

Craniosynostosis Panel Sequencing and Deletion/Duplication Analysis

Craniosynostosis Panel Gene List: *ALPL, ALX4, ASXL1, CDC45, CYP26B1, EFNB1, ERF, FGFR1, FGFR2, FGFR3, GLI3, IFT122, IFT43, IL11RA, MASP1, MEGF8, MSX2, P4HB, POR, RAB23, RECQL4*, SEC24D, SKI, TCF12, TGFB1, TGFB2, TMCO1, TWIST1*, WDR35, ZIC1* *deletion/duplication testing not included

Clinical Features:

Craniosynostosis is a condition characterized by premature ossification of one or more of the cranial sutures of the skull resulting in abnormal head growth and shape. The prevalence is approximately 1 in every 2,000-2,500 births.¹ Craniosynostosis (CS) has been classified into two main groups: non-syndromic and syndromic. Nonsyndromic craniosynostosis (NCS) accounts for 70-75% of cases and occurs as an isolated congenital anomaly. NCS is characterized by the sutures involved and includes sagittal (40-58%), metopic (~25%), unicoronal (~15%), bicoronal (~7-8%), and lamboid (1-3%).^{1,2,3} In 25-30% of cases, craniosynostosis presents as part of a genetic syndrome with varying clinical features and inheritance. Clinical features associated with syndromic craniosynostosis may include facial dysmorphism, developmental delays, hand or foot abnormalities, vision problems, brain malformations, and hearing loss.^{1,2}

In part due to the growth restrictions made by the fused sutures, intracranial pressure sometimes develops in patients with craniosynostosis. Increased intracranial pressure most commonly affects individuals with multiple closed sutures and syndromic patients; however, there have been cases of increased intracranial pressure when only one suture has fused.² Increased intracranial pressure can lead to headaches, visual impairment, sleeping difficulties, and intellectual disability.^{1,4,5}

Genetics:

There are currently over 180 different syndromes that involve craniosynostosis.⁶ The more common genes associated with syndromes where craniosynostosis is the predominant phenotype include *FGFR1, FGFR2, FGFR3, TWIST1, EFNB1, MSX2,* and *RAB23*.¹ Other genes such as *FBN1, POR, TGFB1,* and *TGFB2* are associated with syndromes where craniosynostosis is not a major clinical feature or has reduced penetrance.¹ Pathogenic variants in *FGFR2* account for approximately 32% of cases of syndromic craniosynostosis followed by *FGFR3* (25%), *TWIST1* (19%), and *EFNB1* (7%).⁷ The majority of craniosynostosis cases are associated with autosomal dominant inheritance with half of these cases occurring de novo.⁷ However, autosomal recessive inheritance is also observed and pathogenic variants in *EFNB1* associated with craniofrontonasal syndrome are X-linked dominant.

Test Methods:

Using genomic DNA from the submitted specimen, the complete coding regions and splice site junctions of the genes on this panel are enriched using a proprietary targeted capture system developed by GeneDx for next-generation sequencing with CNV calling (NGS-CNV). The enriched targets are simultaneously sequenced with paired-end reads on an Illumina platform. Bi-directional sequence reads are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19. After gene specific filtering, data are analyzed to identify sequence variants and most deletions and duplications involving coding exons. For the *REQL4* and *TWIST1* genes, sequencing but not deletion/duplication analysis is performed. Alternative sequencing or copy number detection methods are used to analyze regions with inadequate sequence or copy number data. Reportable variants include pathogenic variants, likely pathogenic variants and variants of uncertain significance. Likely benign and benign variants, if present, are not routinely reported but are available upon request.

Test Sensitivity:

The clinical sensitivity of this panel depends in part on the patient's clinical phenotype. Approximately 45% of individuals with a clinical diagnosis of craniosynostosis (syndromic and non-syndromic combined) have a genetic cause.⁶ Syndromic craniosynostosis has an identifiable genetic cause in 75-80% of cases.³ The clinical sensitivity for *FGFR*-related craniosynostosis syndromes is greater than 99%. Clinical sensitivity for *TWIST1* variants related to Saethre-Chotzen syndrome is approximately 68% and *GLI3* variants related to Greig cephalopolysyndactyly is approximately 75%. The clinical sensitivity for *ALPL* for hypophosphatasia is approximately 95%.⁸ The sensitivity for the remaining genes on this panel is unknown at this time.

The technical sensitivity of sequencing is estimated to be > 99% at detecting single nucleotide events. It will not reliably detect deletions greater than 20 base pairs, insertions or rearrangements greater than 10 base pairs, or low-level mosaicism. The copy number assessment methods used with this test cannot reliably detect copy number variants of less than 500 base pairs or mosaicism and cannot identify balanced chromosome aberrations. Assessment of exon-level copy number events is dependent on the inherent sequence properties of the targeted regions, including shared homology and exon size.

Genes and Conditions Associated with Craniosynostosis

| Gene | Protein | Inheritance | Disease Associations |
|--------------------------------|---|--------------|---|
| <i>ALPL</i> ⁸ | ALKALINE PHOSPHATASE | AR AD, AR | Infantile Hypophosphatasia Odontohypophosphatasia |
| <i>ALX4</i> ⁹⁻¹¹ | ARISTALESS-LIKE 4, MOUSE, HOMOLOG OF | AR AD | Frontonasal dysplasia 2 Parietal Foramina 2 Craniosynostosis 5 (susceptibility to) |
| <i>ASXL1</i> ¹²⁻¹³ | ADDITIONAL SEX COMBS-LIKE 1 | AD | Bohring-Opitz syndrome |
| <i>CDC45</i> ¹⁴ | CELL DIVISION CYCLE 45 | AR | Meier-Gorlin syndrome 7 |
| <i>CYP26B1</i> ^{3,15} | CYTOCHROME P450, SUBFAMILY XXVIB, POLYPEPTIDE 1 | AR | Craniosynostosis with radiohumeral fusions and other skeletal and craniofacial anomalies |
| <i>EFNB1</i> ¹⁶ | EPHRIN B1 | XLD | Craniofrontonasal dysplasia |
| <i>ERF</i> ¹⁷⁻¹⁸ | ETS2 REPRESSOR FACTOR | AD | Craniosynostosis 4 |
| <i>FGFR1</i> ¹⁹ | FIBROBLAST GROWTH FACTOR RECEPTOR 1 | AD | Pfeiffer syndrome Jackson-Weiss syndrome Trigonocephaly 1 |
| <i>FGFR2</i> ¹⁹ | FIBROBLAST GROWTH FACTOR RECEPTOR 2 | AD | Antley-Bixler syndrome Apert syndrome Beare-Stevenson cutis gyrata syndrome Bent bone dysplasia Crouzon syndrome Jackson-Weiss syndrome Pfeiffer syndrome Saethre-Chatzen syndrome Craniosynostosis, non-specific |
| <i>FGFR3</i> ¹⁹ | FIBROBLAST GROWTH FACTOR RECEPTOR 3 | AD | Crouzon syndrome Muenke syndrome |
| <i>GLI3</i> ²⁰ | GLI-KRUPPEL FAMILY MEMBER 3 | AD | Greig cephalopolysyndactyly syndrome |
| <i>IFT122</i> ²¹⁻²² | INTRAFLAGELLAR TRANSPORT 122, CHLAMYDOMONAS, HOMOLOG OF | AR | Cranioectodermal dysplasia 1 |

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|--------------------------------|---|----|--|
| <i>IFT43</i> ^{21,23} | INTRAFLAGELLAR TRANSPORT 43 | AR | Cranioectodermal dysplasia 3 |
| <i>IL11RA</i> ^{18,24} | INTERLEUKIN 11 RECEPTOR, ALPHA | AR | Craniosynostosis and dental anomalies |
| <i>MASP1</i> ^{25,26} | MANNAN-BINDING LECTIN SERINE PROTEASE 1 | AR | 3MC syndrome 1 |
| <i>MEGF8</i> ^{27,28} | MULTIPLE EPIDERMAL GROWTH FACTOR-LIKE DOMAINS 8 | AR | Carpenter syndrome 2 |
| <i>MSX2</i> ²⁹ | MUSCLE SEGMENT HOMEBOX, DROSOPHILA, HOMOLOG OF, 2 | AD | Craniosynostosis 2 |
| <i>P4HB</i> ^{30,31} | PROCOLLAGEN-PROLINE, 2-OXOGLUTARATE-4-DIOXYGENASE, BETA SUBUNIT | AD | Cole-Carpenter syndrome 1 |
| <i>POR</i> ³² | CYTOCHROME P450 OXIDOREDUCTASE | AR | Antley-Bixler syndrome with genital anomalies and disordered steroidogenesis |
| <i>RAB23</i> ³³⁻³⁴ | RAS-ASSOCIATED PROTEIN RAB23 | AR | Carpenter syndrome |
| <i>RECQL4</i> ³⁵⁻³⁶ | RECQ PROTEIN-LIKE 4 | AR | Baller-Gerold syndrome |
| <i>SEC24D</i> ³⁷ | SEC24-RELATED GENE FAMILY, MEMBER D | AR | Cole-Carpenter syndrome 2 |
| <i>SKI</i> ³⁸⁻³⁹ | V-SKI AVIAN SARCOMA VIRAL ONCOGENE HOMOLOG | AD | Shprintzen-Goldberg syndrome |
| <i>TCF12</i> ⁴⁰⁻⁴¹ | TRANSCRIPTION FACTOR 12 | AD | Craniosynostosis 3 |
| <i>TGFBR1</i> ⁴²⁻⁴³ | TRANSFORMING GROWTH FACTOR-BETA RECEPTOR, I | AD | Loeys-Dietz syndrome 1 |
| <i>TGFBR2</i> ⁴³⁻⁴⁴ | TRANSFORMING GROWTH FACTOR-BETA RECEPTOR, II | AD | Loeys-Dietz Syndrome 2 |
| <i>TMCO1</i> ⁴⁵⁻⁴⁶ | TRANSMEMBRANE AND COILED-COIL DOMAINS PROTEIN 1 | AR | Craniofacial dysmorphism, skeletal anomalies, and mental retardation syndrome |
| <i>TWIST1</i> ^{16,47} | TWIST, DROSOPHILA, HOMOLOG OF, 1 | AD | Craniosynostosis 1 Robinow-Sorauf syndrome Saethre-Chatzen syndrome with or without eyelid anomalies |
| <i>WDR35</i> ^{21,48} | WD REPEAT-CONTAINING PROTEIN 35 | AR | Cranioectodermal dysplasia 2 |
| <i>ZIC1</i> ^{3,49} | ZIC FAMILY, MEMBER 1 | AD | Craniosynostosis 6 |

Abbreviations:

AD – Autosomal dominant
AR – Autosomal recessive
XLD – X-linked Dominant

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