Hypokalemia and Related Disorders Panel

PANEL GENE LIST (38 GENES)

AP2S1, ATP6V0A4, ATP6V1B1, BSND, CA2, CACNA1D, CACNA1H, CACNA1S, CASR, CLCNKA, CLCNKB, CLDN16, CLDN19, CNNM2, EGF, FAM111A, FXYD2, GNA11, HNF1B, HNF4A, HSD11B2, KCNA1, KCNJ1, KCNJ10, KCNJ5, MAGED2, MAGT1, PCBD1, SARS2, SCN4A, SCNN1B, SCNN1G, SLC12A1, SLC12A3, SLC26A3, SLC34A1, SLC4A1, TRPM6

CLINICAL FEATURES AND GENETICS

Hypokalemia refers to the presentation of low levels of potassium in the blood, most often directly related to excess excretion from the kidneys rather than low intake. Potassium is used to establish membrane gradients, which are crucial for multiple cellular processes in the body, particularly the nerves and muscles.[1] Hypokalemic patients can be asymptomatic or have severe manfestations; common symptoms include metabolic acidosis, rhabdomyolysis, leg cramps, weakness, muscle paralysis, constipation, respiratory failure and cardiac arrhythmia.[1] Clinically significant hypokalemia is observed in approximately 1% of all hospitalized patients, and mild hypokalemia can be observed in as many as 14% of outpatient individuals undergoing potassium laboratory testing.[1] Hypokalemia can be caused by and is associated with a wide spectrum of disorders, phenotypes, and treatments. Genetic disorders that may present with hypokalemia that are covered by this panel include Bartter, Gitelman and Liddle syndromes, hypomagnesemia, distal renal tubular acidosis (RTA) and primary aldosteronism.

Bartter syndrome is a group of disorders characterized by renal salt-wasting due to abnormalities of ion transport. As a result of these abnormalities, affected individuals often exhibit low blood pressure, hypokalemic metabolic alkalosis, hypercalciuria, nephrocalcinosis and nephrolithiasis. Bartter syndrome can present prenatally with polyhydramnios, or in the neonatal or childhood period in less severe forms.[2] Four genes (BSND, CLCKNB, KCNJ1 and SLC12A1) have been identified in association with autosomal recessive Bartter syndrome, and one gene (MAGED2) is associated with a transient antenatal X-linked form of Bartter syndrome, which has a later presentation of increased plasma chloride levels and metabolic alkalosis, among other features.[3] Additionaly, a few patients have been reported to have digenic variants in both the CLCNKA and CLCNKB genes; however, more studies are needed to establish the relationship of the CLCNKA gene with Bartter syndrome.[4,5]

Gitelman syndrome is an autosomal recessive disorder with features similar to but milder than Bartter syndrome, including hypokalemia. Gitelman can be distinguished from Bartter by the additional presence of hypocalciuria rather than hypercalciuria as well as hypomagnesemia. It is primarily caused by variants in the SLC12A3 gene, but can also be caused by variants in the CLCNKB gene.[6]

Liddle syndrome is a form of autosomal dominant hypertension that is also characterized by hypokalemia. It may also be referred to as pseudoaldosteronism because, despite causing a similar phenotype to primary aldosteronism, aldosterone secretion is suppressed.[1] Autosomal dominant variants in the SCNN1B and SCNN1G genes result in Liddle syndrome.[7,8] Of note, autosomal recessive variants in SCNN1B and SCNN1G are associated with an allelic disorder, pseudohypoaldosteronism type I, which results in hyperkalemia.

Hypomagnesemia, or low serum levels of magnesium, is associated with muscle cramping and seizures; severely low levels can lead to coma or death.[9] Bartter and Gitelman syndrome cases caused by the BSND, CLCNKB and SLC12A3 genes may present with hypomagnesemia. Other genes that cause hypomagnesemia have been included on this panel to aid in differentials, which may include Bartter and Gitelman syndromes. For example, pathogenic variants in the CLDN16 and CLDN19 genes cause hypercalciuria and nephrocalcinosis (as can be seen in Bartter syndrome), while pathogenic variants in the HNF1B gene can lead to hypomagnesemia and hypocalciuria (as observed in Gitelman syndrome). Additional genes on this panel that cause disorders associated

with hypomagnesemia are the CNNM2, EGF, FAM111A, FXYD2, KCNA1, KCNJ10, PCBD1, SARS2 and TRPM6 genes.

Renal tubular acidosis (RTA) is a disorder that involves the acidification of blood and metabolic acidosis due to the kidney's inability to excrete acids in urine. It is broadly classified into three major types: distal, proximal and hyperkalemic. Distal RTA (dRTA) is characterized by defects of the distal portion of the nephron tubule and is usually diagnosed in childhood. Features of dRTA include vomiting, dehydration, lethargy, short stature, rickets in children and osteomalacia/osteopenia in adults, weakness, failure to thrive and nephrolithiasis. Hearing loss and hypokalemia have been reported in some cases. Pathogenic variants in the ATP6V1B1, ATP6V0A4 and CA2 genes cause autosomal recessive dRTA, and variants in the SLC4A1 gene cause autosomal dominant, and rarely autosomal recessive, RTA.[10] Heterozygous carriers of variants in ATP6V0A4 or ATP6V1B1 may have an increased risk of nephrolithiasis and nephrocalcinosis in adulthood.[10] Proximal RTA is similarly diagnosed in childhood with short stature, lethargy and/or vomiting and respiratory distress in severe cases.[11] However, proximal RTA often resolves after a few years, unlike the lifetime symptoms caused by dRTA. Proximal RTA may also present as part of renal Fanconi syndrome, a broader excretory disease that presents with heightened urine levels of multiple substances such as glucose, phosphates and amino acids, and can present with hypokalemia in some cases. Genetic defects in genes such as HNF4A and SLC34A1 are associated with renal Fanconi syndrome. Finally, hyperkalemic RTA is related to low aldosterone. Patients are often clinically asymptomatic, although they may present with lethargy or postural hypotension. It usually appears in adulthood and the majority of cases are acquired.[11]

Primary aldosteronism is a disorder associated with pathogenic variants in the CACNA1D and CACNA1H genes[12-14] while familial hyperaldosteronism type III is a disorder caused by variants in the KCNJ5 gene.[15] Both disorders are autosomal dominant and characterized by heightened aldosterone levels, hypertension and hypokalemia.

Other genes previously reported to be associated with hypokalemia and/or disorders that may present with hypokalemia that are included in this panel are AP2S1, CASR, CACNA1S, GNA11, HSD11B2, KCNJ10, MAGT1 and SCN4A.

TEST METHODS

Genomic DNA is extracted from the submitted specimen. For skin punch biopsies, fibroblasts are cultured and used for DNA extraction. The DNA is enriched for the complete coding regions and splice site junctions of the genes on this panel 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 at the exon-level; however, technical limitations and inherent sequence properties effectively reduce this resolution for some genes. For the CACNA1D, CACNA1H, CLCNKA, CNNM2, EGF, FAM111A, FXYD2, HSD11B2, KCNA1, KCNJ10, KCNJ5, MAGED2, MAGT1, PCBD1, SARS2, SCN4A, SCNN1B, SCNN1G and TRPM6 genes, sequencing but no copy number testing is performed. Alternative sequencing or copy number detection methods are used to analyze regions with inadequate sequence or copy number data by NGS. 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.

TECHNICAL TEST SENSITIVITY

The technical sensitivity of sequencing is estimated to be >99% at detecting single nucleotide events, but less for 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 identify most deletions and duplications involving coding exons but are less reliable for detecting copy number variants of less than 500 base pairs. Assessment of copy number events also depends on the inherent sequence properties of the targeted regions, including shared homology and exon size. Mosaicism detection is limited and balanced chromosome aberrations cannot be identified.

CLINICAL TEST SENSITIVITY

Hypokalemia-related disorders are a genetically heterogeneous group of diseases. The clinical sensitivity of sequencing and deletion/duplication analysis of the genes included in this panel depends in part on the patient's clinical phenotype and family history. Additional information about the general clinical sensitivity of each gene is included in the table below.

Gene	Protein	Inheritance	Disease association	Sensitivity
AP2S1	Sigma subunit of adaptor related protein complex 2	AD	FHH type II	14-22% FHH patients without CASR variants[16,17]
ATP6V0A4	ATPase H+ transporting V0 subunit a4	AR	dRTA	34% of dRTA patients[18]
ATP6V1B1	ATPase H+ transporting V1 subunit B1	AR	dRTA with deafness	28% of dRTA patients[18]
BSND	Barttin (CLCNK type accessory beta subunit)	AR	Bartter syndrome	Unknown; 2/5 of Bartter syndrome with hearing loss[19]
CA2	Carbonic anhydrase II	AR	CA II deficiency	100% of patients with CA II deficiency[20]
CACNA1D	Calcium voltage-gated channel subunit alpha1D	AD/AR	PASNA (AD), deafness and sinoatrial node dysfunction (AR)	Rare[12]
CACNA1H	Calcium voltage-gated channel subunit alpha1H	AD	Primary aldosteronism, epilepsy and autism	Rare in primary aldosteronism[13,14]
CACNA1S	Calcium voltage-gated channel subunit alpha1S	AD/AR	Hypokalemic PP (AD), congenital myopathy (AR)	40-60% of hypokalemic PP[21]
CASR	Calcium sensing receptor	AD/AR	Disorders of calcium homeostasis	84% of FHH,[22] 42% of ADH,[23] 14-18% of FIH[24,25]
CLCNKA	Chloride voltage-gated channel Ka	AR	Bartter syndrome (possibly digenic)	Rare, possibly digenic with CLCNKB[4,5]
CLCNKB	Chloride voltage-gated channel Kb	AR	Bartter syndrome, Gitelman syndrome	Rare in Bartter,[26,27] ~3% in Gitelman[6]
CLDN16	Claudin 16	AR	Familial hypomagnesemia with hypercalciuria and nephrocalcinosis	23/25 (92%) families with FHHNC[28]

CLDN19	Claudin 19	AR	Familial hypomagnesemia with hypercalciuria, nephrocalcinosis, and severe ocular involvement	26/27 (91%) families with FHHNC with ocular involvement[29]
CNNM2	Cyclin and CBS domain divalent metal cation transport mediator 2	AD/AR	Hypomagnesmia (AD), hypomagnesemia, seizures, and mental retardation (AD/AR)	Rare[30,31]
EGF	Epidermal growth factor	AR	Hypomagnesemia	Rare[32]
FAM111A	Family with sequence similarity 111 member A	AD	Kenny-Caffey syndrome type 2, osteocraniostenosis	5/5 individuals with KCS type 2[9,33]
FXYD2	FXYD domain containing ion transport regulator 2	AD	Hypomagnesemia	Rare[34]
GNA11	G protein subunit alpha 11	AD	Hyper- and hypocalcemia	2/10 (20%) with hypocalciuric hypercalcemia and 2/8 (25%) with hypocalcemia, all negative for CASR variants[35]
HNF1B	HNF1 homeobox B	AD	MODY, renal malformations, hypomagnesemia and hypocalciuria resembling Gittleman	21/91 (23%) renal malformation cases, among which 8/66 (12%) had hypomagnesemia and a variant;[36] ~5% of MODY[37]
HNF4A	Hepatocyte nuclear factor 4 alpha	AD	One variant (R63W) reported in association with renal Fanconi syndrome, hyperinsulinism and macrosomia; MODY	Rare in renal Fanconi syndrome,[38] ~5% of MODY[39]
HSD11B2	Hydroxysteroid 11-beta dehydrogenase 2	AR	AME	Rare[40]
KCNA1	Potassium voltage-gated channel subfamily A member 1	AD	Episodic ataxia type 1/myokymia syndrome	>90% of episodic ataxia type 1[41]
KCNJ1	Potassium voltage-gated channel subfamily J member 1	AR	Bartter syndrome type II	8/14 (57%) families with antenatal Bartter syndrome[42]
KCNJ10	Potassium inwardly rectifying channel subfamily J member 10	AR	EAST/SeSAME syndrome	Rare: >30 patients, some related, all with biallelic variants[43]

KCNJ5	Potassium voltage-gated channel subfamily J member 5	AD	FH type III, Long QT syndrome	Rare cause of FH[15]
MAGED2	MAGE family member D2	XL	Transient antenatal Bartter syndrome	9% of antenatal Bartter[3]
MAGT1	Magnesium transporter 1	XL	Immunodeficiency with magnesium defect, EBV infection and neoplasia (XMEN disease)	Rare - 7 total patients with XMEN[44]
PCBD1	Pterin-4 alpha-carbinolamine dehydratase 1	AR	Transient hyperphenylalaninemia, MODY-like diabetes with hypomagnesemia	Rare[45,46]
SARS2	Seryl-tRNA synthetase	AR	HUPRA syndrome	Rare[47,48]
SCN4A	Sodium voltage-gated channel alpha subunit 4	AD/AR	Hypokalemic PP type 2, hyperkalemic PP types 1 and 2, paramyotonia congenital, and potassium-aggravated myotonia (AD); congenital myasthenia syndrome, fetal hypokinesia, and congenital myopathy (AR)	7-14% of hypokalemic PP[21]
SCNN1B	Sodium channel epithelial 1 beta subunit	AD/AR	Liddle syndrome (AD); pseudohypoaldosteronism type I (AR)	Rare cause of Liddle syndrome,[7] PHAI sensitivity unknown due to small sample sizes[49]
SCNN1G	Sodium channel epithelial 1 gamma subunit	AD/AR	Liddle syndrome (AD); pseudohypoaldosteronism type I (AR)	Rare cause of Liddle syndrome,[8] PHAI sensitivity unknown due to small sample sizes[50]
SLC12A1	Solute carrier family 12 member 1	AR	Bartter syndrome type 1	9/13 (69%) families with antenatal Bartter syndrome[51]
SLC12A3	Solute carrier family 12 member 3	AR	Gitelman syndrome	339/448 (76%) patients[6]
SLC26A3	Solute carrier family 26 member 3	AR	Congenital chloride diarrhea	Majority of patients[52,53]
SLC34A1	Solute carrier family 34 member 1	AD/AR	Renal Fanconi syndrome, IIH	Rare in renal Fanconi,[54] 14/126 (11%) IHH patients without CYP24A1 variants[55]
SLC4A1	Solute carrier family 4 member 1	AD/AR	dRTA	10% of dRTA patients[18]

TRPM6	Transient receptor potential cation channel subfamily M member 6	AR	Hypomagnesemia with secondary hypocalcemia	Rare[9]

Abbreviations:
AD – Autosomal dominant
ADH - autosomal dominant hypocalcemia
AME – apparent mineralocorticoid excess
AR – Autosomal recessive
dRTA – distal renal tubular acidosis
FHH – familial hypocalciuric hypercalcemia
FIH – familial isolated hyperparathyroidism
HUPRA - hyperuricemia, pulmonary hypertension, renal failure, and alkalosis
IIH - idiopathic infantile hypercalcemia
MODY – Maturity-onset diabetes of the young
PASNA - primary aldosteronism, seizures, and neurologic abnormalities
PP – periodic paralysis
XL – X-linked

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