The Blueprint Genetics NCL and Progressive Myoclonic Epilepsy Panel is a 28 gene test for genetic diagnostics of patients with clinical suspicion of neuronal ceroid lipofuscinoses or progressive myoclonic epilepsy.

The panel covers genes for several entities including but not restricted to neuronal ceroid lipofuscinoses (NCL), Lafora disease, Unverricht-Lundborg disease and sialidoses. Most progressive myoclonic epilepsies (PMEs), but not all, are monogenic, autosomal recessive inherited diseases, and most diseases genes, but not all, encode lysosomal proteins. This panel is part of The Comprehensive Epilepsy Panel.

About NCL and Progressive Myoclonic Epilepsy

The progressive myoclonic epilepsies (PME) are a group of rare inherited disorders characterized by seizures, myoclonus, and progressive neurological degeneration. Patients may also exhibit cerebellar ataxia, dementia, neuropathy, and myopathy. It encompasses different diagnostic entities and the common forms include neuronal ceroid lipofuscinoses (NCLs), Unverricht-Lundborg disease and Lafora disease among other more rare forms of PMEs. Recently, a recurrent de novo mutation in KCNC1 was identified as a new major cause for PME (PubMed 25401298). NCLs are the largest group of PME. The overall prevalence of NCL is reported to be approximately 1.5-9 per million. In the pregenetic era, clinical phenotypes of NCL have been characterized according to three main features: age-of-onset, the order of presentation of the three main symptoms (myoclonus and seizures, cognitive and motor decline, and retinal pathology and visual loss), and electron microscopic (EM) findings. There is genetic and allelic heterogeneity. The majority of NCLs are inherited in an autosomal recessive manner. CLN4 is the only autosomal dominant NCL. Pathogenic variants in thirteen genes (PPT1, TPP1, CLN3, CLN5, CLN6, MFSD8, CLN8, CTSD, DNAJC5, CTSF, ATP13A2, GRN, KCTD7) are known to cause NCL. Unverricht-Lundborg disease (ULD) starts usually in childhood but improves in adulthood when myoclonus lessens in intensity, seizures tend to stop or are readily controllable. ULD is inherited in autosomal recessive manner and is due to mutations in CSTB. The Panel does not detect the expansion of a 12-nucleotide repeat (rs193922905) in the promoter region of CSTB. ULD patients who do not have the dodecamer repeat expansion on one of their alleles have other damaging, but not wholly inactivating mutations. Lafora disease is characterized by myoclonus and generalized tonic-clonic seizures, visual hallucinations, and progressive neurologic degeneration leading to death in 4-10 years after the onset. Lafora disease is inherited in an autosomal recessive manner and is caused by mutations in EPM2A or NHLRC1. The diagnostic yield of these genes is reported to be 84-97%. Prevalence of both ULD and Lafora disease varies significantly between countries.

Availability

Results in 3-4 weeks. We do not offer a maternal cell contamination (MCC) test at the moment. We offer prenatal testing only for cases where the maternal cell contamination studies (MCC) are done by a local genetic laboratory. Read more: http://blueprintgenetics.com/faqs/#prenatal

Gene set description

<table>
<thead>
<tr>
<th>Gene</th>
<th>Associated phenotypes</th>
<th>Inheritance</th>
<th>ClinVar</th>
<th>HGMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFG3L2*</td>
<td>Spastic ataxia, Spinocerebellar ataxia</td>
<td>AD/AR</td>
<td>19</td>
<td>25</td>
</tr>
<tr>
<td>ASAH1</td>
<td>Spinal muscular atrophy with progressive myoclonic epilepsy, Farber lipogranulomatosis</td>
<td>AR</td>
<td>11</td>
<td>53</td>
</tr>
<tr>
<td>ATP13A2</td>
<td>Parkinson disease (Kufor-Rakeb syndrome)</td>
<td>AR</td>
<td>11</td>
<td>35</td>
</tr>
<tr>
<td>CERS1</td>
<td>Epilepsy, progressive myoclonic</td>
<td>AR</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>CLN3</td>
<td>Ceroid lipofuscinosis, neuronal</td>
<td>AR</td>
<td>69</td>
<td>64</td>
</tr>
</tbody>
</table>
*Some regions of the gene are duplicated in the genome leading to limited sensitivity within the regions. Thus, low-quality variants are filtered out from the duplicated regions and only high-quality variants confirmed by other methods are reported out.

**Gene**, refers to HGNC approved gene symbol; **Inheritance** to inheritance patterns such as autosomal dominant (AD), autosomal recessive (AR) and X-linked (XL); **ClinVar**, refers to a number of variants in the gene classified as pathogenic or likely pathogenic in ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/); **HGMD**, refers to a number of variants with possible disease association in the gene listed in Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/). The list of associated (gene specific) phenotypes are generated from CDG (http://research.nhgri.nih.gov/CGD/) or Orphanet (http://www.orpha.net/) databases.

## Non-coding disease causing variants covered by the panel

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genomic location HG19</th>
<th>HGVS</th>
<th>RefSeq</th>
<th>RS-number</th>
<th>Comment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPT1</td>
<td>Chr1:40539203</td>
<td>c.*526_529delATCA</td>
<td>NM_000310.3</td>
<td>rs386833624</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

https://blueprintgenetics.com/
Test performance

Blueprint Genetics offers a comprehensive NCL and Progressive Myoclonic Epilepsy Panel that covers classical genes associated with action myoclonus-renal failure syndrome, neuronal ceroid lipofuscinosis, North Sea progressive myoclonus epilepsy, progressive myoclonic epilepsy, sialidoses type I and II and spinal-muscular atrophy-progressive myoclonic epilepsy. The genes are carefully selected based on the existing scientific evidence, our experience and most current mutation databases. Candidate genes are excluded from this first-line diagnostic test. The test does not recognise balanced translocations or complex inversions, and it may not detect low-level mosaicism. The test should not be used for analysis of sequence repeats or for diagnosis of disorders caused by mutations in the mitochondrial DNA.

Analytical validation is a continuous process at Blueprint Genetics. Our mission is to improve the quality of the sequencing process and each modification is followed by our standardized validation process. Average sensitivity and specificity in Blueprint NGS Panels is 99.3% and 99.9% for detecting SNPs. Sensitivity to for indels vary depending on the size of the alteration: 1-10bps (96.0%), 11-20 bps (88.4%) and 21-30 bps (66.7%). The longest detected indel was 46 bps by sequence analysis. Detection limit for Del/Dup (CNV) analysis varies through the genome depending on exon size, sequencing coverage and sequence content. The sensitivity is 71.5% for single exon deletions and duplications and 99% for three exons’ deletions and duplications. We have validated the assays for different starting materials including EDTA-blood, isolated DNA (no FFPE) and saliva that all provide high-quality results. The diagnostic yield varies substantially depending on the used assay, referring healthcare professional, hospital and country. Blueprint Genetics’ Plus Analysis (Seq+Del/Dup) maximizes the chance to find molecular genetic diagnosis for your patient although Sequence Analysis or Del/Dup Analysis may be cost-effective first line test if your patient’s phenotype is suggestive for a specific mutation profile.

Bioinformatics

The sequencing data generated in our laboratory is analyzed with our proprietary data analysis and annotation pipeline, integrating state-of-the art algorithms and industry-standard software solutions. Incorporation of rigorous quality control steps throughout the workflow of the pipeline ensures the consistency, validity and accuracy of results. The highest relevance in the reported variants is achieved through elimination of false positive findings based on variability data for thousands of publicly available human reference sequences and validation against our in-house curated mutation database as well as the most current and relevant human mutation databases. Reference databases currently used are the 1000 Genomes Project (http://www.1000genomes.org), the NHLBI GO Exome Sequencing Project (ESP; http://evs.gs.washington.edu/EVS), the Exome Aggregation Consortium (ExAC; http://exac.broadinstitute.org), ClinVar database of genotype-phenotype associations (http://www.ncbi.nlm.nih.gov/clinvar) and the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk). The consequence of variants in coding and splice regions are estimated using the following in silico variant prediction tools: SIFT (http://sift.jcvi.org), Polyphen (http://genetics.bwh.harvard.edu/pph2/), and Mutation Taster (http://www.mutationtaster.org).

Through our online ordering and statement reporting system, Nucleus, the customer can access specific details of the analysis of the patient. This includes coverage and quality specifications and other relevant information on the analysis. This represents our mission to build fully transparent diagnostics where the customer gains easy access to crucial details of the analysis process.

Clinical interpretation

In addition to our cutting-edge patented sequencing technology and proprietary bioinformatics pipeline, we also provide the customers with the best-informed clinical report on the market. Clinical interpretation requires fundamental clinical and genetic understanding. At Blueprint Genetics our geneticists and clinicians, who together evaluate the results from the sequence analysis pipeline in the context of phenotype information provided in the requisition form, prepare the clinical statement. Our goal is to provide clinically meaningful statements that are understandable for all medical professionals, even without training in genetics.

Variants reported in the statement are always classified using the Blueprint Genetics Variant Classification Scheme modified from the ACMG guidelines (Richards et al. 2015), which has been developed by evaluating existing literature, databases and with thousands of clinical cases analyzed in our laboratory. Variant classification forms the corner stone of clinical interpretation and following patient management decisions. Our statement also includes allele frequencies in reference populations and in silico predictions. We also provide PubMed IDs to the articles or submission numbers to public databases that have been used in the interpretation of the detected variants. In our conclusion, we summarize all the existing information
and provide our rationale for the classification of the variant.

A final component of the analysis is the Sanger confirmation of the variants classified as likely pathogenic or pathogenic. This does not only bring confidence to the results obtained by our NGS solution but establishes the mutation specific test for family members. Sanger sequencing is also used occasionally with other variants reported in the statement. In the case of variant of uncertain significance (VUS) we do not recommend risk stratification based on the genetic finding. Furthermore, in the case VUS we do not recommend use of genetic information in patient management or genetic counseling. For some cases Blueprint Genetics offers a special free of charge service to investigate the role of identified VUS.

We constantly follow genetic literature adapting new relevant information and findings to our diagnostics. Relevant novel discoveries can be rapidly translated and adopted into our diagnostics without delay. These processes ensure that our diagnostic panels and clinical statements remain the most up-to-date on the market.

CPT codes

SEQ 81479
DEL/DUP 81479

ICD codes

Commonly used ICD-10 codes when ordering the NCL and Progressive Myoclonic Epilepsy Panel

<table>
<thead>
<tr>
<th>ICD-10</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>E75.4</td>
<td>Neuronal ceroid lipofuscinosis</td>
</tr>
</tbody>
</table>

Accepted sample types

- EDTA blood, min. 1 ml
- Purified DNA, min. 5μg
- Saliva (Oragene DNA OG-500 kit)

Label the sample tube with your patient’s name, date of birth and the date of sample collection.

Note that we do not accept DNA samples isolated from formalin-fixed paraffin-embedded (FFPE) tissue.

Resources

- Batten Disease Support and Research Association
- CURE: Citizens United for Research in Epilepsy
- Epilepsy Foundation
- International League Against Epilepsy
- Intractable Childhood Epilepsy Alliance
- NORD - Progressive Myoclonus Epilepsy
- NORD - Batten Disease
- NORD - Kufs Disease
- NORD - Santavuori Disease
- Gene Reviews - Neuronal Ceroid Lipofuscinosis
- Gene Reviews - Action Myoclonus - Renal Failure Syndrome
- Gene Reviews - Unverricht-Lundborg Disease
- Gene Reviews - Lafora Disease