- Clinically affected patients should have their diagnosis confirmed by biochemical analysis (including disease subtype e.g. A); this should be arranged either locally or with the Enzyme Unit, Great Ormond Street Hospital (tel: 0207 4059200 ext 1785/6751). Such patients may then be referred for genetic analysis. If the necessary patient samples are unavailable genetic testing can be undertaken in the parents of the affected child.
- Carrier testing can be offered to the adult relatives of affected patients once a pathogenic variant has been identified.

Prenatal testing
Prenatal testing is available for families in whom pathogenic variants have been identified or in whom appropriate family studies have been undertaken. This service is also offered by biochemical analysis. Please contact the laboratory to discuss.

Service offered
- Analysis of the appropriate gene by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is not currently validated to detect large deletions / duplications. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported.
- Detection of known pathogenic variants in relatives of patients with confirmed MPS3A or MPS3B pathogenic variants by Sanger sequencing.

Target reporting time
8 weeks for routine screen in index case. 4 weeks for carrier testing of specific pathogenic variants. Please contact the laboratory for urgent cases.
Neuronal Ceroid-Lipofuscinosis type 1 (NCL1)
Infantile neuronal ceroid-lipofuscinosis (INCL)

Contact details
Regional Genetics Service
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37 Queen Square
London, WC1N 3BH
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F: +44 (0) 20 7813 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
Neuronal ceroid-lipofuscinosis type 1 (NCL1 / Batten disease) (MIM #256730) is a rare autosomal recessive neurodegenerative disorder caused by pathogenic variants in the \( PPT1 \) gene, which encodes the enzyme palmitoyl–protein thioesterase-1 (\( PPT1; \) MIM 600722). NCL1 is one of at least 14 genetically distinct diseases associated with the NCL disease spectrum. Onset is typically infantile (INCL) however juvenile and adult onset cases have also been described. The differential diagnosis of NCL1 from other NCL types is based on age of onset, clinical phenotype, ultrastructural characterisation of the storage material and \( PPT1 \) enzyme activity. NCL1 is characterised by the accumulation of auto fluorescent lipopigment in granular osmiophilic deposits (GROD) in neurons and other cell types using electron microscopy and loss of palmitoyl protein thioesterase-1 (\( PPT1 \)) enzyme activity in leucocytes and fibroblasts. Typical clinical features of INCL are retarded head growth from about 5 months, hyper excitability (including sleep problems), muscular hypotonia and reduced development of fine motor skills between 10-18 months of age. INCL usually progresses with visual loss (by 18 months - 2 years), loss of motor skills, and premature death between 8-13 years.

The \( PPT1 \) gene (1p32) consists of 9 exons and pathogenic variants have been found throughout the gene. The four most common \( PPT1 \) pathogenic variants are \( p.(\text{Arg122Trp}) \) (Finnish-specific), \( p.(\text{Arg151}^*) \), \( p.(\text{Thr75Pro}) \) and \( p.(\text{Leu10}^*) \). The \( p.(\text{Arg151}^*) \) and \( p.(\text{Leu10}^*) \) pathogenic variants may account for up to 75% of pathogenic variants in certain populations.

Referrals
- Clinically affected patients should, wherever possible, have their diagnosis confirmed by analysis of \( PPT1 \) enzyme activity in leucocytes and fibroblasts. This should be arranged locally or with the Enzyme Unit at Great Ormond Street Hospital; (tel: 0207 4059200 ext 1785/6751). Such patients may then be referred for genetic analysis. If the necessary patient samples are unavailable genetic testing can be undertaken in the parents of a child.
- Carrier testing can be offered to the adult relatives of NCL1 patients once a pathogenic variant has been identified.

Prenatal testing
Prenatal testing is available, if required, for families where specific pathogenic variants have been identified - please contact the laboratory to discuss.

Service offered
- Level 1 analysis: detection of recurrent pathogenic variants \( p.(\text{Arg151}^*) \) and \( p.(\text{Leu10}^*) \) by Sanger sequencing analysis.
- Analysis of the \( PPT1 \) gene by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is not currently validated to detect large deletions / duplications. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported.
- Detection of known pathogenic variants in relatives of patients with confirmed NCL1 pathogenic variants by Sanger sequencing.

Target reporting time
4 weeks for routine level 1 screen in index case. 8 weeks for NGS screen. 4 weeks for carrier testing of specific pathogenic variants. Please contact the laboratory for urgent cases.
Neuronal Ceroid-Lipofuscinosis type 2 (NCL2)
Late infantile neuronal ceroid-lipofuscinosis (LINCL)

Introduction
Neuronal ceroid-lipofuscinosis type 2 (NCL2 / Batten disease) (MIM #204500) is a rare autosomal recessive neurodegenerative disorder caused by pathogenic variants in the TPP1 gene which encodes the lysosomal enzyme tripeptidyl peptidase 1. NCL2 is one of at least 14 genetically distinct diseases associated with the NCL disease spectrum. NCL2 is generally referred to as late-infantile NCL (LINCL) due to typical onset of symptoms between the ages of 2 and 4 years. Variant forms of LINCL (vLINCL) have been reported to be caused by pathogenic variants in the CLN1, CLN5, CLN6, CLN7 and CLN8 genes. Clinical features of LINCL are normal development until the onset of seizures, ataxia and myoclonus between 2 and 4 yrs. LINCL usually progresses with visual loss (by 5-6 yrs), chair bound by 4-6 yrs with poor prognosis. The differential diagnosis of NCL2 from the other NCL types is based on age of onset, clinical phenotype, ultra structural characterisation of the storage material and TPP1 enzyme levels. A clinical diagnosis of NCL2 is confirmed biochemically by loss of tripeptidylpeptidase I (TPP1) enzyme activity in leucocytes and fibroblasts or accumulation of auto fluorescent lipopigment with a curvilinear profile in neurons and other cell types.

TPP1 (11p15) consists of 13 exons. The two most common pathogenic variants are c.509-1G>C (~33% of LINCL chromosomes) and p.(Arg208*) (~26% of LINCL chromosomes). Other pathogenic variants are family specific and found throughout the gene.

Referrals
- Clinically affected patients should, wherever possible, have their diagnosis confirmed by analysis of TPP1 enzyme activity in leucocytes and fibroblasts. This should be arranged locally or with the Enzyme Unit, Great Ormond Street Hospital; (tel: 0207 4059200 ext 1785/6751). Biochemically confirmed patients can be referred for genetic analysis. If the necessary patient samples are unavailable genetic testing can be undertaken in the parents of an affected child.
- Carrier testing can be offered to the adult relatives of NCL2 patients once a pathogenic variant has been identified.

Prenatal testing
Prenatal testing is available, if required, for families where specific pathogenic variants have been identified - please contact the laboratory to discuss.

Service offered
- Level 1 analysis: detection of the recurrent pathogenic variants c.509-1G>C and p.(Arg208*) by Sanger sequencing analysis.
- Analysis of the TPP1 gene by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is not currently validated to detect large deletions / duplications. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported.
- Detection of known pathogenic variants in relatives of patients with confirmed TPP1 pathogenic variants by Sanger sequencing.

Target reporting time
4 weeks for routine level 1 screen in index case, 8 weeks for NGS screen. 4 weeks for carrier testing using pathogenic variant specific tests. Please contact the laboratory for urgent cases.
Neuronal Ceroid-Lipofuscinosis type 3 (NCL3)
Juvenile neuronal ceroid-lipofuscinosis (JNCL)

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
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T +44 (0) 20 7762 6888
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Samples required
• 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
• Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
• Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
• A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
Neuronal ceroid-lipofuscinosis type 3 (NCL3 / Batten disease) (MIM #204200) is a rare autosomal recessive neurodegenerative disorder caused by pathogenic variants in the CLN3 gene. NCL3 is one of at least 14 genetically distinct diseases associated with the NCL disease spectrum. NCL3 is generally referred to as juvenile NCL (JNCL) due typical onset of symptoms between the ages of 4 and 7 years. A rare variant form of JNCL (vJNCL) has been associated with pathogenic variants in the CLN1 gene (usually associated with the infantile form of the disease). NCL3 is typically characterised by normal development until the onset of visual failure due to retinal degeneration between 4 and 7 yrs. Progression of visual loss is usually rapid. Other clinical features include seizures and psychomotor deterioration; prognosis is poor. The differential diagnosis of NCL3 from the other NCL types is based on age of onset, clinical phenotype and ultra-structural characterisation of the storage material. NCL3 is characterised by the accumulation of auto fluorescent lipopigment with a fingerprint profile in neurons and other cell types and the presence of vacuolated lymphocytes in blood smears.

The CLN3 gene (16p12) consists of 15 exons spanning 15kb of genomic DNA. A 1kb deletion (introns 6-8) is reported to account for ~69% of JNCL alleles (~85% in Finnish population). Other pathogenic variants are family specific and found throughout the gene.

Referrals
• Clinical and histopathological review of the affected patient is recommended to indicate a diagnosis of NCL3. Please supply details of histopathological testing undertaken, clinical details and any relevant pedigree. If the necessary patient samples are unavailable genetic testing can be undertaken in the parents of an affected child.
• Carrier testing can be offered to the adult relatives of NCL3 patients once a pathogenic variant has been identified.

Prenatal testing
Prenatal testing is available for families in which the diagnosis of NCL3 has been confirmed by genetic analysis. Please contact the laboratory to discuss.

Service offered
• Level 1: Testing for the common 1kb deletion.
• Analysis of the CLN3 gene by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is not currently validated to detect large deletions / duplications. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported.
• Detection of known pathogenic variants in relatives of patients with confirmed NCL1 pathogenic variants by Sanger sequencing.

Target reporting time
4 weeks for routine level 1 screen in index case, 8 weeks for NGS screen. 4 weeks for carrier testing using pathogenic variant specific tests. Please contact the laboratory for urgent cases.
Variant Neuronal Ceroid-Lipofuscinosis (CLN5, 6, 7 & 8)

Introduction

Variant neuronal ceroid-lipofuscinosis (also generally referred to as variant late-infantile Batten disease) is a rare autosomal recessive neurodegenerative disorder which can be caused by pathogenic variants in one of several genes including CLN5, CLN6, MFSD8 (CLN7) and CLN8. The neuronal ceroid-lipofuscinoses are a group of at least 14 genetically distinct diseases associated with a similar phenotype but variable age of onset.

Variant late-infantile NCL (vLINCL) is so called due to the similarity of clinical presentation and age of onset to the classic late-infantile form of NCL. The differential diagnosis of variant NCL from other NCL types is based on age of onset, clinical phenotype and ultra-structural characterisation of the storage material. Characteristic accumulation of auto fluorescent lipopigment with mixed fingerprint/curvilinear/rectilinear profiles is seen in neurons and other cell types and there is an absence of vacuolated lymphocytes on a blood smear (differentiating this type of NCL from NCL3).

The CLN5 gene (13q21.1-q32) consists of 4 exons, CLN6 (15q21-q23) consists of 7 exons, MFSD8 (CLN7) (4q28.2) consists of 13 exons, and CLN8 (8pter-p22) consists of 3 exons. Pathogenic variants are generally family specific and found throughout each gene.

Referrals

- Clinical and histopathological review of the affected patient is recommended to indicate a diagnosis of variant NCL. Please supply details of histopathological testing undertaken, clinical details and any relevant pedigree. If the necessary patient samples are unavailable genetic testing can be undertaken in the parents of an affected child.
- Carrier testing can be offered to the adult relatives of variant NCL patients once a pathogenic variant has been identified.

Prenatal testing

Prenatal testing is available, if required, for families where pathogenic variants have been identified - please contact the laboratory to discuss.

Service offered

- Analysis of the CLN5, CLN6, MFSD8 (CLN7), and CLN8 genes by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is not currently validated to detect large deletions / duplications. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported.
- Detection of known pathogenic variants in relatives of patients with confirmed pathogenic variants by Sanger sequencing.

Target reporting time

8 weeks for NGS screening. 4 weeks for routine testing of specific pathogenic variant. Please contact the laboratory for urgent cases.
Introduction

The neuronal ceroid lipofuscinoses (NCL; CLN; Batten disease) are a clinically and genetically heterogeneous group of neurodegenerative disorders characterized by the intracellular accumulation of autofluorescent lipopigment storage material in different patterns ultrastructurally. There are a total of at least 14 genetically distinct diseases associated with a similar phenotype but with variable age of onset. The genes include CLN1/PPT1, CLN2/TPP1, CLN3, CLN4/DNAJC5, CLN5, CLN6, MFSD8/CLN7, CLN8, CLN10/CTSD, CLN11/GRN, CLN12/ATP13A2, CLN13/CTSF, CLN14/KCTD7, and CLCN6. Several genes, including CLN4, CLN6, CLN11/GRN, CLN13/CTSF, and CLCN6, are associated with an adult-onset NCL, which includes Kufs type A (CLN6) and Kufs type B (CLN13/CTSF). Pathogenic variants in CLN10/CTSD cause cathepsin D deficiency, which has a neonatal onset.

Referrals

- Referrals can be accepted from any patient where a diagnosis of NCL is suspected. Clinical, biochemical and histopathological review of the affected patient is recommended to indicate the subtype of NCL (see profile sheets for NCL1, NCL2, NCL3, and variant NCL), so that a specific gene can be targeted. However, testing of all of the 14 known genes can be carried out without this information. If the necessary patient samples are unavailable genetic testing can be undertaken in the parents of an affected child.

- Carrier testing can be offered to the adult relatives of NCL patients once a pathogenic variant has been identified.

Prenatal testing

Prenatal testing is available, if required, for families where specific pathogenic variants have been identified - please contact the laboratory to discuss.

Service offered

- Analysis of the CLN1/PPT1, CLN2/TPP1, CLN3, CLN4/DNAJC5, CLN5, CLN6, MFSD8/CLN7, CLN8, CLN10/CTSD, CLN11/GRN, CLN12/ATP13A2, CLN13/CTSF, CLN14/KCTD7, and CLCN6 genes by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is not currently validated to detect large deletions / duplications. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported.

- Detection of known pathogenic variants in relatives of patients with confirmed pathogenic variants by Sanger sequencing.

Target reporting time

8 weeks for NGS screening. 4 weeks for routine testing of specific pathogenic variants. Please contact the laboratory for urgent cases.
Ornithine transcarbamylase (OTC) deficiency

Introduction

Ornithine transcarbamylase (OTC) deficiency (MIM 311250) is a rare X-linked recessive disorder. Females also frequently manifest the condition, presumably due to non-random X chromosome inactivation in their liver cells. Deficiency of OTC causes a defect in the urea cycle and results in hyperammonemia, leading to lethargy, vomiting, coma and premature death. The clinical presentation is variable. In males, there are generally accepted to be two forms of OTC deficiency - a neonatal form, where patients have severe hyperammonemia which is fatal in the first few days of life and a late onset form which occurs at any point after this initial neonatal period and can be exacerbated by infection, metabolic stress or excessive protein intake. Female carriers can also experience this full range of clinical symptoms, varying from apparently unaffected to neonatal death.

The OTC gene (Xp21.1) consists of 10 exons and family pathogenic variants are found throughout the gene, although some recurrent pathogenic variants at CpG sites in exons 1, 3, 5 and 9 are reported and some late onset specific pathogenic variants are known. A whole gene deletion accounts for approximately 10% of OTC cases.

Referrals

- Prior to genetic analysis, clinically affected patients should, wherever possible, be confirmed as having OTC deficiency by enzyme analysis on a liver biopsy or by finding elevated orotic acid levels by biochemical analysis. Allopurinol or protein load tests can be used to indicate female carrier status, but these are not always conclusive. Genetic analysis can be requested in the affected proband of a family, please supply details of biochemical testing undertaken, clinical details and any relevant pedigree.
- If there is no sample available from an affected individual testing can be undertaken in the mother of an affected child (however, it should be noted that, unless there are additional affected family members, they are not necessarily pathogenic variant carriers).
- Carrier testing can be offered to the female relatives of OTC patients once a pathogenic variant has been identified.

Prenatal testing

Prenatal testing is available for families in whom specific pathogenic variants have been identified or in whom appropriate family studies have been undertaken - please contact the laboratory to discuss.

Service offered

Entire gene deletions account for ~10% of neonatal OTC cases and are tested for by MLPA analysis in both males and females. This is then followed by analysis of the OTC gene by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is not currently validated to detect large deletions / duplications. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported. Pathogenic variant specific testing for previously identified pathogenic variants is also available in family members by Sanger sequencing or MLPA.

Target reporting time

8 weeks for genetic screen including MLPA in index case. 4 weeks for carrier testing using pathogenic variant specific tests. Please contact the laboratory for urgent cases.
Osteopetrosis

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7613 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
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Introduction
Autosomal recessive malignant osteopetrosis (MIM 259700) is a rare congenital disorder of bone resorption affecting 1/200,000 individuals. The condition is caused by failure of osteoclasts to resorb immature bone. This results in abnormal bone marrow cavity formation and bone marrow failure.

Clinical features of osteopetrosis include fractures (especially of the long bones), visual impairment, nerve compression resulting in headaches, blindness and deafness, haematological difficulties, unusual dentition, frequent infections, failure to thrive, and growth retardation. It is diagnosed immediately/shortly after birth and death can occur by 2 years due to severe anaemia, bleeding and/or infection.

Osteopetrosis is generally diagnosed through skeletal X-rays. Bones appear unusually dense on X-rays with a chalky white appearance. Bone density tests and bone biopsies can also confirm a diagnosis. At present, bone marrow transplantation is the only treatment that has been proven to significantly alter the course of the disease.

The TCIRG1 gene located at 11q13, consists of 20 exons and encodes an a3 subunit of the vacuolar pump, which mediates acidification of bone/osteoclast interface. Pathogenic variants of this gene have been found in ~50% of autosomal recessive osteopetrosis patients.

Referrals
- Clinically affected patients should if possible have their diagnosis confirmed by X-ray analysis, bone density tests and bone biopsies.
- Carrier testing can be offered to adult relatives of affected patients once a pathogenic variant has been identified.

Prenatal testing
Prenatal testing is available for families in whom pathogenic variants have been identified or in whom appropriate family studies have been undertaken- please contact the laboratory to discuss.

Service offered
Analysis of the TCIRG1 gene by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is not currently validated to detect large deletions / duplications. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported.

Pathogenic variant specific testing for previously identified pathogenic variants is also available in family members by Sanger sequencing.

Target reporting time
8 weeks for screen in index case. 4 weeks for carrier testing using pathogenic variant specific tests. Please contact the laboratory for urgent cases.
Pyridoxine-Dependent Epilepsy (Antiquitin – ALDH7A1)

Introduction

Pyridoxine-dependent epilepsy (PDE MIM 266100) is an autosomal recessive disease caused by pathogenic variants in the alpha-aminoacidic semialdehyde dehydrogenase (ALDH7A1 – antiquitin) gene located on chromosome 5q31. Alpha-aminoacidic semialdehyde (AASA) dehydrogenase is involved in the cerebral lysine degradation pathway. Deficiency of the enzyme leads to the accumulation of AASA and piperidine-6-carboxylate (P6C), elevated levels of which can be detected in urine, plasma, and CSF of affected patients. Patients usually present with neonatal seizures that are unresponsive to conventional anti-convulsant therapy, but which can be controlled by treatment with pyridoxine (vitamin B6). Less commonly, later-onset cases present during the second and third years of life. Patients also display varying degrees of developmental delay, which may, in part, be independent of the timing of pyridoxine therapy. In affected patients, P6C inactivates pyridoxalphosphate (PLP), the active vitamer of pyridoxine, leading to severe PLP deficiency. As PLP is a cofactor of various enzymes in the CNS, seizures in PDE are most probably due to perturbation in the metabolism of cerebral amino acids and neurotransmitters. Pyridoxine is a precursor of PLP and it can therefore reduce the effect of PLP deficiency in PDE patients.

The ALDH7A1 gene consists of 18 exons and pathogenic variants have been found throughout the gene. Two pathogenic variants, c.834G>A, p.(=) and c.1279G>C, p.(Glu427Gln), account for approximately 5% and 33%, respectively, of disease alleles in the Caucasian population.

Referrals

- Clinically affected patients, if possible, should have their diagnosis confirmed by urinary analysis of alpha-aminoacidic semialdehyde (AASA); this should be arranged either locally or with Dr Philippa Mills, Biochemistry Department, UCL Institute of Child Health (tel: 0207 242 9789 (ext 2108)). Biochemically confirmed patients can be referred for genetic analysis.
- If the necessary patient samples are unavailable genetic testing can be undertaken in the parents of the affected child. Information regarding ethnic origin is useful.
- Carrier testing can be offered to adult relatives of affected patients once a pathogenic variant has been identified.

Prenatal testing

Prenatal testing is available for families in whom pathogenic variants have been identified or in whom appropriate family studies have been undertaken - please contact the laboratory to discuss.

Service offered

- Level 1 analysis: detection of recurrent pathogenic variants c.834G>A and c.1279G>C by Sanger sequencing analysis.
- Level 2 analysis: Analysis of the GLA gene by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is not currently validated to detect large deletions / duplications. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported.
- Pathogenic variant specific testing for previously identified family pathogenic variants is also available by Sanger sequencing.

Target reporting time

4 weeks for routine level 1 screen in index case. 8 weeks for level 2 screen. 4 weeks for carrier testing using pathogenic variant specific tests. Please contact the laboratory for urgent cases.
**Schindler Disease**

**Contact details**
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37 Queen Square  
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T +44 (0) 20 7762 6888  
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**Samples required**
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)  
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.  
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis  
- A completed DNA request card should accompany all samples

**Patient details**
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

**Introduction**
Schindler disease (MIM 609241) is a rare autosomal recessive lysosomal storage disease, which is caused by a deficiency of the enzyme, alpha-N-acetylgalactosaminidase (NAGA). NAGA is a lysosomal glycohydrolase that cleaves alpha-N-acetylgalactosaminidase moieties from glycoconjugates inside lysosomes. Schindler disease is clinically heterogeneous with 3 main phenotypes; type 1 is an infantile-onset neuroaxonal dystrophy; type 2, also known as Kanzaki disease (MIM 609242), is an adult onset disorder characterised by angiookeratoma corporis diffusum and mild intellectual impairment; and type 3 is an intermediate disorder with mild to moderate neurological manifestations.

Affected patients have an abnormal urinary oligosaccharide and glycopeptide profile and the diagnosis is confirmed by a deficiency of the NAGA enzyme in plasma, leucocytes, or fibroblasts.

The NAGA gene is located on chromosome 22q13.2 and consists of 9 exons, and family specific pathogenic variants are found throughout the gene. To date, 14 patients from ten families are known and ethnic specific pathogenic variants are recognised, information regarding ethnic origin is therefore a useful indicator.

**Referrals**
- Clinically affected patients should have their diagnosis confirmed by biochemical analysis; this should be arranged either locally or with the Enzyme Unit, Great Ormond Street Hospital; (tel: 0207 4059200 ext 1785/6751). Such patients may then be referred for genetic analysis. If the necessary patient samples are unavailable genetic testing can be undertaken in the parents of the affected child.
- Carrier testing can be offered to the adult relatives of affected patients once a disease causing mutation has been identified.

**Prenatal testing**
Prenatal testing is available for families in whom pathogenic variants have been identified or in whom appropriate family studies have been undertaken – please contact the laboratory to discuss.

**Service offered**
Analysis of the NAGA gene by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is not currently validated to detect large deletions / duplications. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported.

**Target reporting time**
8 weeks for routine mutation screen in index case. 4 weeks for carrier testing using pathogenic variant specific tests. Please contact the laboratory for urgent cases.
CFHR5 Nephropathy

Contact details
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Levels 4-6, Barclay House
37 Queen Square
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T +44 (0) 20 7762 6888
F +44 (0) 20 7813 5785

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
Affected individuals display persistent microscopic haematuria with episodes of macroscopic haematuria associated with intercurrent infections (commonly of the respiratory tract). Renal biopsy demonstrates C3 glomerulonephritis (C3GN, a type of mesangiocapillary glomerulonephritis (MCGN)). 80% men and 20% affected women develop renal failure by the 8th decade of life. The incidence of CFHR5 nephropathy in the Cypriot population is estimated at 1/1000 to 1/8000; prevalence in the UK Caucasian population is low (<1:100,000) since C3GN is a very rare diagnosis. This autosomal dominant condition is caused by a pathogenic variant in the Complement Factor H-Related gene 5 (CFHR5; MIM: *608593). The gene is homologous to Complement Factor H and Complement Factor-H Related genes 1-4 which lie at neighbouring loci. CFHR5 consists of 10 exons which code for 9 homologous short consensus repeat domains, each of which has two internal disulphide bridges. The protein product of CFHR5 has the ability to co-localise with (and regulate activation of) complement C3 in the kidney.

The most common CFHR5 gene pathogenic variant is a duplication of exons 2 and 3 (c.59-1808_430+3242dup) described in the Cypriot population. Only one other pathogenic variant has been published, a frameshift in exon 4 identified in one non-Cypriot C3GN patient.

Referrals
Referrals are accepted from Consultant Clinical Geneticists and Consultant Nephrologists in the following patients:
- Cypriot origin with unexplained haematuria or renal disease.
- Patients of any ethnicity may be referred if C3GN or MCGN is present.
- At risk family members where the familial mutation is known.

Service offered
Detection of exon 2-3 duplication: A single PCR reaction incorporates primers that amplify both a 298bp fragment of the wild type sequence and a 222bp fragment unique to the duplication.

Detection of other pathogenic variants: Analysis of the CFHR5 gene by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is not currently validated to detect large deletions / duplications. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported.

Target reporting time
4 weeks for duplication analysis. 8 weeks for next generation sequencing analysis of CFHR5. Please contact the laboratory for urgent cases.
Cystinosis

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
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T +44 (0) 20 7762 6888
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Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
Cystinosis (MIM 219800, 219900 and 219750) is a rare autosomal recessive disorder affecting 1/175,000 individuals. The condition is caused by the failure to transport the amino acid cysteine out of the lysosomes. Cystine (a dimer of two cysteine molecules) accumulates forming crystals which causes cell and tissue destruction in all systems of the body. Excess cystine can be detected by cystine binding protein assays which can be used to confirm a clinical diagnosis. Three forms of cystinosis have been defined by age of onset and severity of symptoms. Most common form is infantile nephropathic cystinosis (95% of cases) which has an age of onset of 6-12 months. Features include renal proximal tubular dysfunction (renal Fanconi syndrome), without treatment affected children suffer worsening growth retardation and develop end stage renal failure by ~10 years. The juvenile form of cystinosis occurs in around 4-5% of affected individuals. Age of onset is between 12-15 years and individuals usually present with proteinuria and glomerular renal impairment, but do not suffer from such profound tubular dysfunction or growth retardation. The benign form of cystinosis occurs in adulthood: individuals do not suffer from any renal disease and grow normally. They require no treatment and have a normal life expectancy and quality, except perhaps for photophobia due to cystine crystals in the cornea. The CTNS gene consists of 12 exons. The most common pathogenic variant is a 57kb deletion which accounts for approximately 60% of alleles in North Europeans, and makes up around one third of all pathogenic variants found in individuals with cystinosis. The rest of the pathogenic variants reported are spread throughout the coding area of the gene and include insertions, small deletions, nonsense, splicing and missense pathogenic variants.

Referrals
- Clinically affected patients should have their diagnosis confirmed by biochemical analysis; this should be arranged either locally or with the Enzyme Unit, Great Ormond Street Hospital; (tel: 0207 4059200 ext 1785/6751). Biochemically confirmed patients can be referred for genetic analysis.
- Carrier testing can be offered to adult relatives of affected patients once a pathogenic variant has been identified.

Prenatal testing
Prenatal testing is available for families in whom specific pathogenic variants have been identified or in whom appropriate family studies have been undertaken - please contact the laboratory to discuss.

Service offered
- Level 1 genetic analysis: testing for the common 57kb deletion by PCR analysis.
- Level 2 genetic analysis: Analysis of the CTNS gene by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is not currently validated to detect large deletions / duplications. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported.
- Detection of known pathogenic variants in relatives of patients with confirmed CTNS pathogenic variants by Sanger sequencing or deletion PCR analysis.

Target reporting time
4 weeks for routine level 1 screen in index case, 8 weeks for level 2 screen. 4 weeks for routine testing of specific pathogenic variants. Please contact the laboratory for urgent cases.
Juvenile nephronophthisis (NPH1)

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
Familial juvenile nephronophthisis (NPH1, MIM 256100) is an autosomal recessive condition accounting for 2-10% of childhood chronic renal failure. It is caused by pathogenic variants in the NPHP1 gene on chromosome 2. Approximately 80% of familial, and 65% of sporadic nephronophthisis patients with purely renal symptoms have been shown to be homozygous for a 250kb deletion of chromosome 2q13, including almost the entire NPHP1 gene.

A subset of individuals affected with a mild form of Joubert syndrome have also been reported to have this homozygous deletion (Parisi MA et al. Am J Hum Genet. 2004 Jul;75(1):82-91).

Referrals
We offer testing for confirmation of diagnosis in affected probands.
Carrier testing is NOT available.

Prenatal testing
Prenatal testing may be available for families following analysis of the affected proband - please contact the laboratory to discuss.

Service offered
Detection of the homozygous 250kb deletion in affected probands only.

Target reporting time
4 weeks for routine analysis of the NPHP1 deletion. Please contact the laboratory for urgent cases.
**Steroid-Resistant Nephrotic Syndrome (NPHS2)**

**Contact details**
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

**Samples required**
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

**Patient details**
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

**Introduction**
Nephrotic syndrome is a condition that is caused by any of a group of diseases that damage the kidneys’ filtering system, the glomeruli. The structure of the glomeruli prevents most protein from getting filtered through into the urine. Nephrotic syndrome is characterised by proteinuria (abnormally high loss of protein in the urine), albuminemia and hypercholesterolemia. Ultimately, there is rapid progression to end-stage renal disease where the kidneys are irreversibly damaged, resulting in death if untreated.

Treatment is based on relieving symptoms, preventing complications and delaying progressive kidney damage. Patients with nephrotic syndrome are typically treated with steroids, of which about 80% have a good response; the rest are considered to be steroid-resistant and may require renal transplant. One of the main features in steroid-resistant nephrotic syndrome (SRNS; MIM 600995) is focal segmental glomerulosclerosis (FSGS).

Pathogenic variants in the podocin gene, NPHS2, are associated with autosomal recessive steroid-resistant nephrotic syndrome (SRNS), including focal segmental glomerulosclerosis (FSGS). Around half of familial forms and 10-30% of sporadic forms of SRNS are found to have NPHS2 pathogenic variants in both alleles. The gene is located on chromosome 1q25-31 and consists of 8 exons.

**Referrals**
Affected patients should fulfill the following criteria:
Presence of nephrotic syndrome (serum albumin < 25g/l and urine albumin > 4 mg/m2/h or urine albumin/creatinine ratio > 100 mg/mmol ), that is either:
- resistant to treatment with steroids, or
- present in the first 3 months of life, or
- have a histological picture of FSGS on biopsy.

Please also send a completed clinical information sheet (available on our laboratory website).

Carrier testing can be offered to adult relatives of affected patients once a pathogenic variant has been identified.

**Prenatal testing**
Prenatal testing may be available for families following analysis of the affected proband - please contact the laboratory to discuss.

**Service offered**
Analysis of the NPHS2 gene by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is not currently validated to detect large deletions / duplications. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported.

**Target reporting time**
8 weeks for routine screen in index case. 4 weeks for carrier testing using pathogenic variant specific tests. Please contact the laboratory for urgent cases.
Renal tubulopathies

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
The renal tubule is responsible for maintenance of acid-base balance, blood pressure and the correct concentration of electrolytes in the body. Consequently, disorders in renal tubular function are typically associated with abnormalities in these critical physiological parameters. Depending on the severity of the disorder, these abnormalities can be life-threatening and/or associated with severe complications, including sudden death, prematurity, critically high or low blood pressure, heart arrhythmia, failure-to-thrive and kidney stones, or only manifest in mild abnormalities seen on blood tests.

Referrals
Patients with a strong clinical suspicion of a monogenic predisposition to a renal tubulopathy.
Referrals will be accepted from clinical geneticists and consultant nephrologists.

Prenatal testing
Prenatal testing may be available for families following analysis of the affected proband - please contact the laboratory to discuss.

Service offered
Variant screening is carried out by next generation sequencing with library preparation using the Agilent focused clinical exome +1 kit followed by sequencing on the Illumina platforms. Data is analysed using an in-house pipeline with all pathogenic variants confirmed by Sanger sequencing. Screening of the 37 gene panel or one of the following sub-panels may be requested:

- Magnesium related renal tubulopathy 10 gene panel (FXYD2, TRPM6, EGF, HNF1B, CLDN16, CLDN19, KCNA1, KCNJ10, SLC12A3, CLCNKB)
- Dent disease 2 gene panel (CLCN5, OCRL)
- Hypokalaemic alkalosis (Bartter; Gitelman; EAST; Liddle) 8 gene panel (SLC12A3, SLC12A1, KCNJ1, CLCNKB, BSND, KCNJ10, SCN1B, SCN11G)
- Hyperkalaemic acidosis (PHA1/2) 8 gene panel (WNK1, WNK4, KLHL3, CUL3, SCN11A, SCN11B, SCN11G, NR3C2)
- Hypophosphatemia with hypercalciuria 3 gene panel (SLC9A3R1, SLC34A1, SLC34A3)
- Autosomal dominant interstitial kidney disease 2 gene panel (UMOD, REN)
- Nephrogenic diabetes insipidus 2 gene panel (AQP2, AVPR2)
- Calcium related renal tubulopathy 3 gene panel (CASR, GNA11, AP2S1)
- Renal tubular acidosis 4 gene panel (SLC4A4, SLC4A1, ATP6V1B1, ATP6V0A4)

Target reporting time
4 months for next generation sequencing screening in an index case. 4 weeks for familial testing.
Please contact the laboratory for urgent cases.
Autoimmune lymphoproliferative syndrome (ALPS)

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
ALPS is a rare immunodeficiency disorder associated with inherited pathogenic variants in the FAS gene encoding Fas (also known as Apo-I or CD95) receptor protein (ALPS type IA); others have pathogenic variants in the FASLG gene encoding Fas ligand (ALPS type IB), or in the CASP10 and CASP8 genes encoding caspase 10 and 8 protease (ALPS type IIa and IIb, respectively).

Most patients have ALPS type IA (MIM 134637) due mainly to dominant-negative highly penetrant pathogenic variants in the Fas death domain encoded by exon 9 of the FAS gene. ALPS is characterised by splenomegaly, defective lymphocyte apoptosis, lymphadenopathy, hypergammaglobulinaemia (IgG and IgA), autoimmunity and accumulation of a polyclonal population of T cells called double-negative CD4-CD8- T cells. Affected individuals can be diagnosed on the basis of the presence of these double-negative α/β T cells.

Referrals
- Affected patients should be referred to the Molecular Immunology laboratory at GOSH for T cell analysis. This requires prior arrangement and completion of specific request forms (contact Dr Kimberly Gilmour - see details below). We liaise closely with this department and will undertake gene screening in appropriate patients.
- Carrier testing can be offered to relatives of ALPS patients once a disease causing variant has been identified.

Prenatal testing
Prenatal testing is available for families in which a clearly pathogenic variant has been identified - please contact the laboratory to discuss.

Service offered
Molecular screening of the 9 exons of the FAS gene in affected individuals found to have double-negative α/β T cells and based on their clinical details. Cases found to have normal numbers of double-negative α/β T cells may have further investigations such as functional apoptosis assays (contact Prof. Adrian Thrasher - see details below). If DNA from an affected individual is unavailable the parents can be screened for variants where appropriate. Testing of family members for known clearly pathogenic variants is also available.

Technical
Molecular screening is undertaken by sequence analysis of exons 1 to 9 of the FAS gene.

Target reporting time
8 weeks for routine full screen in index case and 4 weeks for familial testing for known clearly pathogenic variants. For urgent samples please contact the laboratory.

To arrange double-negative α/β T cell analysis, or functional apoptosis studies, please contact:
Dr. Kimberly Gilmour, Molecular Immunology, Great Ormond Street Hospital tel.: +44 (0) 20 7829 8835 email: Kimberly.Gilmour@gosh.nhs.uk
Activated PI3 Kinase Delta Syndrome

**Contact details**
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

**Samples required**
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

**Patient details**
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

**Introduction**
Activated PI3 Kinase Delta Syndrome (APDS) is a Primary Immunodeficiency (PID). It is an autosomal dominant disorder due to gain of function pathogenic variants in the catalytic subunit phosphoinositide 3-kinase δ (p110δ) encoded by the PIK3CD gene.

Activation of p110δ can induce cell growth, proliferation and many other cellular functions. APDS is a clinically heterogeneous condition with variable penetrance among affected individuals. The most common manifestation is susceptibility to recurrent infections, progressive lung disease, splenomegaly and lymphoproliferative manifestation with mainly antibody deficiency.

Three recurrent heterozygous gain-of-function pathogenic variants in exon 8, 13 and 24 of the PIK3CD gene have been reported in patients affected by APDS; c.1002C>A p.(Asn334Lys); c.1573G>A p.(Glu525Lys) and c.3061G>A p.(Glu1021Lys) respectively.

**Referrals**
Confirmation of diagnosis in a patient with Activated PI3K-delta syndrome (APDS) / Primary B cell immunodeficiency.
Family members with previously identified PIK3CD clearly pathogenic variant.

**Prenatal testing**
Prenatal diagnosis may not be appropriate - please contact the laboratory to discuss families in whom specific pathogenic variants have been identified or in whom appropriate family studies have been undertaken.

**Service offered**
Testing is performed by targeted direct Sanger sequence analysis of the coding exons 8, 13, 24 of the PIK3CD gene.

**Technical**
Molecular screening is carried out by direct sequencing analysis.

**Target reporting time**
The target reporting time is 8 weeks. Please contact the laboratory for urgent cases.
Cartilage hair hypoplasia (CHH)

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
Pathogenic variants in the untranslated RMRP gene on chromosome 9p13-p12 (encoding the RNA component of RNase MRP endoribonuclease) lead to a wide spectrum of autosomal recessive skeletal dysplasias, ranging from the milder phenotypes metaphyseal dysplasia without hypotrichosis (MDWH) and cartilage hair hypoplasia (CHH) to the severe anauxetic dysplasia (AD). This clinical spectrum includes different degrees of short stature, hair hypoplasia, defective erythrogenesis, and immunodeficiency.

RMRP pathogenic variants are found in both the transcribed region and the promoter region (from the TATA box to the transcription initiation site). A founder pathogenic variant, 70A>G, is present in 92% of Finnish and 48% of non-Finnish patients with CHH (Thiel et al (2007) Am. J. Hum. Genet 81: 519-529).

Nomenclature: Please note, although HGVS recommendations for the description of DNA sequence variants (den Dunnen JT and Antonarakis SE (2000). Hum.Mutat. 15: 7-12) state that numbering should start with 1 at the first nucleotide of the database reference file (genomic Reference Sequence M29916.1), it is common practice in the literature for RMRP numbering to start with 1 at the transcription start site.

Referrals
- Patients with suspected RMRP-related disorder for a full screen of RMRP.
- Adult relatives of patients with RMRP clearly pathogenic variants for carrier status.
- Testing is available for minor siblings to establish carrier status prior to bone marrow/stem cell donation.

Prenatal testing
Prenatal testing is available for families in whom specific clearly pathogenic variants have been identified or in whom appropriate family studies have been undertaken-please contact the laboratory to discuss.

Service offered
Molecular screening of the RMRP gene. Familial testing for relatives of patients with confirmed RMRP clearly pathogenic variants.

Technical
Direct sequencing analysis of the RMRP gene - promoter region and the transcribed region.

Target reporting time
8 weeks for routine full screen in index case.
4 weeks for routine testing of specific pathogenic variants.
For urgent samples please contact the laboratory.
### Familial hemophagocytic lymphohistiocytosis (FHL)

#### Contact details
Regional Genetics Service  
Levels 4-6, Barclay House  
37 Queen Square  
London, WC1N 3BH  
T +44 (0) 20 7762 6888  
F +44 (0) 20 7813 8578

#### Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

#### Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

#### Introduction
Familial hemophagocytic lymphohistiocytosis (FHL) due to perforin gene defects (MIM 603553) is a rare autosomal recessive immunodeficiency characterised by defective or absent T and natural killer (NK) cell cytotoxicity. Affected individuals can be diagnosed on the basis of very low or absent perforin protein. The perforin gene, PRF1 has 3 exons of which exons 2 and 3 are coding. Pathogenic variants are found throughout the gene with some evidence of founder variants. Only 20-40% of FHL cases are due to defects in the perforin gene. FHL disease-causing variants have been identified in several other genes: UNC13D (FHL3), STX11 (FHL4), and STXBP2 (FHL5).

#### Referrals
- Affected patients should be referred to the Molecular Immunology laboratory at GOSH for perforin protein analysis. This requires prior arrangement and completion of specific request forms (see contact information below). We work closely with this department and will undertake molecular analysis in appropriate patients.
- Familial testing can be offered to the relatives of FHL/PRF1 patients once a clearly pathogenic variant has been identified. However, due to the rarity of the disorder partner screening is not offered unless there is consanguinity or a family history of FHL in the partner.

#### Prenatal testing
Prenatal testing is available for families in which a clearly pathogenic variant has been identified - please contact the laboratory to discuss.

#### Service offered
Molecular screening of the PRF1 gene in affected individuals found to have absent or abnormal perforin expression. For cases where there is strong clinical indication of FHL but where evaluation of perforin protein is either not possible or where expression is normal, a full screen test may still be undertaken but will be considered on a case by case basis. If DNA from the affected individual is unavailable and there is a strong clinical indication of FHL, then screening can be undertaken in the parents. Familial known variant testing for clearly pathogenic variants is also available.

#### Technical
Molecular screening is undertaken by sequence analysis of the PRF1 gene.

#### Target reporting time
8 weeks for routine full screen in index case and 4 weeks for familial testing. For urgent samples please contact the laboratory.

To arrange perforin expression studies please contact Dr Kimberly Gilmour in Molecular Immunology, GOSH - Tel: +44 (0) 20 7829 8835,  
Email: Kimberly.Gilmour@gosh.nhs.uk
IL7R-alpha severe combined immunodeficiency (IL7RαSCID)

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
Interleukin 7 receptor alpha severe combined immunodeficiency (IL7Rα-SCID, MIM 608971) is a rare autosomal recessive immunodeficiency characterised by a lack of circulating T cells, but normal B and natural killer cells (T B-NK+). Affected individuals can be diagnosed on the basis of an abnormality or deficiency of the IL7Rα protein. The IL7Rα gene has 8 exons and pathogenic variants have been found throughout the gene.

Referrals
- Affected patients should be referred to the Molecular Immunology laboratory at GOSH for IL7Rα protein analysis. This requires prior arrangement and completion of specific request forms (see contact information below). We work closely with this department and will undertake gene screening in appropriate patients.
- Carrier testing can be offered to the relatives of IL7Rα-SCID patients once a clearly pathogenic variant has been identified. However, due to the rarity of the disorder, partner screening is not offered unless there is consanguinity or a family history of IL7Rα-SCID in the partner.

Prenatal testing
Prenatal testing is available for families in whom known clearly pathogenic variants have been identified or in whom appropriate family studies have been undertaken - please contact the laboratory to discuss.

Service offered
Molecular screening of the IL7Rα gene in affected individuals found to have absent or abnormal IL7Rα expression. For cases where there is a strong clinical indication of IL7Rα-SCID but where evaluation of IL7Rα protein is either not possible or where expression is normal, testing may still be undertaken but will be considered on a case-by-case basis. If DNA from an affected individual is unavailable then screening can be undertaken in the parents. Familial testing for known pathogenic variants is also available.

Technical
Molecular screening is undertaken by sequence analysis of the IL7Rα gene.

Target reporting time
8 weeks for routine full screen in index case and 4 weeks for familial testing. For urgent samples please contact the laboratory.

To arrange IL7Rα expression studies please contact Dr Kimberly Gilmour in Molecular Immunology, GOSH – Tel: +44 (0) 20 7829 8835, Email: Kimberly.Gilmour@gosh.nhs.uk
JAK3-deficient severe combined immunodeficiency (JAK3-SCID)

Introduction

JAK3-SCID (MIM 600802) is an autosomal recessive immunodeficiency characterised by a lack of circulating T cells. Affected individuals can be diagnosed on the basis of an abnormality or deficiency of the Janus 3 kinase protein (JAK3). The JAK3 gene has 24 exons (23 coding) and pathogenic variants are found throughout the gene.

Referrals

- Affected patients should be referred to the Molecular Immunology laboratory at GOSH for JAK3 protein analysis. This requires prior arrangement and completion of specific request forms (see contact information below). We work closely with this department and will undertake gene screening in appropriate patients.
- Familial testing can be offered to the relatives of JAK3 patients once a clearly pathogenic variant has been identified, however due to the rarity of the disorder, partner screening is not offered unless there is consanguinity or a family history of JAK3-SCID in the partner.

Prenatal testing

Prenatal testing is available for families in whom clearly pathogenic variants have been identified - please contact the laboratory to discuss.

Service offered

Molecular screening of the JAK3 gene in affected individuals found to have absent or abnormal JAK3 expression. Cases found to have JAK3 expression may be screened if there is a strong clinical indication for a diagnosis of JAK3-SCID. If DNA from the affected individual is unavailable screening can be undertaken in the parents. Familial testing for known clearly pathogenic variants is available.

Technical

Molecular screening is undertaken by sequence analysis of the JAK3 gene.

Target reporting time

8 weeks for routine full screen in index case and 4 weeks for familial testing. For urgent samples please contact the laboratory.

To arrange JAK3 expression studies please contact Dr Kimberly Gilmour in Molecular Immunology, GOSH

Tel: +44 (0) 20 7829 8835
Email: Kimberly.Gilmour@gosh.nhs.uk
## Introduction

EBV-associated autosomal lymphoproliferative syndrome (ITK deficiency) presents with a similar but not identical phenotype to X-linked lymphoproliferative disease (XLP) characterised by lymphoproliferation and severe immune dysregulation following Epstein-Barr virus (EBV infection) and is inherited in an autosomal recessive manner. As in XLP, not all 3 classic clinical manifestations (fulminant infectious mononucleosis, lymphoproliferative disorder and dysgammaglobulinaemia) may develop and patients can develop different immune mediated syndromes including fatal haemophagocytosis, hypogammaglobulinaemia and autoimmune phenomena presenting as renal disease. While in XLP the predominant lymphoproliferation is Burkitt’s lymphoma, 4 out of 5 published patients with ITK deficiency had Hodgkin’s disease. Infection with EBV is a universal component of the phenotype.

## Referrals

- Affected patients should be referred to the Molecular Immunology laboratory at GOSH for ITK protein analysis. This requires prior arrangement and completion of specific request forms (see contact information below). We work closely with this department and will undertake gene screening in appropriate patients.
- Familial testing can be offered to relatives of ITK patients once a clearly pathogenic variant has been identified. However, due to the rarity of the disorder, partner screening is not offered unless there is consanguinity or a family history of ITK in the partner.

## Prenatal testing

Prenatal testing is available for families in whom clearly pathogenic variants have been identified. Please contact the laboratory to discuss.

## Service offered

Molecular screening of the ITK gene in affected individuals found to have absent or abnormal ITK expression. Cases found to have ITK expression will be screened due to lack of sensitivity data for the protein test.

## Technical

Molecular screening is undertaken by sequence analysis of the 17 exons and exon/intron boundaries.

MLPA analysis for deletion/duplication testing.

## Target reporting time

8 weeks for routine full screen in index case and 4 weeks for familial testing. For urgent samples please contact the laboratory.

## To arrange ITK expression studies please contact

Dr Kimberly Gilmour, Molecular Immunology, GOSH

Tel: +44 (0) 20 7829 8835

Email: Kimberly.Gilmour@gosh.nhs.uk
Introduction

Netherton syndrome (Comèl-Netherton syndrome) (NS) (MIM 256500) is an autosomal recessive multisystemic disorder characterised by localised or generalised congenital ichthyosis, hair shaft abnormalities (trichorrhexis invaginata), atopic diathesis, immune deficiency and markedly elevated IgE levels. The condition predominantly affects females. Some infants with Netherton syndrome develop progressive hypernatremic dehydration, failure to thrive, and enteropathy. These complications can be fatal. NS is caused by mutations in the SPINK5 gene on chromosome 5q32, encoding the serine protease inhibitor LEKTI.

Referrals

- Confirmation of diagnosis in individuals clinically suspected of having Netherton syndrome.
- Carrier testing can be offered to relatives of affected patients once the disease causing mutations in the family have been identified.

Prenatal testing

Prenatal testing is available for couples in whom specific mutations have been identified - please contact the laboratory to discuss.

Service offered

1) Bi-directional sequence analysis of all 34 coding exons and intron-exon boundaries in SPINK5.
2) The c.2468dup, p.LysGlufs*4 pathogenic variant can be detected by fluorescent fragment analysis of the (A)_{10} homopolymer tract in exon 26 of the SPINK5 gene. Two alternative forward primers are used to allow accurate sizing of the repeat region.

Target reporting time

8 weeks for routine sequence analysis of the SPINK5 gene and fragment analysis for the c.2468dup, p.LysGlufs*4 variant. 4 week turnaround time for testing of a known familial pathogenic variant. Please contact the laboratory if urgent or prenatal testing is required.
Introduction

Severe combined immunodeficiency (SCID) is a group of genetically and phenotypically heterogeneous disorders that can be immunologically classified by the absence or presence of T, B, and natural killer (NK) cells. The most severe form of SCID has the T-B-NK+ phenotype, accounting for ~20% of all cases in which patients present with a virtual absence of both circulating T and B cells, while maintaining a normal level and function of NK cells. This form of SCID is caused by autosomal recessive pathogenic variants in at least three primary genes necessary for V(DJ) recombination, RAG1, RAG2, and DCLRE1C (ARTEMIS). DCLRE1C pathogenic variants cause a T and B cell deficient form of SCID that is clinically indistinguishable from a RAG1/RAG2 disorder. Infants present with severe recurrent viral, bacterial or fungal infections and failure to thrive. Defects in DCLRE1C can be distinguished from RAG defects because the former has the additional feature of increased sensitivity to ionising radiation in bone marrow and fibroblast cells. The DNA crosslink repair 1C gene (DCLRE1C; MIM 605988) encodes ARTEMIS which is an essential factor of V(DJ) recombination during lymphocyte development and in the repair of DNA double-strand breaks (DSB) by the non-homologous end joining (NHEJ) pathway. Patients with pathogenic variants in the DCLRE1C gene, suffer from radiosensitive SCID (RS-SCID; MIM 602450) or radiosensitive Omenn syndrome (MIM 603554). The DCLRE1C gene (10p13) has 14 exons and pathogenic variants have been found throughout the gene. The most frequent pathogenic variants reported are gross deletions (59%) due to homologous recombination of the wild-type DCLRE1C gene with a pseudo-DCLRE1C gene located 61.2 kb 5' to the DCLRE1C start codon.

Referrals

- Patients with suspected RS-SCID.
- Carrier testing can be offered to the relatives of RS-SCID or Omenn syndrome patients once a clearly pathogenic variant has been identified.
- Radiation sensitivity testing can be performed by MRC Sussex (Professor Penny Jeggo). Please contact the laboratory to discuss.

Prenatal testing

Prenatal testing is available for families in whom specific clearly pathogenic variants have been identified or in whom appropriate family studies have been undertaken. Please contact the laboratory to discuss.

Service offered

- Sequencing analysis is performed of the coding regions and splice sites of the DCLRE1C gene.
- MLPA analysis for deletion/duplication testing

Target reporting time

8 weeks for routine sequencing screen in index case and 4 weeks for familial testing for known pathogenic variants. For urgent samples please contact the laboratory.

Contact details for Molecular Immunology / Enzyme unit:

Dr Kimberly Gilmour, Molecular Immunology, GOSH - Tel: +44 (0) 20 7829 8835,
Email: Kimberly.Gilmour@gosh.nhs.uk
Enzyme Unit, Chemical Pathology, Great Ormond Street Hospital, London WC1N 3JH Tel: +44 (0) 207405 9200 (x2509)
RAG-deficient severe combined immunodeficiency (RAG-SCID)

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
• 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
• Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
• Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
• A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
RAG-deficient severe combined immunodeficiency (RAG-SCID, MIM 601457) is an autosomal recessive immunodeficiency characterised by a lack of circulating T and B cells. Affected individuals can be diagnosed on the basis of an abnormality or deficiency of the V(D)J recombinase subunits, RAG1 and RAG2. The RAG1 and RAG2 genes have 2 exons of which exon 2 is coding. Clearly pathogenic variants have been found throughout the coding region of both genes. Omenn syndrome (MIM 603554) is a leaky TlowB-SCID characterised by reticuloendotheliosis and eosinophilia. It is caused by pathogenic variants in RAG1 and RAG2 that result in a partially functional recombinase.

Referrals
• Patients with suspected RAG-SCID.
• Familial testing can be offered to the relatives of RAG-SCID or Omenn syndrome patients once a clearly pathogenic variant has been identified. However, due to the rarity of the disorder, partner screening is not offered unless there is consanguinity or a family history of RAG-SCID or Omenn syndrome in the partner.
• RAG1 and RAG2 protein analysis can be performed by the Molecular Immunology laboratory at GOSH but requires a bone marrow sample from affected patients. This requires prior arrangement and completion of specific request forms (see contact information below).

Prenatal testing
Prenatal testing is available for families in whom clearly pathogenic variants have been identified or in whom appropriate family studies have been undertaken. Please contact the laboratory to discuss.

Service offered
Molecular screening of the RAG1 and RAG2 genes in affected individuals. Due to the requirement of a bone marrow sample for protein analysis, a full screen will be carried out in the absence of protein testing if clinically indicated. If DNA from the affected individual is unavailable then screening can be undertaken in the parents.

Technical
Molecular screening is undertaken by sequence analysis of the RAG1 and RAG2 genes.

Target reporting time
8 weeks for routine full screen in index case and 4 weeks for targeted familial testing. For urgent samples please contact the laboratory.

To arrange RAG expression studies please contact
Dr Kimberly Gilmour, Molecular Immunology, GOSH
Tel: +44 (0) 20 7829 8835,
Email: Kimberly.Gilmour@gosh.nhs.uk
**Wiskott-Aldrich syndrome (WAS)**

**Contact details**
Regional Genetics Service  
Levels 4-6, Barclay House  
37 Queen Square  
London, WC1N 3BH  
T +44 (0) 20 7762 6888  
F +44 (0) 20 7813 8578

**Samples required**
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

**Patient details**
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

**Introduction**

WAS (MIM 301000) is an X-linked immunodeficiency characterised by thrombocytopenia and abnormal B- and T-cell functions. In carrier women this manifests as a skewed X-inactivation pattern in their whole blood. Affected individuals can be diagnosed on the basis of an abnormality or deficiency of the Wiskott-Aldrich syndrome protein (WASP). The WAS gene (encoding WASP) has 12 exons and pathogenic variants are found throughout the gene.

**Referrals**
- Affected patients should be referred to the Molecular Immunology laboratory at GOSH for WAS protein analysis. This requires prior arrangement and completion of specific request forms (see contact information below). We work closely with this department and will undertake molecular screening in appropriate patients.
- Carrier testing can be offered to the female relatives of WAS patients once a clearly pathogenic variant has been identified.

**Prenatal testing**
Prenatal testing is available for families in whom clearly pathogenic variants have been identified or in whom appropriate family studies have been undertaken. Please contact the laboratory to discuss.

**Service offered**
Molecular screening of the WAS gene in affected individuals found to have absent or abnormal WASP expression. Cases found to have WASP expression may be screened if there is a strong clinical indication for a diagnosis of WAS. If DNA from an affected male is unavailable the mother can be tested for her X-inactivation status and subsequent variant screening carried out where appropriate.

**Technical**
Molecular screening is undertaken by sequencing analysis.

**Target reporting time**
8 weeks for routine full screen in index case. 4 weeks for familial testing. For urgent samples please contact the laboratory.

**To arrange WASP expression studies please contact**
Dr Kimberly Gilmour, Molecular Immunology, GOSH  
Tel: +44 (0) 20 7829 8835,  
Email: Kimberly.Gilmour@gosh.nhs.uk
X-linked agammaglobulinaemia (XLA)

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
XLA (MIM 300300) is an X-linked immunodeficiency characterised by a lack of circulating B cells. In carrier women this manifests as a skewed X-inactivation pattern in their B cells. Affected individuals can be diagnosed on the basis of an abnormality or deficiency of the Bruton’s tyrosine kinase protein (BTK). The BTK gene has 19 exons and pathogenic variants are found throughout the gene.

Referrals
- Affected patients should be referred to the Molecular Immunology laboratory at GOSH for BTK protein analysis. This requires prior arrangement and completion of specific request forms (see contact number below). We work closely with this department and will undertake molecular screening in appropriate patients.
- Carrier testing can be offered to the female relatives of XLA patients once a clearly pathogenic variant has been identified.

Prenatal testing
Prenatal testing is not available for families in whom pathogenic variants have been identified as maternal B cells circulate in newborns for 3-6 months. However, neonatal testing for the familial variant is available.

Service offered
Molecular screening of the BTK gene in affected individuals found to have absent or abnormal BTK expression. Cases found to have BTK expression may be screened if there is a strong clinical indication for a diagnosis of XLA. If DNA from an affected male is unavailable the mother can be tested for her X-inactivation status and subsequent variant screening carried out where appropriate.

Technical
Molecular screening is undertaken by sequence analysis of the 19 exons and exon/intron boundaries of the BTK gene.
MLPA analysis for deletion/duplication testing.

Target reporting time
8 weeks for routine full screen in index case. 4 weeks for familial testing. For urgent samples please contact the laboratory.

To arrange BTK expression studies please contact
Dr Kimberly Gilmour, Molecular Immunology, GOSH
Tel: +44 (0) 20 7829 8835,
Email: Kimberly.Gilmour@gosh.nhs.uk
X-linked hyper IgM syndrome (X-HIGM)

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis.
- A completed DNA request card should accompany all samples.

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician.

Introduction
Hyper IgM syndrome is a primary immunodeficiency characterised by an inability to produce immunoglobulin isotypes other than IgM and IgD resulting in susceptibility to bacterial and opportunistic infections. The disease is genetically heterogeneous with both X-linked recessive and autosomal recessive forms. X-linked HIGM (MIM 308230) is the most common form and affected individuals can be diagnosed on the basis of an abnormality or deficiency of the CD40 ligand protein, CD154. The CD40LG gene (MIM 300386) has 5 exons and pathogenic variants are found throughout the gene.

Referrals
- Affected patients should be referred to the Molecular Immunology laboratory at GOSH for CD154 protein analysis. This requires prior arrangement and completion of specific request forms (see contact information below). We work closely with this department and will undertake molecular screening in appropriate patients.
- Carrier testing can be offered to the female relatives of X-linked HIGM patients once a clearly pathogenic variant has been identified.

Prenatal testing
Prenatal testing is available for families in whom specific clearly pathogenic variants have been identified. Please contact the laboratory to discuss.

Service offered
Molecular screening of the CD40LG gene in affected individuals found to have absent or abnormal CD154 expression. Cases found to have CD154 expression may be screened if there is a strong clinical indication for a diagnosis of HIGM. If DNA from an affected male is unavailable screening can be undertaken in the mother.

Technical
Molecular screening is undertaken by sequence analysis of the 5 exons and exon/intron boundaries of the CD40LG gene.

Target reporting time
8 weeks for routine full gene screen in index case and 4 weeks for familial testing. For urgent samples please contact the laboratory.

To arrange CD154 expression studies please contact
Dr Kimberly Gilmour, Molecular Immunology, GOSH
Tel: +44 (0) 20 7829 8835,
Email: Kimberly.Gilmour@gosh.nhs.uk
X-linked lymphoproliferative disease (XLP)

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
XLP 1 (MIM 308240) and XLP 2 (MIM 300635) are X-linked immunodeficiencies characterised by extreme sensitivity to the Epstein Barr virus (EBV). Affected individuals can be diagnosed on the basis of an abnormality or deficiency of the SLAM associated protein (SAP) or XIAP protein. The SH2D1A gene (encoding SAP) has 4 exons and the XIAP gene has 7 exons (6 coding).

Referrals
- Affected patients should be referred to the Molecular Immunology department at GOSH for SAP/XIAP protein analysis. This requires prior arrangement and completion of specific request forms (see contact information below). We work closely with this department and are able to undertake variant screening in appropriate patients.
- Carrier testing can be offered to the female relatives of XLP patients once a clearly pathogenic variant has been identified.

Prenatal testing
Prenatal testing is available for families in whom clearly pathogenic variants have been identified or in whom appropriate family studies have been undertaken. Please contact the laboratory to discuss.

Service offered
Molecular screening of the SH2D1A or XIAP genes in affected individuals found to have absent or abnormal SAP or XIAP expression. Cases found to have SAP or XIAP expression may be screened if there is a strong clinical indication for a diagnosis of XLP. If DNA from an affected male is unavailable screening can be undertaken in the mother. Familial testing is also available.

Technical
Molecular screening is undertaken by sequence analysis of the 4 exons and exon/intron boundaries for the SH2D1A gene. This detects approximately 43% of variants in patients shown to have abnormal or deficient SAP. This suggests that there is an as yet unidentified molecular defect in some of these patients, which may or may not be in the SH2D1A gene.
Molecular screening of the 7 exons of the XIAP gene is undertaken by sequence analysis.
MLPA analysis for deletion/duplication analysis.

Target reporting time
8 weeks for routine full screen in index case and 4 weeks for familial testing. For urgent samples please contact the laboratory.

To arrange SAP/XIAP expression studies please contact
Dr Kimberly Gilmour, Molecular Immunology, GOSH
Tel: +44 (0) 20 7829 8835,
Email: Kimberly.Gilmour@gosh.nhs.uk
X-linked severe combined immunodeficiency (XSCID)

Introduction

XSCID (MIM 300400) is an X-linked immunodeficiency characterised by a lack of circulating T cells. In carrier women this manifests as a skewed X-inactivation pattern in their T cells. Affected individuals can be diagnosed on the basis of an abnormality or deficiency of the common gamma chain protein (\(\gamma_c\)). The \(IL2RG\) gene (encoding \(\gamma_c\)) has 8 exons and pathogenic variants are found throughout the gene.

Referrals

- Affected patients should be referred to the Molecular Immunology laboratory at GOSH for \(\gamma_c\) protein analysis. This requires prior arrangement and completion of specific request forms (see contact information below). We work closely with this department and will undertake gene screening in appropriate patients.
- Carrier testing can be offered to the female relatives of XSCID patients once a clearly pathogenic variant has been identified.

Prenatal testing

Prenatal testing is available for families in whom clearly pathogenic variants have been identified or in whom appropriate family studies have been undertaken. Please contact the laboratory to discuss.

Service offered

Molecular screening of the \(IL2RG\) gene in affected individuals found to have absent or abnormal \(\gamma_c\) expression. Cases found to have \(\gamma_c\) expression may be screened if there is a strong clinical indication for a diagnosis of XSCID. If DNA from an affected male is unavailable the mother can be tested for her X-inactivation status and subsequent molecular screening where appropriate.

Technical

Molecular screening is undertaken by sequence analysis of the 8 exons and exon/intron boundaries. This detects approximately 90% of mutations in patients shown to have abnormal or deficient \(\gamma_c\).

Target reporting time

8 weeks for routine full screen in index case. 4 weeks for familial testing. For urgent samples please contact the laboratory.

To arrange \(\gamma_c\) expression studies please contact

Dr Kimberly Gilmour, Molecular Immunology, GOSH
Tel: +44 (0) 20 7829 8835,
Email: Kimberly.Gilmour@gosh.nhs.uk

Contact details

Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required

- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician.
Aortopathy

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request form should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
Aortopathies are a group of conditions linked by disease of the aorta: aortic aneurysm, dilatation and dissection are cardinal features. Other features include abnormality of the aortic valves (bicuspid aortic valve). Aortopathies can be associated with syndromic connective tissue disorders such as Marfan syndrome, Loeys-Dietz syndrome and Ehlers-Danlos syndrome.

Aortopathies can be inherited in autosomal dominant, recessive or X-linked manner.

Referrals
- Patients with a strong clinical suspicion of a monogenic predisposition to aortopathy
- Referrals will be accepted from clinical geneticists and consultants in cardiology.

Prenatal testing
Prenatal diagnosis may be offered as appropriate where pathogenic variants have been identified in accordance with expected inheritance pattern and where appropriate parental testing and counselling has been conducted.

Service offered
Analysis of coding regions and intron/exon boundaries of the following genes. If no clearly pathogenic variant is identified, re-analysis of the clinical exome data may be offered for other loci as appropriate.
Aortopathy panel (AORT_v3):
ACTA2, COL3A1, EFEMP2, FBN1, FBN2, FLNA, LOX, MYH11, MYLK, PLOD1, PRKG1, SKI, SLC2A10, SMAD3, SMAD4, TGFB2, TGFB3, TGFBR1, TGFBR2

Technical
Variant screening is carried out by next generation sequencing with library preparation using the Agilent focused clinical exome +1 kit followed by sequencing on the Illumina platforms. Data is analysed using an in-house pipeline with all pathogenic variants confirmed by Sanger sequencing.

Target reporting time
4 months for next generation sequencing screening in an index case. 4 weeks for familial testing.

Please contact the laboratory for urgent cases.
Ciliopathies Gene Panel

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request form should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
The ciliopathies are a heterogeneous group of conditions with considerable phenotypic overlap. These inherited diseases are caused by defects in cilia; hair-like projections present on most cells, with roles in key human developmental processes via their motility and signalling functions. Ciliopathies are often lethal and multiple organ systems are affected. Ciliopathies are united in being genetically heterogeneous conditions and the different subtypes can share many clinical features, predominantly cystic kidney disease, but also retinal, respiratory, skeletal, hepatic and neurological defects in addition to metabolic defects, laterality defects and polydactyly. Their clinical variability can make ciliopathies hard to recognise, reflecting the ubiquity of cilia. Gene panels currently offer the best solution to tackling analysis of genetically heterogeneous conditions such as the ciliopathies. Ciliopathies affect approximately 1:2,000 births.

Ciliopathies are generally inherited in an autosomal recessive manner, with some autosomal dominant and X-linked exceptions.

Referrals
- Patients presenting with a ciliopathy; due to the phenotypic variability this could be a diverse set of features. For guidance contact the laboratory or Dr Hannah Mitchison (h.mitchison@ucl.ac.uk) / Prof Phil Beales (p.beales@ucl.ac.uk)
- Referrals will be accepted from clinical geneticists and consultants in nephrology, metabolic, respiratory and retinal diseases.
- Testing for Bardet-Biedl syndrome is NCG-funded for patients resident in England and Scotland.

Prenatal testing
Prenatal diagnosis may be offered as appropriate where pathogenic variants have been identified in accordance with expected inheritance pattern and where appropriate parental testing and counselling has been conducted.

Service offered
Analysis of coding regions and intron/exon boundaries of up to 123 genes. Analysis may be based on a sub-panel appropriate to the presentation (preferable), or if diagnosis is unclear, analysis of the entire panel may be requested. See next page for gene lists.

Sub-panels:
- Primary ciliary dyskinesia (PCD) and reduced generation of multiple motile cilia (RGMC) syndrome
- Bardet Biedl syndrome (BBS)
- Visceral Heterotaxy
- Orofaciodigital syndrome (OFDS)
- Alstrom syndrome
- Meckel syndrome
- Skeletal ciliopathies
- Polycystic kidney disease, nephronophthisis and related disorders
- Joubert syndrome and Senior Löken syndrome

Technical
Variant screening is carried out by next generation sequencing with library preparation using the Agilent focused clinical exome +1 kit followed by sequencing on the Illumina platforms. Data is analysed using an in-house pipeline with all pathogenic variants confirmed by Sanger sequencing.
Ciliopathies Gene Panel

Target reporting time

4 months for next generation sequencing screening in an index case. 4 weeks for familial testing. Please contact the laboratory for urgent cases.

Full gene list (CILI_v3):
ACVR2B, AH1, ALMS1, ANKS6, ARL13B, ARL6, ARMC4, B9D1, B9D2, BBIP1, BBS1, BBS10, BBS12, BBS2, BBS4, BBS5, BBS7, BBS9, C1ORF59, C2CD3, C5ORF42, CC2D2A, CCDC103, CCDC114, CCDC151, CCDC28B, CCDC39, CCDC40, CCDC65, CNON, CE120, CEP12, CEP164, CEP290, CEP41, CEP83, CFAP53, CFC1*, CRELD1, CSP1, DCCD2, DDX59, DNAF1, DNAF2, DNAF3, DNAF5, DNAH11, DNAH5, DNAI1, DNAI2, DNAL1, DRCL1, DYX2H1, DYX1C1, EVC, EVCD, GAS8, GDF1, GL13, GLIS2, HNF1B, HYLS1, IFT122, IFT140, IFT172, IFT27, IFT43, IFT80, INPP5E, INVS, IQCB1, KIAA0586, KIF7, LBR, LRRC6, LZTL1, MCIDAS, MKS, MKS1, NEK1, NEK8, NDE8, NODAL, NPHP1, NPHP3, NPHP4, OFD1, OFD6D, PKD1*, PKD2, PKHD1, POC1B, PRKCSH, RPGR*, RPGRIP1L, RPSPH1, RPSPH4A, RPSPH9, SBDS, SCL1, SDCCAG8, SEC63, SPAG1, TBC1D2, TCTN1, TCTN2, TCTN3, TMEM138, TMEM216, TMEM231, TMEM237, TMEM67, TRIM32, TT21B, TT28, UMOD, WDPCP, WDR19, WDR34, WDR35, WDR60, ZC3, ZMYND10, ZNF423

Primary ciliary dyskinesia (PCD) and reduced generation of multiple motile cilia (RGMC) syndrome (PCD_v3)
ARM4C, C1ORF59, CCDC103, CCDC114, CCDC151, CCDC39, CCDC40, CCDC65, CNON, DNAF1, DNAF2, DNAF3, DNAF5, DNAH11, DNAH5, DNAI1, DNAI2, DNAL1, DRCL1, DYX1C1, GAS8, LRRC6, MCIDAS, NDE8, OFD1, RPGR*, RPSPH1, RPSPH4A, RPSPH9, SPAG1, ZMYND10

Bardet Biedl syndrome (BBS)
ARL6, BBIP1, BBS1, BBS2, BBS4, BBS5, BBS7, BBS9, BBS10, BBS12, CCDC28B, CE290, IFT27, LZTFL1, MKS, MKS1, SDCCAG8, TRIM32, TT28, WDPCP

Visceral Heterotaxy (HETEROTAXY_v2)
ACVR2B, CFAP53, CFC1, CRELD1, GDF1, NODAL, ZIC3

Orofaciodigital syndrome (OFDS_v2)
C2CD3, C5ORF42, DDX59, OFD1, SCL1, TBC1D2, TCTN3

Alström syndrome (ALMS)
ALMS1

Meckel syndrome (MECKEL)
B9D1, B9D2, CE290, HYLS1, MKS1, NPHP3, TCTN2, TMEM216, TMEM67

Skeletal ciliopathies (SKELCIL)
C5orf42, CE120, CSP1, DDX59, DYX2H1, EVC, EVCD, IFT43, IFT80, IFT122, IFT140, IFT172, LBR, NEK1, OFD1, SBDS, TCTN3, TMEM216, TT21B, WDR19, WDR34, WDR35, WDR60

Polycystic kidney disease, nephronophthisis and related disorders (PKDNeph_v3)
ANKS6, CE164, CE293, DCCD2, GLIS2, HNF1B, IFT43, INVS, NEK8, NPHP1, NPHP3, NPHP4, PKD1*, PKD2, PKHD1, PRKCSH, SEC63, TMEM67, TT21B, UMOD, WDR19, ZNF423

Joubert syndrome and Senior Loken syndrome (JOUBERT_v2)
AH1, ARL13B, C5ORF42, CC2D2A, CE41, CSP1, GLI3, INPP5E, IQCB1, KIAA0586, KIF7, NPHP4, OFD1, PDE6D, POC1B, RPGRIP1L, SDCCAG8, TCTN1, TCTN3, TMEM138, TMEM216, TMEM231, TMEM237, WDR19, ZNF423

*Genes marked with an asterisk have low (<90%) horizontal coverage due to sequence context and/or the presence of highly homologous regions in the genome.
**GOSHome Gene Panel**

**Contact details**
Regional Genetics Service  
Levels 4-6, Barclay House  
37 Queen Square  
London, WC1N 3BH  
T +44 (0) 20 7762 6888  
F +44 (0) 20 7813 8578

**Samples required**
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request form should accompany all samples

**Patient details**
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

**Introduction**
Testing is now available using a supplemented clinical exome. This test targets around 5000 known disease-causing genes. In collaboration with clinical and research groups in the UCL Institute of Child Health and Great Ormond Street Hospital we have developed curated virtual panels covering a wide range of genetically heterogeneous conditions.

Testing is currently available for the aortopathies, ciliopathies, dermatological conditions and ocular disorders.

Patients are sequenced across the full panel, but only genes from the selected virtual panel will be analysed, avoiding incidental findings and minimising variants of uncertain significance. Following initial testing, expansion of the analysis may be offered where no clearly causative mutation is identified.

Testing of gene groups outside of the curated panels, or individual genes, may be requested where there is substantive evidence linking the phenotype and gene(s) requested.

**Referrals**
- Referrals will be accepted from clinical geneticists and consultants in appropriate specialties

**Service offered**
Analysis of coding regions and intron/exon boundaries of all genes in the requested virtual panel.
Gene lists are available in the individual panel service packs and coverage of virtual panels and genes is available upon request.

**Technical**
Variant screening is carried out by next generation sequencing with library preparation using the Agilent focused clinical exome +1 kit followed by sequencing on the Illumina NextSeq. Data is analysed using an in-house pipeline with all pathogenic variants confirmed by Sanger sequencing.

**Target reporting time**
4 months for next generation sequencing screening in an index case. 4 weeks for familial testing.
Please contact the laboratory for urgent cases.
Dermatology Gene Panel

Introduction

Inherited conditions affecting the skin are a diverse group of genetically heterogeneous disorders. Inheritance may be autosomal dominant, recessive, or X-linked, and mosaic dermatological conditions are increasingly recognised. Where conditions are highly heterogeneous a panel of known causative genes are tested to maximise clinical sensitivity and minimise time to diagnosis.

Referrals

- Patients presenting with symptoms consistent with the genes and conditions listed.
- Referrals will be accepted from clinical geneticists and consultants in dermatology and paediatrics

Prenatal testing

Prenatal diagnosis may be offered as appropriate where pathogenic variants have been identified in accordance with expected inheritance pattern and appropriate parental testing and counselling has been conducted.

Service offered

Analysis of coding regions and intron/exon boundaries of the genes listed in the sub-panels. If no clearly pathogenic variant is identified, re-analysis of the clinical exome data may be offered for other dermatology sub-panels or other loci as appropriate.

Dermatology virtual panels (see page 2 for gene lists):

- Mendelian disorders of cornification / palmoplantar keratodermas
- Ectodermal disorders
- Connective/adipose tissue disorders
- RASopathies and pigmentary disorders
- Cutaneous vascular disorders
- Inflammatory skin disorders
- Progeroid/premature ageing syndromes
- DNA repair disorders
- Epidermolysis bullosa/skin fragility

Technical

Variant screening is carried out by next generation sequencing (NGS) with library preparation using the Agilent focused clinical exome +1 kit followed by sequencing on the Illumina NextSeq. Data is analysed using an in-house pipeline with all likely or clearly pathogenic variants confirmed by Sanger sequencing.

Target reporting time

4 months for a full NGS screen in an index case. 4 weeks for familial testing.

Please contact the laboratory for urgent cases
Dermatology virtual panels:

Mendelian disorders of cornification and palmoplantar keratodermas (CORN_v2):

AAAGAB, ABCA12, ABHD5, ADAM17, ALDH3A2, ALOX12B, ALOX3, AP1S1, AQP5, ARSE, CDSN, CERS3, CLDN1, CSTA, CTSC, CYP4F22, DSC2, DSC3, DSG1, DSG4, DSBP, ELOVL4, FLG, GJB2, JUP, KANK2, KRT1, KRT10, KRT12, KRT16, KRT3, LIPN, LOR, MBTPS2, NIPAL4, PEX7, PHYH, PKP1, PNPLA1, RHBDL2, SASH1, SLC27A4, SLURP1, SNAP29, SPINK5, ST14, STS, TGM1, TRPV3, VPS33B

Ectodermal disorders (ECTODERM_v2):

ADAM10, ARSE, ATP2A2, ATP7A, ATR, CAST, CDH3, CDSN, DCLRE1C, DCK1, DSC2, DSC3, DSG1, DSG4, DSP, EDA, EDA2R, EDAR, EDARADD, GA1, GJB2, GJB3, GJB4, GJB6, GRHL2, HOXC13, HR, IKBKG*, JUP, KANK2, KRT1, KRT14, KRT16, KRT17, KRT5, KRT6A, KRT6B, KRT85, LAMA3, LAMB3, LIPN, LPAR6, MBTPS2, MSX1, NFKBIA, PKP1, POFUT1, POGLUT1, PORCN, PVR1L, PVRL4, RAG1, RAG2, RECOL4, RIN2, SHOC2, SLC29A3, SMARCAD1, SOX18, TP63, TWIST2, USB1, WNT10A

Connective/adipose tissue disorders (CAD_v2):

ABCC6, ADAMTS2, ALDH1A1, ATP6V0A2, B3GALTL6*, B4GALT7, BANF1, CECR1, CHST14, COL1A1, COL1A2, COL5A1, DSE, ECM1, EFEMP2, ELN, FBLN5, FBNI1, FKB14, LEMD3, LMNA, PLOD1, PRDM5, PYCR1, RAG1, RAG2, RECOL4, RIN2, SLC39A13, TGFB1, TGFBR2, TNXB*, ZMPSTE24, ZNF468

RASopathies and Pigmentary disorders (PIGMENT_v3):

ABCB6, ABCD4, ADAM10, ADAR, AP3B1, ARSE, BAP1, BRAF, CBL, CDK4, CDKN2A, DCK1, EDN3, EDNRB, ENPP1, FGFR2, FLNA, GALNT3, GAJ1, GJB3, GJB4, GNA11, GNAQ, GNAS, HPS1, HRAS, KIT, KITLG, KRAS, KRT14, KRT5, LYST, MAP2K1, MAP2K2, MC1R, MITF, MLH1, MS2H, MS6H, MTO1, MYO5A, NF1, NF2, NOP10, NRAS, OCA2, OSIR, PALB2, PAX3, P53CA, PMS2, POFUT1, POGLUT1, PORCN, PRKAR1A, PRKAR1B, PTEN, PTPN11, RAB27A, RAF1, RECOL4, RIT1, SAMD9, SASH1, SHOC2, SLC24A5, SLC29A3, SLC45A2, SLX4, SNA2, SOD1, SOX10, SOX18, SPRED1, STK11, TERC, TERT, TINF2, TSC1, TSC2, TY3, TYK1, USB1, WRAP53

Vascular disorders (VASULAR_v2):

ATM, ATR, CBEB1, ENG, F12, FLT4, FOXC2, GLMN, GNA11, GNAO, KDR, KRT1, KRT3CA, PIK3R2, RASA1, SCND1, SMAD4, SOX18, TEK, TME173

Inflammatory skin disorders (ISD_v2):

ABCC6, ABCD4, ADAM10, ADAR, AP3B1, ARSE, BAP1, BRAF, CBL, CDK4, CDKN2A, DCK1, EDN3, EDNRB, ENPP1, FGFR2, FLNA, GALNT3, GAJ1, GJB3, GJB4, GNA11, GNAQ, GNAS, HPS1, HRAS, KIT, KITLG, KRAS, KRT14, KRT5, LYST, MAP2K1, MAP2K2, MC1R, MITF, MLH1, MS2H, MS6H, MTO1, MYO5A, NF1, NF2, NOP10, NRAS, OCA2, OSIR, PALB2, PAX3, P53CA, PMS2, POFUT1, POGLUT1, PORCN, PRKAR1A, PRKAR1B, PTEN, PTPN11, RAB27A, RAF1, RECOL4, RIT1, SAMD9, SASH1, SHOC2, SLC24A5, SLC29A3, SLC45A2, SLX4, SNA2, SOD1, SOX10, SOX18, SPRED1, STK11, TERC, TERT, TINF2, TSC1, TSC2, TY3, TYK1, USB1, WRAP53

Progeria/premature ageing (PROGERIA_v2):

ABCC6, B3GALT7*, B4GALT7, BANF1, DCX1, ERCC2, ERCC4, ERCC5, ERCC6, LMNA, RECL4

DNA repair disorders (DRD_v3):

ALAS2, BLM, BRCA2, BRIP1, CDAN1, DDB2, ERCC2, ERCC3, ERCC4, ERCC5, ERCC6, ERCC8, FANCA, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCL, GTTF2H5, MLH1, MLLIP, MS2H, MS6H, NOP10, PALB2, PMS2, POLH, RAD51C, SLX4, TERC, TERT, TINF2, WRAP53, XPA, XPC

Epidermolysis bullosa/skin fragility (EBF_v2):

CAST, CD151, CDSN, COL17A1, COL7A1, CSTA, DSC2, DSC3, DSG1, DSG4, DSP, DST, EXPH5, FERMT1, ITGA3, ITGA6, ITGB4, JUP, KRT14, KRT5, LAMA3, LAMB3, LAMC2, PKP1, PLEC, TGM5

*Genes marked with an asterisk have low (<90%) horizontal coverage due to sequence context and/or the presence of highly homologous regions in the genome.
Inherited hearing loss panel

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

Introduction
Inherited or familial hearing loss can present in isolated (non-syndromic) form or as syndromic hearing loss, in combination with other specific phenotypic features.

The genes included in this test contribute to non-syndromic hearing loss as well as those associated with the following syndromic forms: Usher Syndrome, Waardenburg Syndrome, Pendred Syndrome, Perrault Syndrome, Chudley-McCullough Syndrome, Wolfram Syndrome and Branchio-oto-renal Syndrome.

The presentation for patients with these conditions is variable and in some cases may appear to be isolated hearing loss. Prognosis is therefore also variable.

Inheritance of familial hearing loss is gene dependent, and may show autosomal dominant (inherited or de novo), autosomal recessive or X-linked inheritance.

The incidence of pre-lingual severe hearing loss at birth or during early childhood is approximately one per 1000 with a further 1/1000 children becoming deaf before adulthood.

Referrals
- Patients presenting with moderate, severe or profound hearing loss in isolation or in combination with syndromic features consistent with one of the syndromes listed above
- Referrals must originate from either clinical genetics or audiology departments
- All referrals should be accompanied by a completed proforma, which is available from our website (www.labs.gosh.nhs.uk/laboratory-services/genetics/molecular-genetics-service)

Prenatal testing
Please contact the laboratory regarding prenatal testing if it is considered appropriate.

Service offered
Analysis of coding regions and intron/exon boundaries of 108 genes (see list below); variant confirmation and familial tests by Sanger sequencing.

Please note that genes implicated in Usher Syndrome (indicated with an asterisk below) may be requested separately, or in addition to the other genes on this panel. They are not analysed without specific indication.

ACTG1, ATP2B2, BP1, CACBP, CDC50, CD164, CDH23*, CEACAM16, CIB2*, CLDN14, CLC5, CLPP, CLRN1*, COCH, COL11A2, COL4A6, CRYM, DFN5, DFNB31*, DFNB59, DIABLO, DIAPH1, DIAPH3, EDN3, EDNRB, EPS8, ESPN, ESRRB, EYA1, EYA4, GATA3, GIPC3, GJB2, GJB3, GJB6, GPR98*, GPRM2, GRHL2, GRXCR1, HARS*, HARS2, HGF, HSD17B4, IRLR1, KARS, KCE1, KCNJ10, KCNQ1, KCNQ4, KIT, LARS2, LHPP5, LOXHD1, LRTOMT, MARVELD2, MIR96, MTF, MSRB3, MYH14, MYH9, MYO15A, MYO3A, MYO7A*, NARS2, OSSBP2, OTOA, OTOF, OTOG, OTOGL, P2RX2, PAX3, PCDH15*, PDZD7*, PNPT1, POUS3, POUS4, PRPS1, PTPTQ, RDX, RPRG*, SERPINB6, SFX1, SFX5, SLC17A8, SLC26A4, SLC26A5, SLC4A11, SMPX, SNAI2, SOX10, STC, SYNE4, STOR2, TBC1D24, TEATA, TJP2, TMC1, TMIE, TMRPSS3, TNC, TPRN, TRIOBP, TSPEAR, USH1C*, USH1G*, USH2A*, WFS1

Technical
Testing is carried out by next generation sequencing with library preparation using an Agilent SureSelect kit followed by sequencing on the Illumina MiSeq or NextSeq. Data is analysed using an in-house pipeline with all clinically relevant variants confirmed by Sanger sequencing.

Target reporting time
The target reporting time is 16 weeks for a diagnostic screen and 4 weeks for familial testing. Please contact the laboratory for urgent cases.
Introduction

Ocular conditions are highly heterogeneous and show considerable phenotypic overlap. 1 in 2,500 children in the UK are diagnosed as blind or severely visually impaired by the time they reach one year old. As many as half of these cases are likely to be inherited and remain undiagnosed due to the vast number of genes involved in these conditions. Many congenital eye disorders causing visual impairment or blindness at birth or progressive visual impairment also include syndromic conditions involving additional metabolic, developmental, physical or sensory abnormalities. Gene panels offer the enhanced probability of diagnosis as a very large number of genes can be interrogated.

Ocular birth defects include all inheritance modalities. Autosomal dominant and recessive diseases as well as X-linked dominant and recessive diseases are seen. These conditions can also be caused by de novo variants.

Referrals

- Patients presenting with a phenotype appropriate for the requested sub-panel
- Referrals will be accepted from clinical geneticists and consultants in ophthalmology.

Prenatal testing

Prenatal diagnosis may be offered as appropriate where pathogenic variants have been identified in accordance with expected inheritance pattern and where appropriate parental testing and counselling has been conducted.

Service offered

Analysis of coding regions and intron/exon boundaries of the genes listed in the sub-panels. If no clearly pathogenic variant is identified, re-analysis of the clinical exome data may be offered for other ocular sub-panels or other loci as appropriate.

Sub-panels:

- Eye malformations (includes ASD, cataract and expanded MAC panels) (EYEMALF)
- Microphthalmia, anophthalmia and coloboma (MAC) spectrum and aniridia
- Anterior segment dysgenesis (ASD) and glaucoma
- Retinal dystrophies (RETNAL)
- Ocular albinism, photophobia and nystagmus (ALB)
- Cataract, congenital, or lens malformations, congenital (CATARACT)
- Optic atrophy, childhood onset (OPTICATR)
- Eye movement disorders (EMD)

For gene lists see below.

Technical

Variant screening is carried out by next generation sequencing with library preparation using the Agilent focused clinical exome +1 kit followed by sequencing on the Illumina platforms. Data is analysed using an in-house pipeline with all pathogenic variants confirmed by Sanger sequencing.

Target reporting time

4 months for next generation sequencing screening in an index case. 4 weeks for familial testing.

Please contact the laboratory for urgent cases.
Genes marked with an asterisk have low (<90%) homologous regions in the genome.
**Primary immunodeficiency (PID)**

**Contact details**
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

**Samples required**
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

**Patient details**
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

**Introduction**
The primary immunodeficiencies (PID) are a heterogeneous group of >150 disorders that result from defects in immune system development and/or function. PIDs are broadly classified as disorders of adaptive immunity (i.e., T-cell, B-cell or combined immunodeficiencies) or of innate immunity (e.g., phagocyte and complement disorders).

The clinical presentation of PIDs is highly variable; however, most disorders involve increased susceptibility to infection. The type and pattern of infection depends on which part(s) of the host defences are missing or defective since some defences are more important against some pathogens than others. Defects in phagocyte function or humoral immunity (B cell deficiency) result in infections with common and unusual bacteria. Defects in T cell immunity usually present with recurrent viral, fungal, or protozoal infections. Very serious inherited immunodeficiencies become apparent shortly after birth or in the first year of life, and can lead to death if untreated. Others (usually the milder forms) may not present until people reach their twenties and thirties.

The minimum incidence of PID peaked in 2000–08 at 12.5 per 100,000 live births.

**Referrals**
Patients with primary immunodeficiency. A completed proforma should accompany all referrals and is available from our website (http://www.labs.gosh.nhs.uk/laboratory-services/genetics/molecular-genetics-service). Testing can be offered to the relatives of PID patients once a pathogenic variant has been identified.

**Service offered**
Next generation sequencing of 71 genes (see list below) with mutation confirmation by Sanger sequencing.

**List of genes included in panel**
ADA, AICDA, AIRE, BTK, CASP8, CASP10, CD27, CD247, CD3D, CD3E, CD3G, CD40, CD40LG, CIITA, CORO1A, CYBA, CYBB, DCLRE1C, DOCK8, FAS, FASLG, FOXP3, HPS1, HPS4, HPS6, ICOS, IL2RG, IL7R, IL10, IL10RA, IL10RB, ITK, JAK3, LIG4, LRBA, LYST, MAGT1, MYO5B, NCF2, NCF4, NHEJ1, NRAS, ORAI1, PIK3R1, PNP, PRF1, PRKDC, PTPRC, RAB27A, RAG1, RAG2, RFX5, RFXANK, RFXAP, RMRP, SH2D1A, STAT1, STAT3, STAT5A, STAT5B, STX11, STXB2, TAP1, TAP2, TAPBP, TNFRSF13B, UNC13D, UNG, WAS, XIAP, ZAP70

**Technical**
Molecular screening is carried out by next generation sequencing with library preparation using a Sure Select XT custom kit followed by sequencing on the Illumina MiSeq. Data is analysed using an in-house pipeline with all mutations confirmed by Sanger sequencing.

**Target reporting time**
4 months for a full screen in an index case (next generation sequencing).
4 weeks for familial pathogenic variant testing.

Please contact the laboratory for urgent cases.
**Contact details**
Regional Genetics Service  
Levels 4-6, Barclay House  
37 Queen Square  
London, WC1N 3BH  
T +44 (0) 20 7762 6888  
F +44 (0) 20 7813 8578

**Samples required**
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

**Patient details**
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

**Introduction**
Very early onset inflammatory bowel disease (VEO-IBD) affects the gut but also other tissues and as a consequence of persistent inflammation and treatment side effects the function of organs such as the liver may be impeded. Children are often not able to tolerate food and frequently rely on parenteral nutrition. Data is sparse but suggests that patients presenting in the first two years of life generally have a very poor prognosis with high mortality and morbidity (1). VEO-IBD (children with disease-onset before 6 years of age) has an estimated incidence of 4.37/100,000 and a prevalence of 14/100,000. Recent discoveries and published reviews (2) suggest that many monogenic diseases can present with an IBD-like phenotype (monogenic IBD).

Ref:

**Referrals**
Referrals are accepted from Consultant Clinical Geneticists and Consultant Paediatric Gastroenterologists with presentation:
- Patients aged under 6 years of age at onset with bloody diarrhoea & severe failure to thrive
- Severe intestinal inflammation (macro- and microscopic) on upper and/or lower endoscopy
- Histology consistent with chronic inflammatory intestinal pathology
- All referrals should be accompanied by a completed proforma which is available from our website (www.labs.gosh.nhs.uk/laboratory-services/genetics/molecular-genetics-service)

For clinical enquiries, please contact Dr Neil Shah, Gastroenterology, GOSH  
Tel: +44 (0) 20 7405 9200 ext 5949, email: Neil.Shah@gosh.nhs.uk

**Prenatal testing**
Prenatal testing is available for families in whom specific mutations have been identified or in whom appropriate family studies have been undertaken - please contact the laboratory to discuss.

**Service offered**
Analysis of coding regions and intron/exon boundaries of 41 genes (see list below); variant confirmation and familial tests by Sanger sequencing.

ADA, ADAM17, AICDA, BTK, CD3G, CD40LG, CYBA, CYBB, DCLRE1C, DOCK8, EPCAM, FOXP3, GUCY2C, HPS1, HPS4, HPS6, ICOS, IL10, IL10RA, IL10RB, IL2RG, ITGB2, LIG4, LRBA, MYO5B, NCF2, NCF4, PIK3R1, PLCG2, RAG2, RET, SH2D1A, SKIV2L, SLC37A4, STAT1, STAT3, STXB2, TTC37, WAS, XIAP, ZAP70

**Technical**
Molecular screening is carried out by next generation sequencing with library preparation using a Sure Select XT custom kit followed by sequencing on the Illumina MiSeq. Data is analysed using an in-house pipeline with all mutations confirmed by Sanger sequencing.

**Target reporting time**
4 months for a full screen in an index case (next generation sequencing). 4 weeks for familial mutation testing. Please contact the laboratory for urgent cases.
Neonate testing (rapid) for karyotypic sex and common aneuploidies

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- 1-2ml venous blood in plastic lithium heparin tube
- 1-2ml venous blood in plastic EDTA tube if microarray testing is required due to the clinical features
- A completed request card should accompany all samples. Please provide clinical details

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address), details of any relevant family history (including proband’s details) and full contact details for the referring clinician

Introduction
Neonatal testing for karyotypic sex and common aneuploidies is performed upon specific clinician request (using the referral form) in order to provide a rapid result.

Referrals
- Neonates (<3 months of age) with clinical features suggestive of trisomy 13 (Patau syndrome), trisomy 18 (Edwards syndrome), or trisomy 21 (Down syndrome)
- Neonates (<3 months of age) with ambiguous genitalia

NB: testing is not offered for a phenotypically normal baby on the basis of an increased risk of Down syndrome suggested by prenatal screening

Service offered
Testing is offered to neonates with appropriate clinical features (as above).

Technical
The testing is usually performed on interphase cells using FISH probes specific to the chromosomes/regions of interest.
As an alternative to interphase FISH, aneuploidy testing can be performed by karyotyping metaphase cells.
The testing is targeted, and therefore does not exclude the possibility of additional chromosome abnormalities, or investigate the possibility of mosaicism.
If the aneuploidy result is abnormal, karyotyping of metaphase cells will be performed to confirm the result. If the aneuploidy result is normal, further testing will be performed to identify the presence of other chromosome abnormalities; this will be done by karyotyping or microarray depending on the sample received and the clinical phenotype of the patient.

Target reporting time
3 calendar days from date of sample receipt for rapid result
Postnatal Microarray: Developmental delay / Learning Difficulties / Dysmorphism / Multiple Congenital Abnormalities / Epilepsy

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- 2ml venous blood in plastic EDTA bottles (>1ml from neonates) and 2ml in plastic Lithium Heparin bottles.
- A completed request card should accompany all samples. Phenotype information must be provided in order to allow clinical interpretation of the microarray.

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician.

Please contact the laboratory for urgent cases.

Introduction
Genome-wide chromosomal microarray (CMA) detects pathogenic copy number variants (pCNV) in 15-20% of patients with developmental delay, intellectual disability or congenital abnormalities.

CMA may also detect copy number variants of uncertain clinical significance which may require parental follow up testing to aid interpretation.

CMA may also detect pCNVs which are not associated with the presenting phenotype (incidental findings).

Referrals
- Children with developmental delay or learning difficulties with or without dysmorphism.
- Infants or Children with multiple congenital abnormalities.
- Children with epilepsy or neurological disorders.

Service offered
Whole genome microarray analysis at a practical resolution of 200kb for copy number variants (chromosomal deletions and duplications) and additional SNP based identification of uniparental isodisomy and ploidy level changes.

Technical
Whole genome chromosomal microarray analysis using the Affymetrix 750K microarray is performed on DNA extracted from EDTA venous blood. The microarray design uses both single nucleotide polymorphic (SNP) probes and non-polymorphic probes to enable consistent genomic coverage. Copy number variations (CNV) and regions with absence of heterozygosity (AOH) are identified using infoQuant Fusion software. The estimated practical resolution is 200 kb; CNVs below this threshold may not be identified. The microarray will not detect balanced structural chromosome anomalies and may not detect mosaicism. AOH may indicate uniparental isodisomy or regions identical by descent. The CNV identified by the CMA are compared to databases of known genetic variation and to reports of known pathogenic changes. Variants which are not known to have a pathogenic effect or do not have a high risk of pathogenicity may not be reported. AOH of non-imprinted chromosomal regions will not be reported.

pCNVs identified by microarray are confirmed using karyotype, qPCR or Fluorescence In Situ Hybridisation (FISH). These targeted tests are then applied to family members for carrier testing and for prenatal analysis in future pregnancies. Please contact the laboratory for further details.

If pCNV or variants of unknown significance are reported, further samples may be requested (EDTA venous blood and/or lithium heparin venous blood from parents) to aid clinical interpretation and provide a recurrence risk for the family.

Target reporting time
Routine analysis – 28 days.
## Disorders of sexual development/infertility

### Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

### Samples required
- 1-2ml venous blood in plastic lithium heparin tube
- A completed request card should accompany all samples. Please provide clinical details

### Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address), details of any relevant family history (including proband’s details) and full contact details for the referring clinician

### Introduction
Cytogenetic testing can be requested if the patient has abnormal sexual development (e.g. delayed puberty), clinical features suggestive of a sex chromosome abnormality (e.g. Turner syndrome, Klinefelter syndrome), or is experiencing infertility.

### Referrals
- Ambiguous genitalia/indeterminate gender (for neonates, can be in conjunction with ‘rapid’ testing for karyotypic sex)
- Delayed puberty or inappropriate secondary sexual development
- Short stature or amenorrhoea in females
- Oligozoospermia or azoospermia in males
- Premature ovarian failure
- Sperm and egg donors for NHS funded patients
- Couples undergoing assisted conception funded by the NHS

### Service offered
The format of the service offered will vary depending on the patient’s clinical features. The testing is performed by karyotyping (full or targeted), and additional techniques (e.g. fluorescent in situ hybridisation) may be used if an abnormality is suspected or detected during karyotyping.

### Technical
For karyotyping, blood cultures are grown and harvested to yield metaphase cells which are analysed using light microscopy.

If the reason for referral is consistent with the possibility of a sex chromosome abnormality but not an autosomal abnormality (e.g. short stature in a female; query Klinefelter syndrome) a targeted 30 cell score for the sex chromosomes is performed (not a full karyotype). A full karyotype is performed if the sex chromosomes and autosomes need to be examined.

### Target reporting time
Routine analysis – 28 days
If clinical need indicates that an urgent result is required (e.g. current ongoing pregnancy) – 10 days. Please contact the laboratory for urgent cases.
Introduction

Multi-ligation probe amplification (MLPA) and quantitative fluorescence polymerase chain reaction (QF-PCR) are complementary techniques used to identify chromosomal ploidy levels and copy number changes which may explain the pregnancy loss.

When identified, a small number of chromosomal copy number changes will require parental follow-up studies (via blood samples) as the chromosomal copy number change detected in the fetus may have arisen from a balanced rearrangement in one parent. This will provide carrier status information, be useful when planning future pregnancies and may also have further implications for family relatives. It should be noted that in some cases, changes are inherited and are likely to be of no clinical significance.

Referrals

- Testing should be performed on products of conception of the third and subsequent miscarriages (ref: https://www.rcog.org.uk/globalassets/documents/guidelines/gtg_17.pdf).

Service offered

MLPA investigates the subtelomeric regions of all the chromosomes. QF-PCR assesses a number of locations on chromosomes 13, 15, 16, 18, 21, 22, X and Y (these are the commonest trisomies found in pregnancy losses). Used together these techniques will identify monosomy (loss) or trisomy (gain) and triploidy. Mosaicism can be detected if present at significant levels.

Technical

MLPA and QF-PCR are two complimentary techniques that show if there is a chromosomal copy number change that may indicate a cause for a pregnancy loss. Both MLPA subtelomeric analysis using the MRC Holland SALSA P036 subtelomere probe mix and QF-PCR analysis using the Devyser Extend 2 Mix are performed on DNA extracted from foetal derived tissue.

The multiplex PCR method of MLPA uses very small (50-70 nucleotide) sequences as probes. Results are compared with controls and other samples in the run batch to identify gains and losses. Any gains or losses identified that cannot be confirmed by QF-PCR when run at the same time (see below) are run on a second MLPA subtelomere kit, MRC Holland SALSA P070. Both kits must produce concordant results before reporting a loss or a gain. This test will not give information regarding a possible triploid pregnancy.

QF-PCR analysis makes use of small tandem repeats (STRs). STRs can vary in length in individuals. A normal diploid sample has a contribution from two STRs (one from each chromosome). When the STRs are different in size (heterozygous) they give an informative result. When they are the same size (homoygous) the result is not informative, or it may indicate monosomy. There are a minimum of 5 STRs for each of the chromosomes tested by QF-PCR (chromosomes 13, 15, 16, 18, 21, 22, X and Y, as these are the commonest trisomies found in pregnancy losses). Gains, losses, monosomies, trisomies and triploidy can be detected.

A small number of chromosomal copy number changes will require parental follow-up studies to check if they are the result of a parental chromosomal rearrangement. Reports will clearly state if parental bloods are required.

Target reporting time

Routine analysis – 28 days

Please contact the laboratory for urgent cases or private work.
Parental cytogenetic testing

Introduction
Parental cytogenetic testing may be requested following the conception of a cytogenetically abnormal fetus, the birth of a cytogenetically abnormal child, the loss of a pregnancy with a suspected chromosome abnormality, or an unexplained stillbirth/neonatal death.

Referrals
Samples can be referred for a wide variety of reasons (see below):

- parental karyotyping after pregnancy loss of a fetus <24 weeks with multiple congenital abnormalities or severe IUGR, or unexplained stillbirth/neonatal death >24 weeks
- parental testing following MLPA/QF-PCR testing of products of conception if an unbalanced structural chromosomal abnormality is identified or a result is not obtained for technical reasons i.e. a sample was received but failed to produce a result
- parental testing after three or more unexplained miscarriages if the patient is from a hospital with a Service Level Agreement (e.g. Chase Farm, Barnet, Whittington and Royal Free Hospitals); this testing can also be performed on a private patient basis for patients from other hospitals
- parental testing if there have been two identical aneuploidies suggesting parental mosaicism
- parental testing following an abnormal cytogenetic result in a fetus or child NB the proband’s report will state if parental testing is indicated

Please note that parental testing after three or more unexplained miscarriages is not routinely offered (in line with the 2011 RCOG 'green top guidelines').

Service offered
The format of the parental testing will vary depending on the abnormality suspected or detected in the proband. The testing may be performed by karyotyping (full or targeted), fluorescent in situ hybridisation (FISH), qPCR, QF-PCR, MLPA, or rarely microarray. More than one technique may be used.

Technical
If the test is karyotyping or FISH, blood cultures are grown and harvested to yield metaphase cells which are analysed using light microscopy. Extracted DNA is used for qPCR, QF-PCR, MLPA and microarray testing.

Targeted analysis is performed in the following circumstances:-

- Parental follow-up of copy number changes detected in products of conception following pregnancy miscarriage
- Known abnormality or familial structural abnormality (e.g. family history of a known translocation, child has chromosome abnormality) – targeted analysis: If a patient requires testing because of a family history of a structural chromosome abnormality (e.g. translocation, inversion) and a written copy of the abnormal family member’s cytogenetic result including ISCN is available

Target reporting time
Routine analysis – 28 days
If clinical need indicates that an urgent result is required (e.g. current ongoing pregnancy) – 10 days.

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address), details of any relevant family history (including proband’s details) and full contact details for the referring clinician.

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- 2ml venous blood in plastic lithium heparin tube
- 2ml venous blood in plastic EDTA tube if indicated on the proband’s report
- A completed request card should accompany all samples. Please provide clinical details. Gestational age of pregnancy loss should also be supplied.
Introduction

The laboratory routinely cultures fibroblasts, chorionic villi and amniocytes principally for karyotyping purposes such as prenatal diagnosis and mosaicism screening.

The laboratory can also extract and store DNA from established cultures and from the original, uncultured sample material if required.

If requested and clear details are provided, we are able to send cultured cells to other laboratories in the UK and Europe for specialist genetic/metabolic/biochemical testing.

Referrals

- Pregnant women with a family history of a known genetic/metabolic/biochemical problem referred by a specialist consultant
- Children with a suspected (or known) genetic/metabolic/biochemical condition referred by a specialist consultant
- Pregnancy loss material requiring specialist genetic/metabolic/biochemical testing using live cells (specialist consultant referrals)
- Forensic material where DNA and cell storage are required.

Service offered

Extraction and storage of DNA from uncultured and cultured fibroblasts, chorionic villi and amniocytes.

Forwarding of fibroblast cultures for specialist genetic/metabolic/biochemical testing.

Please note that the Regional Genetics Service always attempts to use/recommend accredited laboratories where possible. The Regional Genetics Service accepts no responsibility for results obtained from any other laboratory.

Technical

Culturing of fibroblasts, chorionic villi and amniocytes is performed in the North East Thames Regional Genetics laboratories using fully trained staff and aseptic techniques. Cultures are established by using a synthetic culture media which provides consistency between media aliquots. Cultures are incubated in a 5% carbon dioxide atmosphere which mimics the lower oxygen environment that exists naturally for these types of cells.

Once established, cells can be passaged (divided) to provide extra material if required. Please note, there is a limit to the number of passages possible before cells begin to senesce, and there is a small but increased risk of introducing cultural artefact from prolonged growth of cells.

Target reporting time

Not applicable – cultures will be dealt with as soon as they have grown (usually within 14 days unless other tests are prioritised).

Notification of samples that are failing to grow should be received 10 days after receipt at the laboratory and failed reports issued at approximately 14 days post receipt.

Please contact the laboratory to discuss complex cases.
Rapid invasive prenatal testing for common aneuploidies

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8578

Samples required
- 10-20 ml amniotic fluid
- 10-20mg chorionic villi (after cleaning)
- It is advisable to contact the laboratory prior to sending a sample.

Patient details
Referrals must be made by specialist consultants.
Please provide patient demographic details (full name, date of birth, address and ethnic origin), gestational age at the time of sampling, results of any pre-screening performed and the gestation age it was performed at, details of any relevant family history and full contact details for the referring clinician.

Introduction
Almost all prenatal samples referred to the North East Thames Regional Genetics Service will have rapid invasive prenatal testing in order to test for the commonest aneuploidy conditions (i.e. Down syndrome, Edward’s syndrome, Patau syndrome and Turner syndrome).

A small aliquot of the prenatal sample will be forwarded to Viapath, Genetics Department, 5th Floor Tower Wing, Guy’s Hospital, London, SE1 9RT to be tested by QF-PCR. They will issue QF-PCR results directly to the referring hospital.

Referrals
- Pregnant women with a positive prior screening risk but no scan abnormalities N.B. Women with scan abnormalities will be offered rapid prenatal testing, but will also be offered microarray testing
- Pregnant women with a known family history of a genetic/metabolic/biochemical condition referred by a specialist consultant

Service offered
Forwarding of an aliquot of the prenatal sample to the pan-London testing laboratory at Viapath, Genetics Department, 5th Floor Tower Wing, Guy’s Hospital, London, SE1 9RT.

Based on the reason for referral and the outcome of the QF-PCR test, the following workstreams are applied:
- Normal QF-PCR result and no scan anomalies – no further testing is performed.
- Normal QF-PCR result and ultrasound scan anomalies – microarray testing will be performed by the North East Thames Regional Genetics Service.
- Abnormal QF-PCR results with or without abnormal ultrasound scan anomalies – the QF-PCR result will be confirmed by karyotyping performed by the North East Thames Regional Genetics Service.

Technical
Rapid prenatal testing will be performed using Guy’s in-house QF-PCR test for chromosomes 13, 18 and 21, and where appropriate for the sex chromosomes, X and Y.

Target reporting time
Within 3 days from sample receipt.
Prenatal Microarray: 
Structural fetal abnormalities identified by ultrasound scan (including NT>3.5mm)

Contact details
Regional Genetics Service
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
F +44 (0) 20 7813 8768

Samples required
- 15-20ml amniotic fluid or 12mg chorionic villus biopsy in transport medium.
- A completed request card should accompany all samples. Ultrasound findings including NT measurement and gestation must be provided in order to allow clinical interpretation of the microarray.

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician.

Introduction
Genome-wide chromosomal microarray (CMA) detects pathogenic copy number variants (pCNV) in 4-10% of prenatal samples (amniotic fluid or chorionic villus samples).
CMA may also detect copy number variants of uncertain clinical significance which may require parental follow up testing to aid interpretation.
CMA may also detect pCNV which are not associated with the presenting phenotype (incidental findings).

Referrals
- Samples from pregnancies that have structural abnormalities identified by ultrasound scan where rapid trisomy testing is negative. Note that absent nasal bone and isolated IUGR are not classified as structural fetal abnormalities.
- NT>3.5mm (before 14 weeks gestation) where rapid trisomy testing is negative.

Prenatal reporting
pCNV identified by the microarray are reported as pathogenic if they are associated with fetal scan abnormalities or with fully penetrant intellectual disability.
In line with RCPath guidelines, CNV which are associated with susceptibility to neurodevelopmental disorders (variable penetrance) are reported only if there is a published risk of fetal structural abnormality.
CNV of uncertain clinical significance are reviewed by a panel of Clinical Geneticists and Clinical Scientists and will only be reported if they require segregation analysis (parental carrier status) to aid interpretation of the clinical significance.
pCNV identified by microarray are confirmed using karyotype, qPCR or Fluorescence In Situ Hybridisation (FISH). These targeted tests are then applied to family members for carrier testing and for prenatal analysis in future pregnancies. Please contact the laboratory for further details.

Service offered
Whole genome microarray analysis at a practical resolution of 200kb for copy number variants (chromosomal deletions and duplications) and additional SNP based identification of uniparental isodisomy and ploidy level changes.

Technical
Whole genome chromosomal microarray analysis using the Affymetrix 750K microarray is performed on DNA extracted from uncultured amniotic fluid or chorionic villus samples. The microarray design uses both single nucleotide polymorphic (SNP) probes and non-polymorphic probes to enable consistent genomic coverage. Copy number variations (CNV) and regions with absence of heterozygosity (AOH) are identified using infoQuant Fusion software. The estimated practical resolution is 200kb; CNV below this threshold may not be identified. The microarray will not detect balanced structural chromosome anomalies and may not detect mosaicism. AOH may indicate uniparental isodisomy or regions identical by descent. The CNV identified by the CMA are compared to databases of known genetic variation and to reports of known pathogenic changes. Variants which are not known to have a pathogenic effect or do not have a high risk of pathogenicity may not be reported. AOH of non-imprinted chromosomal regions will not be reported.
If pCNV or variants of uncertain significance are reported, further samples may be requested (EDTA venous blood and/or lithium heparin venous blood from parents) to aid clinical interpretation and provide a recurrence risk for the family.

Target reporting time
Routine analysis – 14 days