Hirschsprung Disease Panel

Test code: MA1801

This panel covers Hirschsprung disease (HSCR) with autosomal recessive and autosomal dominant forms of inheritance. This panel is part of Comprehensive Skeletal / Malformation Syndrome Panel.

About Hirschsprung Disease

Hirschsprung disease (HSCR), or congenital intestinal aganglionosis, is a birth defect characterized by complete absence of neuronal ganglion cells from a portion of the intestinal tract. Nerve cells are critical to the functioning of the colon as they control the regular muscle contractions that keep food moving through the bowels. In HSCR the aganglionic segment includes the distal rectum and a variable length of contiguous proximal intestine. In 80% of individuals, aganglionicosis is restricted to the rectosigmoid colon (short-segment disease), in 15%-20%, it extends proximal to the sigmoid colon (long-segment disease) and in about 5%, aganglionosis affects the entire large intestine (total colonic aganglionosis). Affected infants typically have impaired intestinal motility such as failure to pass meconium within the first 48 hours of life, constipation, emesis, abdominal pain or distention, and occasionally diarrhea in the first two months of life. However, in the milder forms the initial diagnosis of HSCR may be delayed until late childhood or adulthood, and therefore HSCR should be considered in anyone with lifelong severe constipation. Individuals with HSCR are at risk for enterocolitis and/or potentially lethal intestinal perforation. HSCR is considered a neurocristopathy, a disorder of cells and tissues derived from the neural crest, and may occur as an isolated finding or as part of a multisystem disorder. Both syndromic and nonsyndromic causes of HSCR are recognized. Roughly a third of children who have Hirschsprung's disease have other organ system involvement. Examples of monogenic syndromic forms of HSCR (covered by this panel) are Waardenburg syndrome type 4 (autosomal recessive disease due to EDNRB, EDN3 mutations in which HSCR is common and autosomal dominant form with SOX10 mutations in which HSCR is present in almost 100% of cases), Mowat-Wilson syndrome (mutations in ZEB2, HSCR present in 41-71% of cases) and multiple endocrine neoplasia type 2 (MEN 2A and 2B) (mutations in RET). Approximately 50% of familial cases of HSCR are heterozygous for mutations in RET, however the penetrance of these mutations is only 50 to 70%, is gender-dependent, and varies according to the extent of aganglionosis. The incidence of HSCR is approximately 1/5,000 live births, but it varies among different populations.

Availability

Results in 3-4 weeks

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>BDNF</td>
<td>Central hypoventilation syndrome, congenital</td>
<td>AD</td>
<td>1</td>
<td>23</td>
</tr>
<tr>
<td>CELSR3</td>
<td>Hirschsprung disease</td>
<td>AD</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>EDN3</td>
<td>Hirschsprung disease, Central hypoventilation syndrome, congenital, Waardenburg syndrome</td>
<td>AD/AR</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>EDNRB</td>
<td>Hirschsprung disease, ABCD syndrome, Waardenburg syndrome</td>
<td>AD/AR</td>
<td>12</td>
<td>66</td>
</tr>
<tr>
<td>KIF1BP</td>
<td>Goldberg-Shprintzen megacolon syndrome</td>
<td>AR</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>L1CAM</td>
<td>Mental retardation, aphasia, shuffling gait, and adducted thumbs (MASA) syndrome, Hydrocephalus due to congenital stenosis of aqueduct of Sylvius, Spastic, CRASH syndrome, Corpus callosum, partial agenesis</td>
<td>XL</td>
<td>80</td>
<td>292</td>
</tr>
</tbody>
</table>
MITF  Tietz albinism-deafness syndrome, Waardenburg syndrome, Coloboma, osteopetrosis, microphthalmia, macrocephaly, albinism, and deafness (COMMAD)  AD/AR  32  58

NRG1  Nonsyndromic Hirschsprung disease  AD/AR  1  10

NRTN  Hirschsprung disease  AD  2

PAX3  Craniofacial-deafness-hand syndrome, Waardenburg syndrome  AD/AR  54  149

PHOX2B  Central hypoventilation syndrome, congenital, Neuroblastoma, susceptibility to, Neuroblastoma with Hirschsprung disease  AD  11  86

RET  Hirschsprung disease, Central hypoventilation syndrome, congenital, Pheochromocytoma, Medullary thyroid carcinoma, Multiple endocrine neoplasia  AD/AR  122  407

RMRP  Cartilage-hair hypoplasia, Metaphyseal dysplasia without hypotrichosis, Aneutric dysplasia  AR  87  123

SOX10  Peripheral demyelinating neuropathy, central dysmyelination, Waardenburg syndrome, and Hirschsprung disease, Kallmann syndrome  AD  56  148

ZEB2*  Mowat-Wilson syndrome  AD  154  287

*Some regions of the gene are duplicated in the genome. Read more.

# The gene has suboptimal coverage (means <90% of the gene's target nucleotides are covered at >20x with mapping quality score (MQ>20) reads), and/or the gene has exons listed under Test limitations section that are not included in the panel as they are not sufficiently covered with high quality sequence reads.

The sensitivity to detect variants may be limited in genes marked with an asterisk (*) or number sign (#)

Gene refers to the HGNC approved gene symbol; Inheritance refers to inheritance patterns such as autosomal dominant (AD), autosomal recessive (AR), X-linked (XL), X-linked dominant (XLD) and X-linked recessive (XLR); ClinVar refers to the number of variants in the gene classified as pathogenic or likely pathogenic in this database (ClinVar); HGMD refers to the number of variants with possible disease association in the gene listed in Human Gene Mutation Database (HGMD). The list of associated, gene specific phenotypes are generated from CGD or Orphanet 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>
</tr>
</thead>
<tbody>
<tr>
<td>EDN3</td>
<td>Chr20:57875743</td>
<td>c.-125G&gt;A</td>
<td>NM_000114.2</td>
<td></td>
</tr>
<tr>
<td>EDN3</td>
<td>Chr20:57875849</td>
<td>c.-19C&gt;A</td>
<td>NM_000114.2</td>
<td>rs375594972</td>
</tr>
<tr>
<td>L1CAM</td>
<td>ChrX:153128846</td>
<td>c.3531-12G&gt;A</td>
<td>NM_000425.4</td>
<td></td>
</tr>
<tr>
<td>L1CAM</td>
<td>ChrX:153133652</td>
<td>c.1704-75G&gt;T</td>
<td>NM_000425.4</td>
<td></td>
</tr>
<tr>
<td>L1CAM</td>
<td>ChrX:153133926</td>
<td>c.1547-14delC</td>
<td>NM_000425.4</td>
<td></td>
</tr>
<tr>
<td>L1CAM</td>
<td>ChrX:153136500</td>
<td>c.523+12C&gt;T</td>
<td>NM_000425.4</td>
<td></td>
</tr>
<tr>
<td>PAX3</td>
<td>Chr2:223085913</td>
<td>c.958+28A&gt;T</td>
<td>NM_181459.3</td>
<td></td>
</tr>
<tr>
<td>RET</td>
<td>Chr10:43572670</td>
<td>c.-37G&gt;C</td>
<td>NM_020975.4</td>
<td></td>
</tr>
</tbody>
</table>
### Test Strengths

Some patients heterozygous for a de novo polyalanine repeat expansion mutations (PARMs) in the PHOX2B gene have isolated or more commonly syndromic Hirschsprung disease in association with with congenital central hypoventilation syndrome (CCHS, PMID 16888290). Repeat expansions are generally difficult to detect via NGS assays and their clinical validation at large scale is impossible due to lack of publicly available control samples with abnormal repeat expansions. So far, we have been able to detect and confirm stretches of 28 alanines instead of normal amount of 20 (genotype “20/28”). However, we do not know exact sensitivity or detection limit of our assay for these alanine repeats.
The strengths of this test include:

- CAP accredited laboratory
- CLIA-certified personnel performing clinical testing in a CLIA-certified laboratory
- Powerful sequencing technologies, advanced target enrichment methods and precision bioinformatics pipelines ensure superior analytical performance
- Careful construction of clinically effective and scientifically justified gene panels
- Our Nucleus online portal providing transparent and easy access to quality and performance data at the patient level
- Our publicly available analytic validation demonstrating complete details of test performance
- ~2,000 non-coding disease causing variants in our clinical grade NGS assay for panels (please see 'Non-coding disease causing variants covered by this panel' in the Panel Content section)
- Our rigorous variant classification scheme
- Our systematic clinical interpretation workflow using proprietary software enabling accurate and traceable processing of NGS data
- Our comprehensive clinical statements

Test Limitations

Genes with partial, or whole gene, segmental duplications in the human genome are marked with an asterisk (*) if they overlap with the UCSC pseudogene regions. The technology may have limited sensitivity to detect variants in genes marked with these symbols (please see the Panel content table above).

This test does not detect the following:

- Complex inversions
- Gene conversions
- Balanced translocations
- Mitochondrial DNA variants
- Repeat expansion disorders unless specifically mentioned
- Non-coding variants deeper than ±20 base pairs from exon-intron boundary unless otherwise indicated (please see above Panel Content / non-coding variants covered by the panel).

This test may not reliably detect the following:

- Low level mosaicism (variant with a minor allele fraction of 14.6% is detected with 90% probability)
- Stretches of mononucleotide repeats
- Indels larger than 50bp
- Single exon deletions or duplications
- Variants within pseudogene regions/duplicated segments

The sensitivity of this test may be reduced if DNA is extracted by a laboratory other than Blueprint Genetics.

For additional information, please refer to the Test performance section and see our Analytic Validation.

Test performance

Our panels are sectioned from our high-quality, clinical grade NGS assay. Please see our sequencing and detection performance table for details regarding our ability to detect different types of alterations (Table).

Assays have been validated for various sample types including EDTA-blood, isolated DNA (excluding from formalin fixed paraffin embedded tissue), saliva and dry blood spots (filter cards). These sample types were selected in order to maximize the likelihood for high-quality DNA yield. The diagnostic yield varies depending on the assay used, referring healthcare professional, hospital and country. Plus analysis increases the likelihood of finding a genetic diagnosis for your patient, as large deletions and duplications cannot be detected using sequence analysis alone. Blueprint Genetics’ Plus Analysis is a combination of both sequencing and deletion/duplication (copy number variant (CNV)) analysis.
### Performance of Blueprint Genetics high-quality, clinical grade NGS sequencing assay for panels.

<table>
<thead>
<tr>
<th>Variant Type</th>
<th>Sensitivity % (TP/(TP+FN))</th>
<th>Specificity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single nucleotide variants</td>
<td>99.89% (99,153/99,266)</td>
<td>&gt;99.9999%</td>
</tr>
<tr>
<td>Insertions, deletions and indels by sequence analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-10 bps</td>
<td>99.2% (7,745/7,806)</td>
<td>&gt;99.9999%</td>
</tr>
<tr>
<td>11-50 bps</td>
<td>99.13% (2,524/2,546)</td>
<td>&gt;99.9999%</td>
</tr>
<tr>
<td>Copy number variants (exon level dels/dups)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 exon level deletion (heterozygous)</td>
<td>100% (20/20)</td>
<td>NA</td>
</tr>
<tr>
<td>1 exon level deletion (homozygous)</td>
<td>100% (5/5)</td>
<td>NA</td>
</tr>
<tr>
<td>1 exon level deletion (het or homo)</td>
<td>100% (25/25)</td>
<td>NA</td>
</tr>
<tr>
<td>2-7 exon level deletion (het or homo)</td>
<td>100% (44/44)</td>
<td>NA</td>
</tr>
<tr>
<td>1-9 exon level duplication (het or homo)</td>
<td>75% (6/8)</td>
<td>NA</td>
</tr>
<tr>
<td>Simulated CNV detection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 exons level deletion/duplication</td>
<td>98.7%</td>
<td>100.00%</td>
</tr>
<tr>
<td>Size range (0.1-47 Mb)</td>
<td>100% (25/25)</td>
<td></td>
</tr>
</tbody>
</table>

The performance presented above reached by Blueprint Genetics high-quality, clinical grade NGS sequencing assay with the following coverage metrics:

- **Mean sequencing depth**: 143X
- **Nucleotides with >20x sequencing coverage (%)**: 99.86%

### Bioinformatics

The target region for each gene includes coding exons and ±20 base pairs from the exon-intron boundary. In addition, the panel includes non-coding and regulatory variants if listed above (Non-coding variants covered by the panel). Some regions of the gene(s) may be removed from the panel if specifically mentioned in the 'Test limitations' section above. 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. Our pipeline is streamlined to maximize sensitivity without sacrificing specificity. We have incorporated a number of reference population databases and mutation databases including, but not limited to, the 1000 Genomes Project, gnomAD, ClinVar and HGMD into our clinical interpretation software to make the process effective and efficient. For missense variants, in silico variant prediction tools such as SIFT, PolyPhen, MutationTaster are used to assist with variant classification. Through our online ordering and statement reporting system, Nucleus, ordering providers have access to the details of the analysis, including patient specific sequencing metrics, a gene level coverage plot and a list of regions with <20X sequencing depth if applicable. This reflects our mission to...
Clinical interpretation

We provide customers with the most comprehensive clinical report available on the market. Clinical interpretation requires a fundamental understanding of clinical genetics and genetic principles. At Blueprint Genetics, our PhD molecular geneticists, medical geneticists and clinical consultants prepare the clinical statement together by evaluating the identified variants in the context of the phenotypic information provided in the requisition form. Our goal is to provide clinically meaningful statements that are understandable for all medical professionals regardless of whether they have formal training in genetics.

Variant classification is the corner stone of clinical interpretation and resulting patient management decisions. Our classifications follow the Blueprint Genetics Variant Classification Schemes based on the ACMG guideline 2015. Minor modifications were made to increase reproducibility of the variant classification and improve the clinical validity of the report. Our experience with tens of thousands of clinical cases analyzed at our laboratory allowed us to further develop the industry standard.

The final step in the analysis is orthogonal confirmation. Sequence variants classified as pathogenic, likely pathogenic and variants of uncertain significance (VUS) are confirmed using bi-directional Sanger sequencing when they do not meet our stringent NGS quality metrics for a true positive call. Reported heterozygous and homo/hemizygous copy number variations with a size <10 and <3 target exons are confirmed by orthogonal methods such as qPCR if the specific CNV has been seen and confirmed less than three times at Blueprint Genetics.

Our clinical statement includes tables for sequencing and copy number variants that include basic variant information (genomic coordinates, HGVS nomenclature, zygosity, allele frequencies, in silico predictions, OMIM phenotypes and classification of the variant). In addition, the statement includes detailed descriptions of the variant, gene and phenotype(s) including the role of the specific gene in human disease, the mutation profile, information about the gene’s variation in population cohorts and detailed information about related phenotypes. We also provide links to the references, abstracts and variant databases used to help ordering providers further evaluate the reported findings if desired. The conclusion summarizes all of the existing information and provides our rationale for the classification of the variant.

Identification of pathogenic or likely pathogenic variants in dominant disorders or their combinations in different alleles in recessive disorders are considered molecular confirmation of the clinical diagnosis. In these cases, family member testing can be used for risk stratification. We do not recommend using variants of uncertain significance (VUS) for family member risk stratification or patient management. Genetic counseling is recommended.

Our interpretation team analyzes millions of variants from thousands of individuals with rare diseases. Our internal database and our understanding of variants and related phenotypes increases with every case analyzed. Our laboratory is therefore well-positioned to re-classify previously reported variants as new information becomes available. If a variant previously reported by Blueprint Genetics is re-classified, our laboratory will issue a follow-up statement to the original ordering health care provider at no additional cost.

#)

ICD codes

Commonly used ICD-10 codes when ordering the Hirschsprung Disease Panel

<table>
<thead>
<tr>
<th>ICD-10</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q43.1</td>
<td>Hirschsprung disease (HSCR)</td>
</tr>
</tbody>
</table>

Accepted sample types

- EDTA blood, min. 1 ml
- Purified DNA, min. 3μg*
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

- Bowel Group for Kids Inc.
- Cancer.Net
- GeneReviews - Mowat-Wilson Syndrome
- GeneReviews - Waardenburg Syndrome Type I
- GeneReviews - Hirschsprung Disease
- GeneReviews - Mowat-Wilson Syndrome
- GeneReviews - Multiple Endocrine Neoplasia Type 2
- GeneReviews - Waardenburg Syndrome Type I
- Hearing Health Foundation
- International Foundation for Functional Gastrointestinal Disorders
- Mowat Wilson Support Group
- Mowat-Wilson Syndrome Foundation
- NORD - Hirschsprung Disease
- NORD - Mowat-Wilson Syndrome
- NORD - Multiple Endocrine Neoplasia Type 2
- NORD - Waardenburg Syndrome
- National Institute of Diabetes, Digestive & Kidney Diseases