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, TGFBR1, TGFBR2, 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, TGFBR1, and TGFBR2 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 hypophosphastasia 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.

Gene	Protein	Inheritance	Disease Associations
ALPL[8]	ALKALINE PHOSPHATASE	AR AD,AR	Infantile Hypophosphatasia Odontohypophosphatasia
ALX4 [9-11]	ARISTALESS-LIKE 4, MOUSE, HOMOLOG OF	AR AD	Frontonasal dysplasia 2 Parietal Foramina 2 Craniosynostosis 5 (susceptibility to)
ASXL1[12-13]	ADDITIONAL SEX COMBS-LIKE 1	AD	Bohring-Opitz syndrome
CDC45[14]	CELL DIVISION CYCLE 4	AR	Meier-Gorlin syndrome 7
CYP26B1 [3,15]	CYTOCHROME P450, SUBFAMILY XXVIB, POLYPEPTIDE 1	AR	Craniosynostosis with radiohumeral fusions and other skeletal and craniofacial anomalies
EFNB1 [16]	EPHRIN B1	XLD	Craniofrontonasal dysplasia
ERF [17-18]	ETS2 REPRESSOR FACTOR	AD	Craniosynostosis 4

FGFR1 [19]	FIBROBLAST GROWTH FACTOR RECEPTOR 1	AD	Pfeiffer syndrome Jackson-Weiss syndrome Trigonocephaly 1
FGFR2 [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-Chotzen syndrome Craniosynostosis, non-specific
FGFR3 [19]	FIBROBLAST GROWTH FACTOR RECEPTOR 3	AD	Crouzon syndrome Muenke syndrome
GLI3 [20]	GLI-KRUPPEL FAMILY MEMBER 3	AD	Greig cephalopolysyndactyly syndrome
IFT122 [21-22]	INTRAFLAGELLAR TRANSPORT 122, CHLAMYDOMONAS, HOMOLOG OF	AR	Cranioectodermal dysplasia 1
IFT43 [21,23]	INTRAFLAGELLAR TRANSPORT 43	AR	Cranioectodermal dysplasia 3
IL11RA [18,24]	INTERLEUKIN 11 RECEPTOR, ALPHA	AR	Craniosynostosis and dental anomalies
MASP1 [25,26]	MANNAN-BINDING LECTIN SERINE PROTEASE 1	AR	3MC syndrome 1
MEGF8 [27,28]	MULTIPLE EPIDERMAL GROWTH FACTOR-LIKE DOMAINS 8	AR	Carpenter syndrome 2
MSX2 [29]	MUSCLE SEGMENT HOMEOBOX, DROSOPHILA, HOMOLOG OF, 2	AD	Craniosynostosis 2
P4HB [30,31]	PROCOLLAGEN-PROLINE, 2-OXOGLUTARATE-4-DIOXYGENASE, BETA SUBUNIT	AD	Cole-Carpenter syndrome 1

POR [32]	CYTOCHROME P450 OXIDOREDUCTASE	AR	Antley-Bixler syndrome with genital anomalies and disordered steroidogenesis
RAB23 [33-34]	RAS-ASSOCIATED PROTEIN RAB23	AR	Carpenter syndrome
RECQL4 [35-36]	RECQ PROTEIN-LIKE 4	AR	Baller-Gerold syndrome
SEC24D [37]	SEC24-RELATED GENE FAMILY, MEMBER D	AR	Cole-Carpenter syndrome 2
SKI [38-39]	V-SKI AVIAN SARCOMA VIRAL ONCOGENE HOMOLOG	AD	Shprintzen-Goldberg syndrome
TCF12 [40-41]	TRANSCRIPTION FACTOR 12	AD	Craniosynostosis 3
TGFBR1 [42-43]	TRANSFORMING GROWTH FACTOR-BETA RECEPTOR, I	AD	Loeys-Dietz syndrome 1
TGFBR2 [43-44]	TRANSFORMING GROWTH FACTOR-BETA RECEPTOR, II	AD	Loeys-Dietz Syndrome 2
TMCO1 [45-46]	TRANSMEMBRANE AND COILED-COIL DOMAINS PROTEIN 1	AR	Craniofacial dysmorphism, skeletal anomalies, and mental retardation syndrome
TWIST1 [16,47]	TWIST, DROSOPHILA, HOMOLOG OF, 1	AD	Craniosynostosis 1 Robinow-Sorauf syndrome Saethre-Chotzen syndrome with or without eyelid anomalies
WDR35 [21,48]	WD REPEAT-CONTAINING PROTEIN 35	AR	Cranioectodermal dysplasia 2
ZIC1 [3,49]	ZIC FAMILY, MEMBER 1	AD	Craniosynostosis 6

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

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