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For latest version see http://www.ich.ucl.ac.uk/gosh/clinicalservices/Molecular_Genetics/Homepage

Great Ormond Street Hospital for Children NHS Trust

North East Thames Regional Genetics Service

Molecular Genetics Service Pack

North East Thames Regional Molecular Genetics Laboratory

The Regional Molecular Genetics Laboratory was one of the first such diagnostic laboratories established in the UK (1985). It now has a staff of approximately 30, including state registered clinical scientists, genetic technologists and administrative support staff. The staff work closely with clinical colleagues and other healthcare scientists in the pathology directorate and research staff in the Institute of Child Health. The Molecular Genetics Laboratory along with Clinical Genetics and Cytogenetics, forms a strategic Genetics Unit within Great Ormond Street Hospital and also constitutes the North East Thames Regional Genetics Service that serves a population of approximately 5 million.

The service handles approx 8000 samples per year and issues over 6000 reports covering over 50 different disorders. The service repertoire is regularly updated, but includes a number of single gene disorders such as fragile X syndrome, cystic fibrosis, Angelman and Prader-Willi syndromes, deafness, familial hypercholesterolaemia and skeletal dysplasias. It also provides both a National and International service for craniofacial, metabolic and primary immune deficiency disorders. Referrals are received for diagnostic, predictive, carrier and prenatal testing. We also undertake externally funded research projects.

The laboratory provides a DNA banking service and can forward samples to other centres for approved requests providing 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 4.

Contact details

Regional Molecular Genetics Laboratory
Great Ormond Street Hospital NHS Trust
Level 6, York House
37 Queen Square
London WC1N 3BH

Telephone	**+44 (0) 20 7762 6888**
Fax	**+44 (0) 20 7813 8196**

Website http://www.gosh.nhs.uk/gosh/clinicalservices/Molecular_Genetics/

Director of Laboratory	Gail Norbury, FRCPath
Deputy Director	Lucy Jenkins, FRCPath
Laboratory Service Manager	Tony Young

Laboratory Opening Hours (for specimen reception)

The laboratory is open Monday - Friday, 9.00am - 5.00pm excluding bank holidays. Samples arriving outside these hours are refrigerated / frozen prior to processing. Please address samples to address shown above.

Other contacts

Clinical Genetics Unit
Great Ormond Street Hospital (4-5 Long Yard), London. WC1N 3JH

Telephone	+44 (0) 20 7905 2647
Fax	+44 (0) 20 7813 8141
Lead Clinician	Dr Angela Barnicoat

NE London Regional Cytogenetics Laboratory
Level 5, York House, 37 Queen Square London. WC1N 3BH.

Telephone	+44 (0) 20 7829 8870
Fax	+44 (0) 20 7813 8578
Director	Barbara Gibbons

Sample Requirements

It is the responsibility of the patient's clinician to ensure that all requests meet the 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

Blood Sample

5 mls venous blood in plastic EDTA bottles (>2 ml from neonates)

Sample must be labelled with:

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

Prenatal Samples

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.

Minimum criteria:

- Patient's full name (surname/family name and given/individual name)
- Date of birth and/or unique hospital/NHS number

Request form

The clinical molecular genetics laboratory has its own referral cards for use within GOSH. These cards are also distributed to our common users outside of GOSH. It is preferable that these cards are fully completed with the requesting clinicians name and hospital printed. However, referral cards must provide the minimum criteria of:

- Patient's full name (surname/family name and given/individual name)
- Date of birth and hospital/NHS number
- Full name and address of referring clinician/consultant
- Patient's postcode
- **Clearly mark if referral is for a non-NHS patient**

DNA samples

CMGS guidelines recommend at least 2 pieces of identifying information on every sample tube.

- Patient's full name (surname/family name and given/individual name)
- Date of birth and/or unique hospital/NHS number
- Other information provided with referrals should include a pedigree, where appropriate, with the full names of known individuals and correct family identifiers

If results are received e.g. allele numbers, a full explanation of the terminology should be included

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 currently evaluated tests (including a search function and alphabetical list of tests), tests currently undergoing evaluation and tests likely to be submitted in the near future.

The current accreditation status of UK laboratories registered with CPA (UK) Ltd. can be checked at www.cpa-uk.co.uk

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.

Further Information – Click on disease / OMIM number for detailed information

Disorder	Gene	Locus	OMIM disease	OMIM gene	Page
Angelman syndrome		15q11-q13	#105830		8
Cystic Fibrosis	CFTR	7q31.2	#219700	*602421	9
Fragile X syndrome	FMR1	Xq27.3	+309550		10
GNAS disorders	GNAS	20q13.2	+139320		11
Pulmonary Surfactant Metabolism Dysfunction 1, 2 & 3	SFTPB	2p12-p11.2	#265120 (SMDP1)	*178640	12
	SFTPC	8p21	#610913 (SMDP2)	*178620	12
	ABCA3	16p13.3	#610921 (SMDP3)	*601615	12
Popliteal Pterygium syndrome	IRF6	1q32-q41	#119500	*607199	13
Prader-Willi syndrome		15q11-q13	#176270		14
Van der Woude syndrome	IRF6	1q32-q41	#119300	*607199	13

Cardiovascular Disorders

Disorder	Gene	Locus	OMIM disease	OMIM gene	Page
Familial Hypercholesterolaemia	LDLR	19p13.2	#143890	*606945	18
	APOB	2p24	#144010	+107730	18
Hypertrophic Cardiomyopathy	MYBPC3	11p11.2	#192600	*600958	19
	MYH7	14q12		*160760	19
	TNNT2	1q32		*191045	19
	TNNI3	19q13.4		+191044	19

Skeletal Dysplasias

Disorder	Gene	Locus	OMIM disease	OMIM gene	Page
Achondroplasia	FGFR3	4p16.3	#100800	*134934	20
Hypochondroplasia	FGFR3	4p16.3	#146000	*134934	20
Thanatophoric Dysplasia	FGFR3	4p16.3	#187600	*134934	21

Craniosynostosis

Disorder	Gene	Locus	OMIM disease	OMIM gene	Page
Apert syndrome	FGFR2	10q26	#101200	*176943	22
Crouzon syndrome	FGFR2	10q26	#123500	*176943	22
	FGFR3	4p16.3		*134934	22
Muenke syndrome	FGFR3	4p16.3	#602849	*134934	22
Pfeiffer syndrome	FGFR1	8p11	#101600	*136350	22
	FGFR2	10q26		*176943	22
Saethre-Chotzen syndrome	TWIST	7p21	#101400	*601622	22
					22

Deafness

Disorder	Gene	Locus	OMIM disease	OMIM gene	Page
Aminoglycoside induced deafness	MTRNR1	nt648-1601	#580000	*561000	
Branchiootorenal syndrome	EYA1	8q13.3	#113650	*601653	23
Connexin 26 (DFNB1)	GJB2	13q11-q12	#220290	*121011	24
Pendred syndrome	SLC26A4	7q31	#274600	*605646	25

Metabolic Disorders

Disorder	Gene	Locus	OMIM disease	OMIM gene	Page
Carbamoylphosphate synthetase 1 deficiency	CPS1	2q35	#237200	*608307	26
Fabry disease	GLA	Xq22	+301500		27
Gaucher disease	GBA	1q21	#230800 (Type 1) #230900 (Type 2) #231000 (Type 3)	*606463	28 28 28
Glycogen Storage disease type 1a	G6PC	17q21	+232200		29
Glycogen storage disease type 2 (Pompe disease)	GAA	17q25.2-q25.3	#232300	*606800	30
Krabbe disease	GALC	14q31	#245200	*606890	31
Long-chain, deficiency of Acyl-CoA dehydrogenase	HADHA	2p23	#609016	*600890	32
Medium-chain, deficiency of Acyl-CoA dehydrogenase	ACADM	1p31	#201450	*607008	33
Metachromatic Leukodystrophy (incl. pseudodeficiency of arylsulphatase A)	ARSA	22q13.31-qter	#250100	*607574	34
Mucopolysaccharidosis type 1 (Hurler / Scheie)	IDUA	4p16.3	#607014 (Hurler) #607015 (H/S) #607016 (Scheie)	*252800	35
Mucopolysaccharidosis type 2 (Hunter)	IDS	Xq28	+309900		36
Mucopolysaccharidosis type 3 (Sanfilippo)	SGSH NAGLU	17q25.3	#252900 (MPS3A) #252920 (MPS3B)	*605270 *609701	37
Neuronal Ceroid Lipofuscinosis type 1 (incl. infantile Batten disease)	PPT1	1p32	#256730	*600722	38
Neuronal Ceroid Lipofuscinosis type 2 (late-infantile Batten)	CLN2	11p15.5	#204500	*607998	39
Neuronal Ceroid Lipofuscinosis type 3 (juvenile Batten)	CLN3	16p12.1	#204200	*607042	40
Neuronal Ceroid Lipofuscinosis type 5 (variant late-infantile Batten)	CLN5	13q21.1-q32	#256731	*608102	41
Neuronal Ceroid Lipofuscinosis type 6 (variant late-infantile Batten)	CLN6	15q21-q23	#601780	*606725	41
Neuronal Ceroid Lipofuscinosis type 8 (variant late-infantile Batten)	CLN8	8pter-p22	#600143	*607837	41
Ornithine transcarbamylase deficiency	OTC	Xp21.1	#311250	*300461	42
Osteopetrosis, autosomal recessive	TCIRG1	11q13.4-q13.5	#259700	*604592	43

Renal Disorders

Disorder	Gene	Locus	OMIM disease	OMIM gene	Page
Cystinosis (Adult) (Juvenile)	CTNS	17p13	#219800 #219750 #219900	*606272	44
Juvenile Nephronophthisis	NPHP1	2q13	#256100	*607100	45
Steroid-resistant nephrotic syndrome	NPHS2	1q25-q31	#600995	*604766	46

Immunodeficiencies

Disorder	Gene	Locus	OMIM disease	OMIM gene	Page
Autoimmune lymphoproliferative syndrome (ALPS)	TNFRSF6	10q24.1	#601859	*134637	47
Familial hemophagocytic lymphohistiocystosis	PRF1	10q22	#603553	*170280	48
Interleukin 7 receptor severe combined immunodeficiency	IL7R	5p13	#600802	*146661	49
JAK3-deficient severe combined immunodeficiency	JAK3	19p13.1	#600802	*600173	50
RAG-deficient severe combined immunodeficiency	RAG1 RAG2	11p13 11p13	#601457	*179615 *179616	51
Wiskott-Aldrich syndrome	WAS	Xp11.23-p11.22	#301000	*300392	52
X-linked agammaglobulinaemia	BTK	Xq21.3-q22	#307200	+300300	53
X-linked Hyper IgM syndrome (HIGM)	CD40LG	Xq26	#308230	*300386	54
X-linked Lymphoproliferative syndrome	SAP	Xq25	#308240	*300490	55
X-linked Severe combined immunodeficiency	IL2RG	Xq13	#300400	*308380	56

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(ies) 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.

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Angelman syndrome (AS)

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml 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

Introduction
Angelman syndrome (MIM 105830) occurs in 1/15000 - 1/20000 individuals. It is characterized 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 mutations in the imprinting centre (IC) (~5%) that cause abnormal methylation at exon alpha of the SNRPN gene at 15q11-13. These mutations are all detected by disrupted methylation. About 20% of AS patients have a normal methylation pattern and are believed to have a mutation 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 chromosome 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 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). UBE3A mutation analysis is not offered in this laboratory.

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. In AS patients with abnormal methylation, further analysis is recommended to characterise the nature of the mutation. This involves the use of chromosome 15 microsatellite markers from within and flanking the commonly deleted region. Cytogenetic analysis is also helpful in identifying deletions and predisposing parental translocations.
NB Similar analysis is undertaken for Prader Willi syndrome

Target reporting time
Routine analysis - the initial methylation test takes up to 2 weeks. Microsatellite marker analysis takes a further 2 weeks.

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Cystic fibrosis (CF)

Contact details

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GOSH NHS Trust
Level 6
York House
37 Queen Square
London
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Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5mls venous blood in plastic EDTA bottles (>2ml from neonates)

Prenatals should be arranged in advance. 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.

Please state the ethnic origin of the patient

Introduction
Cystic fibrosis (MIM 219700) is an autosomal recessive condition caused by mutations in the cystic fibrosis transmembrane regulator (CFTR) gene. To date over 1000 mutations 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 mutations 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, e.g. congenital bilateral absence of the vas deferens (CBAVD), 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 mutations 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 family mutations 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 specific mutations have been identified - please contact the laboratory to discuss.

Service offered
34-mutation screen and the partially penetrant intron 8 polyT mutation in cases referred for CFTR-related disease such as confirmed CBAVD, bronchiectasis and pancreatitis as well as CF referrals where the p.Arg117His mutation has been detected. 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 and sample availability from the affected individual and appropriate family members.

Technical
The mutation detection system in use in this laboratory is a kit based oligo ligation assay (OLA). As only 34 of the most commonly identified mutations are covered by this analysis failure to identify a mutation cannot exclude affected/carrier status, a residual risk to the individual is therefore calculated and reported wherever possible. In the North European population this system detects approximately 90% of cystic fibrosis mutations. Information regarding the ethnic origin of the patient is important for calculation of residual risk as the mutation spectrum, and hence the detection rate of the assay used, varies in different populations.

Target reporting time
2 weeks for routine analysis of the 34 mutations. 3 days for urgent 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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Fragile X syndrome

Contact details

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Level 6
York House
37 Queen Square
London
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Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

Prenatals 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.

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 are 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 FMR-1 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 FMR-1 gene expression (full mutation expansion). Premutation alleles with 55-200 (CGG)n repeats are unstable at meiosis and can lead to full expansion mutations in subsequent generations. Intermediate alleles (45-55 repeats) are not believed to be associated with Fragile X syndrome, but may display size instability in future generations. FMR-1 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 - preliminary analysis can be carried out on prenatal samples by direct analysis of the FMR-1 (CGG)n repeat and/or by linked marker analysis if samples from the relevant family members are available. Southern blot analysis is carried out to confirm the results of the preliminary screen.

Service offered
Direct analysis of the FMR-1 (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 FMR-1 gene. Southern blot analysis of genomic DNA is carried out using the restriction enzymes NruI and EcoRI and the probe Stb12.3. PCR and Southern blot analysis are unlikely to detect point mutations or deletions within the FMR-1 gene, and are also unable to exclude mosaicism.

Target reporting time
Routine analysis- up to 2 weeks for the initial PCR-based mutation screen, and up to a further 2-4 weeks if Southern blotting is required. For urgent samples please contact the laboratory.

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE
GNAS1 gene mutation disorders
(AHO/PHP1a/PPHP/ McCune Albright's disease)

Contact details

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Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

Prenatals 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.

Introduction:
Albright 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 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 (pseudohyporarathyroidism type 1a, PHP 1a). Both PHP 1a and PPHP are caused by inactivating mutations in the GNAS1 gene. PHP1a usually caused by mutations in maternal GNAS1, PPHP in paternal allele.

McCune Albright's syndrome (MAS) is characterised by precocious puberty, café au lait spots, and polyostic fibrous dysplasia of bone where the normal interior of bone replaced by fibro-osseous connective tissue. McCune Albright is caused by somatic activating mutations in exons 8 and 9 of GNAS1. All MAS patients are mosaics.

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

GNAS1 (located on 20q13.3) has 13 exons, 6 polyadenylation sites 3' and 4 isoforms (due to differential splicing in exon 3). 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 maternal chromosomes.

Referrals
- Patients with clinical symptoms as above.
- Carrier testing for family members for the family mutation

Service offered
AHO: Sequencing analysis of all 13 exons. Approximately 80% of inactivating mutations will be detected by this method.

MAS: Sequencing of exons 8 and 9 plus restriction digest analysis to detect the c.602G>A mutation. Restriction digest analysis is a more sensitive assay for detecting low level mosaicism. DNA from an affected tissue such as bone have given more successful results than DNA extracted from lymphocytes.

Target reporting time
The target reporting time is 2 months. For urgent samples please contact the laboratory

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.

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Pulmonary Surfactant Metabolism Dysfunction 1, 2 and 3 (Genes: SFTPB, SFTPC and ABCA3)

Contact details

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GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

Prenatals 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.

Introduction
Pulmonary Surfactant Metabolism Dysfunction comprises of a genetically heterogeneous group of disorders that resulting 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), or cellular nonspecific interstitial pneumonitis (NSIP).
Type 1 (MIM 265120) is caused by recessive mutations in the pulmonary associated surfactant protein B (SFTPB) gene (MIM 178640) at 2p12-11.2, which encodes the pulmonary-associated surfactant protein B (SPB) a crucial component of pulmonary surfactant, the mixture of lipids and specific proteins, which reduces surface tension at the alveolar air-liquid interface.
Type 2 (MIM 610913) is caused by dominant mutations in the pulmonary associated surfactant protein C (SFTPC) gene (MIM 178620) at 8p21, which encodes pulmonary-associated surfactant protein C (SPC) a crucial component of pulmonary surfactant which also reduces surface tension at the alveolar air-liquid interface.
Type 3 (MIM 610921) is caused by recessive mutations in the ATP binding cassette (ABC), subfamily A, member 3 (ABCA3) (MIM 601615) gene at 16p13.3, which is a member of the ABC transporter family. ABCA3 is expressed predominantly at the limiting membrane of the lamellar bodies in lung alveolar type II cells and is thought to be involved in surfactant secretion.

Referrals
Full term neonates with severe respiratory distress of unknown aetiology. Occasionally older children with respiratory distress of unknown cause. Carrier testing for parents, and other adult relatives. A completed clinical information sheet is required prior to analysis.
Click here for link to clinical information sheet (also available separately on our laboratory website)

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
Direct sequence analysis of the coding regions of the SFTPB gene (exons 1 to 10), SFTPC gene (exons 1 to 5) and of the ABCA3 gene (exons 4-33). Linked marker analysis is also available as appropriate for SFTPB.

Technical
SFTPB: Direct sequencing of exons 1 to 10 and six linked markers are available.
SFTPC: Direct sequencing of exons 1 to 5.
ABCA3: Direct sequencing of exons 4 to 33 (coding exons 1-30).

Target reporting time
2 months for a whole gene screen, 2 weeks for carrier testing. For urgent samples please contact the laboratory.

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Interferon Regulatory Factor 6 (IRF6) gene mutation disorders (Popliteal Pterygium syndrome / Van der Woude syndrome)

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
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Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

Prenatals must be arranged in advance, through a Clinical Genetics department if possible. Amniotic fluid or CV samples for genetic testing should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis Prenatal detection can also be undertaken by biochemical analysis.

A completed DNA request card should accompany all samples.

Introduction
Van der Woude syndrome (VWS; MIM) and Popliteal Pterygium syndrome (PPS) are allelic autosomal dominant disorders caused by mutations 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 mutations commonly identified throughout the IRF6 gene. The features of PPS are thought to result from dominant negative mutations (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 mutations identified in PPS patients. A variety of point mutations and small deletions have been identified in VWS located throughout the IRF6 gene.

Referrals
- Referrals will only be accepted via a Clinical Geneticist or cleft surgeon.
- **A referral criteria form must be completed and must accompany all diagnostic samples.**
  Click here for link to referral criteria form (also available separately on our laboratory website)
- Testing of other family members will be possible upon identification of a causative mutation in the index case.

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

Service offered
- PPS level 1 screen: Testing for recurrent p.Arg84Cys and p.Arg84His mutations by PCR & restriction digest.
- PPS level 2: Direct sequencing of all coding exons (3-9) and intron-exon boundaries as necessary.
- VWS: Direct sequencing of all coding exons (3-9) and intron-exon boundaries.
- Detection of known mutations: In relatives of patients with confirmed PPS / VWS mutations by PCR and restriction digest or sequencing

Target reporting time
2 months for routine mutation screen in index case. 2 weeks for carrier testing using mutation specific tests. For urgent samples please contact the laboratory.

Contact details for Consultant Cleft Geneticist at Great Ormond Street Hospital:
Dr M Lees, Clinical Genetics, Great Ormond Street Hospital, London WC1N 3JH
Tel: +44 (0) 20 7905 2647

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Prader-Willi syndrome (PWS)

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

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. These mutations 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 chromosome 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.

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).

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

Prenatals 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.

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 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 determinate 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.

In PWS patients with abnormal methylation, further analysis is recommended to characterise the nature of the mutation, this affects the recurrence risk for the parents (parental samples required), and involves the use of chromosome 15 microsatellite markers from within the commonly deleted region and markers flanking this region. Cytogenetic analysis is also helpful in identifying deletions and predisposing parental translocations.
NB A similar testing procedure is undertaken for Angelman syndrome

Target reporting time
Routine analysis - the initial methylation test takes up to 2 weeks. Microsatellite marker analysis takes a further 2 weeks.

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Cell-Free Fetal DNA Sex Determination

Contact details
Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

Pregnant Women
10mls venous blood in plastic EDTA bottles

Testing must be arranged in advance, through your Local Clinical Genetics Dept

Or

Fetal Medicine Unit,
EGA,
UCH
London
+44 (0) 20 7380 9872 or
+ (0) 8451555000 ext 5572
email
l.chitty@ich.ucl.ac.uk

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

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. This technique is still relatively new to clinical practice and the results from a European Union quality assurance programme have reported the rare occurrence of a false positive result for a male fetus. In view of this we currently recommend that fetal sex is confirmed when ultrasound is performed at 20 weeks. Our local data for this assay shows a sensitivity, specificity and positive predictive value of 100% (n=90) for samples collected from 7 weeks gestation by ultrasound.

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. The results should be confirmed by ultrasound to avoid the very small risk of an erroneous result. This test may not be applicable in multiple pregnancies including those with a possible vanishing twin.

Male fetuses are 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.

Referrals
All referrals should be made via a Clinical Genetics Department or through the Fetal Medicine Unit at UCLH (see left). Samples are accepted from patients from 7 weeks gestation at which time there should be a sufficient concentration of free fetal DNA in the circulation. Samples may be sent by post to arrive in the laboratory within 24 hours of sampling if possible. The laboratory must be advised in advance because of the need to process the samples as rapidly as possible after collection. 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.
Click here for links to the **audit sheet** and **information for parents sheet** (also available separately on our laboratory website)

Technical
10 mls maternal EDTA blood is separated as rapidly as possible after collection. Cell free DNA is extracted from the plasma and maternal DNA is extracted from the lymphocytes. 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 second sample.

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

X-Inactivation

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

Whole blood analysis (e.g. WAS) requires 5ml venous blood in plastic EDTA bottles (>2ml from neonates)

Samples for B or T cell separation should be arranged locally. If this is not available please contact the laboratory to discuss. Purified (95%) separated cells should always be sent with a sample of whole blood from the same individual.

A completed DNA request card should accompany all samples.

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 one or more loci from the following: androgen receptor (HUMARA) gene, phosphoglycerate kinase (PGK1) gene and M27b locus.

Technical

Methylation sensitive restriction enzymes are used to detect differential methylation patterns between the inactive and active X chromosomes. The methylation sensitive sites are in close proximity to a polymorphic site allowing the two X chromosomes to be distinguished. The androgen receptor is the most informative of the three loci used with a heterozygosity of 90%, followed by M27b and PGK1.

Target reporting time

Routine analysis (immunodeficiency disorders) - 2 months. 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.

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Zygosity testing

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

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.

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

A completed DNA request card should accompany all samples.

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 AmpflSTR 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 2 weeks. For urgent samples please contact the laboratory.

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Familial hypercholesterolaemia (FH)

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

Prenatals 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.

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 mutation in the gene encoding the LDL-receptor (LDLR). LDLR has 18 exons and family specific mutations are found throughout the gene, although some recurrent mutations are reported. Large deletions or duplications encompassing one or more exons accounts for 5% of mutations in LDLR. A clinically indistinguishable disorder, familial defective apolipoprotein B100 (FDB), is due to a mutation 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 mutation, p.Arg3500Gln. Mutations causing FH have also been identified in the PCSK9 gene at 1p34.1, these 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.
- Mutation testing can be offered to the relatives of FH patients once a disease causing mutation has been identified.

Service offered
Mutation screening of the LDLR gene and testing for the p.Arg3500Gln mutation in APOB and p.Asp374Tyr mutation in PCSK9 is undertaken in affected patients. Mutation specific testing for previously identified family mutations is also available in family members.

Technical
ARMs analysis is carried out for a panel of common FH mutations, including the p.Arg3500Gln in APOB and p.Asp374Tyr in PCSK9. Mutation screening of LDLR, for small mutations, is undertaken by dHPLC analysis. MLPA analysis is utilised to look for deletions and duplications of the LDLR gene. In patients meeting the Simon Broome criteria, this testing strategy has a detection rate of approximately 30% in adult referrals and 50% in paediatric referrals, this is likely to reflect the heterogeneity of the condition rather than technical failure.

Target reporting time
2 months for routine mutation screen in index case. 2 weeks for carrier testing using mutation specific 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
NB Include details of most recent lipid profile on request card.

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Hypertrophic Cardiomyopathy
MYBPC3, MYH7, TNNT2, TNNI3 genes

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

Samples for DNA analysis only: 5ml venous blood in plastic EDTA bottles

Prenatals 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.

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 caused by mutations in genes encoding protein components of the cardiac sarcomere. There is a wide heterogeneity with at least thirteen genes responsible for HCM. There is also a variation in expressivity and penetrance

Referrals

- New referrals should fulfil the UKGTN testing criteria
- Confirmatory mutation analysis can be carried out for patients in whom a mutation has been detected in a research laboratory.
- Predictive mutation analysis is available for family members in whom the causative mutation has been confirmed in a CPA accredited laboratory.

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
Sequence of the entire coding region of MYBPC3 (34 exons), MYH7 exons 3-40), TNNT2 (15 exons), TNNI3 (8 exons) genes. Mutations in these four genes are estimated to account for around 80% disease mutations.
Due to the number of cases with more than one mutation (double and compound heterozgyotes), we advise a full screen is completed for all probands.
Estimate of unclassified variants is around 20%
Estimate of double / compound heterozygotes is up to around 5%.
Overall detection rate in referrals received is around 42%.

Technical
Screening is carried out by direct sequencing analysis.

Target reporting time
The target reporting time for each gene is 2 months with a target for a complete panel screen of around 4 months. The target turnaround time for familial mutations (incl. predictive tests) is 2 weeks. For urgent samples please contact laboratory.

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Achondroplasia & Hypochondroplasia (ACH & HCH)

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml 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

Introduction
Achondroplasia (MIM 100800) and hypochondroplasia (MIM 146000) are autosomal dominant skeletal disorders with mutations in the FGFR3 gene on chromosome 4p16.3.

Achondroplasia (ACH) has a birth incidence of between 1/15,000 to 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) is genetically distinct from ACH and is clinically less severe, with no associated craniofacial abnormalities. Because of its mild nature, HCH can be difficult to diagnose and may be genetically heterogeneous. Approximately 60% of HCH patients have one of two mutations in the FGFR3 gene. Of the remaining 40%, some families are reported that do not link to chromosome 4p16.3

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

Prenatal testing
1)Prenatal testing is available to families in whom specific mutations have been identified - please contact the laboratory to discuss.
2)Prenatal testing to confirm a diagnosis of ACH suspected on antenatal ultrasound scan

Service offered
Achondroplasia: Testing for the common p.Gly380Arg (c.1138G>A) and p.Gly380Arg (c.1138G>C) mutations in exon 8 of FGFR3. Together these account for around 99% of mutations.
Hypochondroplasia: Testing for the common p.Asn540Lys (c.1620C>A and c.1620C>G) mutations in exon 11, which account for around 60% of mutations.

Technical
Direct sequence analysis of exons 8 (ACH) and 11 (HCH) detects the common mutations. This will also detect other mutations that may be present in these exons.

Target reporting time
2 weeks for routine analysis. For urgent samples please contact the laboratory.

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Thanatophoric dysplasia (TD) type I and type II

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

Neonates
>2ml venous blood in plastic EDTA bottles
Adults
5ml as above
Other tissue
Please contact the laboratory

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.

Introduction
Thanatophoric Dysplasia (MIM 187600), 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. Mutations in the FGFR3 gene on chromosome 4 have been identified in almost 100% of confirmed cases of TD. A single mutation, p.Lys650Glu, has been identified in all of the TD type II patients reported to date. Several recurrent mutations have been identified in TD type I involving the gain of a cysteine residue, as well as rare mutations.

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

Prenatal testing
1) Prenatal testing is available to families in whom specific mutations have been identified - please contact the laboratory to discuss.
2) Pre-natal testing to confirm a TD diagnosis suspected on antenatal ultrasound scan

Service offered
Testing for the following mutations in FGFR3:

p.Arg248Cys (c.742C>T)	~ 55%	TDI*
p.Ser249Cys (c.746C>G)	~ 5.7%	TDI*
p.Gly370Cys (c.1108G>T)	~ 1.6%	TDI*
p.Tyr373Cys (c.1118A>G)	~ 23.6%	TDI*
p.Lys650Glu (c.1948G>A)	~ 100%	TDII*
p.Lys650Met (c.1949A>T)	~ 3.3%	TDI*
Stop Codon mutations	~ 10%	TDI*

X807Gly (2416T>G)
X807Arg (2416T>C or 2416T>A)
X807Ser (2417G>C)
X807Cys (2418A>T)
Wilcox et al, American J of Medical Genetics 1998; 78:274-281

Technical
The common mutations listed above are detected by direct sequencing analysis of exons 6, 8, 13 and 17. This may also detect other mutations present in these exons.

Target reporting time
2 months for routine analysis. For urgent samples please contact the laboratory.

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 provide full clinical details including information on how the diagnosis of Thanatophoric dysplasia has been made and any relevant family history

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Craniosynostosis

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5mls venous blood in plastic EDTA bottles (>2ml 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.

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

Service offered

- **Muenke Syndrome / Non syndromic craniosynostosis**: p.Pro250Arg in FGFR3 is the only testing offered
- **Pfeiffer Syndrome**: p.Pro250Arg in FGFR3, p.Pro252Arg in FGFR1 and mutation screening initially across exons 8 and 10 of FGFR2 and then exons 3, 5, 11, 14-17
- **Crouzon Syndrome**: p.Pro250Arg in FGFR3, mutation initially across exons 8 and 10 of FGFR2 and then exons 3, 5, 11, 14-17
- **Crouzon Syndrome with acanthosis nigricans**: p.Pro250Arg and p.Ala391Glu in FGFR3, mutation screening initially across exons 8 and 10 of FGFR2 and then exons 3, 5, 11, 14-17
- **Saethre-Chotzen Syndrome**: p.Pro250Arg in FGFR3, mutation screening across exon 1 of TWIST and dosage analysis for TWIST gene deletions
- **Apert Syndrome**: p.Ser252Trp (c.934C>G) and p.Pro253Arg (c.937C>G) in exon 8 of FGFR2

Technical

p.Pro250Arg in FGFR3 and p.Pro252Arg in FGFR1 are detected by restriction enzyme digestion of amplified DNA. Direct sequence analysis of the appropriate exons is carried out to detect the remaining common mutations and to screen for unknown mutations in FGFR2, FGFR3 and TWIST. TWIST gene deletions are detected by fluorescent dosage analysis.

Target reporting time

Routine analysis: 2 weeks for specific mutations and 2 months for mutation screen or dosage analysis in index case. For urgent samples please contact the laboratory

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 provide full clinical details including associated malformation or developmental delay

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Branchio-oto-renal syndrome (BOR)

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

Prenatals 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.

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 is caused by mutations in the EYA1 gene. The gene has 16 exons with most mutations identified in exons 8-16.

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

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

Service offered
Level 1: Dosage analysis of the EYA1 gene to identify deletions and duplications.
Level 2: Mutation screening of exons 8-16 of the EYA1 gene.
Level 3: Mutation screening of exons 1-7 (including alternative exon 1).

A report is issued after each level of testing. Level 3 analysis is only carried out if there is a strong clinical indication for BOR.

Technical
Dosage analysis is by Multiplex Ligation-dependent Probe Amplification (MLPA) analysis. Mutation screening is carried out by direct sequencing.

Target reporting times
Level 1: Dosage analysis - 2 weeks
Level 2: Mutation screening of exons 8-16 - 2 months
Level 3: Mutation screening of exons 1-7 (including alternative exon 1) - 2 months.
Mutation-specific test: 2 weeks.
For urgent samples please contact the laboratory.

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Connexin 26

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

Prenatals 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.

Introduction
Pre-lingual non-syndromic sensorineural hearing loss (NSSNHL) is predominantly due to recessive mutations. 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 mutations may account for 10-30% of sporadic non-syndromic deafness. The c.35delG mutation is the most common GJB2 mutation described so far and is found in the majority of families linked to DFNB1. Other common mutations have been detected in specific ethnic groups. A 342kb deletion upstream of GJB2 and including part of GJB6 (connexin 30) has also been described and causes deafness when inherited on the opposite chromosome to a connexin 26 mutation (del Castillo et al NEJ Med, 346:243-249 (2002)).

Referrals

- Patients with hearing loss for mutation screening of connexin 26
- Adult relatives of patients with connexin 26 mutations for carrier status.
- Please provide information on patient's ethnic origin due to variation in mutation spectrum in different populations.

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
Mutation screening of connexin 26 coding exon 2. Analysis for the 342kb deletion (GJB6-D13S8130), connexin 26 intron 1 splice donor site mutation (c.1-3170G>A) and splice acceptor site mutation (c.1-24A>C) is carried out where necessary. Detection of known mutations in relatives of patients with confirmed connexin 26 mutations.

Technical
Direct sequencing analysis of connexin 26 exon 2 in three overlapping fragments.

Target reporting times
2 months for routine mutation screen in index case. 2 weeks for routine testing of specific mutations. For urgent samples please contact the laboratory.

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Pendred syndrome

Contact details

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GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

Prenatals must be arranged in advance, through a Clinical Genetics department if possible.

Amniotic fluid or chorionic villus samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis

All samples should be accompanied by a completed DNA request card.

Introduction

Pendred syndrome is an autosomal recessive form of hearing loss due to mutations in the *SLC26A4* gene on chromosome 7q31 that presents with other features including goitre, enlarged vestibular aqueducts (EVA) and Mondini malformation. The estimated frequency of Pendred syndrome related hearing loss is 7%.

Mutations in *SLC26A4* disrupt ion exchange activity of the polypeptide, pendrin. Pendrin is expressed in non-sensory epithelia of the inner ear and in thyroid folliculocytes.

SLC26A4 has 21 exons; mutations have been reported across the gene including 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, 7:7 (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 PDS
- Adult relatives of patients with *SLC26A4* mutations for carrier status

Prenatal testing

Prenatal testing may be 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

- Level 1 Mutation Analysis: Detection of more commonly reported mutations. CE-SSCP analysis of exon 10 to detect p.Glu384Gly, p.Thr410Met and p.Thr416Pro. Restriction analysis of exon 6 to detect p.Leu236Pro and p.Gly209Val. Restriction analysis of exon 8 to detect c.1001+1G>A.
- Level 2 Mutation Analysis: CE-SSCP screen of 11 *SLC26A4* exons.
- Level 3 Mutation Analysis: CE-SSCP screen of remaining exons.

Levels 2 and 3 are offered for patients referred with relevant clinical findings in addition to hearing loss or when a single Level 1 mutation is identified.

A report is issued after each level screen.

- Detection of known mutations: In relatives of patients with confirmed *SLC26A4* mutations.

Target reporting time

2 weeks for level 1 screen and 2 months for the extended screen of the index case. 2 weeks for routine testing of specific mutations. For urgent samples please contact the laboratory

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Carbamoylphosphate synthetase 1 (CPS1) deficiency

Contact details

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GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

Prenatals 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.

Introduction
Carbamoylphosphate synthetase 1 deficiency (MIM 237300) is a rare autosomal recessive metabolic disorder. CPSI 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 CPSI may also be exacerbated by infection, metabolic stress or excessive protein intake.

The CPSI gene (2p35) consists of at least 15 exons and at least three family specific mutations have been identified.

Referrals

- Prior to genetic analysis, clinically affected patients should wherever possible be confirmed as having CPSI 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 CPSI patients once an informative haplotype has been identified.

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

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

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

Target reporting time
2 weeks for linkage analysis in the index case and parents. For urgent samples please contact the laboratory.

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Fabry disease (α-galactosidase A deficiency)

Contact details

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Level 6
York House
37 Queen Square
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Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

Prenatals 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.

Introduction

Fabry disease (MIM 301500) is an X-linked recessive lysosomal storage disorder affecting ~1/40000 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 are measured and 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. Female heterozygotes are either asymptomatic or show attenuated classic clinical symptoms. 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 mutations are found throughout the gene, although some recurrent mutations are reported and one mutation, p.Asn215Ser, is commonly found in patients with the cardiac variant.

Referrals

- Clinically affected patients should have their diagnosis confirmed by biochemical analysis; this should be arranged either locally or with the Enzyme Laboratory, Great Ormond Street Hospital. Biochemical confirmed patients can be referred for mutation analysis.
- Carrier testing can be offered to female relatives of affected patients once a disease causing mutation has been identified.

Prenatal testing

Prenatal testing is available, if required, for families in who specific mutations have been identified or in whom appropriate family studies have been undertaken- please contact the laboratory to discuss.

Service offered

Mutation screening of all 7 exons and intron-exon boundaries of the GLA gene is undertaken by direct sequence analysis in affected patients. In patients with the cardiac variant, testing for the p.Asn215Ser mutation by direct sequencing is undertaken as a preliminary step, clinical information to this effect is therefore useful. Mutation specific testing for previously identified family mutations is also available in family members.

Target reporting time

2 months for routine mutation screen in index case. 2 weeks for carrier testing using mutation specific tests. For urgent samples please contact the laboratory.

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Gaucher disease (β-glucocerebrosidase deficiency)

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

Prenatals 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.

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 I) or presence (types 2 and 3) of primary CNS involvement although there is actually likely to be a clinical continuum. Type II is considered to be the most severe form and type I the least severe. All forms are characterised by hepatosplenomegaly and anaemia with bone involvement common in types I and III. Treatment involves bone marrow transplantation or enzyme replacement therapy. Type I is the most prevalent form and is particularly common in the Ashkenazi Jewish population with an incidence of ~1/855 individuals. Type I shows a broad spectrum of severity ranging from severely affected individuals to asymptomatic, presenting in childhood or adulthood. Types II and III are more rare.

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

Referrals

- Clinically affected patients should have their diagnosis confirmed by biochemical analysis; such patients may then be referred for mutation analysis.
- Carrier testing can be offered to adult relatives of affected patients once the disease causing mutations have been identified.

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

Testing is offered for the following recurrent GBA mutations which account for ~86% mutations in the Ashkenazi population and 70% of mutations 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) and del55c.115+1G>A

Please note p.Asn409Ser, p.Leu483Pro, p.Arg502Cys, p.Asp448His, c.84dupG, c.(1263_1319) and del55c.115+1G>A were previously known as N370S, L444P, R463C, D409H, 84GG, c.1263del55 and IVS2+1G>A respectively

Target reporting time

Routine analysis: 2 weeks. For urgent samples please contact the laboratory.

Contact details for biochemistry/ enzyme laboratory:

Enzyme laboratory, Chemical Pathology, 3rd floor, Institute of Child Health,30 Guilford Street, London WC1N 1EH
Tel: +44 (0) 20 7405 9200 (x2509)

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Glycogen storage disease type Ia (GSD1a)

Contact details

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GOSH NHS Trust
Level 6
York House
37 Queen Square
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Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

Prenatals 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.

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 mutations are found throughout the gene, however, ethnic specific mutations are recognised and information regarding ethnic origin is a useful indicator. In the North European Caucasian population two mutations, namely p.Gln347X and p.Arg83Cys account for approximately 62% of all mutations.

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 Laboratory, Great Ormond Street Hospital.
- Affected patients can be referred for mutation 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 disease causing mutation has been identified.

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
The p.Gln347X and p.Arg83Cys mutations account for approximately 62% of mutations in the North European Caucasian population and are tested for by PCR & restriction enzyme digestion as a preliminary screen. Direct sequencing of the 5 exons and intron-exon boundaries is then carried out. Mutation specific testing for previously identified family mutations is also available in family members by PCR & restriction enzyme digest or direct sequencing.

Target reporting time
2 months for routine mutation screen in index case. 2 weeks for carrier testing using mutation specific tests. For urgent samples please contact the laboratory.

Contact details for biochemistry/ enzyme laboratory:
Enzyme laboratory, Chemical Pathology, 3rd floor, Institute of Child Health,30 Guilford Street, London WC1N 1EH
Tel: +44 (0) 20 7405 9200 (x2509)

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Glycogen storage disease type 2 (Pompe disease)

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

Prenatals 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.

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 (Myozyme®) is now available which may slow or reverse symptoms of the disease.

The GAA gene consists of 20 exons (exon 1 non-coding) and family specific mutations are found throughout the gene. Ethnic specific mutations are recognised and information regarding ethnic origin is a useful indicator. A mild splicing mutation in intron 1 (c.-32-13T>G) in combination with a more severe mutation 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 Laboratory, Great Ormond Street Hospital. Biochemical confirmed patients can be referred for mutation 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 disease causing mutations has been identified.

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
Mutation screening of exons 2-20 by direct sequencing analysis. Restriction enzyme analysis for the recurrent late-onset mutation c.-32-13T>G. Mutation specific testing for previously identified family mutations is also available by restriction enzyme digest or direct sequencing.

Target reporting time
2 months for routine mutation screen in index case. 2 weeks for carrier testing using mutation specific tests. For urgent samples please contact the laboratory.

Contact details for biochemistry/ enzyme laboratory:
Enzyme laboratory, Chemical Pathology, 3rd floor, Institute of Child Health,30 Guilford Street, London WC1N 1EH
Tel: +44 (0) 20 7405 9200 (x2509)

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Krabbe disease (Globoid Cell Leukodystrophy)

Contact details

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Level 6
York House
37 Queen Square
London
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Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

Prenatals 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.

Introduction
Krabbe disease (MIM #245200) is an autosomal recessive inborn error of metabolism caused by deficiency of the enzyme galactosyceramidase (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 buildup 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 18 exons (exon 1 non-coding). A recurrent 30 kb 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; such patient may then be referred for mutation 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 disease causing mutations has been identified.

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

Target reporting time
2 weeks for routine deletion mutation test in the index case and family member carrier testing. For urgent samples please contact the laboratory.

Contact details for biochemistry/ enzyme laboratory:
Enzyme laboratory, Chemical Pathology, 3rd floor, Institute of Child Health,30 Guilford Street, London WC1N 1EH
Tel: +44 (0) 20 7405 9200 (x2509)

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Long chain acyl-CoA dehydrogenase (LCHAD) deficiency

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

Prenatals 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.

Introduction
Long chain acyl-CoA dehydrogenase (LCHAD) deficiency is an autosomal recessive disorder of fatty acid metabolism (MIM 201460), caused by a deficiency of the long-chain hydroxyacyl-CoA dehydrogenase (HADHA) enzyme. Tandem mass spectrometry of organic acids in urine, and carnitines in blood spots, allows the diagnosis to be unequivocally determined due to the accumulation of specific undegraded compounds

LCHAD deficiency is clinically heterogeneous but is often characterised by cardiomyopathy, skeletal myopathy, hypoglycemia, pigmentary retinopathy or sudden infant death. 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 gene encoding the HADHA enzyme is located at 2p23. The mutation, c.1528G>C causes the replacement of the amino acid glutamic acid with glutamine at codon 510 (p.Glu510Gln), this results in loss of LCHAD activity and accounts for approximately 87% of mutant alleles.

Referrals

- Clinically affected patients should have their diagnosis confirmed by biochemical analysis. Affected patients can then be referred for mutation testing. 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 affected patients once the disease causing mutation has been identified and partner testing is offered to confirmed carriers.

Prenatal testing
Prenatal testing, by genetic analysis, is available to couples that have both previously been shown to be carriers of the common mutation. Prenatal diagnosis is also offered by biochemistry regardless of mutation. Please contact the laboratory to discuss.

Service offered
Testing for the presence of the common c.1528G>C mutation (p.Glu510Gln) in the HADHA gene by PCR & restriction enzyme digest. Screening the remainder of the gene is not offered.

Target reporting time
Routine analysis - 2 weeks. For urgent samples please contact the laboratory.

Contact details for biochemistry/ enzyme laboratory:
Enzyme laboratory, Chemical Pathology, 3rd floor, Institute of Child Health,30 Guilford Street, London WC1N 1EH
Tel: +44 (0) 20 7405 9200 (x2509)

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Medium chain acyl-CoA dehydrogenase (MCAD) deficiency

Contact details

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37 Queen Square
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Telephone
+44 (0) 20 7762 6888
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+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

Prenatals 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.

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 due to the accumulation of specific undegraded compounds.

MCAD deficiency has an incidence in the UK of between 1/6500 and 1/20000 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 mutation 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 mutant alleles.

Referrals

- Clinically affected patients should have their diagnosis confirmed by biochemical analysis. Affected patients can then be referred for mutation 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 disease causing mutation has been identified and partner testing for the common c.985A>G mutation can be offered if appropriate.

Prenatal testing

Prenatal testing, by genetic analysis, is available to couples that have both previously been shown to be carriers of disease causing mutations. Prenatal diagnosis is also offered by biochemistry regardless of mutation. Please contact the laboratory to discuss.

Service offered

Level 1 Mutation Analysis: Testing for the common c.985A>G mutation by PCR and restriction enzyme digest.
Level 2 Mutation Analysis: Direct sequencing of exons 1 to 12 of the ACADM gene in 11 fragments.

Target reporting time

2 weeks for routine level 1 screen in index case, 2 months for level 2 screen. 2 weeks for routine testing of specific mutations. For urgent samples please contact the laboratory.

Contact details for biochemistry/ enzyme laboratory:

Enzyme laboratory, Chemical Pathology, 3rd floor, Institute of Child Health,30 Guilford Street, London WC1N 1EH
Tel: +44 (0) 20 7405 9200 (x2509)

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Metachromatic Leukodystrophy (MLD) & Pseudodeficiency of arylsulphatase A (PDASA)

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

A completed DNA request card should accompany all samples.

Contact details for enzyme laboratory undertaking ASA activity measurements:

Enzyme laboratory, Chemical Pathology, 3rd floor, Institute of Child Health,30 Guilford Street, London WC1N 1EH

Tel: + 44 (0) 20 7405 9200 (x2509)

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*-Sulphagalactosyl 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 mutations 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 sulphatase deficiency due to mutations in SUMF1. The ARSA gene (22q13.31-qter) comprises 8 exons. Although many novel mutations are known, there are 'common' mutations within the gene, particularly the c.459+1G>A and c.1277C>T (p.Pro426Leu) which account for around 50% of disease alleles in the Northern European population.

Pseudodeficiency of arylsulphatase A (PDASA)
Pseudodeficiency of arysulphatase 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 sequence variants in the ARSA gene, namely PD2 (Poly A) and PD1 (NGly). PD2 (c.2723A>G in the 3'UTR, exon 8) destroys the first downstream polyadenylation site and causes subsequent loss of the 2.1kb mRNA species; this variant is clearly associated with decreased ASA activity. PD2 is almost invariably seen on a background with PD1. PD1 (c.1788 A>G / p.Asn350Ser, exon 6) destroys an N-glycosylation site, causing a change in protein size, but having little effect on stability or activity. PD1 can occur independently of PD2 and its effect in causing PDASA is controversial.

Referrals
PDASA testing is used to assist the interpretation of arylsulphatase A activity results. Referrals are generally via the GOSH enzyme laboratory however referrals may be accepted from other centres 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 mutations have been identified or in whom appropriate family studies have been undertaken. Prenatal testing for PDASA may also be requested by the enzyme laboratory.

Service offered

- PDASA: Testing for the presence of PD1 and PD2 by PCR and restriction enzyme digest.
- MLD Level 1 Analysis: Detection of the common mutations c.459+1G>A and c.1277C>T (p.Pro426Leu) by PCR and restriction enzyme digest.
- MLD Level 2 Analysis: Direct sequencing of all 8 coding exons and intron-exon boundaries.

Target reporting time
2 weeks for PDASA testing. 2 weeks for routine level 1 screen in index case, 2 months for level 2 screen. 2 weeks for routine testing of specific mutations. For urgent samples please contact the laboratory.

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Mucopolysaccharidosis I (MPSI) (Hurler / Scheie syndrome)

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

Prenatals must be arranged in advance, through a Clinical Genetics department if possible. Amniotic fluid or CV samples for genetic testing should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis Prenatal detection can also be undertaken by biochemical analysis.

A completed DNA request card should accompany all samples.

Introduction
MPSI (MIM 252800) is an autosomal recessive, lysosomal storage disorder, otherwise known as Hurler syndrome (severe) or Scheie syndrome (milder variant). The condition is caused by a deficiency of the enzyme a-L-iduronidase (IDUA), which is required for lysosomal degradation of the glycosaminoglycans, heparan 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 amongst their clinical symptoms. 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 mutations identified in the IDUA gene, although many novel mutations are known, there are 'common' mutations within the gene.

The IDUA gene (4p16.3) has 14 exons and mutations have been found throughout the gene. The recurrent mutations p.Gln70X, p.Ala327Pro and p.Trp402X account for approx. 70% of disease alleles in the Northern European population. The p.Trp402X and p.Gln70X are the most common mutations seen in Hurler patients. p.Arg89Gln and 678-7G>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 laboratory, Great Ormond Street Hospital. Biochemical confirmed patients can be referred for mutation 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 disease causing mutation has been identified.

Prenatal testing
Prenatal testing is available for families in whom mutations 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 Mutation Analysis: Detection of commonly reported mutations p.Gln70X, p.Ala327Pro and p.Trp402X by PCR and restriction enzyme digest.
- Level 2 Mutation Analysis: Direct sequencing of all 14 coding exons and intron-exon boundaries.
- Detection of known mutations: In relatives of patients with confirmed MPS1 mutations by PCR and restriction digest or sequencing

Target reporting time
2 weeks for routine level 1 screen in index case, 2 months for level 2 screen. 2 weeks for routine testing of specific mutations. For urgent samples please contact the laboratory.

Contact details for biochemistry/ enzyme laboratory:
Enzyme laboratory, Chemical Pathology, 3rd floor, Institute of Child Health,30 Guilford Street, London WC1N 1EH
Tel: + 44 (0) 20 7405 9200 (x2509)

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Mucopolysaccharidosis type 2 (MPS2) (Hunter syndrome)

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

Prenatals must be arranged in advance, through a Clinical Genetics department if possible. Amniotic fluid or CV samples for genetic testing should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis Prenatal detection can also be undertaken by biochemical analysis.

A completed DNA request card should accompany all samples.

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 in the IDS enzyme activity; 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 mutations are found throughout the gene. Homologous recombination between the IDS gene and an adjacent 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;such patients may then be referred for mutation 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 disease causing mutation has been identified, or appropriate linked markers identified.
- Carrier testing can be offered to female relatives of affected patients once a disease causing mutation has been identified, or appropriate linked markers identified.

Prenatal testing
Prenatal testing is available for families in whom specific mutations have been identified or in whom appropriate family studies have been undertaken. 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 a common inversion, which has been shown to occur in ~10% of Hunter patients. Mutation screening is then undertaken in the remainder of the gene. Mutation specific testing for previously identified family mutations is also available in family members. In families where we are unable to identify a mutation linked markers are available.

Technical
Point mutations, small deletions and insertions are screened for by direct sequence analysis on exons 1 to 9 of the IDS gene. The presence of the homologous recombination event is detected by PCR amplification of the regions involved. This strategy detects >90% mutations in biochemical confirmed Hunter patients. In cases where we are unable to identify the mutation linked marker analysis may be useful for family studies, please contact the laboratory to discuss.

Target reporting time
2 weeks for inversion test. 2 months for routine mutation screen in index case. 2 weeks for carrier testing using mutation specific tests. For urgent samples please contact the laboratory.

Contact details for biochemistry/ enzyme laboratory:
Enzyme laboratory, Chemical Pathology, 3rd floor, Institute of Child Health,30 Guilford Street, London WC1N 1EH
Tel: +44 (0) 20 7405 9200 (x2509)

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Mucopolysaccharidosis type 3 (MPS3) (Sanfilippo syndrome)

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

Prenatals must be arranged in advance, through a Clinical Genetics department if possible. Amniotic fluid or CV samples for genetic testing should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis Prenatal detection can also be undertaken by biochemical analysis.

A completed DNA request card should accompany all samples.

Introduction
Mucopolysaccharidosis type 3 (MPS3 / Sanfilippo syndrome MIM #252900) is an autosomal recessive lysosomal storage disorder due to impaired degradation of heparan sulfate (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 (primarily from aspiration pneumonia). 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 heparin 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 a deficiencies of the enzymes N-sulfoglucosamine sulfohydrolase (SGSH) and alpha-N-acetylglucosaminidase (NAGLU) respectively. There have been several recurrent mutations 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 laboratory, Great Ormond Street Hospital. Such patients may then be referred for mutation 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 mutations 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
- Mutation screening: Direct sequencing of all coding exons and intron-exon boundaries.
- Detection of known mutations: In relatives of patients with confirmed MPS3A or MPS3B mutations by PCR and restriction digest or sequencing

Target reporting time
2 months for routine mutation screen in index case. 2 weeks for carrier testing using mutation specific tests. For urgent samples please contact the laboratory.

Contact details for biochemistry/ enzyme laboratory:
Enzyme laboratory, Chemical Pathology, 3rd floor, Institute of Child Health,30 Guilford Street, London WC1N 1EH
Tel: +44 (0) 20 7405 9200 (x2509)

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Neuronal Ceroid-Lipofuscinosis type 1 (NCL1)
(incl. Infantile Batten disease (INCL))

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

Prenatals 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.

Introduction
Neuronal ceroid-lipofuscinosis type 1 (NCL1; MIM #256730) is a rare autosomal recessive neurodegenerative disorder caused by mutations in the PPT1 gene which encodes the enzyme palmitoyl–protein thioesterase-1 (PPT1; MIM 600722). NCL1 is one of at least eight genetically distinct diseases associated with the NCL disease spectrum. Onset is typically infantile (INCL) however juvenile and adult (1) onset cases have also been described.

The differential diagnosis of NCL1 from other NCL types is based on age of onset, clinical phenotype, ultra structural 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 neurones and other cell types using electron microscopy and loss of palmitoyl protein thioesterase (PPT) 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 yrs), loss of motor skills, and premature death between 8-13 yrs.

The PPT1 gene (1p32) consists of 9 exons and mutations have been found throughout the gene. The four most common PPT1 mutations are p.Arg122Trp (Finnish-specific), p.Arg151X, p.Thr75Pro and p.Leu10X. The p.Arg151X and p.Leu10X mutations may account for up to 75% of mutations in certain populations.

(1) van Diggelen et al. (2001) Ann Neurol 50:269-272

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 laboratory at Great Ormond Street Hospital. Such patients may then be referred for mutation 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 disease-causing mutation has been identified.

Prenatal testing
Prenatal testing is available for families in whom the diagnosis of NCL1 has been confirmed by the identification of a mutation or in whom appropriate family studies have been undertaken - please contact the laboratory to discuss.

Service offered

- Level 1 Mutation Analysis: Detection of recurrent mutations p.Arg151X, p.Thr75Pro & p.Leu10X by PCR and restriction enzyme digest.
- Level 2 Mutation Analysis: Direct sequencing of all 9 coding exons and intron-exon boundaries.
- Detection of known mutations: In relatives of patients with confirmed NCL1 mutations by PCR and restriction digest or sequencing

Target reporting time
2 weeks for routine level 1 screen in index case, 2 months for level 2 screen. 2 weeks for routine testing of specific mutations. For urgent samples please contact the laboratory.

Contact details for biochemistry/ enzyme laboratory:
Enzyme laboratory, Chemical Pathology, 3rd floor, Institute of Child Health,30 Guilford Street, London WC1N 1EH
Tel: +44 (0) 20 7405 9200 (x2509)

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 also supply details of biochemical testing and histopathological review.

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Neuronal Ceroid-Lipofuscinosis type 2 (NCL2)
(Late infantile neuronal ceroid-lipofuscinosis (LINCL))

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

Prenatals 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.

Introduction
Neuronal ceroid-lipofuscinosis type 2 (NCL2; MIM #204500) is a rare autosomal recessive neurodegenerative disorder caused by mutations in the CLN2 gene which encodes the lysosomal enzyme tripeptidyl peptidase (TPP1). NCL2 is one of at least eight genetically distinct diseases associated with the NCL disease spectrum. NCL2 is generally referred to as late-infantile NCL (LINCL) due typical onset of symptoms between the ages of 2 and 4 years. Variant forms of LINCL (vLINCL) have been reported caused by mutations in the CLN1, CLN5, CLN6 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 neurones and other cell types.

CLN2 (11p15) consists of 13 exons. The two most common mutations are c.509-1 G>C ~33% of LINCL chromosomes) and p.Arg208X (~26 % of LINCL chromosomes). Other disease causing mutations are family specific and found throughout the gene.

Referrals

- Clinically affected patients should, wherever possible, have their diagnosis confirmed by analysis of TTP1 enzyme activity in leucocytes and fibroblasts. This should be arranged locally or with the Enzyme Laboratory, Great Ormond Street Hospital. Biochemical confirmed patients can be referred for mutation 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 disease causing mutation has been identified.

Prenatal testing
Prenatal testing is available for families in whom the diagnosis of NCL2 has been confirmed by the identification of a mutation or in whom appropriate family studies have been undertaken - please contact the laboratory to discuss.

Service offered
Testing for the two commonly reported mutations, c.509-1G>C and p.Arg208X, is carried out by sequence analysis of exon 6 of the CLN2 gene. These two mutations account for around 60% of LINCL chromosomes.

Target reporting time
2 weeks for routine analysis. For urgent samples please contact the laboratory.

Contact details for biochemistry/ enzyme laboratory:
Enzyme laboratory, Chemical Pathology, 3rd floor, Institute of Child Health,30 Guilford Street, London WC1N 1EH
Tel: +44 (0) 20 7405 9200 (x2509)

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 also supply details of biochemical testing and histopathological review.

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Neuronal Ceroid-Lipofuscinosis type 3 (NCL3)
(Juvenile Batten disease (JNCL))

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

Prenatals 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.

Introduction
Neuronal ceroid-lipofuscinosis type 3 (NCL3; MIM #204200) is a rare autosomal recessive neurodegenerative disorder caused by mutations in the CLN3 gene. NCL3 is one of at least eight 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 mutations 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 neurones and other cell types and the presence of vacuolated lymphocytes on a blood smear.

The CLN3 gene (16p12) consists of 15 exons spanning 15kb of genomic DNA. A 1.02kb deletion (introns 6-8) is reported to account for ~85% of JNCL chromosomes (Finnish figures). Other disease causing mutations 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. Testing for the common 1.02kb deletion can then be requested; please supply details of biochemical and 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 disease causing mutation has been identified.

Prenatal testing
Prenatal testing is available for families in whom the diagnosis of NCL3 has been confirmed by the identification of the 1.02kb deletion mutation or in whom appropriate family studies have been undertaken - please contact the laboratory to discuss.

Service offered
Testing for the common 1.02kb deletion by three-primer PCR analysis. Other disease causing mutations are highly heterogeneous and testing is not currently offered as part of this diagnostic service. Testing can be offered to family members once the 1.02kb deletion has been identified. In families where an affected patient is found to have a heterozygous deletion and where the second mutation has not been identified, linked markers may be useful. This requires the diagnosis of NCL3 to be confirmed by histopathological analysis. Due to clinical and genetic heterogeneity of the NCLs linked marker analysis is not recommended in non-consanguineous families where no mutation has been identified.

Target reporting time
2 weeks for routine 1.02kb deletion mutation test in the index case and family member carrier testing. For urgent samples please contact the laboratory.

Contact details for histopathology laboratory:
Histopathology, Level 3, Camelia Botnar Laboratories, Great Ormond Street Hospital, London WC1N 3JH.
Tel: +44 (0) 20 7405 9200 (x7907)

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Variant Neuronal Ceroid-Lipofuscinosis (NCL types 5, 6 & 8)
(variant late-infantile Batten disease (vLINCL))

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

Prenatals 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.

Introduction
Variant type 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 mutations in one of several genes including CLN5, CLN6 and CLN8. The neuronal ceroid-lipofuscinoses are a group of at least eight genetically distinct diseases associated with a similar phenotype but variable age of onset. Disease associated with the CLN5, CLN6 and CLN8 genes can sometimes be referred to as Finnish, Czech and Turkish variant late-infantile Batten disease respectively due to mutations being more frequently identified in these populations.

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 neurones 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 and CLN8 (8pter-p22) consists of 3 exons. Mutations are generally family specific and found throughout the gene.

Referrals

- Clinical and histopathological review of the affected patient is recommended to indicate a diagnosis of variant NCL. Please supply details of biochemical and 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 disease causing mutation has been identified.

Prenatal testing
Prenatal testing is available for families in whom the diagnosis of variant NCL has been confirmed by the identification of a mutation or in whom appropriate family studies have been undertaken - please contact the laboratory to discuss.

Service offered
Direct sequencing analysis of all coding exons and intron-exon boundaries of the CLN5, 6 and 8 genes. Genes will be sequenced sequentially in the order CLN5, CLN8 then CLN6 reflecting mutation pickup to date.

Target reporting time
2 months for routine mutation screening. 2 weeks for routine testing of specific mutations. For urgent samples please contact the laboratory.

Contact details for histopathology laboratory:
Histopathology, Level 3, Camelia Botnar Laboratories, Great Ormond Street Hospital, London WC1N 3JH.
Tel: +44 (0) 20 7405 9200 (x7907)

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Ornithine transcarbamylase (OTC) deficiency

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

Prenatals 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.

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 specific mutations are found throughout the gene, although some recurrent mutations at CpG sites in exons 1, 3, 5 and 9 are reported and some late onset specific mutations 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. Mutation 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 mutation carriers).
- Carrier testing can be offered to the female relatives of OTC patients once a disease causing mutation has been identified.

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
Entire gene deletions account for ~10% of neonatal OTC cases and are tested for by multiplex PCR analysis in males. Dosage analysis for females by MLPA is currently in development. Mutation screening for small mutations is undertaken by direct sequence analysis of the 10 exons and intron-exon boundaries. This testing strategy detects approximately 84% of mutations in patients with enzymatically confirmed OTC deficiency. In cases where we are unable to identify the mutation, linked marker analysis may be useful for family studies, please contact the laboratory to discuss. Mutation specific testing for previously identified family mutations is also available in family members by PCR & restriction enzyme digest or direct sequencing.

Target reporting time
2 months for mutation screen in index case. 2 weeks for carrier testing using mutation specific tests. For urgent samples please contact the laboratory.

Contact details for biochemistry/ enzyme laboratory:
Enzyme laboratory, Chemical Pathology, 3rd floor, Institute of Child Health,30 Guilford Street, London WC1N 1EH
Tel: +44 (0) 20 7405 9200 (x2509)

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Osteopetrosis

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

Prenatals 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.

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 TCIRGI (ATP6i) gene located at 11q13, consists of 20 exons and encodes an a3 subunit of the vacuolar pump, which mediates acidification of bone/osteoclast interface. Mutations 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 disease causing mutation has been identified.

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
Mutation screening by direct sequencing is offered for exons 2 to 20 in affected individuals. If no sample is available from the affected individual testing can be undertaken in their mother and father. Mutation specific testing for previously identified family mutations is also available in family members by PCR & restriction enzyme digest or direct sequencing.

Target reporting time
2 months for mutation screen in index case. 2 weeks for carrier testing using mutation specific tests. For urgent samples please contact the laboratory.

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Cystinosis

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

Prenatals 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.

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. The most common form is infantile nephropathic cystinosis (95% of cases) that 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 mutation is a 57 kb deletion which is found in ~76% of northern Europeans and makes up one third of all mutations found in individuals with cystinosis. The rest of the mutations reported are spread throughout the coding area of the gene and include insertions, small deletions, nonsense, splicing and missense mutations. No mutation hotspots have been identified.

Referrals
- Clinically affected patients should have their diagnosis confirmed by biochemical analysis; this should be arranged either locally or with the Enzyme Laboratory, Great Ormond Street Hospital. Biochemical confirmed patients can be referred for mutation analysis.
- Carrier testing can be offered to adult relatives of affected patients once a disease causing mutation has been identified.

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
Level 1 Mutation Analysis: Testing for the common 57 kb deletion by PCR analysis.
Level 2 Mutation Analysis: Direct sequencing of exons 3 to 12 of the CTNS gene (exons 1 & 2 are non-coding)

Target reporting time
2 weeks for routine level 1 screen in index case, 2 months for level 2 screen. 2 weeks for routine testing of specific mutations. For urgent samples please contact the laboratory.

Contact details for biochemistry/ enzyme laboratory:
Enzyme laboratory, Chemical Pathology, 3rd floor, Institute of Child Health,30 Guilford Street, London WC1N 1EH
Tel: +44 (0) 20 7405 9200 (x2509)

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Juvenile nephronophthisis (NPH1)

Contact details

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GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

Prenatals 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.

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 mutations 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.

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.

Technical
The deletion test consists of a polymerase chain reaction (PCR) amplification of exon 5 of NPHP1 on 2q13, together with a control DNA marker located on 2q35 outside the 250kb deletion. A homozygous deletion is detected as failure to amplify the exon 5 fragment, but with normal amplification of the control marker. Homozygosity for the deletion is confirmed by amplifying a second marker within the deleted region, 187.41, with the same control DNA marker.

Target reporting time
2 weeks for routine analysis of the NPHP1 deletion. For urgent samples please contact the laboratory.

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Steroid-Resistant Nephrotic Syndrome (NPHS2)

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>2ml from neonates)

Prenatals 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.

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).

Mutations 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 mutations in both alleles. The gene is located on chromosome 1q25-31 and consists of eight exons.

Referrals
Affected patients should fulfil 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:

1) resistant to treatment with steroids, or
2) present in the first 3 months of life, or
3) has a histological picture of FSGS on biopsy.

Please also send a completed clinical information sheet.
Click here for link to clinical information sheet (also available separately on our laboratory website)

Carrier testing can be offered to adult relatives of affected patients once a disease causing mutation 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
Mutation screening of the eight exons and exon/intron boundaries of the NPHS2 gene by direct sequencing.

Target reporting time
2 months for routine screen in index case. 2 weeks for routine testing of specific mutations. For urgent samples please contact the laboratory.

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Autoimmune lymphoproliferative syndrome (ALPS)

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

For DNA analysis only: 5ml venous blood (pre-BMT) in plastic EDTA bottles (>2ml 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.

Introduction
ALPS is a rare immunodeficiency disorder associated with inherited mutations in the *TNFRSF6* gene encoding Fas (also known as Apo-I or CD95) receptor protein (ALPS type IA); others have mutations in the *TNFSF6* 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 mutations in the Fas death domain encoded by exon 9 of the *TNFRSF6* 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

- Patients should initially be referred to Prof. Adrian Thrasher (see details below) for clinical assessment prior to any testing. Affected patients will 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 mutation screening in appropriate patients.
- Carrier testing can be offered to the relatives of ALPS patients once a disease causing mutation has been identified.

Prenatal testing
Prenatal testing is available for families in whom the family specific mutation has been identified - please contact the laboratory to discuss.

Service offered
Mutation screening of the 9 exons of the *TNFRS6* 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 mutations where appropriate. Mutation-specific tests for family mutations are also available.

Technical
Mutation screening is undertaken by sequence analysis of exons 1 to 9 of the *TNFRS6* gene.

Target reporting time
2 months for routine mutation screen in index case. 2 weeks for carrier testing for known family mutations. For urgent samples please contact the laboratory.

For clinical assessment and to arrange functional apoptosis studies please contact: Prof. Adrian Thrasher, Molecular Immunology, Institute of Child Health
tel.: +44 (0) 20 7813 8490 email: A.Thrasher@ich.ucl.ac.uk

To arrange double-negative α/β T cells analysis please contact:
Dr. Kimberly Gilmour, Molecular Immunology, Great Ormond Street Hospital
tel.: +44 (0) 20 7829 8835 email: GilmoK@gosh.nhs.uk

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Familial hemophagocytic lymphohistiocystosis/Perforin

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

For DNA analysis only: 5ml venous blood (pre-BMT) in plastic EDTA bottles (>2ml 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.

Introduction

Familial hemophagocytic lymphohistiocystosis (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, *PRF*1 has 3 exons of which exons 2 and 3 are coding. Mutations are found throughout the gene with some evidence of founder mutations. Only 20-40% of FHL cases are due to defects in the perforin gene. The guidelines for the NCG service for immunodeficiencies apply to the molecular analysis of perforin (details on request).

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 mutation screening in appropriate patients.
- Carrier testing can be offered to the relatives of FHL/*PRF*1 patients once a disease causing mutation 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.

Service offered

Mutation screening of the *PRF*1 gene in affected individuals found to have no/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, mutation testing 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. Mutation-specific tests for family mutations are also available.

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.

Technical

Mutation screening is undertaken by sequence analysis (detection rate unknown at present). In cases where we are unable to identify the mutation, no further analysis is currently available.

Target reporting time

2 months for routine mutation screen in index case. 2 weeks for carrier testing using mutation-specific tests. 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: gilmok@gosh.nhs.uk

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

IL7R-alpha severe combined immunodeficiency (IL7RαSCID)

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

For DNA analysis only: 5ml venous blood (pre-BMT) in plastic EDTA bottles (>2ml 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.

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 family specific mutations have been found throughout the gene. The guidelines for the NCG service for immunodeficiencies apply to the molecular analysis of IL7Rα-SCID (details on request).

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 mutation screening in appropriate patients.
- Carrier testing can be offered to the relatives of IL7Rα-SCID patients once a disease causing mutation 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 specific mutations have been identified or in whom appropriate family studies have been undertaken - please contact the laboratory to discuss.

Service offered
Mutation screening of the IL7Rα gene in affected individuals found to have no/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, mutation 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. Mutation-specific tests for family mutations are also available.

Technical
Mutation screening is undertaken by sequencing analysis (detection rate unknown at present). In cases where we are unable to identify the mutation, no further analysis is currently available.

Target reporting time
2 months for routine mutation screen in index case. 2 weeks for carrier testing using mutation-specific tests. 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: gilmok@gosh.nhs.uk

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.

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

JAK3-deficient severe combined immunodeficiency (JAK3-SCID)

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

For DNA analysis only: 5ml venous blood (pre-BMT) in plastic EDTA bottles (>2ml 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.

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 23 exons and family specific mutations are found throughout the gene. The guidelines for the NCG service for immunodeficiencies apply to the molecular analysis of JAK3-SCID (details on request).

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 mutation screening in appropriate patients.
- Carrier testing can be offered to the relatives of JAK3 patients once a disease causing mutation 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 specific mutations have been identified or in whom appropriate family studies have been undertaken - please contact the laboratory to discuss.

Service offered
Mutation screening of the JAK3 gene in affected individuals found to have no/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. Mutation-specific tests for family mutations and linkage analysis are also available.

Technical
Mutation screening is undertaken by sequencing analysis. In cases where we are unable to identify the mutation, linkage analysis may be used for family studies - please contact the laboratory to discuss.

Target reporting time
2 months for routine mutation screen in index case. 2 weeks for carrier testing using mutation-specific tests. 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: gilmok@gosh.nhs.uk

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

RAG-deficient severe combined immunodeficiency (RAG-SCID)

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

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+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

For DNA analysis only: 5ml venous blood (pre-BMT) in plastic EDTA bottles (>2ml from neonates)

Prenatals 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.

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. Family specific mutations 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 mutations in RAG1 and RAG2 that result in a partially functional recombinase. The guidelines for the NCG service for immunodeficiencies apply to the molecular analysis of RAG-SCID (details on request).

Referrals

- Affected patients should be referred to the Molecular Immunology laboratory at GOSH for RAG1 and RAG2 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 mutation screening in appropriate patients.
- Carrier testing can be offered to the relatives of RAG-SCID or Omenn syndrome patients once a disease-causing mutation 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.

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

Mutation screening of the RAG1 and RAG2 genes in affected individuals found to have no/abnormal RAG1 or RAG2 expression. Due to the requirement of a bone marrow sample for protein analysis, the undertaking of mutation screening in the absence of protein testing will be considered on a case-by-case basis. If DNA from the affected individual is unavailable then screening can be undertaken in the parents. Mutation-specific tests for family mutations are also available.

Technical

Mutation screening is undertaken by sequencing analysis. In cases where we are unable to identify the mutation, no further analysis is currently available.

Target reporting time

2 months for routine mutation screen in index case. 2 weeks for carrier testing using mutation-specific tests. For urgent samples please contact the laboratory.

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

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Wiskott-Aldrich syndrome (WAS)

Contact details

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Level 6
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37 Queen Square
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WC1N 3BH

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+44 (0) 20 7762 6888
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+44 (0) 20 7813 8196

Samples required

For DNA analysis only: 5ml venous blood (pre-BMT) in plastic EDTA bottles (>2ml 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.

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 for WASP) has 12 exons and family specific mutations are found throughout the gene. The guidelines for the NCG service for immunodeficiencies apply to the molecular analysis of WAS (details on request).

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 mutation screening in appropriate patients.
- Carrier testing can be offered to the female relatives of WAS patients once a disease causing mutation has been identified.

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
Mutation screening of the WAS gene in affected individuals found to have no/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 mutation screening carried out where appropriate. Mutation-specific tests for family mutations and linked marker analysis are also available.

Technical
Mutation screening is undertaken by sequencing analysis. In cases where we are unable to identify the mutation, linked marker or X-inactivation analysis may be used for family studies - please contact the laboratory to discuss.

Target reporting time
2 months for routine mutation screen in index case. 2 weeks for carrier testing using mutation-specific tests. For urgent samples please contact the laboratory.

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

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

X-Linked agammaglobulinaemia (XLA)

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

For DNA analysis only: 5ml venous blood (pre-BMT) in plastic EDTA bottles (>2ml 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.

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 (encoding for BTK) has 19 exons and family specific mutations 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 mutation screening in appropriate patients.
- Carrier testing can be offered to the female relatives of XLA patients once a disease causing mutation has been identified.

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

Mutation screening of the Btk gene in affected individuals found to have no/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 mutation screening carried out where appropriate. Mutation-specific tests and linked marker analysis are also available.

Technical

Mutation screening is undertaken by sequencing analysis. In cases where we are unable to identify the mutation, linked marker or X-inactivation analysis may be used for family studies - please contact the laboratory to discuss.

Target reporting time

2 months for routine mutation screen in index case. 2 weeks for carrier testing using mutation-specific tests. For urgent samples please contact laboratory.

To arrange BTK expression studies please contact Dr Kimberly Gilmour in Molecular Immunology, GOSH - Tel: +44 (0) 20 7829 8835, Email: gilmok@gosh.nhs.uk

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

X-linked Hyper IgM syndrome (HIGM)

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

For DNA analysis only: 5ml venous blood (pre-BMT) in plastic EDTA bottles (>2ml 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.

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 family specific mutations are found throughout the gene. The guidelines for the NCG service for immunodeficiencies apply to the molecular analysis of X-linked HIGM (details on request).

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 mutation screening in appropriate patients.
- Carrier testing can be offered to the female relatives of X-linked HIGM patients once a disease causing mutation has been identified.

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
Mutation screening of the CD40LG gene in affected individuals found to have no/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. Mutation-specific tests and linked marker analysis are also available.

Technical
Mutation screening is undertaken by sequence analysis of the 5 exons and exon/intron boundaries. In cases where we are unable to identify the mutation, linked marker analysis may be used for family studies – Please contact the laboratory to discuss.

Target reporting time
2 months for routine mutation screen in index case. 2 weeks for carrier testing using mutation-specific tests. For urgent samples please contact laboratory.

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

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

X-Linked lymphoproliferative disease (XLP)

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

For DNA analysis only: 5ml venous blood (pre-BMT) in plastic EDTA bottles (>2ml 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.

Introduction
XLP (MIM 308240) is an X-linked immunodeficiency 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). The SH2D1A gene (encoding for SAP) has 4 exons and family specific mutations are found throughout the gene. The guidelines for the NCG service for immunodeficiencies apply to the molecular analysis of XLP (details on request).

Referrals

- Affected patients should be referred to the Molecular Immunology department at GOSH for SAP 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 mutation screening in appropriate patients.
- Carrier testing can be offered to the female relatives of XLP patients once a disease causing mutation has been identified.

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
Mutation screening of the SH2D1A gene in affected individuals found to have no/abnormal SAP expression. Cases found to have SAP 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. Mutation-specific tests for family mutations and linked marker analysis are also available.

Technical
Mutation screening is undertaken by sequence analysis of the 4 exons and exon/intron boundaries. This detects approximately 43% of mutations 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. In cases where we are unable to identify the mutation, linked marker analysis may be used to indicate carrier status and for prenatal diagnosis - please contact the laboratory to discuss.

Target reporting time
2 months for routine mutation screen in index case. 2 weeks for carrier tests for known family mutations. For urgent samples please contact the laboratory.

To arrange SAP expression studies please contact Dr Kimberly Gilmour in Molecular Immunology, GOSH – Tel: +44 (0) 20 7829 8835, Email: gilmok@gosh.nhs.uk

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

X-Linked severe combined immunodeficiency (XSCID)

Contact details

Molecular Genetics
GOSH NHS Trust
Level 6
York House
37 Queen Square
London
WC1N 3BH

Telephone
+44 (0) 20 7762 6888
Fax
+44 (0) 20 7813 8196

Samples required

For DNA analysis only: 5ml venous blood (pre-BMT) in plastic EDTA bottles (>2ml 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.

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 (γc). The IL2Rγc gene (encoding for γc) has 8 exons and family specific mutations are found throughout the gene. The guidelines for the NCG service for immunodeficiencies apply to the molecular analysis of XSCID (details on request).

Referrals

- Affected patients should be referred to the Molecular Immunology laboratory at GOSH for γc 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 mutation screening in appropriate patients.
- Carrier testing can be offered to the female relatives of XSCID patients once a disease causing mutation has been identified.

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

Mutation screening of the IL2Rγc gene in affected individuals found to have no/abnormal γc expression. Cases found to have γc 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 mutation screening where appropriate. Mutation-specific tests for family mutations and linkage analysis are also available.

Technical

Mutation 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 γc. In cases where we are unable to identify the mutation, linkage or X-inactivation analysis may be used for family studies - please contact the laboratory to discuss.

Target reporting time

2 months for routine mutation screen in index case. 2 weeks for carrier testing for known family mutations. For urgent samples please contact the laboratory.

To arrange γc expression studies please contact Dr Kimberly Gilmour in Molecular Immunology, GOSH – Tel: +44 (0) 20 7829 8835, Email: gilmok@gosh.nhs.uk

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

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Document Approved by: | Date:

Q-Pulse Location: Molecular Genetics/Mol Gen Policies/Laboratory/Profile Sheets

Changes in this version

New address and telephone details
Header changed to show link to website
Changed re-need for proforma to meeting test criteria for HCM