



Next Generation Sequencing Panels for Primordial Dwarfism

Clinical Features:

Primordial dwarfism (PD) is a group of conditions characterized by profound pre- and postnatal growth retardation. Most individuals with primordial dwarfism also have a reduction in head size in proportion to, or smaller than, their body size (1). Microcephalic primordial dwarfism is a heterogeneous group of disorders that include Seckel Syndrome, microcephalic osteodysplastic primordial dwarfism (MOPD) and Meier-Gorlin syndrome. Core features of these groups include severe intrauterine and postnatal growth deficiency, severe postnatal short stature, primary microcephaly, characteristic facial features and variable intellectual disability.

Seckel syndrome (OMIM#210600) is characterized by severe proportionally short stature with severe microcephaly (mean postnatal growth retardation is -7 SD with a range from -5 to -13 SD; mean OFC is -9SD with a range from -4 to -14 SD), a 'bird like' profile include a receding forehead, large eyes, beak-like protusion of the nose, narrow face, receding lower jaw and micrognathia, and intellectual disability (2, 3).

Microcephalic osteodysplastic primordial dwarfism type II (MOPD II, OMIM#210720) is differentiated from Seckel syndrome by more severe growth retardation, radiological abnormalities, and absent or mild mental retardation. The radiological abnormalities in MOPD II are short limbs with preferential distal involvement, coxa vara, epiphysiolysis and metaphyseal flaring with V-shaped distal femora metaphyses (2, 3). Typically, intellect is well preserved.

Microcephalic osteodysplastic primordial dwarfism type I (MOPD I, OMIM#210710) overlaps clinically with other types of primordial dwarfism, however it is differentiated by the presence of brain malformations and early lethality (4). There is considerable intra- and interfamilial phenotypic variability with regard to the type and severity of brain malformations, the degree of developmental delay, and total lifespan (4).

Meier-Gorlin syndrome is defined by absent/hypoplastic patellae and markedly small ears. Many cases have normal intellect with proportionate microcephaly. Growth failure is variable and can be mild (1).

3M syndrome is a form of primordial dwarfism characterized by severe pre- and postnatal growth retardation, distinctive facial features, and variable skeletal findings including prominent heels, long slender tubular bones, delayed bone age, winged scapulae, and joint hypermobility. Patients with 3M syndrome have normal intellectual development (5).

Our Seckel Syndrome Sequencing and Deletion/Duplication Panels include analysis of the 11 genes listed below.

Our Meier-Gorlin Syndrome Sequencing Deletion/Duplication Panels include analysis of the 6 genes listed below.

Our 3-M Syndrome Sequencing Panel includes analysis of the 3 genes listed below.

Our Comprehensive Primordial Dwarfism Sequencing and Deletion/Duplication Panels includes analysis of the 28 genes listed below.

Seckel Syndrome		Meier-Gorlin Syndrome	3M Syndrome	MOPD I & II	Primordial Dwarfism	
ATR	LIG4	CDC6	CCDC8	PCNT	CRIP1	XRCC4
CDK5RAP2	NIN	CDT1	CUL7	RNU4ATAC	LARP7	
CENPJ	PCNT	GMNN	OBSL1		POC1A	
CEP152	RBBP8	ORC1			PLK4	
CEP63	TRAIP	ORC4			RTTN	
DNA2		ORC6			TRIM37	

Gene	Molecular Pathology
ATR [OMIM#601215] CDK5RAP2 [OMIM#604804] CENPJ [OMIM#609279] CEP63 [OMIM#614728] CEP152	Several genes have been reported to cause Seckel syndrome in a small number of families: <ul style="list-style-type: none"> ➤ A homozygous synonymous mutation in <i>ATR</i> has been identified in two consanguineous Pakistani families with Seckel syndrome (6). <i>ATR</i> is a central player in a signaling response to DNA damage. ➤ Homozygous splice site mutations in <i>CDK5RAP2</i> have been identified in two consanguineous families with a mild form of Seckel syndrome (7). <i>CDK5RAP2</i> deficiency in primary fibroblasts showed severe alterations of centrosomal structures and abnormalities of cell morphology during interphase (7).

<p>[OMIM#613529] <i>NIN</i> [OMIM#608684] <i>RBBP8</i> [OMIM#604124] <i>TRAIP</i> [OMIM#605958]</p>	<ul style="list-style-type: none"> ➤ A homozygous splicing mutation in <i>CENPJ</i> has been identified in a consanguineous Saudi Arabian family with Seckel syndrome (8). <i>CENPJ</i> is a centrosomal protein and may be involved in microtubule production during mitosis (9). ➤ A homozygous nonsense mutation was identified in <i>CEP63</i> in a consanguineous family of Pakistani descent with three members with primary microcephaly and proportionate short stature, clinically consistent with mild Seckel syndrome (10). The <i>CEP63</i> protein forms a complex with <i>CEP152</i>, and helps to maintain normal centrosome numbers within cells (10). ➤ Homozygous and heterozygous mutations in <i>CEP152</i> have been identified in Turkish, Italian and South African families with Seckel syndrome (11). Splicing, frameshift and missense mutations have been reported. <i>CEP152</i> is a core protein of the centrosome. ➤ Compound heterozygous missense mutations have been identified in the <i>NIN</i> gene in 2 sisters with Seckel syndrome (12). Ninein is a centrosomal protein required for the centrosome to function as a microtubule-organizing center. ➤ A homozygous splicing mutation in <i>RBBP8</i> was identified in four siblings affected by Seckel syndrome from a consanguineous Iraqi family (13). The <i>RBBP8</i> protein (also known as CtlP) is involved in the process of DNA double-strand break repair (13). ➤ Harley, <i>et al.</i>, 2016, identified a homozygous nonsense mutation in the <i>TRAIP</i> gene in two patients with Seckel syndrome. A homozygous missense mutation was identified in a third patient with Seckel syndrome (14). <p><i>CENPJ</i> and <i>CEP152</i> are also genes implicated in autosomal recessive primary microcephaly (MCPH). <i>RBBP8</i> mutations have also been implicated in Jawad syndrome [OMIM#251255], which has clinical overlap with Seckel syndrome.</p>
<p><i>CRIP1</i> [OMIM# 604594]</p>	<p>Homozygous mutations in the <i>CRIP1</i> gene have been reported in two affected individuals with features of primordial dwarfism, including prenatal and postnatal growth deficiency and microcephaly (15). The patients also had facial dysmorphisms including frontal bossing, high forehead and sparse hair and eyebrows (15).</p>
<p><i>DNA2</i> [OMIM# 601810]</p>	<p>A homozygous mutation in <i>DNA2</i> has been described in one consanguineous family with features of Seckel syndrome, including growth restriction with prenatal onset, and severe microcephaly (15).</p>
<p><i>LARP7</i> [OMIM#612026]</p>	<p>Alazami <i>et al.</i>, 2012 identified a homozygous 7bp duplication in <i>LARP7</i> in a consanguineous Saudi family with facial dysmorphism, intellectual disability and primordial dwarfism (16). All reported patients had growth parameters at least 3.5 SD below the mean. Consistent dysmorphic features included malar hypoplasia, deep-set eyes, broad nose, short philtrum and macrostomia.</p>
<p><i>LIG4</i> [OMIM#601837]</p>	<p>Biallelic truncating <i>LIG4</i> mutations have been identified in a series of 11 patients with microcephalic primordial dwarfism (17). The position of truncating mutations has been observed to correlate with phenotypic severity (17). <i>LIG4</i> mutations have been recurrently reported in Ligase IV syndrome (OMIM#606593), originally identified in a typically-developing 14-year-old with acute lymphoblastic leukemia (18). Bone marrow failure has occurred in 70% of patients reported to date (17). Other patients with <i>LIG4</i> syndrome are described as having immunodeficiency, developmental delay, Seckel or “bird-like” facies, microcephaly, and growth restriction (19).</p>
<p><i>ORC1</i> [OMIM#601902] <i>ORC4</i> [OMIM#603056] <i>ORC6</i> [OMIM#607213] <i>CDT1</i> [OMIM#605525] <i>CDC6</i> [OMIM#602627] <i>GMNN</i> [OMIM#602842]</p>	<p>Mutations in <i>ORC1</i> have been identified in 4/33 individuals with Meier-Gorlin syndrome (MGS) (20). Mutation analysis of other genes of this pre-replication complex showed mutations in <i>ORC4</i>, <i>ORC6</i>, <i>CDT1</i> and <i>CDC6</i> in 14 individuals from nine families with MGS (21). Guernsey <i>et al</i> identified mutations in <i>ORC1</i>, <i>ORC4</i> and <i>CDT1</i> in 8 individuals from five families with MGS (21). While most affected individuals described had typical features of MGS, a considerable wide phenotypic variation was observed and no clear genotype-phenotype correlation has been elucidated. Mutations in genes from the pre-replication complex are expected to disturb the process of DNA replication (22). Heterozygous, <i>de novo</i> mutations in <i>GMNN</i> have also been identified in three patients with MGS (23). All three mutations reported to date were identified in exon 2. The <i>GMNN</i> gene encodes geninin, which is a DNA replication inhibitor that interacts with <i>CDT1</i> (23).</p>
<p><i>CCDC8</i> [OMIM#614145] <i>CUL7</i> [OMIM#609577] <i>OBSL1</i> [OMIM#610991]</p>	<p>3-M syndrome, which is named for the initials of the first three authors to describe the condition, is characterized by pre- and postnatal growth deficiency, distinctive craniofacial features, and skeletal abnormalities including slender long bones, tall vertebral bodies, and short thorax with pectus carinatum or pectus excavatum (24). Affected individuals have normal intellect. 3-M syndrome is caused by homozygous or compound heterozygous mutations in one of three genes: <i>CCDC8</i>, <i>CUL7</i>, or <i>OBSL1</i>. <i>CUL7</i> assembles an E3 ubiquitin ligase complex and promotes ubiquitination (25). <i>OBSL1</i> acts as a cytoskeletal adaptor. Hanson <i>et al.</i>, 2009, demonstrated that loss of <i>OBSL1</i> leads to downregulation of <i>CUL7</i> (26). <i>CCDC8</i> is a protein of undefined function that has been shown to interact with <i>OBSL1</i> (25).</p>

<i>PCNT</i> [OMIM#605925]	Mutations in <i>PCNT</i> have been identified in patients with MOPD II/Seckel syndrome. <i>PCNT</i> is located at 21q22.3 and studies have shown that the absence of <i>PCNT</i> results in disorganized mitotic spindles and missegregation of chromosomes. Rauch <i>et al.</i> (2008) identified 29 different homozygous or compound heterozygous mutations in the <i>PCNT</i> gene in 25 patients with MOPD II (27). Williams <i>et al.</i> (2010) identified 12 homozygous and 1 heterozygous mutation in the <i>PCNT</i> gene in 8/8 patients with MOPDII and 5/16 patients diagnosed with Seckel syndrome (3). Clinical analysis of Seckel cases with <i>PCNT</i> mutations showed that they all presented with minor skeletal changes and clinical features compatible with a MOPDII diagnosis.
<i>PLK4</i> [OMIM# 616171]	<i>PLK4</i> mutations have been reported in families with features of primordial dwarfism, including severe primary microcephaly and growth retardation (28). Patients can have ocular abnormalities including microphthalmia, microcornea, and cataracts.
<i>POC1A</i> [OMIM#614783]	A homozygous <i>POC1A</i> nonsense mutation has been identified in 5 children from 3 consanguineous Saudi families with primordial dwarfism and distinctive facial features (SOFT syndrome, OMIM#614813) (29). SOFT syndrome is characterized by severely short long bones, characteristic facies with paucity of hair and nail anomalies. Facial dysmorphism include a long triangular face with prominent nose and small ears. Affected individuals have an unusual high-pitched voice.
<i>RNU4ATAC</i> [OMIM#210710]	Mutations in <i>RNU4ATAC</i> have been identified in multiple families with MOPD I, including one large Amish cohort with a founder mutation [9]. <i>RNU4ATAC</i> encodes U4atac, which is a small nuclear RNA that is a crucial component of the minor spliceosome, and is required for proper excision of the U12-dependent class of introns, which are found in many essential genes (30).
<i>RTTN</i> [OMIM#]	Shamseldin, <i>et al.</i> , 2015, identified a homozygous non-canonical splicing variant in <i>RTTN</i> in a consanguineous family with primary microcephaly and severe growth retardation (31). Several additional families have subsequently been identified with homozygous or compound heterozygous sequence changes in <i>RTTN</i> (31-33). Reported affected individuals have severe microcephaly with head circumference between -4.4 and -11 SD. Severe growth retardation is evident pre- and postnatally. Affected individuals additionally have variable brain malformations including pachygyria, polymicrogyria, lissencephaly, cerebral hypoplasia, and cerebellar hypoplasia (31-33).
<i>TRIM37</i> [OMIM#605073]	Mulibrey (MUscle, LIver, BRain, EYes) nanism is a rare form of primordial dwarfism characterized by prenatal onset progressive growth failure, hypotonia, constrictive pericarditis, and hepatomegaly. Yellow discoloration in the eyes is typically present. Most affected individuals have normal intelligence. Homozygous or compound heterozygous mutations in <i>TRIM37</i> have been identified in patients with Mulibrey nanism (34).
<i>XRCC4</i> [OMIM#194363]	Murray <i>et al.</i> , 2015, identified homozygous or compound heterozygous mutations in <i>XRCC4</i> in six patients with microcephalic primordial dwarfism. The majority of mutations reported were protein truncating. Patients exhibited pre- and postnatal growth retardation and microcephaly. Postnatally patients developed severe microcephaly, with a median head circumference of -8.15SD. Some but not all patients exhibited developmental delays (35).

Inheritance:

The majority of the genes listed above exhibit autosomal recessive inheritance. The exception is *GMNN*-related Meier-Gorlin syndrome, which is due to heterozygous, typically *de novo* mutations in *GMNN*.

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.

Deletion/duplication analysis is available for 14 of the genes listed above and is performed by oligonucleotide array-CGH. Partial exonic copy number changes and rearrangements of less than 400 bp may not be detected by array-CGH. Array-CGH will not detect low-level mosaicism, balanced translocations, inversions, or point mutations that may be responsible for the clinical phenotype. The sensitivity of this assay may be reduced when DNA is extracted by an outside laboratory.

Comprehensive Primordial Dwarfism Sequencing Panel (28 genes sequence analysis)

Sample specifications:	3 to 10 cc of blood in a purple top (EDTA) tube
Cost:	\$2000
CPT codes:	81407
Turn-around time:	8 weeks

Note: We cannot bill insurance for this test.

Comprehensive Primordial Dwarfism Deletion/Duplication Panel (28 genes deletion/duplication analysis)

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	\$1545
CPT codes:	81407
Turn-around time:	6 weeks

Seckel Syndrome Sequencing Panel (11 genes sequence analysis)

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	\$2000
CPT codes:	81407
Turn-around time:	8 weeks

Note: We cannot bill insurance for this test.

Seckel Syndrome Deletion/Duplication Panel (11 genes sequence analysis)

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	\$1545
CPT codes:	81407
Turn-around time:	6 weeks

Meier-Gorlin Syndrome Sequencing Panel (6 genes sequence analysis)

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	\$2000
CPT codes:	81407
Turn-around time:	8 weeks

Note: We cannot bill insurance for this test.

Meier-Gorlin Syndrome Deletion/Duplication Panel (6 genes deletion/duplication analysis)

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	\$1545
CPT codes:	81407
Turn-around time:	6 weeks

3M Syndrome Sequencing Panel (3 genes sequence analysis)

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	\$1500
CPT codes:	81407
Turn-around time:	8 weeks

Note: We cannot bill insurance for this test.

Results:

Results, along with an interpretive report, are faxed to the referring physician as soon as they are completed. Additional reports are available as requested. All abnormal results are 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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