

Abnormal Mineralization Panel

Disorder also known as: Chondrocalcinosis 2; Dent disease; Familial hypocalciuric hypercalcemia, type 1; Hypocalcemia; Hypophosphatasia; Neonatal severe hyperparathyroidism; Hypophosphatemic nephrolithiasis/osteoporosis; Hypophosphatemic rickets; Tyrosinemia; Vitamin D-dependent rickets

Abnormal Mineralization Panel Gene List: *ALPL, ANKH, AP2S1, CASR, CLCN5, CYP27B1, CYP2R1, DMP1, ENPP1, FAH, FGF23, GNA11, PHEX, SLC34A1, SLC34A3, SLC9A3R1, VDR*

Clinical Features:

Abnormal mineralization disorders result from defects in the uptake of calcium and phosphorus by bones and teeth. The two main kinds of mineralization disorders are hypophosphatasia and hypophosphatemia, also called hypophosphatemic rickets. Hypophosphatasia is a rare genetic skeletal dysplasia that occurs due to pathogenic variants in the *ALPL* gene that result in loss of function of alkaline liver phosphatase. Hypophosphatasia can be inherited in an autosomal dominant or autosomal recessive manner. Clinical features of hypophosphatasia range from severe perinatal onset of respiratory insufficiency and hypercalcemia to adult onset of stress fractures and pseudofractures in middle age.¹ Hypophosphatasia can also present as odontohypophosphatasia, which includes premature loss of primary teeth and dental caries without other skeletal involvement.¹ Low serum alkaline phosphatase levels suggest a diagnosis of hypophosphatasia.

Hypophosphatemia results from low levels of phosphate in the blood, which can cause rickets and osteomalacia. It may be present with or without hypercalcemia. Hypophosphatemia is a disorder that causes insufficient resorption of phosphate in the renal tubule, leading to low bone density and clinical findings such as bowing of the long bones, bone pain, muscle weakness short stature, craniosynostosis, and extraskelton ossification.² Secondary complications may occur involving hyperparathyroidism, hypercalcemia and hypercalciuria, and nephrocalcinosis. Variants in the *CLCN5, CYP27B1, CYP2R1, DMP1, ENPP1, FGF23, PHEX, SLC34A3*, and *VDR* genes are associated with various types of hypophosphatemic rickets.²⁻¹¹ The most common form of hypophosphatemic rickets is inherited in an X-linked manner and is caused by variants in the *PHEX* gene. Hypophosphatemic rickets and osteoporosis can also be inherited in an autosomal recessive or autosomal dominant manner due to dysregulation of calcium and/or phosphate levels, resulting in decreased bone mineralization.² Individuals with tyrosinemia type I caused by variants in the *FAH* gene can also develop rickets secondary to renal dysfunction.¹²

Familial hypocalciuric hypercalcemia is an autosomal dominant condition characterized by mild lifelong elevated serum calcium levels, hypermagnesemia, normal parathyroid hormone, and a decreased renal calcium/creatinine clearance ratio.¹³⁻¹⁵ Pathogenic variants in the *CASR*, *AP2S1*, and *GNA11* genes result in familial hypocalciuric hypercalcemia.¹³⁻¹⁵ *CASR* variants may also result in neonatal hyperparathyroidism which may be autosomal dominant or autosomal recessive in inheritance. Neonatal hyperparathyroidism is a life-threatening condition characterized by marked symptomatic hypercalcemia, bony changes due to hyperparathyroidism, and neurodevelopmental deficits if these symptoms go untreated.¹⁷ Gain of function *CASR* and *GNA11* variants have been associated with autosomal dominant hypocalcemia which results in mild hypocalcemia and few other symptoms, though seizures and hyperphosphatemia have been reported.^{15,17}

Chondrocalcinosis, also known as calcium pyrophosphate dehydrate deposition disease, is a condition characterized by joint pain and arthritis due to calcium deposition in the cartilage of joints. Pathogenic variants in the *ANKH* gene have been reported in association with chondrocalcinosis.¹⁸⁻¹⁹

Hypophosphatemic nephrolithiasis/osteoporosis types 1 and 2 are autosomal dominant conditions that result from pathogenic variants in the *SLC34A1* and *SLC9A3R1* genes, respectively.²⁰⁻²¹

Genetics:

Autosomal dominant, autosomal recessive, and X-linked

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. 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.

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.

Test Sensitivity:

The clinical sensitivity of the Abnormal Mineralization Panel depends in part on the patient's clinical phenotype. The clinical sensitivity for *ALPL* variants associated with hypophosphatasia is approximately 95%.¹ Variants in the *PHEX* gene account for up to 84% of cases with hypophosphatemic rickets.⁹ It is currently unknown what proportion of individuals with a clinical diagnosis of hypophosphatemic rickets is a result of a pathogenic variant in the *CLCN5*, *CYP27B1*, *CYP2R1*, *DMP1*, *ENPP1*, *FGF23* and *SLC34A3*, *VDR* genes.³⁻¹¹ However, about 60% of individuals with a clinical diagnosis of Dent disease will have an identifiable *CLCN5* variant.²²

Among patients with clinical features suggestive of familial hypocalciuric hypercalcemia (FHH), neonatal hyperparathyroidism (NSHPT), or autosomal dominant hypocalcemic (ADH) disorders, the overall *CASR* mutation detection rate is 29% (26% in FHH and NSHPT; 41% in ADH).²³ Arg15 is a likely mutational hotspot, and *AP2S1* missense variants located at this residue are detectable in more than 20% of FHH patients who are negative for *CASR* pathogenic variants.^{14,24} As few cases have been documented in the literature, the detection rate for pathogenic variant in *GNA11* among individuals with FHH or ADH is unknown.

Among patients with clinical features suggestive of tyrosinemia type I, greater than 95% will have an identifiable variant in the *FAH* gene.¹²

The clinical sensitivity for *ANKH* variants associated with chondrocalcinosis 2 is unknown.¹⁸

Variants in *SLC34A1* and *SLC9A3R1* associated with hypophosphatemic nephrolithiasis/osteoporosis are rare, and therefore, the clinical sensitivity is also unknown.^{20,21,25}

Gene	Protein	Inheritance	Disease Associations
<i>ALPL</i> ¹	ALKALINE PHOSPHATASE, LIVER	AD/AR	Hypophosphatasia
<i>ANKH</i> ¹⁸	ANKH INORGANIC PYROPHOSPHATE TRANSPORT REGULATOR	AD	Chondrocalcinosis 2 Craniometaphyseal dysplasia
<i>AP2S1</i> ¹⁴	ADAPTOR-RELATED PROTEIN COMPLEX 2, SIGMA-1 SUBUNIT	AD	Hypocalciuric hypercalcemia, type III

<i>CASR</i> ¹³⁻¹⁷	CALCIUM SENSING RECEPTOR	AD/AR	Hypocalciuric hypercalcemia, familial, type 1 Hypocalcemia, autosomal dominant 1 Hypocalcemia, autosomal dominant 1 with Bartter syndrome Hyperparathyroidism, neonatal severe
<i>CLCN5</i> ³	CHLORIDE VOLTAGE-GATED CHANNEL 5	XLR	Dent disease 1, Hypophosphatemic rickets Nephrolithiasis, type I Proteinuria, low molecular weight, with hypercalciuric nephrocalcinosis
<i>CYP27B1</i> ⁴	CYTOCHROME P450 FAMILY 27 SUBFAMILY B MEMBER 1	AR	Vitamin D-dependent rickets, type 1A
<i>CYP2R1</i> ⁵	CYTOCHROME P450 FAMILY 2 SUBFAMILY R MEMBER 1	AR	Vitamin D-dependent rickets, type 1B
<i>DMP1</i> ⁶	DENTIN MATRIX ACIDIC PHOSHOPROTEIN 1	AR	Hypophosphatemic rickets, autosomal recessive, 1
<i>ENPP1</i> ⁷	ECTONUCLEOTIDE PYROPHOSPHATASE/PHOSPHO DIESTERASE 1	AR	Hypophosphatemic rickets, autosomal recessive, 2 Arterial calcification, generalized, of infancy
<i>FAH</i> ¹²	FUMARYLACETOACETATE HYDROLASE	AR	Tyrosinemia, type 1
<i>FGF23</i> ⁸	FIBROBLAST GROWTH FACTOR 23	AD/AR	Hypophosphatemic rickets, autosomal dominant Tumoral calcinosis, hyperphosphatemic, familial
<i>GNA11</i> ¹⁵	GUANINE NUCLEOTIDE-BINDING PROTEIN, ALPHA-11	AD	Hypocalcemia, autosomal dominant 2 Hypocalciuric hypercalcemia, type II
<i>PHEX</i> ⁹	PHOSPHATE REGULATING ENDOPEPTIDASE HOMOLOG, X-LINKED	XLD	Hypophosphatemic rickets, X-linked dominant
<i>SLC34A1</i> ²⁰	SOLUTE CARRIER FAMILY 34 MEMBER 1	AD/AR	Nephrolithiasis/osteoporosis, hypophosphatemic, 1 Hypercalcemia, infantile, 2 Fanconi renal tubular syndrome 2
<i>SLC34A3</i> ¹⁰	SOLUTE CARRIER FAMILY 34 MEMBER 3	AR	Hypophosphatemic rickets with hypercalciuria, hereditary
<i>SLC9A3R1</i> ²¹	SLC9A3 REGULATOR 1	AD	Nephrolithiasis/osteoporosis, hypophosphatemic, 2

<i>VDR</i> ¹¹	VITAMIN D (1,25-DIHYDROXYVITAMIN D3) RECEPTOR	AR	Vitamin D-dependent rickets, type 2A
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Abbreviations:

- AD – Autosomal dominant
- AR – Autosomal recessive
- XLD = X-linked dominant
- XLR = X-linked recessive

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