THE UNIVERSITY OF CHICAGO | Genetic Services Laboratories

Next Generation Sequencing Panel for Craniofacial Development

Clinical Features:
Craniofacial disorders occur when there is an abnormality in the development or growth of the skull and/or facial bones. Individuals with these conditions may present with only craniofacial abnormalities or may have extracranial abnormalities, such as anomalies of the limbs or digits. Examples of craniofacial disorders include craniosynostosis (premature fusion of one or more cranial sutures), frontonasal dysplasia, and cranioectodermal dysplasia.

Our Craniofacial Panel includes analysis of the 29 genes listed below.

Craniofacial Panel						
Craniosynostosis syndromes				**Cranioectodermal dysplasia**	**Frontonasal dysplasia**	**Other**
FGFR1	TCF12	GLI3	RECQL4	IFT122	ALX1	FAM20C
FGFR2	ERF	IL11RA	SKI	WDR35	ALX3	RUNX2
FGFR3	ZIC1	MEGF8	TGFBR1	IFT43	ALX4	
TWIST1	DPH1	POR	TGFBR2	WDR19	EFNB1	
MSX2	FLNA	RAB23				

Craniosynostosis syndromes

One of the most clinically important developmental disorders of the skull vault is craniosynostosis — the premature fusion of one or more cranial sutures — which affects 1 in 2000/2,500 individuals. This can be associated with increased intracranial pressure, difficulties with vision, hearing and breathing, and facial deformity [1]. Craniosynostosis may be an isolated finding or may be a feature of a syndrome with other clinical findings and/or congenital anomalies.

Gene	Clinical Features	Details
FGFR1 FGFR2 FGFR3 TWIST1	Craniosynostosis	Gain-of-function mutations in *FGFR1* to *3* have been associated with Pfeiffer, Apert, Crouzon, Beare-Stevenson, Jackson-Weiss, and Muenke syndromes. These syndromes are characterized by bicoronal craniosynostosis or cloverleaf skull, distinctive facial features, and variable hand and foot findings. The majority of patients with Saethre-Chotzen syndrome have loss-of-function mutations in *TWIST1* [2]. Mutations in these genes account for approximately 20% of cases of craniosynostosis.
MSX2	Craniosynostosis-2	Mutations in the *MSX2* gene are a very rare cause of craniosynostosis. A gain of function missense mutation in *MSX2* has been described in a few families with autosomal dominant "Boston type" craniosynostosis, whose features are highly variable [3].
TCF12	Craniosynostosis-3	Mutations in the *TCF12* gene occur predominantly in patients with coronal synostosis, accounting for 32% and 10% of individuals with bilateral and unilateral pathology, respectively [4].
ERF	Craniosynostosis-4	Exome sequencing identified a nonsense mutation in *ERF* in a family with craniosynostosis. Subsequent sequencing of *ERF* in 411 samples from unrelated individuals with craniosynostosis identified 8 heterozygous mutations in 11 additional families with synostosis of variable suture [5]
ZIC1	Craniosynostosis-6	Heterozygous truncating variants in *ZIC1* have been described in individuals with bicoronal synostosis with brachycephaly and learning disabilities [6].
DPH1	Developmental delay with short stature, dysmorphic features, and sparse hair	Homozygous mutations in *DPH1* have been identified in individuals with intellectual disability with short stature and craniofacial and ectodermal anomalies, including scaphocephaly with or without craniosynostosis and sparse hair on scalp, eyebrows and eyelashes [7].
FLNA	Oculopalatodigital spectrum disorders	Gain of function mutations in the X-linked gene *FLNA* are associated with a group of skeletal and connective tissue related disorders known as otopalatodigital spectrum disorders. Craniofacial abnormalities including hypertelorism, cleft palate, micrognathia, oligohypodontia, and downslanting

		palpebral fissures are frequently seen in affected individuals. Skeletal anomalies may include bowing of the long bones, overlapping fingers, and terminal osseous dysplasia. Some forms of OPDS are male lethal, while others, such as otopalatodigito syndrome type 1, result in a phenotype in both males and females, with females exhibiting a milder phenotype than males [8]. Craniosynostosis has also been reported in several patients [8-10].
GLI3	Greig cephalopolysyndactyly syndrome	Greig cephalopolysyndactyly syndrome is characterized by frontal bossing, scaphocephaly and hypertelorism associated with pre/postaxial polydactyly and variable syndactyly. The phenotype can also include craniosynostosis. Heterozygous *GLI3* mutations and deletions have been identified in patients with Greig cephalopolysyndactyly syndrome [11].
IL11RA	Craniosynostosis and dental anomalies	Homozygous mutations in *IL11RA* are associated with an autosomal recessive form of craniosynostosis with dental anomalies. The craniosynostosis associated with *IL11RA* mutations can involve the metopic, sagittal, coronal, and/or lambdoid sutures [12]. Dental anomalies include maxillary hypoplasia, delayed tooth eruption and supernumerary teeth [12]. Keupp, *et al.,*2013, noted that the features associated with mutations in *IL11RA* may exhibit significant clinical overlap with Crouzon syndrome [13].
POR	Antley-Bixler syndrome (ABS)	ABS is a rare craniosynostosis syndrome characterized by radiohumeral synostosis. Additional features include midface hypoplasia, choanal stenosis/atresia, multiple joint contractures, genitourinary anomalies and impaired steroidogenesis. Individuals with ABS and normal steroidogenesis can have *FGFR* mutations, while those with ABS and impaired steroidogenesis have mutations in *POR* [14].
RAB23 MEGF8	Carpenter syndrome	Carpenter syndrome is characterized by acrocephaly with variable synostosis. Additional clinical features include characteristic facies, brachydactyly of the hands with syndactyly, preaxial polydactyly and syndactyly of the feet, congenital heart defects, growth retardation, intellectual disability, hypogenitalism and obesity. Homozygous and compound heterozygous mutations in *RAB23* have been identified in patients with Carpenter syndrome type 1 [15]. Carpenter syndrome type 2 is caused by homozygous or compound heterozygous mutations in *MEGF8* [5].
RECQL4	Baller-Gerold syndrome	Cardinal features of Baller-Gerold syndrome, which has phenotypic overlap with Saethre-Chotzen syndrome, include craniosynostosis and radial aplasia. Mutations in *RECQL4* have been identified in patients with Baller-Gerold syndrome, as well as two additional overlapping conditions, Rothmund-Thompson syndrome and RAPADILINO syndrome [16].
SKI	Shprintzen-Goldberg syndrome	Shprintzen-Goldberg syndrome (SGS) is a connective tissue disorder characterized by craniosynostosis of the coronal, sagittal, or lambdoid sutures, in addition to Marfanoid body habitus and developmental delays. Individuals with SGS exhibit distinctive craniofacial features, including dolichocephaly, downslanting palpebral fissures, proptosis, and hypertelorism[17]. Approximately 90% of patients with a clinical diagnosis of SGS have a mutation in the *SKI* gene. To date, all reported mutations have been in exon 1 of the *SKI* gene[18, 19].
TGFBR1 TGFBR2	Loeys-Dietz syndrome	Loeys-Dietz syndrome (LDS) is a connective tissue disorder characterized by arterial aneurysms and/or dissections in addition to skeletal manifestations such as pectus abnormalities, scoliosis, and joint laxity [20]. Craniofacial findings in LDS can include widely spaced eyes, cleft palate, and craniosynostosis. Sagittal synostosis is the most common type of craniosynostosis associated with LDS, though coronal synostosis and metopic synostosis have also been described [20]. Approximately 90% of cases of LDS are caused by heterozygous mutations in *TGFBR1* or *TGFBR2.*

Cranioectodermal dysplasia

Cranioectodermal dysplasia is a multi-system ciliopathy characterized by skeletal involvement, ectodermal features, joint laxity, growth retardation and characteristic facial features. Craniosynostosis is a primary feature that distinguishes cranioectodermal dysplasia from other ciliopathies. Nephronophthisis is a major cause of morbidity and mortality. Brain malformations and developmental delay may also occur.

Gene	Clinical Features	Details
IFT122 WDR35 IFT43 WDR19	Cranioectodermal dysplasia 1,2,3,4	All mutations in individuals with cranioectodermal dysplasia occur in genes that encode members of the IFT-A hexamere protein complex. Most molecularly confirmed individuals have biallelic missense mutations that affect highly conserved nucelotides, or a combination of a missense mutation with a severe,

		truncating mutation. Mutations in *IFT122, WDR35, IFT43* and *WDR19* account for approximately 40% of affected individuals with cranioectodermal dysplasia

Frontonasal dysplasia

Frontonasal dysplasia is characterized by combinations of hypertelorism, abnormal nasal configuration, and oral, palatal, or facial clefting, sometimes associated with facial asymmetry, skin tags, ocular or cerebral malformations, widow's peak, and anterior cranium bifidum [21].

Gene	Clinical Features	Details
ALX3	Frontonasal dysplasia 1	Mutations in *ALX3* have been identified in patients with Frontonasal dysplasia 1. Missense, nonsense, frameshift and splice site mutations have been reported. Genotype-phenotype correlations have not been elucidated to date.
ALX4	Frontonasal dysplasia 2	A homozygous nonsense mutation in the *ALX4* gene was identified in a consanguineous family with Frontonasal dysplasia 2 [22]. ALX4 plays a critical role in craniofacial development as well as in skin and hair follicle development
ALX1	Frontonasal dysplasia 3	A homozygous splice site mutation in the *ALX1* gene was identified in a consanguineous Turkish family with Frontonasal dysplasia 3 [23]. ALX1 plays a crucial role in the early phase of chondrocyte development.
EFNB1	Craniofrontonasal syndrome	Craniofrontonasal syndrome is an X-linked developmental disorder that shows greater severity in heterozygous females than in hemizygous males. Females have frontonasal dysplasia, craniofacial asymmetry, craniosynostosis, bifid nasal tip, grooved nails, wiry hair, and abnormalities of the thoracic skeleton, whereas males typically show only hypertelorism. Mutations in *EFNB1* are identified in about 87% of cases of craniofrontonasal syndrome[24]

Other

Gene	Clinical Features	Details
FAM20C	Raine syndrome	Raine syndrome is a neonatal osteosclerotic bone dysplasia that typically results in early death. Radiographic studies show generalized increase in the density of all bones and a marked increase in the ossification of the skull, which underlies the characteristic facial features, and midface hypoplasia. Homozygous and compound heterozygous mutations in *FAM20C* have been identified in patients with Raine syndrome [25].
RUNX2	Cleidocranial dysplasia	Cleidocranial dysplasia is characterized by persistently open skull sutures with bulging calvaria, hypoplasia/aplasia of the clavicles, dental anomalies and vertebral malformations. Heterozygous loss of function mutations in *RUNX2* have been identified in patients with cleidocranial dysplasia [26]

Test methods:

Comprehensive sequence coverage of the coding regions and splice junctions of all genes in this panel is performed. Targets of interests are enriched and prepared for sequencing using the Agilent SureSelect system. Sequencing is performed using Illumina technology and reads are aligned to the reference sequence. Variants are identified and evaluated using a custom collection of bioinformatic tools and comprehensively interpreted by our team of directors and genetic counselors. All pathogenic and likely pathogenic variants are confirmed by Sanger sequencing. The technical sensitivity of this test is estimated to be >99% for single nucleotide changes and insertions and deletions of less than 20 bp.

Our CNV detection algorithm was developed and its performance determined for the sole purpose of identifying deletions and duplications within the coding region of the gene(s) tested. Partial exonic copy number changes and rearrangements of less than 400 bp may not be detected by this methodology. Regions of high homology and repetitive regions may not be analyzed. This methodology will not detect low level mosaicism, balanced translocations, inversions, or point mutations that may be responsible for the clinical phenotype. The sensitivity of our deletion/duplication assay may be reduced when DNA extracted by an outside laboratory is provided.

Craniofacial Panel (29 genes)

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	$3000
CPT codes:	81406 81407
Turn-around time:	8 weeks

Note: We cannot bill insurance for the above test.

Results:

Results, along with an interpretive report, will be faxed to the referring physician. Additional reports will be provided as requested. All abnormal results will be reported by telephone.

For more information about our testing options, please visit our website at dnatesting.uchicago.edu or contact us at 773-834-0555.

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