THE UNIVERSITY OF CHICAGO | Genetic Services Laboratories

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 Panel includes mutation analysis of the 11 genes listed below.
Our Meier-Gorlin Syndrome Panels includes mutation analysis of the 6 genes listed below.
Our 3-M Syndrome Sequencing Panel includes mutation analysis of the 3 genes listed below.
Our Primordial Dwarfism Panel includes mutation analysis of the 28 genes listed below.

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

Gene	Molecular Pathology
ATR [OMIM#601215] *CDK5RAP2* [OMIM#604804] *CENPJ* [OMIM#609279] *CEP63* [OMIM#614728] *CEP152* [OMIM#613529]	Several genes have been reported to cause Seckel syndrome in a small number of families: ➢ A homozygous synonymous mutation in *ATR* has been identified in two consanguineous Pakistani families with Seckel syndrome (6). *ATR* is a central player in a signaling response to DNA damage. ➢ Homozygous splice site mutations in *CDK5RAP2* have been identified in two consanguineous families with a mild form of Seckel syndrome (7). CDK5RAP2 deficiency in primary fibroblasts showed severe alterations of centrosomal structures and abnormalities of cell morphology during interphase (7). ➢ A homozygous splicing mutation in *CENPJ* has been identified in a consanguineous

NIN [OMIM#608684] *RBBP8* [OMIM#604124] *TRAIP* [OMIM#605958]	Saudi Arabian family with Seckel syndrome (8). CENPJ is a centrosomal protein and may be involved in microtubule production during mitosis (9). ➢ A homozygous nonsense mutation was identified in *CEP63* 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 CEP63 protein forms a complex with CEP152, and helps to maintain normal centrosome numbers within cells (10). ➢ Homozygous and heterozygous mutations in *CEP152* have been identified in Turkish, Italian and South African families with Seckel syndrome (11). Splicing, frameshift and missense mutations have been reported. CEP152 is a core protein of the centrosome. ➢ Compound heterozygous missense mutations have been identified in the *NIN* 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 *RBBP8* was identified in four siblings affected by Seckel syndrome from a consanguinous Iraqi family (13). The RBBP8 protein (also known as CtIP) is involved in the process of DNA double-strand break repair (13). ➢ Harley, *et al.*, 2016, identified a homozygous nonsense mutation in the *TRAIP* gene in two patients with Seckel syndrome. A homozygous missense mutation was identified in a third patient with Seckel syndrome (14). *CENPJ* and *CEP152* are also genes implicated in autosomal recessive primary microcephaly (MCPH). *RBBP8* mutations have also been implicated in Jawad syndrome [OMIM#251255], which has clinical overlap with Seckel syndrome.
CRIPT [OMIM# 604594]	Homozygous mutations in the *CRIPT* 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).
DNA2 [OMIM# 601810]	A homozygous mutation in *DNA2* has been described in one consanguineous family with features of Seckel syndrome, including growth restriction with prenatal onset, and severe microcephaly (15).
LARP7 [OMIM#612026]	Alazami *et al*, 2012 identified a homozygous 7bp duplication in *LARP7* 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.
LIG4 [OMIM#601837]	Biallelic truncating *LIG4* 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). *LIG4* 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 occured in 70% of patients reported to date (17). Other patients with LIG4 syndrome are described as having immunodeficiency, developmental delay, Seckel or “bird-like” facies, microcephaly, and growth restriction (19).
ORC1 [OMIM#601902] *ORC4* [OMIM#603056] *ORC6* [OMIM#607213] *CDT1* [OMIM#605525] *CDC6* [OMIM#602627] *GMNN* [OMIM#602842]	Mutations in *ORC1* 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 *ORC4, ORC6, CDT1 and CDC6* in 14 individuals from nine families with MGS (21). Guernsey *et al* identified mutations in *ORC1, ORC4* and *CDT1* 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, *de novo* mutations in *GMNN* have also been identified in three patients with MGS (23). All three mutations reported to date were identified in exon 2. The *GMNN* gene encodes geninin, which is a DNA replication inhibitor that interacts with CDT1 (23).
CCDC8 [OMIM#614145] *CUL7* [OMIM#609577] *OBSL1* [OMIM#610991]	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: *CCDC8*, *CUL7*, or *OBSL1.* CUL7 assembles an E3 ubiquitin ligase complex and promotes ubiquitination (25). OBSL1 acts as a cytoskeletal adaptor. Hanson *et al.*, 2009, demonstrated that loss of OBSL1 leads to downregulation of CUL7 (26). CCDC8 is a protein of undefined function that has been shown to interact with OBSL1 (25).

PCNT [OMIM#605925]	Mutations in *PCNT* have been identified in patients with MOPD II/Seckel syndrome. *PCNT* is located at 21q22.3 and studies have shown that the absence of PCNT results in disorganized mitotic spindles and missegregation of chromosomes. Rauch *et al.* (2008) identified 29 different homozygous or compound heterozygous mutations in the *PCNT* gene in 25 patients with MOPD II (27). Williams *et al* (2010) identified 12 homozygous and 1 heterozygous mutation in the *PCNT* gene in 8/8 patients with MOPDII and 5/16 patients diagnosed with Seckel syndrome (3). Clinical analysis of Seckel cases with *PCNT* mutations showed that they all presented with minor skeletal changes and clinical features compatible with a MOPDII diagnosis.
PLK4 [OMIM# 616171]	*PLK4* 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.
POC1A [OMIM#614783]	A homozygous *POC1A* 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.
RNU4ATAC [OMIM#210710]	Mutations in *RNU4ATAC* have been identified in multiple families with MOPD I, including one large Amish cohort with a founder mutation [9]. *RNU4ATAC* 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).
RTTN [OMIM#	Shamseldin, *et al.*, 2015, identified a homozygous non-canonical splicing variant in *RTTN* 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 *RTTN* (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).
TRIM37 [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 *TRIM37* have been identified in patients with Mulibrey nanism (34).
XRCC4 [OMIM#194363]	Murray *et al.*, 2015, identified homozygous or compound heterozygous mutations in *XRCC4* 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.

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.

Comprehensive Primordial Dwarfism Panel (28 genes)

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	$2000
CPT codes:	81406
	81407

Turn-around time: 8 weeks
Note: We cannot bill insurance for this test.

Seckel Syndrome Panel (11 genes)
Sample specifications: 3 to10 cc of blood in a purple top (EDTA) tube
Cost: $2000
CPT codes: 81406
81407
Turn-around time: 8 weeks
Note: We cannot bill insurance for this test.

Meier-Gorlin Syndrome Panel (6 genes)
Sample specifications: 3 to10 cc of blood in a purple top (EDTA) tube
Cost: $2000
CPT codes: 81406
81407
Turn-around time: 8 weeks
Note: We cannot bill insurance for this test.

3M Syndrome Panel (3 genes)
Sample specifications: 3 to10 cc of blood in a purple top (EDTA) tube
Cost: $1500
CPT codes: 81406
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.

References:

1. Klingseisen A, Jackson AP. Mechanisms and pathways of growth failure in primordial dwarfism. Genes Dev 2011: 25: 2011-2024.
2. Faivre L, Cormier-Daire V. Seckel Syndrome. Orphanet encyclopedia, 2005.
3. Willems M, Geneviève D, Borck G et al. Molecular analysis of pericentrin gene (PCNT) in a series of 24 Seckel/microcephalic osteodysplastic primordial dwarfism type II (MOPD II) families. J Med Genet 2010: 47: 797-802.
4. Abdel-Salam GM, Abdel-Hamid MS, Issa M et al. Expanding the phenotypic and mutational spectrum in microcephalic osteodysplastic primordial dwarfism type I. Am J Med Genet A 2012: 158A: 1455-1461.
5. Clayton PE, Hanson D, Magee L et al. Exploring the spectrum of 3-M syndrome, a primordial short stature disorder of disrupted ubiquitination. Clin Endocrinol (Oxf) 2012: 77: 335-342.
6. O'Driscoll M, Ruiz-Perez VL, Woods CG et al. A splicing mutation affecting expression of ataxia-telangiectasia and Rad3-related protein (ATR) results in Seckel syndrome. Nat Genet 2003: 33: 497-501.
7. Yigit G, Brown KE, Kayserili H et al. Mutations in CDK5RAP2 cause Seckel syndrome. Mol Genet Genomic Med 2015: 3: 467-480.
8. Al-Dosari MS, Shaheen R, Colak D et al. Novel CENPJ mutation causes Seckel syndrome. J Med Genet 2010: 47: 411-414.
9. Woods CG, Bond J, Enard W. Autosomal recessive primary microcephaly (MCPH): a review of clinical, molecular, and evolutionary findings. Am J Hum Genet 2005: 76: 717-728.
10. Sir JH, Barr AR, Nicholas AK et al. A primary microcephaly protein complex forms a ring around parental centrioles. Nat Genet 2011: 43: 1147-1153.
11. Kalay E, Yigit G, Aslan Y et al. CEP152 is a genome maintenance protein disrupted in Seckel syndrome. Nat Genet 2011: 43: 23-26.
12. Dauber A, Lafranchi SH, Maliga Z et al. Novel microcephalic primordial dwarfism disorder associated with variants in the centrosomal protein ninein. J Clin Endocrinol Metab 2012: 97: E2140-2151.
13. Qvist P, Huertas P, Jimeno S et al. CtIP Mutations Cause Seckel and Jawad Syndromes. PLoS Genet 2011: 7: e1002310.
14. Harley ME, Murina O, Leitch A et al. TRAIP promotes DNA damage response during genome replication and is mutated in primordial dwarfism. Nat Genet 2016: 48: 36-43.
15. Shaheen R, Faqeih E, Ansari S et al. Genomic analysis of primordial dwarfism reveals novel disease genes. Genome Res 2014: 24: 291-299.
16. Alazami AM, Al-Owain M, Alzahrani F et al. Loss of function mutation in LARP7, chaperone of 7SK ncRNA, causes a syndrome of facial dysmorphism, intellectual disability, and primordial dwarfism. Hum Mutat 2012: 33: 1429-1434.

17. Murray JE, Bicknell LS, Yigit G et al. Extreme growth failure is a common presentation of ligase IV deficiency. Hum Mutat 2014: 35: 76-85.
18. Riballo E, Critchlow SE, Teo SH et al. Identification of a defect in DNA ligase IV in a radiosensitive leukaemia patient. Curr Biol 1999: 9: 699-702.
19. O'Driscoll M, Cerosaletti KM, Girard PM et al. DNA ligase IV mutations identified in patients exhibiting developmental delay and immunodeficiency. Mol Cell 2001: 8: 1175-1185.
20. Bicknell LS, Walker S, Klingseisen A et al. Mutations in ORC1, encoding the largest subunit of the origin recognition complex, cause microcephalic primordial dwarfism resembling Meier-Gorlin syndrome. Nat Genet 2011: 43: 350-355.
21. Bicknell LS, Bongers EM, Leitch A et al. Mutations in the pre-replication complex cause Meier-Gorlin syndrome. Nat Genet 2011: 43: 356-359.
22. Guernsey DL, Matsuoka M, Jiang H et al. Mutations in origin recognition complex gene ORC4 cause Meier-Gorlin syndrome. Nat Genet 2011: 43: 360-364.
23. Burrage LC, Charng WL, Eldomery MK et al. De Novo GMNN Mutations Cause Autosomal-Dominant Primordial Dwarfism Associated with Meier-Gorlin Syndrome. Am J Hum Genet 2015: 97: 904-913.
24. Holder-Espinasse M. 3-M Syndrome. In: Pagon R, Adam M, Ardinger H, eds. GeneReviews [Internet]. Seattle, WA: University of Washington, Last update 2012.
25. Hanson D, Murray PG, Black GC et al. The genetics of 3-M syndrome: unravelling a potential new regulatory growth pathway. Horm Res Paediatr 2011: 76: 369-378.
26. Hanson D, Murray PG, Sud A et al. The primordial growth disorder 3-M syndrome connects ubiquitination to the cytoskeletal adaptor OBSL1. Am J Hum Genet 2009: 84: 801-806.
27. Rauch A, Thiel CT, Schindler D et al. Mutations in the pericentrin (PCNT) gene cause primordial dwarfism. Science 2008: 319: 816-819.
28. Shaheen R, Al Tala S, Almoisheer A et al. Mutation in PLK4, encoding a master regulator of centriole formation, defines a novel locus for primordial dwarfism. J Med Genet 2014: 51: 814-816.
29. Shaheen R, Faqeih E, Shamseldin HE et al. POC1A truncation mutation causes a ciliopathy in humans characterized by primordial dwarfism. Am J Hum Genet 2012: 91: 330-336.
30. Nagy R, Wang H, Albrecht B et al. Microcephalic osteodysplastic primordial dwarfism type I with biallelic mutations in the RNU4ATAC gene. Clin Genet 2012: 82: 140-146.
31. Shamseldin H, Alazami AM, Manning M et al. RTTN Mutations Cause Primary Microcephaly and Primordial Dwarfism in Humans. Am J Hum Genet 2015: 97: 862-868.
32. Rump P, Jazayeri O, van Dijk-Bos KK et al. Whole-exome sequencing is a powerful approach for establishing the etiological diagnosis in patients with intellectual disability and microcephaly. BMC Med Genomics 2016: 9: 7.
33. Grandone A, Torella A, Santoro C et al. Expanding the phenotype of RTTN variations: a new family with primary microcephaly, severe growth failure, brain malformations and dermatitis. Clin Genet 2016: 90: 445-450.
34. Karlberg N, Jalanko H, Lipsanen-Nyman M. Growth and growth hormone therapy in subjects with mulibrey nanism. Pediatrics 2007: 120: e102-111.
35. Murray JE, van der Burg M, IJspeert H et al. Mutations in the NHEJ component XRCC4 cause primordial dwarfism. Am J Hum Genet 2015: 96: 412-424.