Version 4.5 (Oct 2011)

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 pathology 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 approximately 13000 samples per year and issues over 8000 reports covering around 60 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 provided funding is available. A complete list of in-house services and corresponding information sheets can be found in this service pack. Further details regarding tests which may be available from other laboratories can be found on page 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.labs.gosh.nhs.uk/**

Head of Regional Molecular Genetics Lucy Jenkins, FRCPath
Deputy Head Regional Molecular Genetics Sam Loughlin DipRCPath

Laboratory Opening Hours
The laboratory is staffed Monday - Friday, 8.00am - 6.00pm excluding bank holidays. Sample reception is open from 8.30am to 5.30pm. Specimens arriving outside these hours are refrigerated / frozen prior to processing. There is no out-of-hours service. Please send samples to the address shown above.

Other contacts
Director of Genetics Laboratories Nick Lench PhD FRCPath
Finance / Admin Manager Jonathan Northfield
IT Manager Mike Tinsley

Clinical Genetics Unit
Great Ormond Street Hospital, Level 4, York House, 37 Queen Square, London. WC1N 3BH.
Telephone: +44 (0) 20 7762 6831 Fax: +44 (0) 20 7813 8141
Lead Clinician Dr Jane Hurst

NE Thames Regional Cytogenetics Laboratory
Great Ormond Street Hospital, Level 5, York House, 37 Queen Square London. WC1N 3BH.
Telephone: +44 (0) 20 7829 8870 Fax: +44 (0) 20 7813 8578
Head of Cytogenetics Jonathan Waters PhD FRCPath

Sample Requirements

It is the responsibility of the patient's clinician to ensure that all requests meet testing criteria, that samples are correctly labelled and request forms are completed to a minimum standard.
In submitting samples the clinician confirms that consent for testing and possible storage has been obtained

Samples

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

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

Samples must be labelled with:

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

Prenatal Samples

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

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

Cell Free Fetal DNA Analysis: 20ml of maternal blood in EDTA is required. Sample date & gestation as confirmed by ultrasound scan must be provided along with a valid clinical indication for early gender determination. This test does not apply to twin pregnancies. Please see page 25 for further details.

Minimum sample labelling criteria:

- Patient's full name and date of birth
- Unique hospital/NHS number

Request form

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

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

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

Sending samples

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

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

Pricing

NHS Provider to Provider and Private / Overseas test prices

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

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 http://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		9
Bardet-Biedl syndrome	Various under development		#209900		10
Beckwith Wiedemann syndrome		11p15.5	#130650		11
Familial breast/ovarian cancer	BRCA1 BRCA2	17q21 13q13.1	#604370 #612555	+113705 *600185	12
Cartilage Hair Hypoplasia	RMRP	9p21-p12	#250250	*157660	13
Cystic Fibrosis	CFTR	7q31.2	#219700	*602421	14
Fragile X syndrome	FMR1	Xq27.3	+309550		15
GNAS disorders	GNAS	20q13.2	+139320		16
Kabuki syndrome	MLL2	12q13.12	# 147920		17
Pulmonary Surfactant Metabolism Dysfunction 1, 2 & 3	SFTPB	2p12-p11.2	#265120 (SMDP1)	*178640	18
	SFTPC	8p21	#610913 (SMDP2)	*178620	18
	ABCA3	16p13.3	#610921 (SMDP3)	*601615	18
Popliteal Pterygium syndrome	IRF6	1q32-q41	#119500	*607199	19
Prader-Willi syndrome		15q11-q13	#176270		20
Primary congenital glaucoma	CYP1B1	2p22.2	#231300	*601771	21
Rhabdoid tumour predisposition syndrome	SMARCB1	22q11.23	#609322	*601607	22
Rett syndrome	MECP2	Xq28	#312750	*300005	23
Silver Russell syndrome		11p15.5 7p11.2	#180860		24
Van der Woude syndrome	IRF6	1q32-q41	#119300	*607199	19

Cardiovascular Disorders

Disorder	Gene	Locus	OMIM disease	OMIM gene	Page
Familial Hypercholesterolaemia	LDLR	19p13.2	#143890	*606945	28
	APOB	2p24	#144010	+107730	28
Hypertrophic Cardiomyopathy	MYBPC3	11p11.2	#192600	*600958	29
	MYH7	14q12		*160760	29
	TNNT2	1q32		*191045	29
	TNNI3	19q13.4		+191044	29
Loeys Dietz syndrome	TGFBR1	9q22.33	#609192	*190181	30
	TGFBR2	3p22	#608967 #610168 #610380	+190182	30

Skeletal Dysplasias

Disorder	Gene	Locus	OMIM disease	OMIM gene	Page
Achondroplasia	FGFR3	4p16.3	#100800	*134934	31
Hypochondroplasia	FGFR3	4p16.3	#146000	*134934	31
Thanatophoric Dysplasia	FGFR3	4p16.3	#187600	*134934	32

Craniosynostosis

Disorder	Gene	Locus	OMIM disease	OMIM gene	Page
Apert syndrome	FGFR2	10q26	#101200	*176943	33
CATSHL	FGFR3	4p16.3	#610474	*134934	33
Crouzon syndrome	FGFR2	10q26	#123500	*176943	33
	FGFR3	4p16.3		*134934	
Crouzon with acanthosis nigricans	FGFR3	4p16.3	#612247	*134934	33
Muenke syndrome	FGFR3	4p16.3	#602849	*134934	33
Pfeiffer syndrome	FGFR1	8p11	#101600	*136350	33
	FGFR2	10q26		*176943	
Saethre-Chotzen syndrome	TWIST	7p21	#101400	*601622	33

Deafness

Disorder	Gene	Locus	OMIM disease	OMIM gene	Page
Aminoglycoside induced deafness	MTRNR1	nt648-1601	#580000	*561000	34
Branchio-oto-renal syndrome	EYA1	8q13.3	#113650	*601653	35
	SIX1				35
	SIX5				35
Connexin 26 (DFNB1)	GJB2	13q11-q12	#220290	*121011	36
EAST syndrome	KCNJ10	1q23.2	#612780	*602208	37
Pendred syndrome	SLC26A4	7q31	#274600	*605646	38
Waardenburg syndrome Types 1 & 3	PAX3	2q35	#193500	*606597	39
			#148820		39

Metabolic Disorders

Disorder	Gene	Locus	OMIM disease	OMIM gene	Page
Carbamoylphosphate synthetase 1 deficiency	CPS1	2q35	#237200	*608307	40
Fabry disease	GLA	Xq22	+301500		41
Gaucher disease	GBA	1q21	#230800 (Type 1) #230900 (Type 2) #231000 (Type 3)	*606463	42 42 42
Glycogen Storage disease type 1a	G6PC	17q21	+232200		43
Glycogen storage disease type 2 (Pompe disease)	GAA	17q25.2-q25.3	#232300	*606800	44
Krabbe disease	GALC	14q31	#245200	*606890	45
Long-chain, deficiency of Acyl-CoA dehydrogenase	HADHA	2p23	#609016	*600890	46
Medium-chain, deficiency of Acyl-CoA dehydrogenase	ACADM	1p31	#201450	*607008	47
Metachromatic Leukodystrophy (incl. pseudodeficiency of arylsulphatase A)	ARSA	22q13.31-qter	#250100	*607574	48
Mucopolysaccharidosis type 1 (Hurler / Scheie)	IDUA	4p16.3	#607014 (Hurler) #607015 (H/S) #607016 (Scheie)	*252800	49 49 49
Mucopolysaccharidosis type 2 (Hunter)	IDS	Xq28	+309900		50
Mucopolysaccharidosis type 3 (Sanfilippo)	SGSH NAGLU	17q25.3	#252900 (MPS3A) #252920 (MPS3B)	*605270 *609701	51 51
Neuronal Ceroid Lipofuscinosis type 1 (incl. infantile Batten disease)	PPT1	1p32	#256730	*600722	52
Neuronal Ceroid Lipofuscinosis type 2 (late-infantile Batten)	TPP1	11p15.5	#204500	*607998	53
Neuronal Ceroid Lipofuscinosis type 3 (juvenile Batten)	CLN3	16p12.1	#204200	*607042	54
Neuronal Ceroid Lipofuscinosis type 5 (variant late-infantile Batten)	CLN5	13q21.1-q32	#256731	*608102	55
Neuronal Ceroid Lipofuscinosis type 6 (variant late-infantile Batten)	CLN6	15q21-q23	#601780	*606725	55
Neuronal Ceroid Lipofuscinosis type 8 (variant late-infantile Batten)	CLN8	8pter-p22	#600143	*607837	55
Ornithine transcarbamylase deficiency	OTC	Xp21.1	#311250	*300461	56
Osteopetrosis, autosomal recessive	TCIRG1	11q13.4-q13.5	#259700	*604592	57

Renal Disorders

Disorder	Gene	Locus	OMIM disease	OMIM gene	Page
CFHR5 Nephropathy	CHFR5	1q31.3		*608593	58
Cystinosis (Adult) (Juvenile)	CTNS	17p13	#219800 #219750 #219900	*606272	59 59 59
Juvenile Nephronophthisis	NPHP1	2q13	#256100	*607100	60
Steroid-resistant nephrotic syndrome	NPHS2	1q25-q31	#600995	*604766	61

Immunodeficiencies

Disorder	Gene	Locus	OMIM disease	OMIM gene	Page
Autoimmune lymphoproliferative syndrome (ALPS)	TNFRSF6	10q24.1	#601859	*134637	62
Familial hemophagocytic lymphohistiocystosis	PRF1	10q22	#603553	*170280	63
Interleukin 7 receptor alpha severe combined immunodeficiency	IL7Ra	5p13	#600802	*146661	64
JAK3-deficient severe combined immunodeficiency	JAK3	19p13.1	#600802	*600173	65
Netherton Syndrome	SPINK5	5q32	#256500	*605010	66
Radiation-sensitive SCID	Artemis (DCLREC1C)	10p	#602450	*605988	67
RAG-deficient severe combined immunodeficiency	RAG1 RAG2	11p13 11p13	#601457	*179615 *179616	68 68
Wiskott-Aldrich syndrome	WAS	Xp11.23-p11.22	#301000	*300392	69
X-linked agammaglobulinaemia	BTK	Xq21.3-q22	#307200	+300300	70
X-linked Hyper IgM syndrome (HIGM)	CD40LG	Xq26	#308230	*300386	71
X-linked Lymphoproliferative syndrome	SAP XIAP	Xq25 Xq25	#308240 #300635	*300490 *300079	72 72
X-linked Severe combined immunodeficiency	IL2RG	Xq13	#300400	*308380	73

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 (>1ml from neonates)

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

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

A completed DNA request card should accompany all samples

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 4 weeks. Microsatellite marker analysis takes 4 weeks from receipt of parental samples. Please contact the laboratory for urgent cases.

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Bardet-Biedl Syndrome (BBS)

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 (>1ml 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
Bardet-Biedl syndrome (BBS, OMIM #209900) is an autosomal recessive disorder comprised of postaxial polydactyly, retinitis pigmentosa, genito-urinary and renal abnormalities, obesity and learning difficulties. Patients may also have diabetes, Hirschprung disease, hypertension and neurological deficits. One third of patients will develop renal failure and about 10% will progress to end-stage renal failure requiring dialysis and/or transplantation. Diagnosis of the condition is often delayed until early to mid-teens only when visual impairment emerges.
The condition is rare with an estimated incidence of 1 in 160,000 in the UK although a higher incidence is seen in communities in which consanguineous marriage is common.
BBS is a heterogeneous condition with at least 15 different genes associated with the syndrome.

Referrals
NCG funded analysis is available to patients resident in England and Scotland. For non-NCG referrals, please contact the laboratory.

Prenatal testing
Prenatal testing may be available for families following molecular confirmation of diagnosis in the proband - please contact the laboratory to discuss.

Service offered
Level 1 screening – Testing for four common BBS mutations, c.1169T>G (p.Met390Arg) in BBS1, c.72C>G (p.Tyr24X) and c.823C>T (p.Arg275X) in BBS2 and c.271dup (p.Cys91fsX5) in BBS10. These mutations account for approximately 30% of North European BBS mutant alleles. If any patient is found to carry a single heterozygous mutation, the coding region of the relevant gene will be screened by sequencing in order to identify a second mutation.

Level 2 screening – patients who do not have a mutation on level 1 screening will be screened for mutations in 13 BBS genes by Next Generation sequencing (in development).

Technical
Level 1 screening is carried out by sequencing of exon 12 of BBS1, exons 1 and 8 of BBS2 and exon 2A of BBS10.

Target reporting time
Level 1 screening takes 4 weeks. As level 2 screening is still in development, target reporting times are not yet available. 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

Beckwith-Wiedemann Syndrome (BWS)

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 (>1ml from neonates)

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

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

A completed DNA request card should accompany all samples

Introduction
Beckwith-Wiedemann Syndrome (MIM 130650) occurs in approximately 1/13,700 individuals. Beckwith-Wiedemann Syndrome is a growth disorder characterised by macrosomia, macroglossia, visceromegaly, embryonal tumors, hemihyperplasia, omphalocele, neonatal hypoglycemia, ear creases/pits, adrenocortical cytomegaly and renal abnormalities.

BWS is a complex, multigenic disorder caused by alterations in growth regulatory genes on chromosome 11p15. Approximately 85% of BWS cases are sporadic and are mainly caused by epigenetic alterations in one of the two imprinting control regions on chromosome 11p15 (ICR1 and ICR2). Decreased methylation at KvDMR (ICR2) accounts for majority of BWS cases (~50%). Approximately 2-7% of BWS patients have increased methylation at the H19DMR (ICR1) locus. Paternal uniparental disomy of 11p15.5 results in increased methylation at H19DMR and decreased methylation at KvDMR and accounts for 10-20% of patients. Mutations in the CDKN1C gene are a further cause of BWS and are inherited in autosomal dominant manner; these account for 5-10% of sporadic and 40% of familial BWS cases. Parent of origin specific chromosome rearrangements involving 11p15 are also associated with the BWS phenotype. Translocations and inversions show maternal inheritance whereas duplications typically show paternal inheritance. Chromosomes rearrangements account for less than 1% of cases.

Referrals
- Confirmation of clinically suspected BWS in children/adults
- To confirm paternal UPD (parental samples required)

Prenatal testing
Prenatal testing to confirm a diagnosis of BWS suspected on antenatal ultrasound scans. Please contact the laboratory to discuss, prior to sending prenatal samples.

Service offered
Confirmation of BWS by dosage and methylation analysis of chromosome 11p15. Chromosome 11p15 microsatellite dosage analysis is also offered to confirm paternal UPD as a cause of BWS (parental samples required). CDKN1C mutation analysis is not offered in this laboratory.

Technical
For diagnostic referrals the initial test involves methylation and dosage analysis in chromosome 11p15 region using methylation specific MLPA analysis. Unaffected individuals will show normal dosage and methylation at both imprinting control regions. Patients with BWS will show either increased methylation at ICR1 or reduced methylation at ICR2. Patients with BWS caused by paternal UPD11p15.5 will show increased methylation at ICR1 and reduced methylation at ICR2. In BWS patients with paternal UPD, further analysis using chromosome 11 microsatellite dosage markers is recommended. Cytogenetic analysis can also be helpful in identifying chromosomal rearrangements.

Reporting time
Routine analysis - the initial methylation and dosage (MS-MLPA) test takes up to 4 weeks. Microsatellite marker analysis takes 4 weeks from receipt of parental samples. Please contact the laboratory if urgent testing is required.

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

BRCA1 / BRCA2

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
Hereditary breast and ovarian cancer due to mutations in BRCA1 and BRCA2 genes is the most common cause of hereditary forms of both breast and ovarian cancer. The prevalence of BRCA1/2 mutations is ~1/400 to 1/800, however this varies depending on ethnicity. Notably, in Ashkenazi Jewish populations there are three well-described founder mutations and their combined frequency in this population is 1/40.
Mutations in the BRCA1 and BRCA2 genes are also associated with other forms of cancer including fallopian tube carcinoma and primary papillary serous carcinoma of the peritoneum. The risk of prostate cancer in male BRCA1 carriers is increased with a relative risk of ~1.8, however the age at diagnosis is comparable to that of sporadic prostate cancer. Prostate cancer is more strongly associated with BRCA2 mutations, with a relative risk of 4.6 in male carriers as well as evidence of earlier age of onset.

Referrals
Referrals are accepted only via a Consultant Clinical Geneticist.

Samples required

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

Service offered
Mutation screening is currently offered for the common Ashkenazi Jewish mutations mutations (BRCA1: c.68_69delAG and c.5266dupC and BRCA2: c.5946delT) and the common Polish mutations (BRCA1: c.181T>G, c.4035delA and c.5266dupC). This is carried out by direct sequence analysis.

Predictive testing can be offered to individuals who have a known family mutation in either the BRCA1/BRCA2 gene. A family mutation control is required for analysis. This is carried out by either direct sequence analysis or MLPA analysis as appropriate.

Target reporting time
4 weeks for either the Ashkenazi Jewish and Polish mutation panels.
2 weeks for predictive testing where the mutation is known in the family.

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

Cartilage-hair hypoplasia (CHH) (RMRP gene)

Contact details

Molecular Genetics
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

5ml venous blood in plastic EDTA bottles (>1ml 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

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

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

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

Referrals

- Patients with suspected *RMRP*-related disorder for mutation screening of *RMRP.*
- Adult relatives of patients with *RMRP* mutations for carrier status.
- Testing is available for minor siblings to establish carrier status prior to bone marrow/stem cell donation.

Prenatal testing

Prenatal testing is available for families in whom specific 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 *RMRP* gene. Detection of known mutations in relatives of patients with confirmed *RMRP* mutations.

Technical

Direct sequencing analysis of the *RMRP* gene in two overlapping fragments which cover the promoter region and the transcribed region.

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

Cystic fibrosis (CF)

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 (>1ml 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

32 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 32 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 32 mutations. Please contact the laboratory if urgent or prenatal testing is required.

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Fragile X syndrome

Contact details

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York House
37 Queen Square
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WC1N 3BH

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

Samples required

5ml venous blood in plastic EDTA bottles (>1ml 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 *FMR1* gene on chromosome Xq27.3 (FRAX A cases). Expansion of the (CGG)n repeat sequence to >200 repeats accompanied by methylation of the adjacent CpG island extinguishes the *FMR1* gene expression (full mutation expansion). Premutation alleles with 59-200 (CGG)n repeats are unstable at meiosis and can lead to full expansion mutations in subsequent generations. Intermediate alleles (50-58 repeats) are not believed to be associated with fragile X syndrome, but may display size instability in future generations. *FMR1* point mutations and deletions are rare causes of the syndrome. Premutation allele carriers can display additional phenotypes such as premature ovarian failure (POF) and a neurodegenerative disorder of older adults, fragile X associated tremor/ataxia syndrome (FXTAS).

Referrals

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

Prenatal testing

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

Service offered

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

Technical

DNA is analysed by PCR of the (CGG)n repeat within the 5' untranslated region of the *FMR1* gene. Southern blot analysis of genomic DNA is carried out using the restriction enzymes *Nru*I and *Hind*III and the probe StB12.3. PCR and Southern blot analyses are unlikely to detect point mutations or deletions within the *FMR1* gene, and are also unable to exclude mosaicism.

Target reporting time

Routine analysis- 2 weeks for the initial PCR-based mutation screen, and 8 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 (>1ml 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's hereditary osteodystrophy (AHO) is an autosomal dominant disorder characterised by short stature, obesity, brachydactyly, subcutaneous ossifications and mental defects. There is a 2:1 ratio of affected females to males. AHO can present in one of two ways: with the somatic features of AHO alone (pseudopseudohypoparathyroidism, PPHP); or with AHO plus resistance to multiple hormones which increase cAMP in their target organs (pseudohypoparathyroidism type 1a, PHP 1a). Both PHP 1a and PPHP are caused by inactivating mutations in the GNAS1 gene. PHP1a is usually caused by mutations in maternal GNAS1, PPHP in paternal allele.
McCune Albright syndrome (MAS) is characterised by precocious puberty, café au lait spots and polyostic fibrous dysplasia of bone where the normal interior of bone is replaced by fibro-osseous connective tissue. McCune Albright syndrome is caused by somatic activating mutations in exons 8 and 9 of GNAS1. All MAS patients are mosaics. GNAS1 encodes 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 of exons 3 and 4). There are two alternatively spliced transcripts using exons upstream of GNAS1 (termed XLαs and NESP55) spliced to GNAS1 ex2-13 (+/- exon 3) expressed in most fetal tissue. Although the gene is biallelically expressed in most fetal tissue, XLαs is only expressed from the paternal chromosome and NESP55 only expressed from the maternal chromosome.

Referrals
- Patients with clinical symptoms as above.
- Carrier testing for family members for the familial 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 has given more successful results than DNA extracted from lymphocytes.

Target reporting time
The target reporting time is 2 months for a GNAS1 mutation screen and 2 weeks for carrier testing. 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

Kabuki Syndrome (KS)

Contact details

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

Samples required

5mls venous blood in plastic EDTA bottles (>1ml 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
Kabuki syndrome (KS, or Niikawa-Kuroki syndrome; MIM# 147920) is a rare syndrome characterized by intellectual disability, distinct dysmorphic features and growth retardation. Kabuki Syndrome is an autosomal dominant condition that occurs in 1 in approximately 32,000 live births. Most cases appear to be de novo, but familial cases are reported. Kabuki syndrome is caused by mutations in the MLL2 gene, encoding an H3K4 histone methyl transferase which acts as an epigenetic transcriptional activator during growth and development. A majority of the reported mutations are truncating mutations (nonsense or frameshift mutations) but missense and splicing mutations have also been identified in Kabuki Syndrome patients

Referrals
Prospective patients should have a probable diagnosis of Kabuki syndrome based on the presence of:
Characteristic facial appearance suggestive of Kabuki Syndrome, e.g.
Long palpebral fissures with eversion of the lateral portion of the lower eyelid
Broad, arched eyebrows with lateral sparseness
Short columella with depressed nasal tip
Large, prominent or cupped ears
AND developmental delay
OR In newborns only: a combination of congenital abnormalities such that Kabuki Syndrome is the most likely diagnosis.
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
Mutation screening of all 54 exons and intron-exon boundaries of the *MLL2* gene is undertaken by direct sequence analysis in affected patients. Mutation specific testing for previously identified family mutations is also available in family members.

Technical
Mutation screening is carried out by direct fluorescent sequencing.

Target reporting time
Mutation screening: 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
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 (>1ml 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 a genetically heterogeneous group of disorders that result in severe respiratory insufficiency or failure in full-term neonates or infants. These disorders are associated with various pathologic entities, including pulmonary alveolar proteinosis (PAP), desquamative interstitial pneumonitis (DIP), 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.

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 coding exons 1 to 10. Six linked markers are available.
SFTPC: Direct sequencing of coding exons 1 to 5.
ABCA3: Direct sequencing of coding exons 4 to 33.

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

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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 (>1ml 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.
- 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
- Direct sequencing of all coding exons (3-9) and intron-exon boundaries.
- Testing for known mutations in relatives of patients with confirmed PPS / VWS mutations by sequencing.

Target reporting time
2 months for routine mutation screen in index case. 2 weeks for carrier testing. 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 7762 6831

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

Samples required

5ml venous blood in plastic EDTA bottles (>1ml 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
Prader-Willi syndrome (PWS) (MIM 176270), occurring in 1/15000 - 1/20000 individuals, is characterised by diminished fetal activity, obesity, muscular hypotonia, developmental delay, short stature, hypogonadotropic hypogonadism, and small hands and feet. The PWS phenotype results from the lack of a paternal contribution at 15q11-q13. This can be caused by a deletion (~70%), maternal uniparental disomy (UPD) (25-30%) and rarely due to mutations in the imprinting centre (IC) that cause abnormal methylation at exon alpha of the SNRPN locus. These 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).

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 4 weeks. Microsatellite marker analysis takes 4 weeks from receipt of parental samples. Please contact the laboratory for urgent cases.

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE
Primary Congenital Glaucoma (PCG)

Contact details

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

Introduction:
Primary congenital glaucoma (PCG; MIM: #231300) is a congenital or infantile onset condition, characterised by raised intraocular pressure. Signs of this may include enlargement of the globe (buphthalmos), cloudy/hazy corneas, lacrimation and optic disc changes. The condition causes irreversible optic nerve damage and blindness if untreated.
This autosomal recessive condition has an incidence of 1/10,000 and is caused by mutations in the *CYP1B1* gene (MIM:*601771) located at 2p22.2 It consists of 3 exons, 2 of which are coding (NM_000104.3).

Patients with primary congenital glaucoma (PCG) are defined by elevated intraocular pressure (IOP) >21 mm Hg and/or signs consistent with elevated IOP, including

- disc cupping >0.3 or disc asymmetry ≥0.2
- progressive disc cupping
- buphthalmos (prominent, enlarged eye)
- enlarged corneal diameter (>11 mm in newborn, >12 mm in a child <1 year, or >13 mm in a child >1 year)
- corneal edema
- Descemet's membrane splits (Haab's striae)
- visual field defects
- progressive myopia in a child < 2 years of age.

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

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

Samples required

5ml venous blood in plastic EDTA bottles (>1ml 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.

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

- Patients with the symptoms detailed above
- Carrier testing in family members for familial mutations

Service offered
Analysis for point mutations and small insertions / deletions is by direct sequencing of the coding region in 6 amplicons. A wide variety of mutations have been reported, occurring throughout the coding region but no common mutations have been described. A single case has been identified with a whole gene deletion, however analysis for large deletions and duplications is not currently available.

Target reporting time
The target reporting time is 2 months for a *CYP1B1* mutation screen and 2 weeks for carrier testing. 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

Rhabdoid tumour predisposition syndrome

Contact details

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+44 (0) 20 7762 6888
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Samples required

5mls venous blood in plastic EDTA bottles (>1ml 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
Rhabdoid tumour predisposition syndrome (MIM# 609322) is a rare syndrome occurring in infancy and early childhood. The incidence is not currently known but is thought to occur in ~ 0.1-05 per million children per year. Rhabdoid tumours are highly malignant neoplasms that develop in the brain and spinal cord (atypical teratoid rhabdoid tumour), kidney and/or soft tissue (malignant rhabdoid tumour or extra-renal rhabdoid tumour). Patients may present with apparently sporadic tumours in one site or with multiple tumours arising in the brain, kidney and/or soft tissues. Approximately 1/3 of rhabdoid tumours are due to germline mutations in the *SMARCB1* gene. The *SMARCB1* gene encodes the INI-1/hSNF5 protein which is a member of the SW1/SNF chromatin re-modelling complex and functions in controlling gene transcription.
Rhabdoid tumours are highly resistant to conventional chemotherapies and radiotherapy, patients frequently succumb rapidly to disease.
Loss of protein expression of SMARCB1 gene can be detected by immunohistochemistry, however a small number of tumour show expression

Referrals
Referrals are accepted only via a Consultant Clinical Geneticist or Consultant Neurologist

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

Service offered
Mutation screening by direct sequencing is offered for the 9 exons of the *SMARCB1* gene. MLPA analysis is available to look for large deletions / duplications.

Target reporting time
8 weeks for mutation screen in index case. 2 weeks for targeted 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

Rett Syndrome (RTT)

Contact details

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

Samples required

5mls venous blood in plastic EDTA bottles (>1ml 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.

Introduction

Rett syndrome (MIM 312750) is an X-linked dominant neurodevelopmental condition caused by mutations in the methyl-CpG-binding-protein 2 *(MECP2)* gene on Xq28 which contains 4 exons. 8 common mutations account for around 45% of cases of RTT. Large deletions of *MECP2* account for around 38% of classical and 7.5% of atypical RTT where no mutation is identified by sequencing. *MECP2* mutations have also been reported in individuals with autism, Angelman-like syndrome and non-specific mental retardation.

Referrals

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

Prenatal testing

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

Service offered

MECP2 is analysed by **(1) bi-directional sequence analysis**, covering the 5'UTR, all four exons and intron-exon boundaries and extending 300bp into the 3'UTR. It includes the alternatively spliced 5' end of the *MECP2_e1 and MECP2_e2* isoforms but not mutations in the 3' untranslated or promoter regions of the gene. **(2) Large deletions and duplications** can be detected by MLPA using kit P015-E1 from MRC-Holland where 13 *MECP2* probes cover all 4 exons.

Nomenclature

The MECP2 translational initiation site (TIS) was originally identified in exon 2 (*MECP2_e2/ MECP2A*) but a second TIS in exon 1 (identified in 2004) results in a transcript (*MECP2_e1*/*MECP2B*) containing exons 1, 3 & 4, encoding a protein that is 17 amino acids longer than the exon 2-containing transcript. The 8.5kb 3'UTR is also alternatively spliced. *MECP2_e1* is the predominant isoform. Mutation nomenclature has historically been based on the *MECP2_e2* transcript which does not include exon 1. Mutations are described using the Human Genome Variation Society (HGVS) guidelines (http://www.hgvs.org) based on reference sequence NM_001110792.1 (MECP2_e1).

Target reporting time

8 weeks for routine sequence and dosage analysis. 2 weeks for known familial mutations. Please contact the laboratory if urgent or prenatal testing is required.

Patient details

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Silver Russell Syndrome (SRS)

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 (>1ml 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

Silver Russell Syndrome (SRS) (MIM 180860), occurring in ~1/100000 individuals, is characterised by severe intrauterine and postnatal growth retardation. The growth failure in SRS is frequently associated with failure to thrive and very low body mass index. Craniofacial symptoms include a characteristic small triangular face with a prominent forehead and micrognathia, down turned corners of the mouth and ear abnormalities. In more than 50% of patients, limb and body asymmetry is present.

SRS is a genetically heterogeneous disorder. Up to 50% of cases are due to abnormal methylation of chromosome 11p15, particularly decreased methylation at the H19DMR imprinting control centre (ICR1). Approximately 5-10% of individuals with SRS will have maternal uniparental disomy of chromosome 7. In 1-2% of SRS patients microscopic chromosome aberrations have been described. Maternal UPD 7 and methylation abnormalities are usually de novo events associated with low recurrence risk however, familial cases have been reported.

Referrals

Confirmation of clinically suspected SRS in children/adults.
Investigation for maternal UPD7 (parental samples required).

Prenatal testing

Due to the low recurrence risk associated with Silver Russell Syndrome prenatal diagnosis is not routinely offered.

Service offered

In the first instance methylation / dosage analysis by MS-MLPA of chromosome 11p15 is carried out in patients referred with possible Silver Russell syndrome. If normal, microsatellite analysis of chromosome 7 is carried out to detect UPD7; samples from both parents are required for microsatellite analysis.

Technical

For diagnostic referrals the initial test involves methylation and dosage analysis in chromosome 11p15 region using methylation specific MLPA analysis. Unaffected individuals will show normal dosage and methylation at both imprinting control regions. Patients with Silver Russell syndrome will show reduced methylation at the H19DMR imprinting control region (ICR1). In patients with normal methylation pattern in the chromosome 11p15 region, further analysis using microsatellite markers for chromosome 7 is recommended to exclude maternal UPD7 as a cause of SRS (parental samples required). Cytogenetic analysis is also helpful in identifying predisposing parental translocations.

Target reporting time

Routine analysis - the initial methylation test takes up to 4 weeks. Microsatellite marker analysis for UPD7 takes 4 weeks from receipt of parental samples. Please contact the laboratory for urgent cases.

Patient details

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

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
2x 10mls venous blood in plastic EDTA bottles*

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

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% (394 tests on 189 pregnancies from 2007 to 2009) 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.

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

Referrals
All referrals should be made via a Clinical Genetics Department, please contact the laboratory in advance of sending a sample. Samples are accepted from patients from 7 weeks gestation (confirmed by scan) at which time there should be a sufficient concentration of free fetal DNA in the circulation.
*From 7 to 9 weeks gestation, 2 x 10ml samples are required, taken one week apart. At 9+ weeks gestation 2 x 10ml samples may be taken at the same time. Samples should be sent to arrive in the laboratory within 24 hours of sampling if possible. Advance notice is required so samples can be processed rapidly upon receipt. Information on the outcomes of the pregnancy will be requested as part of a national ethically approved audit. Information sheets for parents and the audit are available on our laboratory website.

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

Target reporting time
The results of the Y-specific probe analysis should be available within 3 days of the laboratory receiving the second sample.

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 (>1ml 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 the androgen receptor (HUMARA) gene, Xq11-q12 (MIM 313700)

Technical

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

Target reporting time

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

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

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Telephone
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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 (>1ml 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 4 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 (>1ml 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.Arg3527Gln. Mutations causing FH have also been identified in the PCSK9 gene at 1p34.1 that account for a small proportion of cases.

Referrals

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

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

- Patients are separated into two groups, ‘definite FH’ and ‘possible FH’. For a diagnosis of ‘definite FH’ both a) & b) must be present, but for ‘possible’ FH both a) & c) or a) & d) must be observed. Both groups are appropriate for genetic testing.
- 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.Arg3527Gln mutation in APOB and p.Asp374Tyr mutation in PCSK9 in affected patients. Mutation specific testing for previously identified family mutations is available to blood relatives.

Technical
The Fluidigm Dynamic Array for genotyping is used in combination with TaqMan allelic discrimination assays to analyse FH48 panel of mutations associated with FH, with a pick up rate of around 61%. MLPA analysis of the LDLR gene detects around 5% of mutations and is used in combination with FH48 for a first level screen pick-up rate of approximately 66%. Extended mutation screening of the LDLR gene is performed by sequence analysis of the promoter and 18 exons of the LDLR gene, detecting around 34% of mutations.

Target reporting time
4 weeks for first level screen (FH48+MLPA) and 2 months for extended mutation screen in index case. 2 weeks for familial mutation testing.

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

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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

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

Referrals

- 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

Sequencing of the entire coding region of the MYBPC3 (34 exons), MYH7 (exons 3-40), TNNT2 (15 exons) & TNNI3 (8 exons) genes. Mutations in these four genes are estimated to account for up to 80% of disease mutations.
Due to the number of cases reported with more than one mutation (double and compound heterozygotes), we advise that a full gene screen is completed for all probands.

Technical

Screening is carried out by direct sequencing analysis.

Target reporting time

The target reporting time for all four genes is 2 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

Loeys-Dietz syndrome (LDS)

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 (>1ml 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

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

Referrals

- New referrals should fulfil the UKGTN testing criteria.
- Mutation testing can be offered to the relatives of LDS patients once a disease causing mutation has been identified.

Service offered

Mutation screening of all the coding exons of the TGFBR1 and TGFBR2 genes in affected patients. Mutation specific testing for previously identified family mutations is available to other family members.

Technical

Screening is carried out by direct sequencing analysis.

Target reporting time

The target reporting time for both genes is 2 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
NB Include details of most recent lipid profile on request card.

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Achondroplasia (ACH) & Hypochondroplasia (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 (>1ml from neonates)

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

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

A completed DNA request card should accompany all samples

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 and 1/77,000. Around 80-90% of cases are sporadic and there is an association with increased paternal age at the time of conception, suggesting that new mutations are generally of paternal origin. There are rare familial forms, as well as reported cases of germline and somatic mosaicism.

Hypochondroplasia (HCH) 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 70% of HCH patients have one of two mutations in the FGFR3 gene. Of the remaining 30%, some families are reported that do not link to chromosome 4p16.3

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 c.1138G>C) mutations in exon 8 of FGFR3. Together these account for around 99% of mutations.

Hypochondroplasia: Mutation screening of exons 6, 8, 11 and 13 of FGFR3 will detect approximately 75% of reported mutations. Includes testing for the common p.Asn540Lys (c.1620C>A and c.1620C>G) mutations in exon 11 (which account for around 70% of mutations) and the common achondroplasia p.Gly380Arg (c.1138G>A and c.1138G>C) mutations.

Technical

Direct sequence analysis of exons 8 (ACH) and 6, 8, 11 and 13 (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 ACH analysis and 4 weeks for routine HCH 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

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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

Neonates
>1ml 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, a sporadic neonatal lethal skeletal dysplasia, is divided into two subsets based upon radiological findings. TD type I (MIM 187600) is associated with curved femora and variable but milder craniosynostosis and TD type II (MIM 187601) 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) Prenatal testing to confirm a TD diagnosis suspected on antenatal ultrasound scan

Service offered

TD type I - Several recurrent mutations have been identified involving the gain of a cysteine residue, including p.Arg248Cys (55% of cases), p.Tyr373Cys (24%), and p.Ser249Cys (6%). Rarer mutations including p.Lys650Met, p.Gly370Cys, and p.Ser371Cys have also been reported. Mutation of the stop codon accounts for 10% of TD type I patients.

TD type II - A single mutation, p.Lys650Glu, has been identified in all of the TD type II patients reported to date.
Reference: Passos-Bueno *et a*l; Human Mutation 14: 115-125, 1999

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. The common achondroplasia p.Gly380Arg (c.1138G>A and c.1138G>C) mutations will also be detected by this analysis.

Target reporting time
4 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
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

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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

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

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

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

A completed DNA request card should accompany all samples.

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 then mutation screening, 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 then 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 then mutation screening across exon 1 of TWIST and MLPA analysis for TWIST gene deletions
- **Apert Syndrome**: targeted analysis for common mutations: p.Ser252Trp (c.755C>G) and p.Pro253Arg (c.758C>G) in exon 8 of FGFR2

Technical

Direct sequence analysis of the appropriate exons is carried out to detect the common mutations and to screen for unknown mutations in FGFR1, FGFR2, FGFR3 and TWIST. TWIST gene deletions are detected by MLPA dosage analysis.

Target reporting time

Routine analysis: 2 - 4 weeks for targeted mutation testing and 2 months for mutation screen and MLPA 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 associated malformation or developmental delay

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Mitochondrial m.1555A>G Testing

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 (>1ml from neonates)

A completed DNA request card should accompany all samples.

Introduction

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

The homoplasmic mutation m.1555A>G in the mitochondrial MT-RNR1 (12S rRNA) gene has been associated with aminoglycoside-induced and nonsyndromic sensorineural deafness (Estivill X *et al* , Am J Hum Genet 62(1): 27-35, 1998; Prezant TR *et al.,* Nat Genet 4 (3): 289-294, 1993).

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

Referrals

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

Service offered

Mutation analysis for the m.1555A>G mutation.

Technical

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

Target reporting times

2 weeks for routine testing of m.1555A>G mutation in index case. 2 weeks for maternal relatives of patients with the m.1555A>G mutation. 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

Branchio-oto-renal syndrome (BOR)

Contact details

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Introduction

Branchio-oto-renal syndrome (BOR) (MIM 113650) is an autosomal dominant condition that manifests with the following phenotypes:

- Hearing loss
- Preauricular pits ("ear pits")
- Pinnae abnormalities
- Branchial fistulae (lateral fistula of the neck)
- Renal anomalies

BOR has an incidence of approximately 1/40,000, accounts for about 2% of profoundly deaf children, and can be caused by mutations in the EYA1 gene or, more rarely, in SIX1 or SIX5 genes. EYA1 has 16 exons with most mutations identified in exons 8-16. SIX1 and SIX5 have 2 and 3 coding exons respectively.

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.

Samples required

5ml venous blood in plastic EDTA bottles (>1ml 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 testing is available for families in whom specific mutations have been identified - please contact the laboratory to discuss.

Service offered

Level 1 testing involves MLPA analysis of the EYA1 gene to identify larger deletions and duplications and mutation screening of exons 8-16 of the EYA1 gene should MLPA be negative.
Level 2 testing involves mutation screening of exons 1-7 of the EYA1 gene (including alternative exon 1) is available on request.
Patients testing negative for EYA1 mutations can be tested for mutations in the SIX1 and SIX5 genes by sequencing analysis.

Technical

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

Target reporting times

EYA1 testing: MLPA analysis and mutation screening of exons 8-16: 2 months; mutation screening of exons 1-7: 2 months
SIX1 and SIX5 testing: 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

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

Samples required

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

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

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

Referrals

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

Service offered

Mutation screening of connexin 26 coding exon 2. Analysis for the 309kb deletion (GJB6-D13S1830) and 232bk deletion (GJB6-D13S1854), connexin 26 intron 1 splice donor site mutation (c.-23+1G>A) and splice acceptor site mutation (c.1-24A>C). Detection of known mutations in relatives of patients with confirmed connexin 26 mutations.

Technical

Direct sequencing analysis of connexin 26 exon 2 which covers the 681bp coding region and the c.-24A>C splice acceptor site mutation. Size separation assay for the 309kb and 232kb deletions and restriction digest assay for the c.-23+1G>A splice donor site mutation.

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
EAST SYNDROME

Contact details

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Samples required

5ml venous blood in plastic EDTA bottles (>1ml 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
EAST syndrome (OMIM #612780) is an autosomal recessive condition that manifests with the following phenotypes:

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

EAST syndrome is a rare disorder caused by mutations in the KCNJ10 gene (OMIM *602208). KCNJ10 has 1 coding exon.

Referrals
Prospective patients should have a clear clinical phenotype, preferably referred by clinical genetics or specialist renal departments
Asymptomatic (carrier) testing can be offered to relatives of affected patients once 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
Mutation screening of the large coding of the KCNJ10 gene in three overlapping fragments.

Technical
Mutation screening is carried out by direct fluorescent sequencing.

Target reporting times
Mutation screening: 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

Pendred syndrome

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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).

Samples required

5ml venous blood in plastic EDTA bottles (>1ml 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.

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

- Mutation Analysis: Direct sequencing of the 13 exons in which the majority of mutations (approximately 94%) have been detected (exons 2, 3, 4, 6, 8, 9, 10, 11, 12, 13, 16, 17, 19).
- Additional analysis of the remaining 8 *SLC26A4* exons is offered for patients referred with relevant clinical findings in addition to hearing loss or when a single mutation is identified by the initial sequencing.
- Detection of known mutations: In relatives of patients with confirmed *SLC26A4* mutations.

Target reporting time

2 months for mutation screening of an index case.
2 weeks for routine testing of specific mutations (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

Waardenburg Syndrome type 1 and 3 (WS1 & WS3) (PAX3 gene)

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Samples required

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

A completed DNA request card should accompany all samples.

Introduction

Waardenburg syndrome (WS) is an auditory-pigmentary disorder consisting of four clinical subtypes with an annual incidence of 1/270 000 births. WS comprises approximately 3% of congenitally deaf children. WS1 (MIM #193500) and WS3 (MIM #148820) are defined by deafness, depigmentation features and dysmorphology. WS3 individuals also have musculoskeletal abnormalities of the upper limbs.

The Paired Box Gene 3 (PAX3) on chromosome 2q35 is the only gene known to be associated with WS1 and WS3 with point mutations identified in more than 90% of affected individuals. No common mutations are known. Partial and total gene deletions have also been described and may represent 10% of cases without identified point mutations.

WS1 is autosomal dominant with *de novo* or dominant mutations with variable expressivity. In WS3 homozygous and compound heterozygous (severe phenotype) or heterozygous (moderate phenotype) mutations are seen.

Referrals

- Patients with suspected WS1/WS3 for mutation screening and MLPA analysis of PAX3
- Adult relatives of patients with PAX3 mutations for carrier status.

Service offered

Mutation screening of PAX3 gene. Dosage analysis by MLPA. Detection of known mutations in relatives of patients with PAX3 mutations.

Technical

Direct sequencing analysis of PAX3 covering the 5'UTR and all ten exons present in the longest transcript. This analysis also covers the alternatively spliced 3' end of the major isoform.
Large scale deletions detected in PAX3 by MLPA.

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

Carbamoylphosphate synthetase 1 (CPS1) deficiency

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Samples required

5ml venous blood in plastic EDTA bottles (>1ml 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. CPS1 deficiency affects the first enzymatic step in the urea cycle and results in hyperammonemia that can lead to lethargy, vomiting, coma and premature death. The clinical presentation is varied from neonatal onset, where patients have severe hyperammonemia which is fatal in the first few days of life, to a case reported where a woman in her third decade of life collapsed and died after a normal pregnancy and delivery. The onset of CPS1 may also be exacerbated by infection, metabolic stress or excessive protein intake.
The CPS1 gene (2q35) consists of 38 exons.

Referrals

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

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

Prenatal testing

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

Service offered

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

Technical

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

Target reporting time

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)

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Samples required

5ml venous blood in plastic EDTA bottles (>1ml 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. Clinical manifestation in carrier females can range from being asymptomatic to being as severely affected as affected males. Enzyme replacement therapy for Fabry disease is now well established and in wide use.

The gene encoding α-galactosidase A (*GLA*) (Xq22.1) consists of 7 exons and family specific 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 male patients should have their diagnosis confirmed by biochemical analysis; this should be arranged either locally or with the Enzyme Unit, Chemical Pathology, Great Ormond Street Hospital. Biochemically confirmed patients can be referred for mutation analysis.
- Clinically affected female patients can be referred directly for mutation analysis (due to unreliability of heterozygote detection by biochemical testing).
- 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 where specific mutations have been identified - 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. 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

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

Samples required

5ml venous blood in plastic EDTA bottles (>1ml 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 I and III) 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 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

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)del55 and c.115+1G>A.

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

Target reporting time

Routine analysis: 4 weeks. For urgent samples please contact the laboratory.

Contact details for Biochemistry/Enzyme Unit:

Enzyme Unit, Chemical Pathology, Great Ormond Street Hospital, London, WC1N 3JH
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 1a (GSD1a)

Contact details

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Level 6
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London
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+44 (0) 20 7813 8196

Samples required

5ml venous blood in plastic EDTA bottles (>1ml 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 Unit, Chemical Pathology, 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
Direct sequencing of the 5 exons and intron-exon boundaries is carried out. Mutation specific testing for previously identified family mutations is also available in family members by direct sequencing.

Target reporting time
8 weeks 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 Unit:
Enzyme Unit, Chemical Pathology, Great Ormond Street Hospital, London, WC1N 3JH
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)

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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 Unit, Chemical Pathology, Great Ormond Street Hospital. Biochemically 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 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 exons 2-20 by direct sequencing analysis. Mutation specific testing for previously identified family mutations is also available by direct sequencing.

Target reporting time
8 weeks 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 Unit:
Enzyme Unit, Chemical Pathology, Great Ormond Street Hospital, London, WC1N 3JH
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)

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Samples required

5ml venous blood in plastic EDTA bottles (>1ml 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 galactosylceramidase (galactocerebrosidase). Galactosylceramidase (EC 3.2.1.46) is a lysosomal enzyme involved in the catabolism of galactosylceramide, a major lipid in myelin, kidney, and epithelial cells of the small intestine and colon. Enzyme deficiency results in the build-up of undigested fats affecting growth of the nerve's protective myelin sheath and causes severe degeneration of mental and motor skills. The disease may be diagnosed by its characteristic grouping of certain cells (multinucleated globoid cells), nerve demyelination and degeneration, and destruction of brain cells. Special stains for myelin (e.g; luxol fast blue) may be used to aid diagnosis. Definitive testing is by direct enzyme analysis.

Infants with Krabbe disease are normal at birth. Symptoms begin between the ages of 3 and 6 months with irritability, inexplicable crying, fevers, limb stiffness, seizures, feeding difficulties, vomiting, and slowing of mental and motor development. In infants, the disease is generally fatal before age 2. There are also juvenile- and adult-onset cases of Krabbe disease, which have similar symptoms but slower progression and significantly longer lifespan. Although there is no cure for Krabbe disease, bone marrow transplantation has been shown to benefit mild cases early in the course of the disease. The incidence of Krabbe disease is around 1 in 100,000–200,000 births.

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

Referrals

- Clinically affected patients should have their diagnosis confirmed by enzyme analysis; 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. Information regarding ethnic origin is useful.
- 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 the 30kb deletion has been identified or in whom appropriate family studies have been undertaken - please contact the laboratory to discuss.

Service offered
Testing for the common 30kb deletion by three-primer PCR analysis. Other 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 Unit:
Enzyme Unit, Chemical Pathology, Great Ormond Street Hospital, London, WC1N 3JH
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 (>1ml 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.
- Pregnant patients who have AFLP can be referred for carrier testing, along with their partners.
- Carrier testing can be offered to the adult relatives of affected patients once a 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 of the remainder of the gene is not available.

Target reporting time

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

Contact details for Biochemistry/ Enzyme Unit:

Enzyme Unit, Chemical Pathology, Great Ormond Street Hospital, London, WC1N 3JH
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

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 (>1ml 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, 8 weeks for level 2 screen. 2 weeks for routine testing of specific mutations. For urgent samples please contact the laboratory.

Contact details for Biochemistry/ Enzyme Unit:

Enzyme Unit, Chemical Pathology, Great Ormond Street Hospital, London, WC1N 3JH
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 (>1ml from neonates)

A completed DNA request card should accompany all samples.

Contact details for Enzyme Unit undertaking ASA activity measurements:

Enzyme Unit,
Chemical Pathology,
Great Ormond Street Hospital,
London,
WC1N 3JH

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.*96A>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.1788A>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 Enzyme Unit, Great Ormond Street Hospital 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 Unit, Great Ormond Street Hospital.

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
4 weeks for PDASA testing. 4 weeks for routine level 1 screen in index case, 8 weeks 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 1 (MPS1) (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 (>1ml 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
MPS1 (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 alpha-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 c.590-7G>A are generally associated with Scheie syndrome.

Referrals
- Clinically affected patients should have their diagnosis confirmed by biochemical analysis; this should be arranged either locally or with the Enzyme Unit, Great Ormond Street Hospital. Biochemically 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 direct sequence analysis.
- 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 direct sequencing

Target reporting time
4 weeks for routine level 1 screen in index case, 8 weeks for level 2 screen. 2 weeks for routine testing of specific mutations. For urgent samples please contact the laboratory.

Contact details for Biochemistry/ Enzyme Unit:
Enzyme Unit, Chemical Pathology, Great Ormond Street Hospital, London, WC1N 3JH
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 (>1ml 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 of the IDS enzyme; biochemical enzyme analysis utilises these features to confirm a clinical diagnosis.

Hunter syndrome is clinically heterogeneous, but the predominant clinical features include coarse facial features, stiff joints, hepatosplenomegaly, cardiovascular and respiratory disorders, developmental delay and mental retardation. The *IDS* gene consists of 9 exons and family specific 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.

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 including MLPA analysis to detect large deletions and duplications. 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 of 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 biochemically 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. 8 weeks for routine mutation screen and MLPA in index case. 2 weeks for carrier testing using mutation specific tests. For urgent samples please contact the laboratory.

Contact details for Biochemistry/ Enzyme Unit:
Enzyme Unit, Chemical Pathology, Great Ormond Street Hospital, London, WC1N 3JH
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 (>1ml 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 caused by 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 heparan sulphate degradation; biochemical enzyme analysis utilises these features to confirm a clinical diagnosis.

Testing is currently available for types A and B which are due to deficiencies of the enzymes N-sulfoglucosamine sulfohydrolase (SGSH) and alpha-N-acetylglucosaminidase (NAGLU), respectively. There have been several recurrent 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 Unit, 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 direct sequencing.

Target reporting time
8 weeks 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 Unit:
Enzyme Unit, Chemical Pathology, Great Ormond Street Hospital, London, WC1N 3JH
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 (>1ml 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-1 (PPT1) enzyme activity in leucocytes and fibroblasts.

Typical clinical features of INCL are retarded head growth from about 5 months, hyper excitability (including sleep problems), muscular hypotonia and reduced development of fine motor skills between 10-18 months of age. INCL usually progresses with visual loss (by 18 months - 2 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 Unit 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 and p.Leu10X by direct sequencing analysis.
- 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 direct sequencing.

Target reporting time

4 weeks for routine level 1 screen in index case, 8 weeks for level 2 screen. 2 weeks for routine testing of specific mutations. For urgent samples please contact the laboratory.

Contact details for Biochemistry/ Enzyme Unit:

Enzyme Unit, Chemical Pathology, Great Ormond Street Hospital, London, WC1N 3JH
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 (>1ml 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 *TPP1* gene which encodes the lysosomal enzyme tripeptidyl peptidase. 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 to be caused by mutations in the *CLN1*, *CLN5*, *CLN6*, *CLN7* and *CLN8* genes.

Clinical features of LINCL are normal development until the onset of seizures, ataxia and myoclonus between 2 and 4 yrs. LINCL usually progresses with visual loss (by 5-6 yrs), chair bound by 4-6 yrs with poor prognosis.

The differential diagnosis of NCL2 from the other NCL types is based on age of onset, clinical phenotype, ultra structural characterisation of the storage material and TPP1 enzyme levels. A clinical diagnosis of NCL2 is confirmed biochemically by loss of tripeptidylpeptidase I (TPP1) enzyme activity in leucocytes and fibroblasts or accumulation of auto fluorescent lipopigment with a curvilinear profile in neurones and other cell types.

TPP1 (11p15) consists of 13 exons. The two most common mutations are c.509-1G>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 TPP1 enzyme activity in leucocytes and fibroblasts. This should be arranged locally or with the Enzyme Unit, Great Ormond Street Hospital. Biochemically 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
- Level 1 Mutation Analysis: Detection of recurrent mutations c.509-1G>C and p.Arg208X in exon 6 by direct sequencing analysis.
- Level 2 Mutation Analysis: Direct sequencing of all 13 coding exons and intron-exon boundaries.
- Detection of known mutations in relatives of patients with confirmed *TPP1* mutations by direct sequencing.

Target reporting time
2 weeks for routine level 1 screen in index case, 8 weeks for level 2 screen. 2 weeks for routine testing of specific mutations. For urgent samples please contact the laboratory.

Contact details for Biochemistry/ Enzyme Unit:
Enzyme Unit, Chemical Pathology, Great Ormond Street Hospital, London, WC1N 3JH
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 (>1ml 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 ~69% of JNCL alleles (~85% in Finnish population). 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's 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 (>1ml 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's 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*, *CLN6* and *CLN8* genes. Genes will be sequenced sequentially in the order *CLN5*, *CLN8* then *CLN6* reflecting mutation pickup to date. However, a particular gene can be tested on request.

Target reporting time
8 weeks 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 (>1ml 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 MLPA analysis in both males and females. This is then followed by mutation screening of the gene 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 direct sequencing or MLPA.

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

Contact details for Biochemistry/ Enzyme Unit:
Enzyme Unit, Chemical Pathology, Great Ormond Street Hospital, London, WC1N 3JH
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 (>1ml 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 *TCIRG1* gene located at 11q13, consists of 20 exons and encodes an a3 subunit of the vacuolar pump, which mediates acidification of bone/osteoclast interface. 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 direct sequencing.

Target reporting time

8 weeks 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
CFHR5 Nephropathy

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
Affected individuals display persistent microscopic haematuria with episodes of macroscopic haematuria associated with intercurrent infections (commonly of the respiratory tract). Renal biopsy demonstrates C3 glomerulonephritis (C3GN, a type of mesangiocapillary glomerulonephritis (MCGN)). 80% men and 20% affected women develop renal failure by the 8th decade of life.
The incidence of *CFHR5* nephropathy in the Cypriot population is estimated at 1/1000 to 1/8000; prevalence in the UK Caucasian population is low (<1:100,000) since C3GN is a very rare diagnosis.
This autosomal dominant condition is caused by mutation in the Complement Factor H-Related gene 5 (CFHR5; MIM: *608593). The gene is homologous to *Complement Factor H* and *Complement Factor-H Related genes 1-4* which lie at neighbouring loci. *CFHR5* consists of 10 exons which code for 9 homologous short consensus repeat domains, each of which has two internal disulphide bridges. The protein product of *CFHR5* has the ability to co-localise with (and regulate activation of) complement C3 in the kidney.
The most common *CFHR5* gene mutation is a duplication of exons 2 and 3 (c.59-1808_430+3242dup) described in the Cypriot population. No other mutations have been published although a frameshift in exon 4 is present in one non-Cypriot C3GN patient and her healthy mother.

Samples required

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

A completed DNA request card should accompany all samples.

Referrals
Referrals are accepted from Consultant Clinical Geneticists and Consultant Nephrologists in the following patients:

- Cypriot origin with unexplained haematuria or renal disease.
- Patients of any ethnicity may be referred if C3GN or MCGN is present.
- At risk family members where the familial mutation is known.

Prenatal testing
Prenatal testing is not generally indicated for this condition.

Service offered
Detection of exon 2-3 duplication: A single PCR reaction incorporates primers that amplify both a 298bp fragment of the wild type sequence and a 222bp fragment unique to the duplication.
Detection of other mutations: PCR amplification of all 10 exons of CFHR5 followed by direct sequence analysis is in development.

Target reporting time
2 weeks for duplication 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
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 (>1ml 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 57kb 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 Unit, 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 57kb 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).
- Detection of known mutations in relatives of patients with confirmed *CTNS* mutations by direct sequencing or deletion PCR analysis.

Target reporting time
2 weeks for routine level 1 screen in index case, 8 weeks for level 2 screen. 2 weeks for routine testing of specific mutations. For urgent samples please contact the laboratory.

Contact details for Biochemistry/ Enzyme Unit:
Enzyme Unit, Chemical Pathology, Great Ormond Street Hospital, London, WC1N 3JH
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

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 (>1ml 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.

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

Referrals

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

Prenatal testing

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

Service offered

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

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 (>1ml 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 8 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 (available 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 8 exons and exon/intron boundaries of the *NPHS2* gene by direct sequencing.

Target reporting time
8 weeks 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 (>1ml from neonates)

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

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

A completed DNA request card should accompany all samples.

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 *TNFRSF6* 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 *TNFRSF6* 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: Adrian.Thrasher@gosh.nhs.uk

To arrange double-negative α/β T cell analysis please contact:
Dr. Kimberly Gilmour, Molecular Immunology, Great Ormond Street Hospital tel.: +44 (0) 20 7829 8835 email: Kimberly.Gilmour@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 (>1ml from neonates)

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

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

A completed DNA request card should accompany all samples.

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.

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: Kimberly.Gilmour@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
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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 (>1ml from neonates)

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

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

A completed DNA request card should accompany all samples.

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: Kimberly.Gilmour@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 (>1ml from neonates)

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

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

A completed DNA request card should accompany all samples.

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

Netherton Syndrome (NS)

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

5mls venous blood in plastic EDTA bottles (>1ml 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.

Introduction

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

Referrals

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

Prenatal testing

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

Service offered

1) Bi-directional sequence analysis of all 34 coding exons and intron-exon boundaries in SPINK5.
2) The c.2468dup, p.LysGlufsX4 mutation can be detected by fluorescent fragment analysis of the $(A)_{10}$ homopolymer tract in exon 26 of the SPINK5 gene. Two alternative forward primers are used to allow accurate sizing of the repeat region.

Target reporting time

8 weeks for routine sequence analysis of the SPINK5 gene and fragment analysis for the c.2468dup, p.LysGlufsX4 mutation. 2 week turnaround time for testing of a known familial mutation. Please contact the laboratory if urgent or prenatal testing is required.

Patient details

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

NE THAMES REGIONAL MOLECULAR GENETICS SERVICE

Radiation-sensitive severe combined immunodeficiency (RS-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 (>1ml from neonates)

Prenatal diagnosis must be arranged in advance, through a Clinical Genetics department if possible. Amniotic fluid or CV samples should be sent to Cytogenetics for dissection and culture, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis

A completed DNA request card should accompany all samples.

Introduction

Severe combined immunodeficiency (SCID) is a group of genetically and phenotypically heterogeneous disorders that can be immunologically classified by the absence or presence of T, B, and natural killer (NK) cells. The most severe form of SCID has the $T^-B^-NK^+$ phenotype, accounting for ~20% of all cases in which patients present with a virtual absence of both circulating T and B cells, while maintaining a normal level and function of NK cells. This form of SCID is caused by autosomal recessive mutations in at least three primary genes necessary for V(D)J recombination, *RAG1, RAG2,* and *DCLRE1C (ARTEMIS)*. DCLRE1C mutations cause a T and B cell deficient form of SCID that is clinically indistinguishable from a RAG1/RAG2 disorder. Infants present with severe recurrent viral, bacterial or fungal infections and failure-to-thrive. Defects in DCLRE1C can be distinguished from RAG defects because the former has the additional feature of increased sensitivity to ionising radiation in bone marrow and fibroblast cells.

The DNA crosslink repair 1C gene (DCLRE1C; MIM 605988) encodes ARTEMIS which is an essential factor of V(D)J recombination during lymphocyte development and in the repair of DNA double-strand breaks (DSB) by the non-homologous end joining (NHEJ) pathway. Patients with mutations in the DCLRE1C gene, suffer from radiosensitive SCID (RS-SCID; MIM 602450) or radiosensitive Omenn syndrome (MIM 603554).

The DCLRE1C gene (10p13) has 14 exons and mutations have been found throughout the gene. The most frequent mutations reported are gross deletions (59%) due to homologous recombination of the wild-type DCLRE1C gene with a pseudo-DCLRE1C gene located 61.2 kb 5' to the DCLRE1C start codon.

Referrals

- A skin biopsy from clinically affected patients can be sent to the enzyme laboratory who will establish a fibroblast line. These are forwarded to Penny Jeggo at the MRC Sussex for radiation sensitivity testing.
- Carrier testing can be offered to the relatives of RS-SCID or Omenn syndrome 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

- Sequencing analysis is performed of the coding regions and splice sites of exons 1-14 of the DCLRE1C gene.
- MLPA Kit P368-A1 has been optimised for deletion/duplication analysis

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 Molecular Immunology / Enzyme unit:

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

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

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 (>1ml 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: Kimberly.Gilmour@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

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 (>1ml from neonates)

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

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

A completed DNA request card should accompany all samples.

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: Kimberly.Gilmour@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 (>1ml from neonates)

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

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

A completed DNA request card should accompany all samples.

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: Kimberly.Gilmour@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 (>1ml from neonates)

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

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

A completed DNA request card should accompany all samples.

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: Kimberly.Gilmour@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

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Level 6
York House
37 Queen Square
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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 (>1ml from neonates)

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

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

A completed DNA request card should accompany all samples.

Introduction
XLP 1 (MIM 308240) and XLP 2 (MIM 300635) are X-linked immunodeficiencies characterised by extreme sensitivity to the Epstein Barr virus (EBV). Affected individuals can be diagnosed on the basis of an abnormality or deficiency of the SLAM associated protein (SAP) or XIAP protein. The SH2D1A gene (encoding for SAP) has 4 exons and family specific mutations are found throughout the gene. The XIAP gene has 7 exons (6 coding). 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/XIAP protein analysis. This requires prior arrangement and completion of specific request forms (see contact information below). We work closely with this department and are able to undertake 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 or XIAP genes in affected individuals found to have no/abnormal SAP or XIAP expression. Cases found to have SAP or XIAP expression may be screened if there is a strong clinical indication for a diagnosis of XLP. If DNA from an affected male is unavailable screening can be undertaken in the mother. 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 for the SH2D1A gene. 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.
Mutation screening of the 7 exons of the XIAP gene is undertaken by sequence analysis.

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: Kimberly.Gilmour@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

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Telephone
+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 (>1ml from neonates)

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

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

A completed DNA request card should accompany all samples.

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: Kimberly.Gilmour@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