Craniosynostosis
Indication-Specific Gene Panel
Including: sequencing and high resolution deletion/duplication analysis

PANEL DESCRIPTION:
Craniosynostosis describes the premature closure of one or more sutures in an individual’s skull, which can cause an abnormal head shape. Craniosynostosis is further characterized by the specific suture(s) prematurely closed: sagittal, coronal, metopic, or lambdoid. Many craniosynostosis syndromes exist and can often have additional features. Craniosynostosis occurs in 1 in 2000-2500 births. This indication-specific panel includes full-gene sequence and high resolution deletion/duplication analysis of 26 genes commonly associated with craniosynostosis.

PANEL DETAILS:
• This panel includes both sequencing and high resolution deletion/duplication analysis of the genes specified.
  o Sequencing is performed using a customized next generation sequencing library. Analysis includes the coding exons of all genes in the panel plus ten bases into the introns and untranslated regions (5' and 3'). Sanger sequencing is performed to confirm variants suspected or confirmed to be pathogenic.
  o Deletion/duplication analysis is performed using a high resolution, custom microarray platform designed to target the genes of interest at the exon level.
• Detection rates are limited to the genes specified; this test does not provide whole genome analysis.
• Gene panels are a more cost-effective approach than single gene testing to confirm or establish a diagnosis. However, if single gene testing is desired for the patient or family members of an individual with a known mutation, that must be ordered separately.

ADDITIONAL TESTING DETAILS:
• If microarray analysis is performed, it will be done using a high resolution, single nucleotide polymorphism (SNP) platform designed to interrogate the whole genome at a resolution much higher than is possible using traditional karyotyping or fluorescence in situ hybridization (FISH) methodologies. Our High Density SNP array contains a total of 2.6 million markers distributed throughout the genome for the detection of both genomic dosage anomalies (deletions and duplications) and regions of homozygosity (ROH; regions lacking typical amounts of genetic variation). This marker density provides a global resolution of 10 Kb to 20 Kb for copy number changes and 5 Mb resolution for ROH.

RECOMMENDED TESTING STRATEGY:
Tests below can be ordered individually, however our laboratory’s recommended Comprehensive Testing for craniosynostosis includes the following two tests:
  Next Generation Sequencing
  • This gene test panel covers genes commonly associated with craniosynostosis and several skeletal dysplasias. Next generation sequencing (NGS) analyzes multiple genes at once, making this a cost-effective method of testing genes known to be as associated with these indications.
  Targeted Deletion/Duplication Analysis
  • If no pathogenic aberrations are detected by NGS, deletion/duplication analysis is performed to identify partial or whole gene deletions and duplications in the associated genes.
  IF INDICATED, also perform SNP Microarray Analysis
  • Chromosomal abnormalities, including microdeletions and microduplications, are found in at least 22% of children with syndromic craniosynostosis. High Density SNP Microarray can be ordered simultaneously or in a tiered fashion to assess for these copy number changes.
DISORDERS INCLUDED IN THIS PANEL:
Several skeletal dysplasias are also covered by this panel because mutations in these genes can inhibit or alter bone growth.

- Antley-Bixler syndrome with genital anomalies and disordered steroidogenesis [POR]
- Baller-Gerold syndrome [RECQL4]
- Bifid nose with or without anorectal and renal anomalies [FREM1]
- Bohring-Opitz syndrome [ASXL1]
- Carpenter syndrome [RAB23]
- Cleidocranial dysplasia [RUNX2]
- Cranioectodermal dysplasia 1 [IFT122]
- Cranioectodermal dysplasia 2 [WDR35]
- Cranioectodermal dysplasia 3 [IFT43]
- Cranioectodermal dysplasia 4 (sagittal craniosynostosis) [WDR19]
- Craniofrontonasal syndrome (CFNS) [EFNB1]
- Craniosynostosis, type 1 [TWIST1]
- Craniosynostosis 2 [MSX2]
- Craniosynostosis 3 [TCF12]
- Craniosynostosis 4 [ERF]
- Craniosynostosis and dental anomalies [IL11RA]
  - Antley-Bixler syndrome without genital anomalies or disordered steroidogenesis [FGFR2]
  - Apert
  - Beare-Stevenson cutis gyrata syndrome
  - Bent bone dysplasia syndrome [FGFR2]
  - CATSHL syndrome [FGFR3]
  - Craniofacial-skeletal-dermatologic dysplasia [FGFR3]
  - Crouzon syndrome with or without acanthosis nigricans [FGFR3]
  - FGFR1-related craniosynostosis
  - FGFR3-related craniosynostosis
  - Isolated coronal synostosis [FGFR3]
  - Hartsfield syndrome [FGFR1]
  - Hypogonadotropic hypogonadism 2 with or without anosmia [FGFR1]
  - Jackson-Weiss [FGFR2]
  - LADD syndrome [FGFR2, FGFR3]
  - Muenke syndrome [FGFR3]
  - Nevus, epidermal, somatic [FGFR3]
  - Osteoglophonic dysplasia [FGFR1]
  - Pfeiffer
  - SADDAN [FGFR3]
  - Saethre-Chotzen [FGFR2]
  - Scaphocephaly, maxillary retrusion, and mental retardation [FGFR2]
  - Trigonocephaly 1 [FGFR1]
- Greig cephalopolysyndactyly syndrome [GLI3]
- Loeys-Dietz syndrome, type 1 [TGFB1]
- Loeys-Dietz syndrome, type 2 [TGFB2]
- Manitoba oculotochoanal syndrome [FREM1]
- Metaphyseal dysplasia with maxillary hypoplasia with/without brachydactyly [RUNX2]
- Multiple epidermal growth factor like domains 8; megf8, Carpenter syndrome [MEGF8]
- Parietal foramina 1 with or without cleidocranial dysplasia [MSX2]
- Polydactyly, postaxial, types A1 and B [GLI3]
- Polydactyly, preaxial, type IV [GLI3]
- RAPADILINO syndrome \([RECQL4]\)
- Robinow-Sorauf syndrome \([TWIST1]\)
- Rothmund-Thomson syndrome \([RECQL4]\)
- Saethre-Chotzen syndrome with or without eyelid abnormalities \([CDC45, FGFR2, TWIST1]\) - GeneReviews®
- Short-rib thoracic dysplasia 5 with or without polydactyly \([WDR19]\)
- Short-rib thoracic dysplasia 7 with or without polydactyly \([WDR35]\)
- Shprintzen-Goldberg syndrome \([SKI]\)
- Thoracic Aortic Aneurysms and Aortic Dissections \([TGFBR2]\)
- Trigonocephaly 2 \([FREM1]\)
- ZIC1-craniosynostosis \([ZIC1]\)

**INDICATIONS FOR TESTING:**

- Craniosynostosis
- Facial features such as proptosis (bulging eyes), ptosis, widely spaced eyes, flattened midface, temporal bossing, frontal bossing
- Syndactyly, clinodactyly, broad toes, broad thumbs
- Short stature or shortened long bones
- Palatal abnormalities (cleft or high palate)
- Bone fusions, especially carpal, tarsal, and radioulnar synostosis
- Developmental delay, hearing loss, or vision concerns in a person suspected to have craniosynostosis or skeletal dysplasia

**SPECIMEN COLLECTION & TRANSPORT:**

Complimentary test kits are available upon request, but are not required.

**SAMPLE TYPE and REQUIREMENTS:**

- **blood, > 3 months of age:** 3-5 ml whole blood in an EDTA tube (purple top)
- **blood, newborn:** 1-3 ml whole blood in an EDTA tube (purple top)
- **buccal swab:** 5 swabs
- **extracted DNA:** 5 µg in a DNA microcentrifuge tube

**SHIPPING:**

- Maintain and ship samples at room temperature.
- Coordinate transport for sample to be received in our laboratory within 24-48 hours of collection.
  - **LOCAL:** Call 402-559-5070 (option 1)
  - **OUT OF AREA:** Prior to shipment, please fax the completed test request form to 402-559-7248, including the FedEx® airbill tracking number.
    - Saturday delivery MUST be checked when sending FedEx® on Friday.
    - Please include Internal Billing Reference # 3155070600 on the FedEx® airbill.
    - **Ship To:** Human Genetics Laboratory – Zip 5440
      UNMC Shipping & Receiving Dock
      601 S. Saddle Creek Road
      Omaha, NE 68106

**REQUIRED FORM:** The following form can be downloaded via our website.
- Postnatal Test Request Form

**OPTIONAL FORM:**
- Informed Consent for Genetic Testing

**POTENTIAL TEST RESULTS:**

Once a variant is confirmed, our laboratory team interprets this information in conjunction with the patient’s clinical findings and the scientific literature in order to classify a finding. There are three possible results:
• A normal result indicates that sequencing or deletion/duplication analysis of the genes analyzed did not find any pathogenic mutations or variants of uncertain clinical significance (or no clinically-significant chromosome anomalies were identified by microarray analysis).

• An abnormal (or pathogenic) result indicates that a pathogenic mutation was identified (or microarray analysis identified a genomic dosage anomaly [deletion or duplication] or ROH that likely provides an explanation for the individual’s clinical findings). Any available information regarding the phenotype associated with that mutation will accompany the technical details on the report.

• In some cases, the clinical significance of an identified sequence variant (or chromosomal anomaly detected by microarray) may not be well understood. These variants (anomalies) will be reported as variants of uncertain clinical significance (UCS). Any available information about the molecular characteristics of the genetic change and the relationship of the genetic change to phenotype will be included on the report. Over time, as more patients are reported, a variant of uncertain clinical significance may be revised to an informative result, and a revised report will be generated.

• Parental testing may be recommended in order to classify the result as de novo or familial for the purpose of recurrence risk calculation.

TURN-AROUND-TIMES:  For all sample types, results are typically available in 2-6 weeks.

BILLING:  Our laboratory offers patient/self-pay, insurance (including Medicare/Medicaid), and client/institution billing options. Verifying coverage requirements or obtaining preauthorization PRIOR TO OR AT THE TIME OF SPECIMEN COLLECTION is often necessary. We provide preauthorization services upon request by calling 402-559-5070 (option 3); the following form is helpful for obtaining the information required by insurance providers and can be downloaded via our website.

• Request for Insurance Preauthorization

In some circumstances, a test may be warranted even though insurance coverage is denied or not guaranteed. For these situations, we request the following form be signed by the patient and submitted with the sample. This helps inform patients of their potential financial responsibility, should the costs of genetic testing not be paid by their insurance provider.

• Advanced Beneficiary Notice of Noncoverage (ABN) – required when billing Medicare

CPT CODES:
• Next Generation Sequencing: 81407(x2)
• Targeted Deletion/Duplication Analysis: 81228

PRICING:  For current costs contact the laboratory billing staff at 402-559-5070 (option 3).

GENE LIST: (expanded panel, version 2_26)
This 26 gene panel is applied to all testing performed on and after April 1, 2016.

| ASXL1 | FREM1 | POR | TGFBR2 |
| CDC45 | GLI3 | RAB23 | TWIST1 |
| EFNB1 | IFT43 | RECOL4 | WDR19 |
| ERF | IFT122 | RUNX2 | WDR35 |
| FGFR1 | IL11RA | SKI | ZIC1 |
| FGFR2 | MEGF8 | TCF12 | |
| FGFR3 | MSX2 | TGFBR1 | |

REFERENCES: