

Hypophosphatasia and Hypophosphatemic Rickets Panel

Disorders also known as: Hypophosphatemic rickets; Vitamin D-resistant rickets, Vitamin D-Dependent Rickets Type I; 1-Alpha Hydroxylase Deficiency, Vitamin D-resistant rickets; Autosomal recessive hypophosphatemia (ARHP)

Gene List: *CLCN5*, *CYP27B1*, *CYP2R1*, *DMP1*, *ENPP1*, *FGF23*, *PHEX*, *SLC34A3*, *VDR*

Clinical Features:

Variants in these genes have been associated with hereditary hypophosphatemic rickets (HHR).¹⁻⁹ HHR is characterized by bony changes including osteomalacia with or without bone and joint pain, leg bowing, and dental defects due to hypophosphatemia. Skeletal changes may be severe and resemble those found in skeletal dysplasia. Other features often include short stature, muscle weakness, craniosynostosis, and extraskeleton ossification. Hearing loss has also been reported. Secondary complications may occur involving hyperparathyroidism, hypercalcemia and hypercalciuria, and nephrocalcinosis.

CLCN5 gene variants are associated with X-linked Dent disease type 1, a disorder characterized by hypercalciuria, nephrocalcinosis, low molecular-weight proteinuria and renal failure.¹ Approximately 15-30% of individuals with Dent disease 1 also have hypophosphatemic rickets or osteomalacia.¹⁰ Males with a *CLCN5* variant are typically affected, while females may be mildly or variably affected.¹⁰

CYP27B1 and *CYP2R1* gene variants have been reported in individuals with hypophosphatemia, hypocalcemia, bone pain, rickets related skeletal abnormalities, low serum 1,25(OH)₂D concentrations, and growth deficiency.^{2,3} Individuals who have *CYP27B1* disease causing variants may also present with seizures and tetany.² Disease associated with the *CYP27B1* gene is referred to as vitamin D 1 α -hydroxylase deficiency, formally called vitamin D dependent rickets type 1A or pseudo-vitamin D deficient rickets. The *CYP2R1* gene is associated with vitamin D 25-hydroxylase deficiency.

DMP1, *ENPP1*, *FGF23* and *PHEX* gene variants result in HHR without hypercalciuria and are associated with elevated plasma FGF23 levels.⁵ *DMP1* and *ENPP1* variants are rare and autosomal recessive. *FGF23* and *PHEX* variants are autosomal dominant and X-linked dominant, respectively. *ENPP1* variants have also presented as generalized arterial calcification of infancy (GACI).⁵ The same homozygous loss of function pathogenic variants in *ENPP1* have been reported to cause GACI with hypophosphatemia in one family member, and HHR without arterial calcification in the other.⁵ Individuals with *FGF23* gain of function variants cause HHR and are phenotypically similar to individuals with *PHEX* variants, the most common

form of inherited rickets.^{6,11} Loss of function variants in *FGF23* result in familial hyperphosphatemic tumoral calcinosis.¹²

SLC34A3 gene variants result in autosomal recessive HHR due to reduced renal phosphate reabsorption and hypercalciuria due to increased serum levels of 1,25-dihydroxyvitamin D.⁸ Other features include muscle weakness and bone pain. *FGFR3* plasma level are generally normal to slightly low.⁸ Heterozygous carriers have been reported to have increased 1,25-dihydroxyvitamin D levels, hypophosphatemia, hypercalciuria or kidney stones.¹³

VDR gene variants result in autosomal recessive vitamin d dependent rickets. Individuals experience early onset rickets, hypocalcemia, secondary hyperparathyroidism and often alopecia of the scalp or entire body.⁹ Variants in the DNA binding domain are more commonly associated with alopecia.¹⁴ Treatment involves high doses of intravenous or oral calcium administration.⁹

The rickets panel may clarify a clinical diagnosis or identify a genetic diagnosis for rickets or a rickets-related disorder. If a genetic diagnosis is found, genetic testing and recurrence risk information would be available for at-risk family members. In addition, having an identified genetic diagnosis may or may not impact medical management or treatment of the condition.

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:

Variants in the *PHEX* gene are the most common cause of hypophosphatemic rickets, making up to 84% of cases.^{7,15-16} It is currently unknown what proportion of individuals with a clinical diagnosis of hypophosphatemic rickets is a result of a pathogenic variant in the genes *CLCN5*, *CYP27B1*, *FGF23*, and *VDR*.^{2,9,11,16-17} Variants in the genes *CYP2R1*, *DMP1*, *ENPP1*, and *SLC34A3* are rare, as they have only been seen in a small subset of families.^{4-5,8,18}

Gene	Protein	Inheritance	Disease Associations
<i>CLCN5</i>	Chloride channel 5	XLR	Hypophosphatemic rickets, Dent disease, hypercalciuric nephrocalcinosis
<i>CYP27B1</i>	Cytochrome P450, subfamily XXVIIIB, Polypeptide 1	AR	Hypophosphatemic rickets (Vitamin D-dependent rickets)
<i>CYP2R1</i>	Cytochrome P450, subfamily IIR, Polypeptide 1	AR	Hypophosphatemic rickets (Vitamin D-dependent rickets)
<i>DMP1</i>	Dentin matrix acidic phosphoprotein 1	AR	Hypophosphatemic rickets
<i>ENPP1</i>	Ectonucleotide pyrophosphatase/phosphodiesterase 1	AR	Hypophosphatemic rickets, GAC1, Cole disease
<i>FGF23</i>	Fibroblast growth factor 23	AD	Hypophosphatemic rickets
<i>PHEX</i>	Phosphate-regulating endopeptidase homolog, XL	XLD	Hypophosphatemic rickets
<i>SLC34A3</i>	Solute carrier family 34, member 3	AR	Hypophosphatemic rickets, hypercalciuria
<i>VDR</i>	Vitamin D receptor	AR	Vitamin-D dependent rickets with or without alopecia

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