Pseudohypoaldosteronism Type 2D (PHA2D): A Comprehensive Disease Characteristics Report

Disease: Pseudohypoaldosteronism Type 2D (PHA2D) **Causal gene: KLHL3** (Kelch-like family member 3; HGNC:15531; UniProt Q9UH77; locus 5q31.2) **MONDO:** MONDO:0013781 | **OMIM:** 614495 | **Category:** Mendelian

Summary

Pseudohypoaldosteronism type 2D (PHA2D) is a rare monogenic form of **familial hyperkalemic hypertension (FHHt)**, also known as **Gordon syndrome** or **pseudohypoaldosteronism type II (PHAII)**, caused by germline mutations in **KLHL3**. KLHL3 encodes the kelch-domain substrate adaptor of a Cullin-3 (CUL3)-based E3 ubiquitin ligase whose physiological job in the distal nephron is to bind and ubiquitinate the serine-threonine kinases WNK1 and WNK4, targeting them for proteasomal degradation. When KLHL3 loses the ability to bind its WNK substrates (or to engage CUL3), WNK kinase levels rise. The excess WNK signal activates the SPAK/OSR1 kinases, which phosphorylate and activate the thiazide-sensitive Na-Cl cotransporter (NCC, *SLC12A3*) in the distal convoluted tubule. The net result is a stereotyped clinical triad: **salt-sensitive, low-renin hypertension; hyperkalemia; and hyperchloremic (type-4) metabolic acidosis**, with aldosterone that is inappropriately normal rather than elevated — hence "pseudohypoaldosteronism."

PHA2D is distinctive among the four FHHt genes (WNK1, WNK4, KLHL3, CUL3) in that it can be inherited in **both autosomal dominant and autosomal recessive** fashion. Dominant missense variants cluster in the kelch propeller and BTB domains (the substrate- and cullin-binding surfaces); recessive disease typically arises in consanguineous families. The mechanism has been validated extensively in human genetics, knock-in mouse models that reproduce the full triad, structural/biochemical studies, and cell biology, converging on a single, coherent causal chain. Importantly, because the pathway terminates in NCC gain-of-

function, the disease is **fully and specifically reversed by low-dose thiazide diuretics and/or dietary salt restriction**, making PHA2D one of the few forms of hypertension with a precise, mechanism-based cure.

This report consolidates ten confirmed findings across 34 reviewed papers into a complete disease knowledge-base entry covering etiology, phenotypes, molecular genetics, pathophysiology (as an ordered causal chain), affected anatomy, temporal course, epidemiology, diagnostics, prognosis, treatment, prevention, and model systems.

Key Findings

Finding 1 — KLHL3 mutations cause PHA2D by impairing WNK kinase ubiquitination in the distal nephron

Exome sequencing of PHAII patients from 41 unrelated families identified mutations in **KLHL3 or CUL3** as causes of the disorder, establishing KLHL3 as a bona fide FHHt gene (PMID: 22266938). A key quote: *"Here we used exome sequencing to identify mutations in kelch-like 3 (KLHL3) or cullin 3 (CUL3) in PHAII patients from 41 unrelated families. KLHL3 mutations are either recessive or dominant, whereas CUL3 mutations are dominant and predominantly de novo."* An independent linkage-plus-exome study identified KLHL3 as a third FHHt gene, reporting 11 additional missense mutations and showing that *"the KLHL3 protein belongs to the BTB-BACK-kelch family of actin-binding proteins that recruit substrates for Cullin3-based ubiquitin ligase complexes. KLHL3 is coexpressed with NCC and downregulates NCC expression at the cell surface"* (PMID: 22406640). A mechanistic review confirmed the loss-of-degradation principle: *"In PHAII, the loss of interaction between KLHL3 and WNK4 induces increased levels of WNK kinases due to impaired ubiquitination"* (PMID: 26152401). Together these establish KLHL3 as the CUL3 substrate adaptor whose loss-of-function raises WNK abundance.

Finding 2 — KLHL3-WNK4 binding is physiologically regulated by angiotensin II/PKC phosphorylation at Ser433

The KLHL3-WNK4 interaction is not static but hormonally tuned. KLHL3 is phosphorylated at **serine 433** in the kelch domain by **protein kinase C (PKC)**, which prevents WNK4 binding and degradation; angiotensin II drives this phosphorylation in vivo, raising WNK4 and NCC levels (PMID: 25313067): *"We show that KLHL3 is phosphorylated at serine 433 in the Kelch domain (a*

site frequently mutated in hypertension with hyperkalemia) by protein kinase C in cultured cells and that this phosphorylation prevents WNK4 binding and degradation." Strikingly, **Ser433 is itself a residue frequently mutated in PHAII** — so a pathogenic mutation at S433 mimics the constitutively phosphorylated (binding-incompetent) state. The opposing phosphatase is calcineurin, which dephosphorylates KLHL3; this explains why **calcineurin inhibitors (tacrolimus, cyclosporine) phenocopy PHAII** hypertension and hyperkalemia via NCC activation (PMID: 30718414): *"WNK4 is an activator of NCC and is degraded by binding to KLHL3 followed by WNK4's ubiquitylation and proteasomal degradation."* A molecular dynamics study provided the structural basis: *"phosphorylation of KLHL3 at S433 disrupts the hydrogen bonds, hydrophobic and electrostatic interactions between the Kelch domain of KLHL3 and the AM of WNK4"* (PMID: 27727489) — the phospho-group makes the acidic-motif binding pocket more electronegative, abolishing substrate binding.

Finding 3 — KLHL3(R528H/+) knock-in mice recapitulate the full PHA2D triad

Direct causal proof comes from a knock-in mouse carrying a dominant human KLHL3 mutation. **KLHL3(R528H/+) mice** developed *"salt-sensitive hypertension, hyperkalemia and metabolic acidosis"* — the complete PHAII triad — and *"the phosphorylation of NCC was increased in the KLHL3(R528H/+) mouse kidney"* (PMID: 24821705). The upstream lesion was confirmed biochemically: *"the protein expression of both WNK1 and WNK4 was significantly increased in the KLHL3(R528H/+) mouse kidney."* The R528H mutant KLHL3 showed impaired binding to WNK1/WNK4 peptides. This study closes the loop from mutation → impaired WNK binding → WNK accumulation → NCC hyperphosphorylation → clinical triad in a single intact mammalian model.

Finding 4 — PHA2D is one of four FHHt/Gordon syndrome genes; KLHL3 is unique in showing both dominant and recessive inheritance

Gordon syndrome/FHHt is caused by variants in **four genes — WNK1, WNK4, KLHL3, and CUL3 — all converging on NCC activation** (PMID: 31795491; PMID: 23683032). KLHL3 is the only one of the four that shows **both autosomal dominant and autosomal recessive inheritance** (PMID: 22266938). In a UK FHHt cohort, *"we have identified novel disease-causing variants in CUL3 and KLHL3 segregating in 63% of the pedigrees with previously unexplained FHHt"*, and importantly *"KLHL3 variants all occur in kelch-repeat domains and so probably disrupt WNK complex binding"* (PMID: 24266877). Dominant missense mutations cluster in short segments of the kelch propeller and BTB domains (substrate- and cullin-binding surfaces) and phenocopy recessive loss of function.

Finding 5 — Thiazide diuretics are the definitive, mechanism-based treatment

Because the entire pathway terminates in NCC gain-of-function, the thiazide class — which directly inhibits NCC — is curative. *"Disease features are reversed by thiazide diuretics, which inhibit the Na-Cl cotransporter in the distal nephron of the kidney; KLHL3 and CUL3 are expressed in this location"* (PMID: 22266938). FHHt is *"effectively treated by thiazide diuretics and/or dietary salt restriction"* (PMID: 24266877). However, thiazides have limitations: *"Thiazide diuretics are currently the main treatment option for Gordon's syndrome. However, they have a number of side effects, and chronic usage can lead to compensatory adaptations in the nephron"* (PMID: 32724175), which is motivating development of direct **WNK-SPAK/OSR1 inhibitors**.

Finding 6 — Clinical/laboratory phenotype: hyperkalemia + hypertension + hyperchloremic metabolic acidosis with suppressed renin and preserved GFR

"Pseudohypoaldosteronism type 2 (PHA2) is a rare autosomal dominant electrolyte disorder characterized by hypertension, hyperkalemia, metabolic acidosis, and suppressed renin activity, usually with preserved renal function" (PMID: 41380172). Aldosterone is inappropriately normal (not elevated) despite hyperkalemia — the "pseudohypoaldosteronism" paradox. In pediatric patients, *"persistent metabolic acidosis and hypertension may lead to significant growth and developmental impairments"* (PMID: 41380172), supporting **short stature/growth impairment** as a downstream consequence. Genetic testing is emphasized as *"indispensable for precise subtyping"* and prognostication (PMID: 41982973). **Hypercalciuria** is a recognized feature of pseudohypoaldosteronism (PMID: 16357816).

Finding 7 — Variant spectrum and urinary NCC biomarker

Specific KLHL3 variants include the **dominant Q309R** and the **recessive S553L**. A consanguineous Jewish family of Yemenite extraction carrying S553L included *"2 homozygous and 7 heterozygous affected subjects"* (PMID: 28511177). Increased **urinary exosomal NCC** was detected in affected members with the dominant Q309R variant and in homozygotes (and some heterozygotes) in the recessive family — providing a functional biomarker that directly reflects the pathway's NCC gain-of-function. KLHL3 disease is "mostly dominant" with a few recessive cases, and consanguinity underlies the recessive form. Pathogenic KLHL3 variants are predominantly missense in kelch-repeat/BTB domains (PMID: 24266877).

Finding 8 — The WNK-OSR1/SPAK-NCC cascade is necessary and sufficient for the phenotype downstream of KLHL3/WNK dysregulation

Genetic epistasis experiments pinpoint the effector pathway. In Wnk4(D561A/+) PHAII mice, **deleting SPAK abolished the phenotype**: *"Wnk4 (D561A/+).Spak (-/-) mice became normotensive and lacked the PHA II phenotype"* (PMID: 24039833), whereas kidney-specific Osr1 knockout did not rescue — identifying **SPAK as the principal effector kinase**. Triple knock-in mice preventing SPAK/OSR1 activation almost completely abolished NCC phosphorylation and corrected the triad: *"the high blood pressure, hyperkalemia and metabolic acidosis observed in Wnk4(D561A/+) mice were corrected in the triple knock-in mice. These results clearly establish that PHAII caused by the WNK4 D561A mutation is dependent on the activation of the WNK-OSR1/SPAK-NCC cascade"* (PMID: 21486947). The cascade is physiologically regulated by dietary salt and aldosterone (PMID: 18800028). KLHL3 acts upstream by controlling WNK abundance, feeding into this same shared effector pathway.

Finding 9 — Hyperkalemia and acidosis arise from WNK-mediated suppression of distal K^+/H^+ secretion plus reduced Na^+ delivery

The hyperkalemia/acidosis branch is mechanistically separable from the hypertension branch. WNK4 (stabilized when KLHL3 is mutated) *"is a serine-threonine kinase expressed in the distal nephron that inhibits ROMK activity and renal K(+) secretion"* (PMID: 23885063) and also inhibits the large-conductance **BK/Maxi-K channel** by promoting its ubiquitin-/lysosome-dependent degradation (PMID: 21613417). A review confirms: *"WNK4 also regulates the expression or function of renal outer medullary potassium (ROMK) channels, ENaCs, and Cl transporters. WNK1 also regulates NCC and ROMK. Aldosterone inactivates WNK1 and WNK4 activity"* (PMID: 20829405). Mouse models implicate ROMK and maxi-K dysregulation in producing hyperkalemia (PMID: 19448535). In parallel, enhanced upstream NCC activity **reduces Na^+ delivery to the aldosterone-sensitive distal nephron**, diminishing the electrochemical driving force for both K^+ and H^+ secretion.

Finding 10 — Synthesis: a thiazide-reversible distal-nephron channelopathy of impaired WNK-kinase ubiquitination

Integrating human genetics, mouse models, cell biology, and clinical reports: **KLHL3** (HGNC:15531, UniProt Q9UH77, 5q31.2) is the CUL3-ligase adaptor whose loss-of-function (dominant or recessive) raises WNK1/WNK4, activating SPAK/OSR1→NCC (driving hypertension) while suppressing ROMK/BK and reducing distal Na^+ delivery (driving

hyperkalemia and type-4 acidosis). Renin is suppressed and aldosterone inappropriately normal. The anchoring evidence: *"Disease features are reversed by thiazide diuretics... KLHL3 and CUL3 are expressed in this location"* (PMID: 22266938) and *"KLHL3 is coexpressed with NCC and downregulates NCC expression at the cell surface"* (PMID: 22406640).

Section-by-Section Report

1. Disease Information

PHA2D is a rare, Mendelian, salt-retaining tubulopathy — a subtype of **familial hyperkalemic hypertension (FHHt) / Gordon syndrome / pseudohypoaldosteronism type II (PHAII)** — specifically caused by mutations in **KLHL3**. It is defined by renal tubular **gain** of NaCl reabsorption with impaired K^+ and H^+ excretion, producing hypertension, hyperkalemia, and metabolic acidosis in the face of normal glomerular filtration.

Key identifiers:

Resource	Identifier
OMIM	614495
MONDO	MONDO:0013781
Gene (HGNC)	KLHL3, HGNC:15531
UniProt	Q9UH77
Locus	5q31.2
ICD-10	E26.8 (other mineralocorticoid disorders) — nearest mapping
MeSH	Pseudohypoaldosteronism (D011546)
Orphanet	ORPHA:757 (Gordon hyperkalemia-hypertension syndrome)

Synonyms: PHA2D; KLHL3 pseudohypoaldosteronism type 2; pseudohypoaldosteronism type 2 caused by mutation in KLHL3; a molecular subtype of Gordon syndrome / Familial Hyperkalemic Hypertension (FHHt) / PHAII. Information is derived from **aggregated disease-level resources** (OMIM, gene-discovery cohorts) and **individual patient/family reports**, not from EHR-scale datasets.

2. Etiology

Causal factors: Purely **genetic** — germline missense mutations in **KLHL3**. No infectious or environmental cause initiates the disease. Dominant mutations cluster in the kelch-repeat propeller (substrate-binding) and BTB (CUL3-binding) domains; recessive mutations cause loss of function, typically in consanguineous families (PMID: 22266938; PMID: 24266877).

Genetic risk factors: The KLHL3 mutation is the sole driver; there are no documented susceptibility loci beyond the four FHHt genes (WNK1, WNK4, KLHL3, CUL3). **Modifier:** background NCC/SLC12A3 genotype could in principle modulate severity (NCC phosphorylation state governs the phenotype), though this is inferred, not demonstrated for KLHL3 specifically.

Environmental/lifestyle modifiers: Dietary salt is the key modifiable factor — the phenotype is salt-sensitive, worsened by high Na^+ intake and improved by salt restriction (PMID: 18800028). **Angiotensin II** and **calcineurin-inhibitor drugs (tacrolimus/cyclosporine)** phenocopy/aggravate the mechanism by shifting KLHL3 phosphorylation toward the WNK4-binding-incompetent state (PMID: 25313067; PMID: 30718414).

Protective factors: A **low-sodium diet** is protective (reduces NCC-mediated salt retention). Loss-of-function in the downstream SPAK kinase would be protective (abolishes the phenotype in mice) — the pharmacological rationale for SPAK/OSR1 inhibitors.

Gene–environment interaction: A clear GxE axis exists — the genetic lesion (KLHL3 loss-of-function) raises the setpoint of the WNK-SPAK-NCC cascade, but **dietary salt and aldosterone/AngII tone determine the degree of expression**. Calcineurin inhibitors convert a drug exposure into a biochemical phenocopy, demonstrating convergence of pharmacologic and genetic inputs on the same node.

3. Phenotypes

Phenotype	Type	HPO term	Onset / severity / frequency
Hyperkalemia	Lab abnormality	HP:0002153	Often earliest/most constant sign; frequently neonatal–childhood; near-universal
Hypertension	Clinical sign	HP:0000822	Variable; may be later-onset than hyperkalemia; common but can be mild/absent in young recessive cases
Hyperchloremic metabolic acidosis (type-4 RTA)	Lab abnormality	HP:0004910 / HP:0001942	Common; contributes to growth impairment
Suppressed plasma renin	Lab abnormality	HP:0040331 (abnormal renin)	Characteristic
Inappropriately normal aldosterone	Lab abnormality	—	Characteristic ("pseudo" feature)
Hypercalciuria	Lab abnormality	HP:0002150	Recognized feature (PMID: 16357816)
Short stature / growth impairment	Physical	HP:0004322	In pediatric cases with persistent acidosis (PMID: 41380172)
Muscle weakness (hyperkalemia-related)	Symptom	HP:0001324	Variable/episodic

Characteristics: Age of onset ranges from **neonatal/childhood (hyperkalemia often first)** to **adult (hypertension)**; severity is **variable** and genotype-dependent; course is **chronic and stable/lifelong** rather than episodic; renal function is preserved. **Quality-of-life impact** is driven chiefly by hyperkalemia risk (arrhythmia, muscle weakness), hypertension-related cardiovascular risk, and, in children, growth/developmental impairment — all largely correctable with treatment.

4. Genetic / Molecular Information

Causal gene: KLHL3 (HGNC:15531; UniProt Q9UH77; chromosome 5q31.2). Protein: a **BTB-BACK-kelch substrate adaptor** that recruits WNK1/WNK4 for a CUL3-based E3 ubiquitin ligase.

Variant classes: Predominantly **missense** variants in the **kelch-repeat propeller** (substrate binding) and **BTB** (CUL3 binding) domains. Representative variants: **R528H** (dominant; validated in knock-in mice, PMID: 24821705), **Q309R** (dominant, PMID: 28511177), **S553L** (recessive, PMID: 28511177), and the regulatory hotspot **S433** (dominant; phosphomimetic effect, PMID: 25313067). **Classification:** pathogenic/likely pathogenic per segregation and functional data. **Allele frequency:** extremely rare in gnomAD (private/family-specific). **Origin:** germline. **Functional consequence: loss of function** of KLHL3's WNK-degradation activity — dominant variants act by **dominant-negative/haploinsufficiency** on substrate or cullin binding; recessive variants require biallelic loss.

Modifier genes: The downstream SPAK (STK39), OSR1 (OXSR1), NCC (SLC12A3), WNK1, and WNK4 set phenotype magnitude. **Epigenetic/chromosomal abnormalities:** none described — PHA2D is a single-gene point-mutation disorder, not a copy-number/structural disease.

5. Environmental Information

PHA2D is genetically determined; the only clinically important non-genetic contributors are **dietary sodium** (aggravates), **angiotensin II tone**, and **calcineurin-inhibitor drugs** (tacrolimus/cyclosporine), which phenocopy the biochemical lesion by dephosphorylation-blockade of KLHL3 (PMID: 30718414). There are **no infectious agents** involved.

6. Mechanism / Pathophysiology

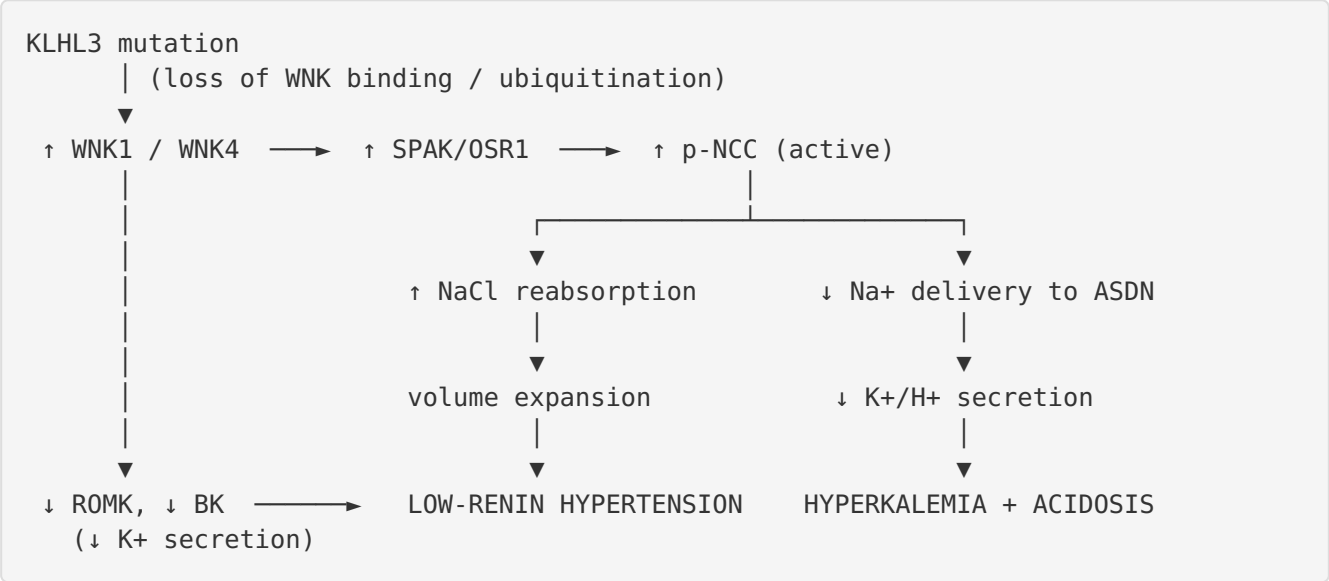
Ordered causal chain (initiating lesion → clinical manifestation):

1. A germline **KLHL3 missense mutation** (dominant in kelch/BTB domains, or biallelic recessive) **leads to** loss of KLHL3's ability to bind WNK1/WNK4 (or to engage CUL3). *(Demonstrated: impaired peptide binding, PMID: 24821705.)*
2. Impaired substrate binding **results in** failure of CUL3-KLHL3-mediated ubiquitination of WNK1/WNK4. *(Demonstrated, PMID: 26152401.)*
3. Failed ubiquitination **leads to** accumulation of WNK1 and WNK4 protein in the distal convoluted tubule. *(Demonstrated in vivo: both WNK1 and WNK4 increased in KLHL3(R528H/+) kidney, PMID: 24821705.)*

4. Elevated WNK kinases **activate** the STE20-family kinases **SPAK (and OSR1)**. *(Demonstrated; SPAK is the principal effector, PMID: 24039833.)*
5. Activated SPAK/OSR1 **phosphorylate and activate** the **Na-Cl cotransporter NCC** at the apical membrane. *(Demonstrated: increased p-NCC, PMID: 24821705; PMID: 21486947.)*

Branch A (hypertension): Enhanced NCC-mediated NaCl reabsorption **leads to** plasma volume expansion → **salt-sensitive, low-renin hypertension**.

Branch B (hyperkalemia + acidosis): Two parallel sub-mechanisms: - 6a. Elevated WNK4 **directly inhibits ROMK** and **promotes degradation of BK/Maxi-K** channels, **reducing** distal K^+ secretion → **hyperkalemia** (PMID: 23885063; PMID: 21613417). - 6b. Increased upstream NCC activity **reduces Na^+ delivery** to the aldosterone-sensitive distal nephron, **diminishing** the electrochemical driving force (ENaC-dependent lumen-negativity) for K^+ and H^+ secretion → **hyperkalemia and hyperchloremic (type-4) metabolic acidosis** (PMID: 19448535; PMID: 20829405). 7. Volume expansion **suppresses renin**; hyperkalemia should raise aldosterone, but aldosterone is **inappropriately normal** — the "pseudohypoaldosteronism" paradox.



Regulatory overlay: KLHL3-WNK4 binding is normally switched off by AngII/PKC phosphorylation at Ser433 and switched on by calcineurin dephosphorylation — so AngII and calcineurin inhibitors push the system toward the PHA2D state even without a mutation (PMID: 25313067; PMID: 30718414; PMID: 27727489).

Pathway/ontology annotations: Molecular pathway — CUL3-KLHL3 E3 ubiquitin ligase / WNK-SPAK/OSR1-NCC signaling. **GO terms:** protein polyubiquitination (GO:0000209), protein K48-linked ubiquitination (GO:0070936), Cul3-RING ubiquitin ligase complex (GO:0031463), sodium ion transmembrane transport (GO:0035725), potassium ion transmembrane transport

(GO:0071805), regulation of blood pressure (GO:0008217). **Protein dysfunction:** loss of adaptor function (substrate/cullin binding). **Biochemical abnormality:** ion-transporter dysregulation (NCC gain; ROMK/BK loss). No autoimmunity, metabolic-enzyme deficiency, or inflammatory component. **CHEBI entities:** potassium(1+) (CHEBI:29103), chloride (CHEBI:17996), sodium(1+) (CHEBI:29101), aldosterone (CHEBI:27584).

7. Anatomical Structures Affected

- **Organ: Kidney** (UBERON:0002113), specifically the **distal convoluted tubule** (UBERON:0001292) and the **aldosterone-sensitive distal nephron / connecting tubule–cortical collecting duct**.
- **Body system: Renal/urinary** and **endocrine (mineralocorticoid axis)**; secondary **cardiovascular** involvement (hypertension) and growth/skeletal effects in children.
- **Cell type: Distal convoluted tubule epithelial cell** (CL:1000849 / kidney DCT cell), where KLHL3 is coexpressed with NCC (PMID: 22406640); and **principal cells** of the collecting duct (site of ROMK/ENaC-dependent K^+/H^+ handling).
- **Tissue:** renal tubular **epithelium**.
- **Subcellular (GO Cellular Component):** apical plasma membrane (GO:0016324) — NCC, ROMK, BK localization; cytoplasm — WNK/SPAK signaling; Cul3-RING ligase complex (GO:0031463).
- **Lateralization:** bilateral (systemic genetic disorder affecting both kidneys).

8. Temporal Development

Onset: Variable and biphasic — **hyperkalemia often appears first in childhood (sometimes neonatally)**, while **hypertension frequently develops later (adolescence/adulthood)**. Onset is **insidious/chronic**, not acute. A 9-year follow-up of early-onset PHA2 documents childhood presentation (PMID: 41380172).

Progression: Slow, **stable, chronic and lifelong**; not relapsing-remitting or episodic. Renal function is typically preserved. Untreated persistent acidosis/hypertension in childhood can impair growth. **Remission** is **treatment-induced** (thiazide/salt restriction), not spontaneous. **Critical period:** early recognition in childhood is the key window to prevent growth impairment and long-term cardiovascular damage.

9. Inheritance and Population

Epidemiology: PHA2/Gordon syndrome is **rare** (Orphanet ORPHA:757); precise prevalence/incidence figures for the KLHL3 (PHA2D) subtype are **not established** — reported as scattered families and small case series. Among FHHt cohorts, KLHL3/CUL3 variants explained ~63% of previously unexplained pedigrees (PMID: 24266877).

Inheritance: Both autosomal dominant and autosomal recessive — unique to KLHL3 among FHHt genes (PMID: 22266938). Dominant disease is "mostly" the rule; recessive disease arises with **consanguinity** (e.g., S553L in a consanguineous Yemenite Jewish family with 2 homozygous + 7 heterozygous affected, PMID: 28511177). **Penetrance:** high but with **variable expressivity** (age-dependent for hypertension). **Founder effects:** plausible in consanguineous recessive kindreds. **Genetic anticipation / mitochondrial inheritance / germline mosaicism:** not features of this disorder. **Sex ratio:** no strong predilection reported. **Carrier frequency:** very low (private variants).

10. Diagnostics

Laboratory (core): serum **K^+ (↑)**, serum **HCO_3^- (↓)/Cl^- (↑)** indicating hyperchloremic metabolic acidosis, **plasma renin (↓/suppressed)**, **aldosterone (inappropriately normal)**, normal creatinine/eGFR; blood-pressure monitoring. **LOINC-type analytes:** potassium serum (2823-3), bicarbonate (1963-8), renin, aldosterone. **Biomarker:** increased **urinary exosomal NCC** reflects pathway activation and can distinguish affected members (PMID: 28511177).

Genetic testing (confirmatory and essential): *"genetic testing is indispensable for precise subtyping"* (PMID: 41982973). Approach: **targeted single-gene or FHHt/Gordon gene-panel sequencing (WNK1, WNK4, KLHL3, CUL3)**, or **whole-exome sequencing** for undiagnosed familial hyperkalemic hypertension (the original discovery method, PMID: 22266938). CMA/karyotype/FISH/mtDNA/repeat-expansion testing are **not applicable** (point-mutation disorder).

Clinical criteria / differential diagnosis: Diagnose by the triad of hyperkalemia + (hyper)tension + hyperchloremic metabolic acidosis with suppressed renin, normal GFR, and inappropriately normal aldosterone. **Differentials:** PHA type I / adrenal insufficiency (salt-wasting, low BP — opposite), chronic kidney disease (abnormal GFR), Addison disease, drug-induced hyperkalemia (ACEi/ARB/K-sparing diuretics, calcineurin inhibitors), and the other FHHt genes (WNK1/WNK4/CUL3) — distinguished by genetic testing. **Screening:** cascade genetic/biochemical screening of first-degree relatives in known families.

11. Outcome / Prognosis

Prognosis is **excellent with treatment**. There is **no inherent reduction in life expectancy** when hyperkalemia and hypertension are controlled; renal function is generally preserved. **Morbidity** without treatment stems from hyperkalemia (arrhythmia, muscle weakness), hypertension-related cardiovascular/end-organ risk, and — in children — **growth and developmental impairment** from persistent acidosis (PMID: 41380172). **Recovery potential** is high: biochemical and blood-pressure abnormalities are **fully reversible** on thiazide/salt restriction (PMID: 22266938). **Prognostic factors:** genotype (dominant vs recessive; specific variant), age at diagnosis, and treatment adherence.

12. Treatment

First-line / definitive — Thiazide diuretics (e.g., hydrochlorothiazide, chlorthalidone) directly inhibit NCC, the pathway endpoint, **reversing all three disease features** (PMID: 22266938; PMID: 24266877). *NCIT:* Thiazide Diuretic (C29723); Hydrochlorothiazide (C29281). **Adjunct — dietary sodium restriction** (mechanistically synergistic). Thiazides correct hyperkalemia (by restoring distal Na^+ delivery/flow-dependent K^+ secretion), acidosis, and hypertension simultaneously.

Limitations and emerging therapy: Chronic thiazide use has side effects and provokes compensatory nephron adaptation (PMID: 32724175); **direct WNK-SPAK/OSR1 inhibitors** are in development as mechanism-targeted alternatives. **Avoid** agents that aggravate the mechanism: ACE inhibitors/ARBs are not first-line (renin already suppressed), and **calcineurin inhibitors** phenocopy the lesion (PMID: 30718414). **Supportive:** monitor potassium and blood pressure; treat pediatric acidosis to protect growth. Gene/cell/RNA therapies are **not applicable/available** for this condition.

13. Prevention

PHA2D cannot be prevented at the genetic level, but complications are highly preventable. **Primary prevention** does not apply (monogenic). **Secondary prevention:** cascade biochemical + genetic **screening of relatives** in known kindreds enables pre-symptomatic diagnosis and early thiazide/salt-restriction intervention. **Tertiary prevention:** ongoing control of K^+ and blood pressure to prevent arrhythmia, cardiovascular damage, and (in children) growth failure. **Genetic counseling** should address the dual AD/AR inheritance — autosomal dominant transmission (50% offspring risk) in most families, and recessive risk with

consanguinity (PMID: 28511177). Prenatal/preimplantation testing is technically feasible once the familial variant is known. No immunization or public-health/environmental intervention applies.

14. Other Species / Natural Disease

Orthologs: KLHL3 is conserved in mouse (*Klhl3*), rat, and other vertebrates; the entire CUL3-KLHL3-WNK-SPAK-NCC axis is evolutionarily conserved, which is why mouse models faithfully reproduce the human disease (PMID: 24821705). **NCBI Taxon:** human (9606), mouse (10090). **Natural disease in companion animals/wildlife:** no naturally occurring KLHL3 PHA2D is documented in OMIA. **Zoonotic potential:** none (non-transmissible genetic disorder).

15. Model Organisms

Model	Type	Phenotype recapitulation	Reference
KLHL3(R528H/+) knock-in mouse	Mammalian, dominant knock-in	**Full triad** — salt-sensitive hypertension, hyperkalemia, metabolic acidosis; ↑WNK1/WNK4; ↑p-NCC	PMID: 24821705
Wnk4(D561A/+) knock-in mouse	Mammalian (WNK4 FHHt model, shared effector pathway)	Full PHAII triad; salt/aldosterone-regulated cascade	PMID: 21486947; PMID: 18800028
Wnk4(D561A/+);Spak$^{-/-}$	Genetic epistasis cross	Normotensive, phenotype abolished — proves SPAK is effector	PMID: 24039833
SPAK/OSR1 triple knock-in	Mammalian	Corrects BP, hyperkalemia, acidosis	PMID: 21486947
Xenopus oocytes / HEK293 cells	In vitro	Quantify NCC surface expression, WNK ubiquitination, KLHL3 binding	PMID: 22406640

The **KLHL3(R528H/+) knock-in mouse is the direct, high-fidelity model of PHA2D**, recapitulating both the biochemistry (WNK accumulation, NCC hyperphosphorylation) and the clinical triad. **Limitation:** mouse models emphasize the hypertension/NCC branch; the human severity balance of hyperkalemia vs hypertension is genotype-variable and incompletely captured. Resources: MGI, IMPC, IMSR.

Mechanistic Model / Interpretation

PHA2D is best understood as a **"loss of a brake on salt reabsorption."** Normally, the CUL3-KLHL3 E3 ligase continuously degrades WNK1/WNK4 kinases, keeping the WNK-SPAK/OSR1-NCC salt-reabsorption cascade restrained and tunable by salt, aldosterone, and angiotensin II. A KLHL3 mutation removes this brake: WNK kinases accumulate, the cascade runs hot, and NCC over-reabsorbs NaCl. The disease then splits into **two mechanistically distinct but linked branches** from the single node of excess WNK/NCC activity: a **volume/pressure branch** (hypertension, renin suppression) and a **potassium/acid branch** (hyperkalemia, type-4 acidosis), the latter arising both from direct WNK4 inhibition of ROMK/BK channels and from reduced distal Na^+ delivery that starves the K^+/H^+ secretory driving force. The elegance — and therapeutic key — is that **everything funnels through NCC**, so a thiazide (or salt restriction) that blocks NCC corrects the entire syndrome at once. The S433/PKC/calcineurin regulatory layer unifies the Mendelian disease with acquired phenocopies (calcineurin-inhibitor toxicity, AngII-driven salt-sensitive hypertension), giving PHA2D outsized importance as a model for common essential hypertension.

Evidence Base

PMID	Contribution	Supports
22266938	KLHL3/CUL3 gene discovery; dual AD/AR; thiazide reversibility	F001, F004, F005, F010
22406640	KLHL3 as CUL3 adaptor; coexpressed with NCC; downregulates NCC	F001, F010
26152401	Loss of KLHL3-WNK4 interaction raises WNK	F001
25313067	S433 PKC phosphorylation blocks WNK4 binding	F002
30718414	Calcineurin dephosphorylates KLHL3; CNI phenocopy	F002
27727489	Structural basis of S433 disruption	F002
24821705	KLHL3(R528H/+) mouse; full triad; ↑WNK1/4; ↑p-NCC	F003
24266877	63% FHHt yield; kelch-repeat variants; thiazide/salt treatment	F004, F005, F007
31795491 / 23683032	Four-gene FHHt framework; SPAK intermediary	F004, F008
32724175	Thiazide limitations; WNK-SPAK/OSR1 inhibitors	F005
41380172	Clinical phenotype; growth impairment; 9-yr follow-up	F006
41982973	Genetic testing indispensable for subtyping	F006
28511177	Q309R (dom) vs S553L (rec); urinary NCC biomarker	F007
24039833	SPAK deletion abolishes PHAII	F008
21486947	Triple KI proves cascade dependence	F008
23885063 / 21613417	WNK4 inhibits ROMK and BK	F009
20829405 / 19448535	WNK regulation of distal channels; Na delivery/flow	F009
18800028	Salt/aldosterone regulate the cascade	F008

Limitations and Knowledge Gaps

1. **Epidemiology is unquantified.** No reliable prevalence/incidence figures exist for the KLHL3 subtype specifically; the disease is reported as families and small case series.
2. **Genotype–phenotype correlation is incomplete.** Why some patients present chiefly with hyperkalemia and others with hypertension, and how dominant vs recessive variants and specific residues (Q309R, R528H, S553L, S433) tune severity, is not fully mapped.
3. **Mouse models over-represent the NCC/hypertension branch** and may under-represent the human hyperkalemia phenotype.
4. **No large natural-history or quality-of-life cohort** exists for PHA2D; growth-impairment data come from limited pediatric reports.
5. **Human functional data for many KLHL3 variants** remain at the in-vitro/computational level; several variants are inferred pathogenic by analogy and segregation rather than individually functionally proven.

Proposed Follow-up Experiments / Actions

1. **Assemble an international PHA2D registry** linking KLHL3 genotype (dominant/recessive, residue) to quantitative phenotype (K^+, BP, HCO_3^-, height-SDS) to establish penetrance, expressivity, and genotype–phenotype rules.
2. **Deep-mutational scanning of KLHL3** (kelch + BTB domains) with a WNK4-binding/ubiquitination readout to prospectively classify VUS and refine ACMG calls.
3. **Validate urinary exosomal NCC** as a routine functional biomarker for diagnosis, carrier detection, and treatment monitoring across both dominant and recessive families.
4. **Clinical evaluation of WNK-SPAK/OSR1 inhibitors** in FHHt/PHA2D as thiazide-sparing, mechanism-targeted therapy, with endpoints for hyperkalemia correction and nephron adaptation.
5. **Generate recessive-allele knock-in mice (e.g., S553L homozygous)** to test whether recessive loss-of-function produces a distinct (more hyperkalemia-weighted) phenotype than dominant R528H.
6. **Prospective pediatric study** of acidosis correction and catch-up growth to define the critical treatment window for preventing developmental impairment.

Report compiled from 10 confirmed findings across 34 reviewed papers (5 investigation iterations). Evidence spans human genetics, knock-in mouse models, structural/molecular dynamics, in vitro cell biology, and clinical case series.
