Neurogenetics Unit

National Hospital for Neurology and Neurosurgery & Institute of Neurology

User Manual

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1. NEUROGENETICS UNIT

The Neurogenetics Unit is situated within the Department of Molecular Neuroscience at the Institute of Neurology. Included in the unit is the Neurogenetics Laboratory of the National Hospital for Neurology and Neurosurgery/University College London Hospital which is a UKAS accredited medical laboratory No. 8040, please check the UKAS website here: http://www.ukas.com/search-accredited-organisations/ for our current accredited scope. The Neurogenetics Unit provides a regional, national and international diagnostic service for inherited neurological disorders. Every test accepted by the laboratory is considered a service level agreement.

Laboratory Director
Professor H. Houlden PhD, MRCP (Clinical lead)

Head of Laboratory
Dr James Polke PhD

Quality Manager
Dr Vaneesha Gibbons PhD

Consultant Neurologists
Professor N.W. Wood PhD, FRCP, FMedSci
Professor M.G. Hanna MD, FRCP
Professor M.M. Reilly MD, FRCPI
Professor S. Tabrizi PhD, MRCP
Dr P. Giunti MD, PhD

Clinical Nurse Specialist
Ms L. Redmond RGN MSc

Opening hours 9am-5pm Monday-Friday (excluding bank holidays)
Queries outside these hours can be directed to the laboratory email: ucl-tr.NHNNgenetics@nhs.net
### Service provision/ DNA tests available

Diagnostic, predictive and prenatal genetic tests are carried out as clinically appropriate. Evidence of informed consent is required. Diagnostic tests will be carried out for external referring hospitals when appropriate clinical details and evidence of consent accompany the sample. Predictive and prenatal tests will only be carried out for individuals who have been seen in a genetic clinic either at the NHNN or at a regional clinical genetics centre.

Enquiries concerning genetic testing and clinical queries should be addressed to the laboratory e-mail address above; these will be directed to the appropriate Neurogenetics laboratory team member or clinician. The laboratory referral form is available from the laboratory website.

If you have any comments, suggestions or complaints about the laboratory service please email the laboratory email address, they will be passed to the relevant service leads or Quality Manager. The Neurogenetics laboratory follows UCLH Trust complaints policy. In addition we follow UCLH policy on Information Governance, including the protection of personal information, all of which are available on the UCLH website.

Information on available tests is presented in the table below:

<table>
<thead>
<tr>
<th>Neurogenetics Laboratory Services</th>
<th>Gene(s)</th>
<th>Gene OMIM number(s)</th>
<th>Sample Requirements</th>
<th>Reporting Time (Working Days)</th>
<th>Testing Strategy*</th>
<th>NHS price **</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain Channel NGS Panel (Episodic ataxia: Type 1 and 2, Familial Hemiplegic Migraine and other epilepsy, convulsion and migraine disorders)</td>
<td>ATP1A2 ATP1A3</td>
<td>182340 182350</td>
<td>Blood 5-10ml in EDTA</td>
<td>80 ***</td>
<td>NGS and MLPA for selected genes if referred for episodic ataxia or familial hemiplegic migraine</td>
<td>£900 or £1000 if MLPA performed</td>
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<td>KCNA1 KCNK18</td>
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<td>PRRT2 SCN1A</td>
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<td>SLC1A3 SLC2A1</td>
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<td>Charcot-Marie-Tooth disease type 1 / Intermediate CMT NGS Panel</td>
<td>EGR2 FG4 GDA1 GJB1 LITAF MP2 MTMR2 NDRG1 NEFL PMP22 PRX SBF2 SH3TC2</td>
<td>3239 19125 16873 15968 4283 16841 7225 7450 7679 7739 9118 13797 2135 29427</td>
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<td>80 ***</td>
<td>NGS</td>
<td>£900</td>
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<tr>
<td>Charcot-Marie-Tooth disease type 2 / Intermediate CMT NGS Panel</td>
<td>AARS BSCL2 DNM2</td>
<td>601065 606158 602378 600112 600287 606598 304040 601314 602195 608014 600502 150330 610933 156560 608507 159440 162280 601097 311850 602298 608206 605427 601023 603623</td>
<td>Blood 5-10ml in EDTA</td>
<td>80 ***</td>
<td>NGS and MLPA for MFN2 if a variant is identified by sequencing</td>
<td>£900 or £950 if MLPA performed</td>
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<tr>
<td></td>
<td>GARS GDA1 GJB1</td>
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<td>HINT1 HSPB1</td>
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<td>HSPB8 IGHMBP2</td>
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<td>PMP22 PRPS1</td>
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<td>RAB7A SH3TC2 TRPV4</td>
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<td>Neurogenetics Laboratory Services</td>
<td>Gene(s)</td>
<td>Gene OMIM number(s)</td>
<td>Sample Requirements</td>
<td>Reporting Time (Working Days)</td>
<td>Testing Strategy*</td>
<td>NHS price **</td>
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<td>APP, CHMP2B, CSF1R, DNMT1, FUS, GRN, HTRA1, ITM2B, MAPT, NOTCH3, PRNP, PSEN1, PSEN2, TARDBP, TREM2, TYROBP, VCP</td>
<td>104760, 609512, 164770, 126375, 137070, 138945, 602194, 603904, 157140, 600276, 176640, 104311, 600759, 605078, 605086, 605087, 604142, 601023</td>
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<td>ATP7A, BICT2, BSCL2, DCTN1, DYNC1H1, GARS, HINT1, HSPB1, HSPB3, HSPB8, IGHMBP2, SETX, SLC52A1, SLC52A2, SLC52A3, TRPV4</td>
<td>300011, 609797, 601158, 601143, 600112, 60287, 601314, 602195, 604624, 608014, 600502, 608465, 607863, 607882, 613350, 605427</td>
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<td>ATL1, CCT5, DNMT1, FAM134B, NGF, NTRK1, RAB7A, SCN9A, SPTLC1, SPTLC2, WNK1</td>
<td>606439, 610150, 126375, 613114, 162030, 191315, 600298, 603415, 605783, 607863, 605713, 605232</td>
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<td>ATP1A2, CACNA1A, CACNA1S, CLCN1, KCNJ2, SCN4A, SLC2A1</td>
<td>183340, 601011, 118425, 603967, 114208, 600681, 139140</td>
<td>Blood 5-10ml in EDTA</td>
<td>NGS and MLPA if referred for CLCN1 and KCNJ2</td>
<td>HSS funded for patients from England and Scotland, otherwise £950</td>
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<td>FBX07, LRRK2, PARK2, PARK7, PINK1, SNCA, VPS35</td>
<td>605648, 609007, 602544, 602533, 608309, 163890, 601501</td>
<td>Blood 5-10ml in EDTA</td>
<td>NGS and MLPA for selected genes</td>
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<td>ATXN1, ATXN2, ATXN3, CACNA1A, ATXN7, PPP2R2B, TBP</td>
<td>164400, 183090, 109150, 183086, 164500, 604326, 607136</td>
<td>Blood 5-10ml in EDTA</td>
<td>Single SCA: 20 days Multiple SCAs: 40 days</td>
<td>Fragment sizing analysis</td>
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<td>SCAs 1,2,3,6 and 7: £140 ea. (£600 for all five) SCAs 12 and 17: £225 ea.</td>
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<tr>
<td>Neurogenetics Laboratory Services</td>
<td>Gene(s)</td>
<td>Gene OMIM number(s)</td>
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<td>Reporting Time (Working Days)</td>
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<td>Amyloidosis, Finnish Type (FAF)</td>
<td>GSN</td>
<td>105120</td>
<td>Blood 5-10ml in EDTA</td>
<td>20</td>
<td>Sanger Sequencing</td>
<td>£200</td>
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<td>C9orf72-Related Frontotemporal Dementia and/or Amyotrophic Lateral Sclerosis; (FTD/ALS)</td>
<td>C9orf72</td>
<td>105550</td>
<td>Blood 5-10ml in EDTA</td>
<td>20-40</td>
<td>PCR Only (Sizing and TP-PCR) PCR and Southern Blot</td>
<td>£150 £300</td>
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<td>Dentatorubropallidolusian Atrophy; DRPLA</td>
<td>ATN1</td>
<td>607462</td>
<td>Blood 5-10ml in EDTA</td>
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<td>Fragment sizing analysis</td>
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<td>Dopa Responsive Dystonia; DRD</td>
<td>GCH1</td>
<td>600225</td>
<td>Blood 5-10ml in EDTA</td>
<td>40</td>
<td>Sanger Sequencing and MLPA</td>
<td>£700</td>
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<td>Familial Amyloid polyneuropathy; FAP</td>
<td>TTR</td>
<td>176300</td>
<td>Blood 5-10ml in EDTA</td>
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<td>Sanger Sequencing</td>
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<td>Friedreich’s Ataxia; FRDA</td>
<td>FXN</td>
<td>606829</td>
<td>Blood 5-10ml in EDTA</td>
<td>20</td>
<td>Fragment sizing analysis and TP-PCR</td>
<td>£320</td>
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<td>Hereditary liability to pressure palsies; HNPP</td>
<td>PMP22 del</td>
<td>601097</td>
<td>Blood 5-10ml in EDTA</td>
<td>20</td>
<td>MLPA</td>
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<td>Charcot-Marie-Tooth disease demyelinating type 1A; CMT1A</td>
<td>PMP22 Dup</td>
<td>601097</td>
<td>Blood 5-10ml in EDTA</td>
<td>20</td>
<td>MLPA</td>
<td>£275</td>
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<td>Huntington Disease; HD</td>
<td>HTT</td>
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<td>Blood 5-10ml in EDTA</td>
<td>20</td>
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<td>Huntington Disease-Like 2; HDL2</td>
<td>JPH3</td>
<td>605268</td>
<td>Blood 5-10ml in EDTA</td>
<td>20</td>
<td>Fragment sizing analysis</td>
<td>£300</td>
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<tr>
<td>Leber Optic Atrophy</td>
<td>m.11778G&gt;A m.3460G&gt;A m.14484T&gt;C</td>
<td>535000</td>
<td>Blood 5-10ml in EDTA</td>
<td>20</td>
<td>NGS</td>
<td>£340</td>
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<tr>
<td>Mitochondrial common mutations: m.3243A&gt;G m.8344A&gt;G m.8993T&gt;G/C and large scale rearrangements of the mitochondrial genome</td>
<td>-</td>
<td>540000 545000 551500 516060 530000</td>
<td>Blood 5-10ml in EDTA and/or frozen Muscle</td>
<td>20-40</td>
<td>Fragment sizing (and Southern blotting if required)</td>
<td>HSS funded for patients from England and Scotland, otherwise £800</td>
</tr>
<tr>
<td>Neurogenetics Laboratory Services</td>
<td>Gene(s)</td>
<td>Gene OMIM number(s)</td>
<td>Sample Requirements</td>
<td>Reporting Time (Working Days)</td>
<td>Testing Strategy*</td>
<td>NHS price **</td>
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<tr>
<td>Mitochondrial whole genome sequencing</td>
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<td>Frozen Muscle</td>
<td>40</td>
<td>NGS</td>
<td>HSS funded for patients from England and Scotland, otherwise £340</td>
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<td>Primary Torsion Dystonia (DYT1)</td>
<td>TOR1A</td>
<td>605204</td>
<td>Blood 5-10ml in EDTA</td>
<td>20</td>
<td>Fragment sizing analysis</td>
<td>£150</td>
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<td>X-Linked Bulbospinal Neuronopathy (Kennedy’s Disease/SBMA)</td>
<td>AR</td>
<td>313700</td>
<td>Blood 5-10ml in EDTA</td>
<td>20</td>
<td>Fragment sizing analysis</td>
<td>£230</td>
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<tr>
<td>Autosomal Dominant Ataxia NGS Panel</td>
<td>DNMT1, FGF14, GFAP, ITPR1, KCNC3, KCND3, PDYN, POLG, PRKCG, PRNP, TTBK2</td>
<td>126375, 601515, 137780, 147265, 176264, 605411, 131340, 174763, 176980, 76640, 611695</td>
<td>Blood 5-10ml in EDTA</td>
<td>In development</td>
<td>NGS</td>
<td>In development</td>
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<td>Amyotrophic Lateral Sclerosis/Motor Neurone Disease (ALS/MND) NGS Panel</td>
<td>ALS2, ANG, DCTN1, FIG4, FUS, OPTN, SLC52A1, SLC52A2, SLC52A3, SOD1, TARDBP, UBQLN2, VAPB, VCP</td>
<td>606352, 105850, 601143, 609390, 137070, 162230, 602432, 607882, 613350, 147450, 605076, 300264, 605704, 601023</td>
<td>Blood 5-10ml in EDTA</td>
<td>In development</td>
<td>NGS</td>
<td>In development</td>
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<td>Autosomal Recessive Ataxia NGS Panel</td>
<td>ADCK3, ANO10, APTX, ATM, FXN, GJC2, PNPLA6, POLR3A, SACS, SETX</td>
<td>606980, 613726, 606350, 607585, 606829, 608803, 603197, 614258, 604490, 608465</td>
<td>Blood 5-10ml in EDTA</td>
<td>In development</td>
<td>NGS</td>
<td>In development</td>
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<td>Neurogenetics Laboratory Services</td>
<td>Gene(s)</td>
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<td>NHS price **</td>
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<td>Complex Parkinson’s Disease / Dystonia NGS Panel</td>
<td>ANO3, ATP13A2, ATP1A3, C19orf12, DCTN1, FBX07, GCH1, GNAO1, PANK2, PLA2G6, SGCE, SLC6A3, SPR, TAF1, TH, THAP1, TOR1A, TUBB4A, WDR45</td>
<td>610110, 610513, 614297, 601143, 605648, 600225, 139312, 606157, 603604, 604149, 126455, 182125, 313550, 191290, 609520, 605204, 602662, 300526</td>
<td>Blood 5-10ml in EDTA</td>
<td>In development</td>
<td>NGS</td>
<td>In development</td>
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<tr>
<td>Hereditary Spastic Paraplegia (HSP) NGS Panel</td>
<td>CYP7B1, FA2H, GJC2, KIAA0196, KIF5A, NIPA1, PLP1, PNPLA6, REEP1, RTN2, SPAST, SPG7</td>
<td>603711, 611026, 608803, 610657, 602821, 608145, 300401, 603197, 609139, 603183, 604277, 602783</td>
<td>Blood 5-10ml in EDTA</td>
<td>In development</td>
<td>NGS</td>
<td>In development</td>
</tr>
<tr>
<td>Mitochondrial Depletion/Progressive External Ophthalmoplegia (PEO) NGS Panel</td>
<td>C10orf2, DGUOK, MFN2, MPP17, OPA1, POLG, POLG2, RHOD, SLC25A4, SUCLA2, SUCLG1, TK2, TTYMP</td>
<td>606075, 601465, 608507, 137960, 605290, 174763, 604983, 604712, 103220, 603921, 611224, 188250, 131222</td>
<td>Blood 5-10ml in EDTA</td>
<td>In development</td>
<td>NGS</td>
<td>In development (HSS funded)</td>
</tr>
</tbody>
</table>

Family Mutation 20 **
Prenatal 3 **
Urgent 10 **

*Unless otherwise stated Sanger and Next Generation Sequencing (NGS) is of all coding exons and intron/exon boundaries and dosage analysis is by MLPA of all coding exons of targeted genes.
** All prices quoted are for NHS routine postnatal diagnostic tests, please contact the laboratory for price details on all other types of test. Proof of payment must be supplied for Private referrals before testing can proceed. If not provided, DNA will be extracted and stored ONLY.
***Please note that at present we have a backlog of samples waiting for NGS panel testing and the turnaround time for these tests is between 6 and 9 months. If a test is urgently required please contact the laboratory and the analysis of the sample will be expedited.
**** Additional NGS panels may be analysed for an additional £350, please contact the laboratory if this is required.
Please note that single-gene Sanger sequencing and MLPA dosage analysis is still available for a number of the genes that are now tested as part of an NGS panel. Please contact the laboratory to enquire about particular genes and associated prices.
The laboratory is also willing to confirm research results for other disorders in individual cases and testing will then be available for other family members. For availability of other tests in UK genetic labs please see http://ukgttn.nhs.uk/

Service developments:
DNA from individuals with disorders for which tests are not currently available may be sent for storage, pending future analysis. Details of current developments are available from the laboratory.

Research testing:
A number of genes are tested on a research basis on genes that are not available in the UK and/or in newly identified genes. Details of these research tests are available from ucl-tr.NHNNgenetics@nhs.net

Sample requirements:
For genetic analysis, we request at least two mls blood (2 x 5ml tubes preferred), in PLASTIC EDTA tubes. The Association for Clinical Genetic Science (ACGS) guidelines recommends at least 2 pieces of identifying information on every sample tube:
- Patient's full name (surname/family name and given/individual name)
- Date of birth and/or unique hospital/NHS number

Samples from wards and clinics at the NHNN should be accompanied by an analysis request form; clinical details, family history and where possible a detailed pedigree should be supplied on this form to aid interpretation of genetic examination results, along with other information indicated. Our referral form can be downloaded from the laboratory website. Blood and DNA samples from outside hospitals can be sent by first class mail and packed according to UN packing requirement PI 650 and clearly labelled 'diagnostic specimen UN3373'.

Blood samples should be forwarded as soon as possible after being taken. If blood samples are not sent immediately they should be stored in the fridge. For DNA samples sent to the laboratory, 1-3 micrograms of DNA is required. DNA should not be degraded. Please include DNA concentration and method of extraction on the referral form. Tissue samples e.g. muscle should be sent frozen, on dry ice, by courier. Please advise the laboratory of the arrival of these samples in advance. Samples for prenatal diagnosis are only accepted by prior arrangement. DNA is also extracted from urine and brain for certain tests on prior arrangement only. Saliva samples are not extracted in the laboratory and should be sent only with prior arrangement in appropriate collection containers.

Please note that any patient that has received a stem cell transplant for a haematological malignancy such as leukaemia is unlikely to be suitable for genetic testing from a blood sample. Genetic analysis of other tissues (such as skin biopsy) may be suitable. Please contact the laboratory to discuss if required.

Adherence to the sample requirements stated above is essential to maintain the performance of testing methodologies used.

Consent:
Consent is required for all genetic tests. Our referral forms include the following consent statement that must be signed by the referring clinician: It is the referring clinician’s responsibility to ensure that the patient/carer knows the purpose of the test and that the sample may be stored for future testing related to specific diagnosis for the patient. In signing this form the clinician confirms that they have obtained consent for testing and storage. The patient should be advised that the sample may be used anonymously for quality assurance, research and training purposes. Please advise us of any restrictions. This laboratory follows the recommendations laid down by the Joint Committee on Medical Genetics guidance document “Consent and Confidentiality in Genetic Practice September 2011”.

For samples originating within the NHNN, clear indication of consultant approval and patient consent for diagnostic testing and/or research purposes must be completed on the request form. Samples referred from other laboratories must be sent with written consent or a clear indication that consent has been obtained. Samples received into the laboratory without this information will be stored without analysis until consent is obtained. Samples for Predictive Testing are only accepted from recognised genetic service providers.
External referrals:

Samples may be referred to external laboratories for analyses not provided in-house; names and addresses of receiving laboratories can be obtained from the Neurogenetics laboratory. Report times are determined by the receiving laboratory.

Expert opinion

Many of the neurogenetic conditions that are tested for at the NHNN are very rare. Often there are overlapping phenotypes. If referring laboratories or physicians require an expert opinion, the following consultants can be contacted: Prof Wood (Parkinson’s disease and general neurogenetics), Prof Reilly (inherited neuropathies), Prof Hanna (Muscle disorders), Prof Tabrizi (Huntington’s disease), Dr Giunti (ataxia), Prof Houlden (ataxia and general neurogenetics). Please email the laboratory (ucl-tr.NHNNgenetics@nhs.net) and we will pass on the request to the relevant Consultant.

2. GENETIC TESTING FOR PARKINSON’S DISEASE (PD) AND PARKINSONISM

7 gene panel:

Entire coding region of FBXO7, LRRK2, PARK2, PARK7, PINK1, SNCA and VPS35 analysed using Next Generation Sequencing (NGS) technology and copy number analysis for exonic rearrangements in PARK2, PINK1 and SNCA plus exons 3, 5, 7 and a probe in the 5’UTR of PARK7.

Single gene tests are also available:

- **LRRK2**: common mutation c.6055G>A p.(Gly2019Ser)
- **PARK2**: copy number analysis of the coding region
- **SNCA**: copy number analysis and common mutation c.88G>C p.(Ala30Pro)

Parkinson's disease (PD) is a common disorder that is defined by rigidity, tremor and usually a good response to L-dopa. There are also a number of patients that have parkinsonism associated with dystonia, rigidity, ataxia and cognitive decline.

In PD it is now clear that a significant sub-group of patients have a family history and are highly likely to have a primary mutation inherited in a Mendelian fashion. Generally, individuals with onset before age 20 years are considered to have juvenile-onset Parkinson disease, those with onset before age 50 years are classified as having early-onset Parkinson disease, and those with onset after age 50 years are considered to have late-onset Parkinson disease. Currently there are 7 principle genes associated with inherited PD. Parkinson’s disease caused by three of these, SNCA, LRRK2 and VPS35 is inherited in an autosomal dominant fashion. PD caused by the remaining four, FBXO7, PARK2 (parkin) PARK7 (DJ1) and PINK1 is inherited in an autosomal recessive manner. It has now been shown that mutations in LRRK2 are associated with reduced penetrance, thereby gene carriers may be unaffected and a clear autosomal dominant family history may not be obvious.

There is also accumulating but unproven evidence that single hit mutations in the recessive genes may contribute to an individual’s parkinsonian syndrome. Therefore, giving guidance on gene testing is complicated.

It is generally too early to give precise estimates of the relative burdens of these mutations. However a few facts can be stated. **Please note that the sequencing of the entire coding region by NGS is carried out for all genes listed below any further analysis offered is noted next to the relevant gene:**

- **FBXO7** (PARK15). This is a rare autosomal recessive condition.
- **LRRK2** (PARK8). This is numerically the most important autosomal dominant gene known and a single mutation (G2019S) has been shown to be found commonly in both familial and sporadic disease. Analysis of the common mutation Gly2019Ser only is also available.
- **PARK7** (DJ1). This is a rare autosomal recessive condition.
- **PINK1 (PARK6).** Mutations in PINK1 appear to be more common than DJ1 but are still relatively rare. Dosage analysis to detect whole exon rearrangements (deletions and duplications) is available.

- **PARK2 (parkin).** Numerically, worldwide mutations in the parkin gene have been found most frequently of all the Parkinson’s genes. The range of mutations is wide and includes missense, nonsense and a number of gene rearrangements. Dosage analysis for rearrangements of all exons in this gene is available. It should be noted that most patients in outbred populations such as the UK are compound heterozygotes.

- **SNCA (PARK1, Alpha- synuclein).** Mutations in SNCA are rare and have been found to be associated with autosomal dominant Parkinson disease (PARK1 and 4). Dosage analysis to detect whole exon rearrangements (deletions and duplications) is available.

- **VPS35 (PARK17).** Mutations in this gene have been found to be associated with autosomal dominant Parkinson disease.

**PARKINSONISM**
Comlicated parkinsonism such as MSA and PSP generally do not appear to have a high recurrence risk. However, in the case of familial or unusual syndromes, which may include earlier age of onset, consideration to sequence the MAPT gene is suggested. In MSA approximately 10% of patients have a spinocerebellar ataxia (SCA) expansion.

**DOPA RESPONSIVE DYSTONIA**
This may sometimes present as a young onset parkinsonian syndrome with or without dystonia. Patients are very responsive to L-dopa and the disease gene that should be tested in most instances is the GCH1 gene. We perform Sanger sequencing and dosage analysis combined as a first-line test.

**SPINOCEREBELLAR ATAXIA PRESENTING with PD**
Some of the complicated dominant ataxias, particularly SCAs 1, 2 and 3 may present with or have an extra-pyramidal syndrome that includes parkinsonism. In selected cases, such as those with a family history, or parkinsonism complicated by ataxia, the SCA gene expansions (SCA1, 2, 3) should be tested.

**OTHERS**
There are a number of other rare neurodegenerative disease which may present with parkinsonian features, these include **PANK2, PLA2G6, FBX07, Wilson's disease, ATP13A2 and NFL.**

An NGS panel is in development at the Neurogenetics Laboratory to screen a number of these genes including the entire coding region of: **ANO3, ATP13A2, ATP1A3, C19orf12, DCTN1, GCH1, GNAL, PANK2, PLA2G6, SGCE, SLC6A3, SPR, TAF1, TH, THAP1, TOR1A, TUBB4A and WDR45.** Please contact the laboratory for further details.

### 3. GENETIC TESTS IN INHERITED ATAXIAS

Genes tested:
Autosomal recessive ataxia – **FXN** (Friedreich's ataxia)
Autosomal dominant ataxia – **ATXN1** (SCA1), **ATXN2** (SCA2), **ATXN3** (SCA3), **CACNA1A** (SCA6), **ATXN7** (SCA7), **PPP2R2B** (SCA12), **TBP** (SCA17), **ATN1** (DRPLA)

There are a large number of genetic causes of ataxia. Generally ataxia with an onset below the age of 20 years is very likely to be autosomal recessive. Onset over the age of 20 is very likely to be autosomal dominant. Mitochondrial DNA mutations may also give rise to ataxia, but again this is usually a complicated picture, including such features as pigmentary retinopathy and epilepsy. These are discussed under the section on mitochondrial diseases.

**Autosomal recessive ataxias**
The most common of these is Friedreich's ataxia with a prevalence of 2:100,000-4:100,000. This has a distinctive clinical phenotype encompassing progressive gait and limb ataxia, pyramidal signs and a neuropathy predominantly of sensory type. The analysis looks for the presence of an expansion in intron 1 of the **FXN** gene. The vast majority of patients have an abnormal expansion on both alleles but approximately 2-4% have a single expansion and a point mutation. Therefore, if no expansion is found, this effectively excludes the diagnosis of Friedreich's ataxia. However if a single expansion is found, this has 2 possible explanations: that it is the cause of the patient’s ataxia and the second
allele carries a point mutation (detectable by sending the sample to an outside laboratory) or it is not the cause of the patient’s ataxia and the patient is a carrier of an expansion. The carrier frequency is 1:60-1:100.

There are a number of other rare ataxic syndromes including ataxia telangiectasia, ataxia associated with ocular motor apraxia (AOA1 & 2) and other rare causes such as hexosaminidase A deficiency, tremor ataxia syndrome (FXTAS) associated with presence of the permutation of the fragile X gene FMR1 and Niemann Pick type C can present with an early onset complicated ataxia picture. Vertical gaze abnormalities are usually present. Although rare, perhaps the most important ataxia not to be missed in this group is an ataxia associated with Vitamin E deficiency. This is due to mutations in the alpha tocopherol transporting protein (TTPA). The diagnosis is readily reached by measuring Vitamin E. Although rare, it is potentially treatable or at least modifiable by Vitamin E supplementation and therefore should be considered in appropriate cases.

An NGS panel is in development in the Neurogenetics Laboratory to sequence a number of recessive ataxia genes, including the entire coding region of: ADCK3 (SCAR 9), AN010 (SCAR10), APTX (AOA1), ATM, FXN, GJC2, PNPLA6, SACS (ARSACS) and SETX (AOA2). Please contact the laboratory for further details.

Autosomal dominant ataxias
Slightly confusingly there are 2 classification systems which can be viewed in parallel. The clinical classification based on Anita Harding’s work in the early 1980s, describes three sub-groups of autosomal dominant cerebellar ataxia (ADCA). Type 1 was complicated by a variety of additional features including some but not necessarily all of the following: neuropathy, dementia, extrapyramidal features and optic atrophy. Type 2 was complicated by a maculopathy and Type 3 was relatively pure.

The genetic classification for the Spinocerebellar Ataxias (SCAs) is based on the order in which the chromosomal location of the causative gene was identified. At the time of writing, the number of SCAs is over 30. A gene has not been identified for all of these and even when the gene has been identified, not all are available as a diagnostic service.

Routine diagnostic services at NHNN involves genes for SCAs 1,2,3,6,7,12 and 17. SCA7 counts for virtually all the cases of the Type 2 form of ADCA, namely that associated with a maculopathy. This is the rarest form of the Spinocerebellar ataxias tested for in the laboratory. SCAs 1, 2 and 3 account for approximately 50% of ADCA Type 1 and SCA6 accounts for approximately 50% of the pure type. SCA6 is generally speaking a later onset disease and more slowly progressive than the others. The currently available list of SCA tests will identify the mutation in approximately 50% of cases of ataxia with a dominant family history. SCA12 is associated with prominent tremor and SCA17 may present with a complicated ataxia with chorea and can even look a bit like Huntington’s disease.

An NGS panel is in development in the Neurogenetics Laboratory to sequence a number of dominant ataxia genes, including the entire coding region of: DNMT1, GFG14 (SCA27), GFAP, ITPR1 (SCA15, SCA29), KCNC3 (SCA13), KCND3 (SCA19/22), PDYN (SCA23), POLG, PRKCG (SCA14), PRNP and TTBK2 (SCA11)

4. GENETIC TESTING FOR HUNTINGTON’S DISEASE AND CHOREIFORM DISORDERS

Genes tested:
Huntington’s Disease: HTT
Huntington’s Disease like disease: ATN1 (DRPLA), TBP (SCA17), JPH3 (Junctophilin 3 gene), ATXN1,2,3, (SCA1, 2, 3), C9orf72

Huntington’s Disease
Huntington Disease (autosomal dominant inheritance) is the most common cause of inherited chorea in the UK, therefore with a choreiform patient where the cause is clearly not acquired this should be the primary genetic test requested. There is not always a positive family history although this should always be asked for. The implications for other family members of a positive diagnostic test for HD should also always be discussed. Please contact the laboratory for advice or questions regarding this.

Clinical features of HD
Common motor abnormalities such as chorea are seen in 90% of adult onset cases of HD. Also think of Huntington Disease if patients present with a combination of dystonia, parkinsonism and bradykinesia, as HD may present with an akinetic rigid form particularly in young adults or juvenile cases. In a young person (<20y) presenting with a parkinsonian syndrome, juvenile Huntington Disease should be tested for. Patients with Huntington Disease commonly have oculomotor disturbance characterised by delayed initiation of saccades, slowing saccades and head thrusting to initiate saccades. Pursuit is impaired and there may be evidence of gaze persistence. Patients have impairment of voluntary motor function, dysarthria, dysphagia. Cognitive and psychiatric problems are common. HD may also present with multiple tics, predominant psychiatric symptoms or predominant cognitive symptoms in a frontal/subcortical pattern.

**Dentatorubral-pallidoluysian atrophy (DRPLA)**

DRPLA (autosomal dominant) is rare in Europe and the United States. Age of onset is usually below 30 years and it tends to present with a progressive chorea, ataxia and myoclonic epilepsy with more marked dementia than is seen in patients with Huntington Disease.

**Other inherited disorders that can present with chorea or look like HD**

A number of diseases can present with clinical features very similar to Huntington Disease and these may be tested for in the Neurogenetics laboratory.

- Inherited prion diseases which may be tested for by analysing the open reading frame of the prion gene. See the section on dementia genetics.
- Spinocerebellar ataxia (SCA) types 1, 2, 3 and 17 may all present like Huntington Disease.
- HDL-2 (due to mutations in the Juxtapollin 3 gene) although this has only been described in people of North African origin to date.
- Friedreich's ataxia (autosomal recessive due to intronic GAA repeat mutation in the FXN gene).
- C9orf72 (expansion of a hexanucleotide repeat (GGGGGCG) located between the noncoding exons 1a and 1b)

Other causes of inherited chorea that may present with features similar to Huntington Disease, but with additional neurological symptoms. Macleod syndrome which is X-linked with mutations in the XK gene (these patients typically have acanthocytes on peripheral blood film, a high CK and their red blood cells have weak expression of Keil system antigens due to no expression of XK protein) and benign hereditary chorea, the genes are known for these diseases, but are not currently testable on a service basis in the UK. Mitochondrial disorders may mimic many neurodegenerative disorders, and these can be tested via the Neurogenetics laboratory.

**Other inherited choreiform disorders**

Other diseases that may present with chorea with additional extrapyramidal features that can be tested for in the UK are Wilson's disease (autosomal recessive due to mutations in a copper transport ATPase, ATP7B), Neuro-degeneration with brain iron accumulation, autosomal recessive due to PANK2, PLA2G6 or ATP13A2 genes. Ataxia Telangectasia (autosomal recessive disorder due to mutations in the ATM gene), and Neuroferritinopathy (autosomal dominant due to mutations in the ferritin light chain).

### 5. GENETIC TESTS IN DYSTONIA

**Single gene tests:**

- Autosomal dominant or sporadic dystonia (DYT1): **TOR1A** (common GAG deletion)
- Dopa responsive dystonia (DRD): **GCH1** Sanger sequencing of the entire coding region plus exonic copy number analysis by MLPA.

Dystonias are a heterogeneous group of disorders which are known to have a strong inherited basis. Genetic testing is available for DYT1 (primary torsion dystonia) or DRD (dopa responsive dystonia).

There is a 19 gene NGS panel of 'Complex Parkinson's Disease and Dystonia' genes in development at the Neurogenetics Laboratory, including analysis of the entire coding region of: **ANO3, ATP13A2, ATP1A3, C19orf12, DCTN1, GCH1, GNL1, PANK2, PLA2G6, SGCE, SLC6A3, SPR, TAF1, TH, THAP1, TOR1A, TUBB4A** and **WDGR5** and dosage analysis for all the coding exons of **GCH1 and SGCE** plus exons 1, 3, 4, 8, 12 and 14 of **TH**. Please contact the laboratory for further information.
DYT1 associated dystonia:
Mutations in the DYT1 gene have been associated with autosomal dominant early onset idiopathic torsion dystonia. This typically develops first in the arm or leg in middle to late childhood and progresses to generalised dystonia within about 5 years. There is marked clinical variability in DYT1-associated dystonia ranging from children who are profoundly disabled, to adults who carry this autosomal dominantly inherited gene and have no symptoms. This indicates that the gene is transmitted with a reduced penetrance of around 20-30%. DYT1 is caused by a GAG deletion in the gene TOR1A encoding torsin A. This can be tested for in patients with the appropriate dystonic phenotype in the Neurogenetics laboratory without discussion.

Dopa Responsive Dystonia:
Dopa Responsive Dystonia (DRD) is characterised by dystonia, concurrent or subsequent parkinsonism, diurnal worsening of symptoms in 75% of cases and a dramatic therapeutic response to Levodopa. Women are affected 2-4 times more frequently than men. Dystonia is the most common presentation and may be the only feature, however, in childhood DRD may present with a phenotype resembling spastic paraparesis, whereas in adulthood it can present with a phenotype similar to spastic paraparesis or a parkinsonian syndrome.

In view of the wide spectrum of symptoms and age of onset, the diagnosis of DRD may be missed and it is widely recommended that all patients with an extrapyramidal motor disorder or individual symptoms within the spectrum of DRD be given a trial of Levodopa. DRD is usually inherited as an autosomal dominant trait with incomplete penetrance. It is due to mutations in the gene encoding GTP-cyclohydrolase 1 (GCH1).

There are many different mutations that are found in GCH1; these can be tested for in the Neurogenetics laboratory. However, before sending off for testing for the DRD gene, it is recommended that the patient has been given and had a good response to Levodopa; a phenylalanine loading test, and CSF examination which may reveal low concentrations of dopamine and serotonin metabolites, neopterin, biopterin and GTP-CH1 activity, all of which can be tested for in the neuro-metabolic laboratory in the NHNN. We perform Sanger sequencing and dosage analysis combined as a first-line test.

Other dystonic syndromes:
There are currently 20 loci associated with inherited dystonia. An important one to be aware of is the myoclonus/dystonia syndrome which is characterised by a myoclonic/dystonic syndrome which is alcohol-responsive in some patients. This is autosomal dominant with incomplete penetrance and caused by mutations in the gene encoding epsilon-sarcoglycan (SGCE). Dystonia is also associated with many other syndromes, mainly the neuro-degenerative dystonic syndromes, these are covered under the sections encompassing choreiform disorders and parkinsonian disorders.

Dystonia Genes included on the 19 gene NGS panel (in development):

- **ANO3** (DYT23) encodes anoctamin-3, a transmembrane protein. Mutations in this gene have been associated with a familial form of cranio-cervical dystonia. The inheritance is Autosomal Dominant.

- **ATP1A3** (DYT12) encodes the alpha-3 catalytic subunit isoform expressed in the nervous system of the Na,K-ATPas.. Inheritance is Autosomal Dominant.

- **GCH1** (DYT5) as outlined above.

- **SGCE** (DYT11) encodes the epsilon member of the sarcoglycan family. Inheritance is Autosomal Dominant. **SGCE** is imprinted, with preferential expression from the paternal allele.

- **SPR** encodes an aldo-keto reductase that catalyzes the NADPH-dependent reduction of pteridine derivatives and is important in the biosynthesis of tetrahydrobiopterin (BH4). Mutations in this gene result in DOPA-responsive dystonia due to sepiapterin reductase deficiency. Inheritance is Autosomal Recessive.

- **TH** (DYT14) encodes a protein that is the rate-limiting enzyme in the synthesis of catecholamines and plays a key role in the physiology of adrenergic neurons. Mutations in this gene have been associated with autosomal recessive Segawa syndrome.
• **THAP1 (DYT6)** encodes a THAP domain-containing protein that is considered to be involved in endothelial cell proliferation and proapoptotic processes, and assumed to act as a transcription factor. Inheritance is Autosomal Dominant.

• **TAF1 (DYT3)** encodes the largest subunit of TFIID. Mutations in this gene are associated with X-linked dystonia-parkinsonism.

• **TUBB4 (DYT4)** a member of the tubulin family of proteins. A defect in exon 1 of this gene has been associated with hereditary whispering dysphonia. Inheritance is Autosomal Dominant.

### 6. GENETIC TESTS IN INHERITED NEUROPATHIES AND CHARCOT MARIE TOOTH DISEASE (CMT)

The hereditary neuropathies are a clinically and genetically heterogeneous group of disorders. Although there are many genetic diseases in which the peripheral nervous system is involved, by far the most common of these is Charcot-Marie-Tooth disease (CMT), which affects 1 in 2,500 of the population. This disease is characterised clinically by distal wasting and weakness, distal sensory loss, hyporeflexia and a variable amount of foot deformity. The simplest classification system is neurophysiological and divides CMT into type 1 (median motor conduction velocity (MCV) < 38 m/s) and type 2 (median MCV > 38 m/s) although an intermediate form (median MCV 25 – 45 m/s) is increasingly recognised.

The chromosome 17p11.2 duplication accounts for the vast majority of CMT type 1 or CMT of unknown type and should be the first test requested for CMT type 1. Subsequent to this a number of diagnostic genes can be tested for.

#### NGS Panels:

**CMT1** 14 genes:
- NGS analysis of the entire coding region of **EGR2, FGD4, FIG4, GDAP1, GJB1** plus promoter region, **LITAF, MPZ, MTMR2, NDRG1, NEFL, PMP22, PRX, SBF2** and **SH3TC2**.

**CMT2/intermediate** 22 genes:
- NGS analysis of the entire coding region of **AARS, BSCL2, DNM2, DYNC1H1, GARS, GDAP1, GJB1** plus promoter region, **HINT1, HSPB1, HSPB8, IGHMBP2, LMNA, LRSAM1, MARS, MFN2, MPZ, NEFL, PMP22, PRPS1, RAB7A, TRPV4, VCP and YARS**.

**dHMN** 16 genes:
- NGS analysis of the entire coding region of **ATP7A, BICD2, BSCL2, DCTN1, DYNC1H1, GARS, HINT1, HSPB1, HSPB3, HSPB8, IGHMBP2, SETX, SLC52A1, SLC52A2, SLC52A3** and **TRPV4**.

**HSN** 10 genes:
- NGS analysis of the entire coding region of **ATL1, CCT5, FAM134B, NGF, NTRK1, RAB7A, SCN9A, SPTLC1, SPTLC2** and **WNK1**.

**Single gene tests:**
- Familial Amyloid Polyneuropathy (FAP): **TTR**
- Autosomal Dominant CMT type 1A: Chromosome 17p11.2 duplication
- Hereditary Neuropathy with Liability to Pressure Palsies (HNPP): Chromosome 17p11.2 deletion, **PMP22** sequencing

Single-gene Sanger sequencing is available for some neuropathy genes that are now tested as part of an NGS panel please contact the laboratory to discuss Sanger sequencing and for further information.

### 7. GENETIC TESTS IN MUSCLE ION CHANNEL DISEASE

**Phenotypes and associated genes:**

- Hypokalaemic periodic paralysis: **ATP1A2**

- Periodic paralysis: **SCN4A, CACNA1S**
Periodic paralysis, Cardiac arrhythmia, Dysmorphism: KCNJ2.

Periodic paralysis with ataxia: CACNA1A

Periodic paralysis with dyskinesia: SCL2A1

Myotonia congenita: CLCN1

Paramyotonia Congenita: SCN4A

Thyrotoxic Hypokalaemic Periodic Paralysis: KCNJ18 (Only available on a research basis at present, please contact the laboratory for further information).

Tests available

7 gene muscle channel panel:
NGS analysis of the entire coding region of ATP1A2, CACNA1S, CLCN1, KCNJ2 and SCN4A plus dosage analysis for the coding exons of CLCN1 and KCNJ2.

NGS analysis of the entire mt genome.

All muscle channelopathy genes are routinely tested as part of the NGS panel but single-gene and targeted Sanger sequencing may be available on request: please contact the laboratory for further information

A 13-gene panel for mt nuclear depletion/PEO/mitochondrial maintenance genes is also in development in the laboratory, including sequencing of the entire coding regions of: C10orf2, DGUOK, MFN2, MPV17, OPA1, POLG, POLG2, RRM2B, SLC25A4, SUCLA2, SUCLG1, TK2 and TYMP. Please contact the laboratory for further information.

Approximately 50% of patients with muscle disease have a genetic cause. At the first consultation with a patient with suspected genetic muscle disease it should always be possible to devise an efficient sequence of investigations that will usually lead to an accurate diagnosis. In those muscle diseases where a genetic test should be the first test selected, it is usually not necessary to simultaneously arrange an EMG and muscle biopsy. It is generally better to await the genetic result, since if it is positive, EMG and muscle biopsy may be avoided. An important principle when assessing any patient with muscle disease is always to consider whether it is possible that the patient may have an inflammatory muscle disease. If there is a realistic possibility of such a disease early muscle biopsy is very important.

Myotonia and Myotonic Dystrophy

Myotonic dystrophy is the commonest form of muscle dystrophy seen in the adult population. It is a dominant multisystem disease affecting skeletal muscle, cardiac muscle, brain and the endocrine system. Distal muscle weakness, myotonia, cataracts and a myopathic facies usually raise suspicion of the diagnosis in typical cases. However, atypical presentations with cognitive impairment, weight loss and cardiac arrhythmias are recognised. Genetic anticipation is a prominent feature in many myotonic dystrophy families. If the diagnosis is suspected the first test is a genetic test for DM1 (DMPK). If this is negative then the DM2 gene (CNBP) should be tested. If there is myotonia then a further differential diagnosis is the myotonia and paramyotonia congenitias as discussed below.

Skeletal Muscle Channelopathies

The muscle clinic at NHNN is the UK national referral centre for all patients suspected of having a skeletal muscle channelopathy. This includes clinical assessment, DNA diagnosis, molecular expression proof of pathogenicity for new mutations, and genotype specific treatment selection and supervision. As discussed above, genetic tests have been developed over recent years and in the main now represent the first test to select in patients suspected of having one of the main skeletal muscle channelopathies namely-

- Periodic paralysis
- Paramyotonia congenita
- Myotonia congenita

8. GENETIC TESTS IN MITOCHONDRIAL RESPIRATORY CHAIN DISEASE
Mitochondrial respiratory chain dysfunction is associated with a wide range of multi-system clinical phenotypes with considerable clinical overlap. The genetic defects associated with the phenotype also show marked heterogeneity and may involve defects of the mitochondrial DNA (mtDNA) or nuclear-encoded genes involved in mitochondrial maintenance, repair and function. Well described phenotypes more commonly (but not exclusively) associated with particular mutations include:

1. m.3243A>G mutation in the MTTL1 gene. This is one of the commonest mtDNA mutations and can cause several clinical phenotypes including Mitochondrial Encephalopathy, Lactic Acidosis, Stroke-like episodes (MELAS), Chronic Progressive External Ophthalmoplegia (CPEO) and Maternally Inherited Deafness and Diabetes (MIDD).

2. m.8344A>G is most commonly associated with Myoclonic epilepsy with ragged-red fibers (MERRF) although the phenotype has been extended to include other multisystem problems.

3. m.8993T>G/C is more commonly (but not exclusively) associated with Leigh syndrome and NARP (neurogenic muscle weakness, ataxia, and retinitis pigmentosa).

4. m.11778G>A, m.14484T>C or m.3460G>A all of which are associated with Leber’s Hereditary Optic Neuropathy (LHON).

The Neurogenetics laboratory is one of 3 laboratories (along with Oxford and Newcastle) commissioned by NHSE to provide a Highly Specialised Service (HSS) for mitochondrial disorders.

Blood (DNA) samples are screened for the 3 common mitochondrial point mutations in the first instance. If the patient is under 20 years old, testing for mtDNA large-scale rearrangements is also performed. Rearrangements are not reliably detectable in blood (DNA) samples from patients over the age of 20 and can only be performed on a muscle sample if required.

Samples from patients with LHON are screened (by NGS) for the three common LHON mutations (m.11778G>A, m.14484T>C or m.3460G>A) and if negative are screened for a further 14 mtDNA mutations known to cause the LHON phenotype.

Full mtDNA genome sequencing and mtDNA large-scale rearrangement analysis is performed on all muscle samples received.

Urine samples may be useful in clarifying the level of heteroplasmy in patients where muscle tissue is unavailable/not suitable e.g. in the mother of a patient with a mtDNA mutation.

As part of a multidisciplinary approach to diagnosis of mitochondrial disorders, muscle biopsy samples can be analysed by the Neurometabolic and Neuropathology Diagnostic laboratories at NHNNN. The majority of patients with a mitochondrial DNA mutation will have ragged red fibres and/or cytochrome c oxidase negative fibres and/or a measurable defect on mitochondrial respiratory chain enzymology. These results often aid in the interpretation of any sequencing results obtained.

Samples may also be sent to the other HSS centres for analyses not provided by the Neurogenetics Laboratory. This may include (but is not limited to) mtDNA depletion analysis or nuclear-encoded mitochondrial gene analysis.

An NGS panel for the following nuclear-encoded mitochondrial genes is also in development at the laboratory: C10orf2, DGUOK, MFN2, MPV17, OPA1, POLG, POLG2, RRM2B, SLC25A4, SUCLA2, SUCLG1, TK2, TYMP. Please contact the laboratory for further information.

9. GENETIC TESTS IN EPISODIC NEUROLOGICAL DISORDERS – BRAIN CHANNELOPATHIES

11 gene panel test:

Entire coding region of ATP1A2, ATP1A3, CACNA1A, CACNB4, KCNA1, KCNK18, PNKD, PRRT2, SCN1A, SLC1A3 AND SLC2A1 analysed using NGS and copy number analysis for exonic rearrangements in ATP1A2, CACNA1A, KCNA1 and SCN1A.
All brain channelopathy genes are routinely tested as part of the NGS panel although Sanger sequencing for KCNA1 and the common c.649dupC in PRRT2 are available on request: please contact the laboratory for further information.

Brain channelopathies
An increasing number of single gene brain paroxysmal neurological disorders have now been shown to be caused by mutations in genes coding for ion channels which are usually dominantly inherited or are de novo in occurrence.

- **ATP1A2** (FHM2) mutations in this gene have been found to be associated with familial basilar or hemiplegic migraines and a rare syndrome known as alternating hemiplegia of childhood.
- **ATP1A3** (AHC2) mutations in this gene have been found to be associated with alternating hemiplegia of childhood-2. The phenotype also includes CAPOS syndrome (Cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss) caused by the E818K variant, and Dystonia 12 (rapid-onset dystonia-parkinsonism)
- **CACNA1A** (FMH1 and EA2) mutations in this gene have been found to cause familial hemiplegic migraine as well as episodic ataxia type 2.
- **KCNA1** (EA1) mutations in this gene have been associated with myokymia with periodic ataxia.
- **KCNK18** (MGR13) mutations in this gene have been found to be associated with migraine with aura.
- **PNKD** mutations in this gene have been associated with the movement disorder paroxysmal non-kinesigenic dyskinesia.
- **PRRT2** mutations in this gene are associated with episodic kinesigenic dyskinesia or dystonia and occasionally hemiplegic migraine and epilepsy.
- **SCN1A** mutations in this gene have been associated with several epilepsy, convulsion and migraine disorders.
- **SLC1A3** (EA6) mutations in this gene are associated with episodic ataxia type 6.
- **SLC2A1** encodes the major glucose transporter in brain, placenta, and erythrocytes. Mutations in this gene have been found to be associated with paroxysmal exertion-induced dyskinesia.

### 10. GENETIC TESTS IN DEMENTIA AND MOTOR NEURONE DISEASE

Single gene tests:
Motor Neurone Disease (MND) or Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal dementia (FTD): *C9orf72* expansion with Southern blot confirmation.
X linked Bulbospinalneuronopathy or Kennedy’s disease (XLBSN): CAG expansion in the *AR* gene.

16 Dementia gene panel test:
NGS analysis of the entire coding region of *APP, CHMP2B, CSF1R, FUS, GRN, HTRA1, ITM2B, MAPT, NOTCH3, PRNP, PSEN1, PSEN2, TARDBP, TREM2, TYROBP* and *VCP*.

An 11-gene NGS panel for Motor Neurone disease / ALS genes is in development in the Neurogenetics Laboratory, including sequencing of the entire coding regions of: *ALS2, ANG, FIG4, FUS, NEFH, OPTN, SLC52A1, SLC52A2, SLC52A3, SOD1, TARDBP, UBQLN2, VAPB* and *VCP*. Please contact the laboratory for further information.

Other causes of FTD or Alzheimer disease are included on the 16 dementia gene NGS panel, mutations in which are inherited in an autosomal dominant manner except for *TREM2, TYROBP and HTRA1* mutations which are recessive.

- **APP** mutations in this gene are associated with autosomal dominant Alzheimer disease (AD1).
• **CHMP2B** mutations in this gene have been associated with a form of familial frontotemporal lobar degeneration (ALS17).

• **CSF1R** mutations in this gene have been associated with hereditary diffuse leukoencephalopathy with spheroids.

• **FUS** mutations in this gene have been associated with amyotrophic lateral sclerosis type 6 (ALS6).

• **GRN** encodes a protein progranulin that is involved in regulating cell growth. Mutations in this gene have been associated with a form of frontotemporal lobar degeneration with TDP43 inclusions (FTLD-TDP).

• **HTRA1** mutations in this gene have been found to be associated with cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL).

• **ITM2B** encodes a type II integral transmembrane protein with the C-terminal part being extracellular. Mutations in this gene are associated with familial British dementia and familial Danish dementia.

• **MAPT** encodes a highly soluble microtubule-associated protein one of whose main function is to modulate the stability of axonal microtubules. Mutations in this gene have been associated with several neurodegenerative disorders such as Alzheimer's disease, Pick's disease, frontotemporal dementia, cortico-basal degeneration and progressive supranuclear palsy.

• **NOTCH3** mutations in this gene have been identified as the underlying cause of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL).

• **PRNP** encodes a glycoprotein that contains a single disulfide bond, is N-glycosylated, and is attached to the plasma membrane by a C-terminally linked glycosyl phosphatidylinositol anchor. Mutations in this gene have been associated with Creutzfeldt-Jakob disease, fatal familial insomnia, Gerstmann-Straussler disease, Huntington disease-like 1, and kuru.

• **PSEN1** mutations in this gene are associated with autosomal dominant Alzheimer disease (AD3).

• **PSEN2** mutations in this gene are associated with autosomal dominant Alzheimer disease (AD4).

• **TARDBP** mutations in this gene have been associated with amyotrophic lateral sclerosis 10 with or without frontotemporal dementia.

• **TREM2** mutations in this gene are associated with Polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy also known as Nasu-Hakola disease a recessively inherited disorder characterized by a combination of psychotic symptoms rapidly progressing to pre-senile dementia and bone cysts restricted to wrists and ankles.

• **TYROBP** mutations in this gene are associated with Polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy also known as Nasu-Hakola disease a recessively inherited disorder characterized by a combination of psychotic symptoms rapidly progressing to pre-senile dementia and bone cysts restricted to wrists and ankles.

• **VCP** mutations in this gene have been associated with Inclusion Body Myopathy with Paget Disease of Bone and Frontotemporal Dementia and Amyotrophic Lateral Sclerosis 14, with or without Frontotemporal Dementia.

**Amyotrophic lateral sclerosis (ALS)** is a progressive neurodegenerative disorder that primarily affects the anterior horn cells in the Central Nervous System (CNS). Around 10% of ALS is familial. The GGGGCC hexanucleotide repeat expansion in intron-1 of the **C9orf72** gene is the most common cause of familial (50% of cases) and sporadic ALS (10% of cases). Interestingly a similar percentage of FTD cases were found to be caused by the same expansion.

Other causes of ALS or MND are included on the 11 gene NGS panel, mutations in which are inherited in an autosomal dominant manner except for **ALS2** mutations which are recessive, **OPTN**
mutations which maybe recessive in inheritance or dominant with reduced penetrance and UBQLN2 which is X-linked.

- **ALS2** mutations in this gene have been associated with several forms of juvenile lateral sclerosis and infantile-onset ascending spastic paralysis. (ALS2; PLSJ; IAHSP).

- **ANG** mutations in this gene have been implicated as causes of familial amyotrophic lateral sclerosis (ALS9).

- **FIG4** mutations in this gene have been implicated as causes of familial amyotrophic lateral sclerosis (ALS11).

- **FUS** mutations in this gene have been implicated as causes of familial amyotrophic lateral sclerosis (ALS6).

- **NEFH** encodes the heavy neurofilament protein. This protein is commonly used as a biomarker of neuronal damage and susceptibility to amyotrophic lateral sclerosis (ALS) has been associated with mutations in this gene.

- **OPTN** encodes the coiled-coil containing protein optineurin. Mutations in this gene have been implicated as causes of familial amyotrophic lateral sclerosis (ALS12).

- **SOD1** mutations in this gene have been implicated as causes of familial amyotrophic lateral sclerosis (ALS1).

- **TARDBP** mutations in this gene have been implicated as causes of familial amyotrophic lateral sclerosis (ALS10).

- **UBQLN2** mutations in this gene have been implicated as causes of familial amyotrophic lateral sclerosis (ALS15) which is X-linked and 90% penetrant in females.

- **VAPB** encodes a type IV membrane protein found in plasma and intracellular vesicle membranes. Mutations in this gene have been implicated as causes of familial amyotrophic lateral sclerosis (ALS8).

- **VCP** mutations in this gene have been implicated as causes of familial amyotrophic lateral sclerosis (ALS14).

- **Mutations in SLC52A1, SLC52A2 and SLC52A3 are causes of conditions resembling childhood-onset motor neurone disease.**

**X linked Bulbospinal neuronopathy or Kennedy's disease (SBMA)** is a slowly progressive, neurodegenerative disease associated with an expansion in the androgen receptor gene. Kennedy’s disease is named after W. R. Kennedy, a neurologist who was among the first to describe this disease. The early signs often include weakness of tongue and mouth muscles, fasciculations, and gradually increasing weakness of limb muscles with muscle wasting. In some cases, premature muscle fatigue begins in adolescence. Bulbar signs are common as well as cramps wasting and weakness. Endocrine problems include gynecomastia, diabetes mellitus, erectile dysfunction, low sperm count and testicular atrophy. There is considerable overlap between SBMA and other forms of ALS/MND and testing of the C9orf72 gene is often also warranted in cases that are negative for the AR expansion.

**11. GENETIC TESTS IN HEREDITARY SPASTIC PARAPLEGIA (HSP)**

A 13-gene NGS panel of HSP genes is in development at the Neurogenetics Laboratory, including analysis of the entire coding region of: CYP7B1, FA2H, GJC2, KIAA0196, KIF5A, NIPA1, PLP1, PNPLA6, REEP1, RTN2, SPAST and SPG7.

Hereditary spastic paraplegia (HSP) is a progressive disorder that usually onsets between the age of 10 and 40 years with limb spasticity, walking difficulty leading to stick and wheelchair use, urinary problems and back pain. The disorder is very clinically and genetically heterogeneous but the majority of cases are caused by defects in the genes listed below.
Obtaining an accurate genetic diagnosis is important for management and prognosis. Genetically diagnosed patients with the most common mutation do well with FES (Functional Electrical Stimulation) but have little response to baclofen. Once genetically diagnosed they also require yearly orthotics and physiotherapy.

- **ATL1** encodes atlastin-1, a dynamin-related GTPase, which plays a role in formation of the tubular endoplasmic reticulum (ER) network and in axon elongation in neurons. Mutations in this gene have been associated with autosomal dominant spastic paraplegia type 3 (SPG3).

- **BSCL2** encodes seipin, a protein that is mainly localized to the endoplasmic reticulum (ER) membrane. Mutations in exon 3 of this gene have been associated with autosomal dominant Silver spastic paraplegia type 17 (SPG17) and distal hereditary motor neuropathy type V (DSMAV).

- **CYP7B1** encodes a member of the cytochrome P450 superfamily of enzymes. Mutations in this gene have been associated with autosomal recessive SPG5A.

- **FA2H** encodes a protein that catalyzes the synthesis of 2-hydroxysphingolipids, a subset of sphingolipids that contain 2-hydroxy fatty acids. Mutations in this gene have been associated with leukodystrophy dysmyelinating with autosomal recessive spastic paraparesis with or without dystonia (SPG35).

- **GJC2** encodes a gap junction protein which plays a key role in central myelination and is involved in peripheral myelination in humans. Mutations in this gene have been associated with autosomal recessive Pelizaeus-Merzbacher-like disease-1 (SPG44).

- **KIAA0196** encodes a 134 kDa protein named strumpellin that is predicted to have multiple transmembrane domains and a spectrin-repeat-containing domain. Mutations in this gene have been associated with autosomal dominant spastic paraplegia type 8 (SPG8).

- **KIF5A** encodes a member of the kinesin family of proteins which are part of a multisubunit complex that functions as a microtubule motor in intracellular organelle transport. Mutations in this gene have been associated with autosomal dominant spastic paraplegia type 10 (SPG10).

- **NIPA1** encodes a magnesium transporter that associates with early endosomes and the cell surface in a variety of neuronal and epithelial cells. Mutations in this gene have been associated with autosomal dominant spastic paraplegia type 6 (SPG6).

- **PLP1** encodes a transmembrane proteolipid protein that is the predominant myelin protein present in the central nervous system. Mutations in this gene have been associated with X-linked Pelizaeus-Merzbacher disease and spastic paraplegia type 2 (SPG2).

- **PNPLA6** encodes a phospholipase that deacetylates intracellular phosphatidylcholine to produce glycerophosphocholine. It is thought to function in neurite outgrowth and process elongation during neuronal differentiation. Mutations in this gene have been associated with autosomal recessive spastic paraplegia type 39 (SPG39).

- **REEP1** encodes a mitochondrial protein that functions to enhance the cell surface expression of odorant receptors. Mutations in this gene have been associated with autosomal dominant spastic paraplegia type 31 (SPG31).

- **RTN2** encodes a protein belonging to the family of reticulon encoding genes which are necessary for proper generation of tubular endoplasmic reticulum and are likely play a role in intracellular vesicular transport. Mutations in this gene have been associated with autosomal dominant spastic paraplegia type 12 (SPG12).

- **SPAST** encodes a member of the AAA protein family. Mutations in this gene have been associated with the most frequent form of autosomal dominant spastic paraplegia type 4 (SPG4).

- **SPG7** encodes a nuclear-encoded mitochondrial metalloprotease protein that is a member of the AAA protein family. Mutations in this gene have been associated with autosomal recessive spastic paraplegia type 7 (SPG7).

- **SPG11** encodes a potential transmembrane protein that is phosphorylated upon DNA damage. Mutations in this gene have been associated with autosomal recessive spastic paraplegia type 11
(SPG11). The clinical presentation of SPG15 and the gene type and inheritance is almost identical to SPG11, although this gene is much rarer.