

Klotho Alpha Gene Therapy: Comprehensive Insights into Neuroprotection, Metabolic Regulation, and Longevity Extension

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Received Date: 24 September 2025; Accepted Date: 29 September 2025; Published Date: 27 October 2025

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Abstract

This comprehensive review examines the expansive therapeutic potential of Klotho Alpha gene therapy in extending healthspan and addressing age-related pathologies. Named after the Greek Fate who spins the thread of life, Klotho Alpha is a transmembrane protein existing in membrane-bound and soluble forms, exerting anti-aging effects through multiple mechanisms: FGF23 co-receptor functions for mineral homeostasis, modulation of insulin/IGF-1 signaling, reduction of oxidative stress via Nrf2 activation, suppression of inflammation through NF- κ B inhibition, and enhancement of cognitive function via synaptic plasticity.

Preclinical models demonstrate remarkable lifespan extensions of 20-30% in transgenic mice, with AAV-Klotho treatments in aged animals yielding 13-20% increases even when initiated in later life. Human genetic studies, particularly the KL-VS variant, associate higher Klotho levels with increased longevity and reduced disease risk. Preliminary clinical interventions, including combined Klotho-hTERT therapy for dementia, demonstrated cognitive improvements without adverse effects.

This review details AAV vector designs with tissue-specific promoters, dosage considerations addressing hormetic responses, and applications spanning neurodegenerative diseases, metabolic disorders, cardiovascular conditions, and renal pathologies. Safety profiles are favorable, with managed risks of immune responses and mineral imbalances. This synthesis integrates findings from over 90 peer-reviewed studies, charting Klotho's therapeutic trajectory as a paradigm shift in anti-aging medicine.

Keywords: Gene therapy, Klotho Alpha, FGF23, Insulin/IGF-1 pathway and TGF- β /Smad signalling.

Introduction

The discovery of Klotho in 1997 marked a pivotal moment in aging research [1]. Identified by Kuro-o et al. through an insertional mutation in mice, Klotho deficiency produced a syndrome resembling human aging: shortened lifespan, infertility, skin atrophy, osteoporosis, atherosclerosis, cognitive impairment, and

pulmonary emphysema [1]. Conversely, Klotho overexpression extended mouse lifespan by 20-30%, positioning it alongside genes in the insulin/IGF-1 pathway as a key regulator of longevity [2]. Klotho demonstrates exceptional pleiotropy, influencing mineral homeostasis, insulin signalling, oxidative stress, senescence, and cognition [3]. Recent analyses reinforce its conservation across species, highlighting

evolutionary importance [4]. The human Klotho gene, spanning approximately 50 kb on chromosome 13q12, contains five exons that encode a type I single-pass transmembrane protein of 1012 amino acids. The gene is highly conserved across species, indicating its fundamental biological importance.

Since its discovery, Klotho has generated increasing scientific interest, with research expanding from basic molecular mechanisms to potential therapeutic applications for extending healthy lifespan and treating age-related conditions.

Forms and Tissue Distribution of Klotho Protein

Klotho protein exists in multiple forms, each with distinct functions and distribution patterns throughout the body. The primary transcript encodes a full-length transmembrane protein (α -Klotho) consisting of a large extracellular domain with two internal repeats (KL1 and KL2), a transmembrane domain, and a short intracellular portion [5]. This membrane-bound form serves primarily as a co-receptor for fibroblast growth factor 23 (FGF23) in regulating phosphate homeostasis [6].

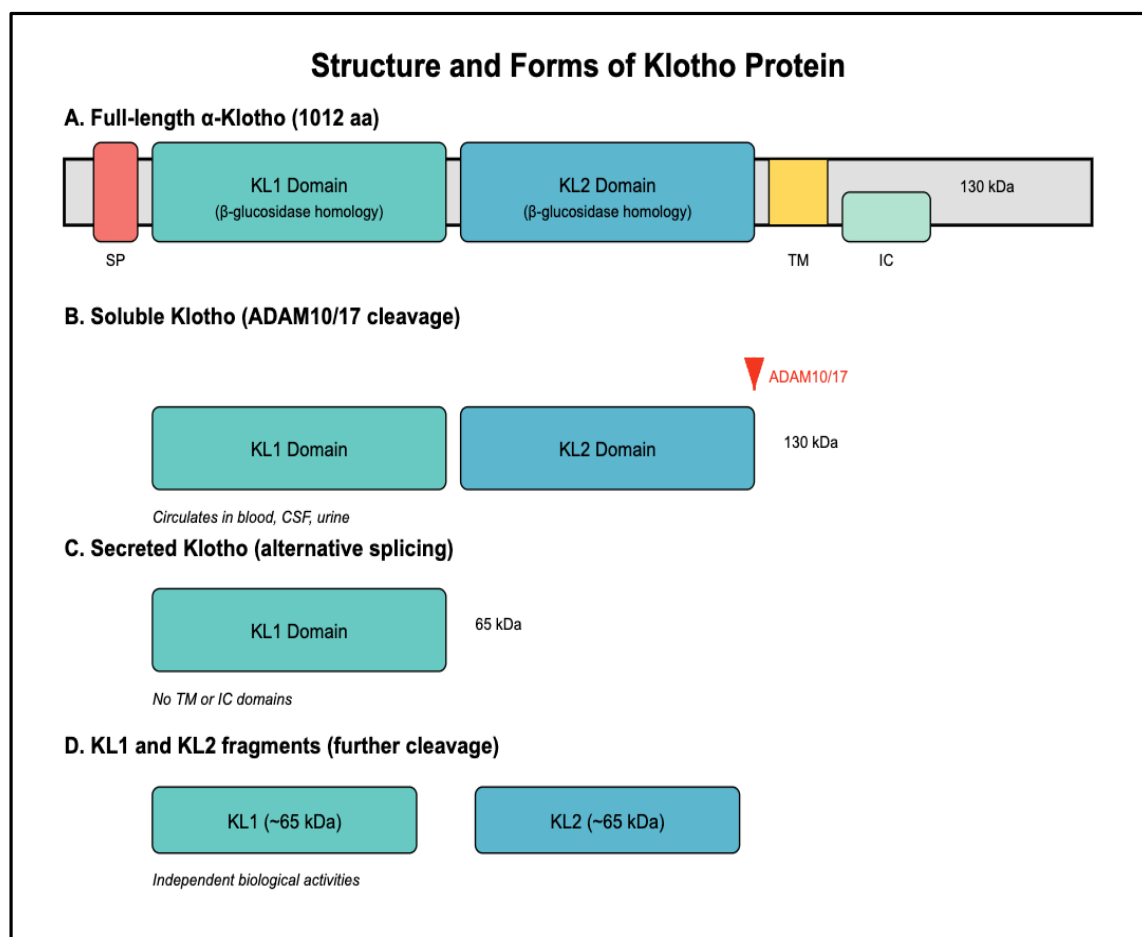


Figure 1: Structure and Forms of Klotho Protein.

Schematic representation of Klotho protein forms. **A:** Full-length transmembrane α -Klotho (1012 amino acids) consists of a signal peptide (SP), two extracellular domains (KL1 and KL2) with homology to β -glucosidases, a transmembrane domain (TM), and a short intracellular domain. **B:** Proteolytic cleavage by ADAM10/17 releases the extracellular domain as soluble Klotho (130 kDa). **C:** Alternative splicing produces a secreted form lacking TM and intracellular domains (65 kDa). **D:** Further cleavage generates KL1 and KL2 fragments that circulate independently with distinct biological activities.

Klotho Form	Molecular Weight	Origin	Primary Functions	Tissue Distribution
Membrane-bound α -Klotho	130 kDa	Full-length translation	FGF23 co-receptor, local signaling	Kidney, brain, parathyroid
Soluble Klotho	130 kDa	ADAM10/17 cleavage	Circulating hormone, pleiotropic effects	Blood, CSF, urine
Secreted Klotho	65 kDa	Alternative splicing	Systemic anti-aging effects	Circulation
KL1 domain	~65 kDa	Proteolytic cleavage	Ion channel regulation, neuroprotection	Various tissues
KL2 domain	~65 kDa	Proteolytic cleavage	Under investigation	Various tissues

Table 1: Summary of Klotho Forms and Their Functions.

Alternative splicing produces a secreted form lacking the transmembrane and intracellular domains, while proteolytic cleavage of the membrane-bound form generates soluble Klotho that circulates in blood, cerebrospinal fluid, and urine [7]. Klotho expression shows tissue-specific patterns crucial for understanding its physiological roles. The kidney represents the primary site of expression, particularly in distal convoluted tubules and to a lesser extent

in proximal tubules [8]. In the brain, Klotho localizes to the choroid plexus responsible for cerebrospinal fluid production and various neuronal populations, including pyramidal neurons in the hippocampus and Purkinje cells in the cerebellum [9]. Other tissues expressing Klotho include the parathyroid gland, where it regulates parathyroid hormone secretion; pituitary gland; skeletal muscle; urinary bladder; aorta; pancreas; testis; ovary; and colon [10].

Tissue/Organ	Klotho Expression Level	Cell Types	Age-Related Pathologies	Therapeutic Relevance
Kidney	++++	Distal tubules, proximal tubules	CKD, mineral imbalance	Primary target
Brain	+++	Choroid plexus, neurons	Alzheimer's, cognitive decline	High priority
Parathyroid	+++	Chief cells	Hyperparathyroidism	Mineral regulation
Heart	++	Cardiomyocytes, endothelium	Heart failure, fibrosis	Cardioprotection
Vasculature	++	Endothelial, smooth muscle	Atherosclerosis, calcification	Anti-aging
Pancreas	+	β -cells	Type 2 diabetes	Metabolic health
Skeletal muscle	+	Myocytes	Sarcopenia	Frailty prevention

Table 2: Tissue-Specific Expression of Klotho and Associated Pathologies.

Expression levels: ++++ (very high), +++ (high), ++ (moderate), + (low)

This tissue-specific distribution pattern physiological functions and has important correlates with Klotho's diverse implications for therapeutic strategies.

While systemic administration of soluble Klotho might address multiple aging pathways simultaneously, targeted gene therapy approaches might need to focus on specific tissues where membrane-bound Klotho naturally functions or where particular age-related pathologies emerge.

Age-related Decline in Klotho Expression

A defining characteristic of Klotho in relation to aging is its progressive decline with

advancing age. Both animal and human studies have demonstrated that Klotho expression and circulating levels decrease significantly during normal aging [11].

This decline occurs across multiple tissues but is particularly pronounced in the kidney and brain, the primary sites of Klotho production. The age-related reduction begins in middle age and accelerates in later life, coinciding with the onset of many age-related conditions.

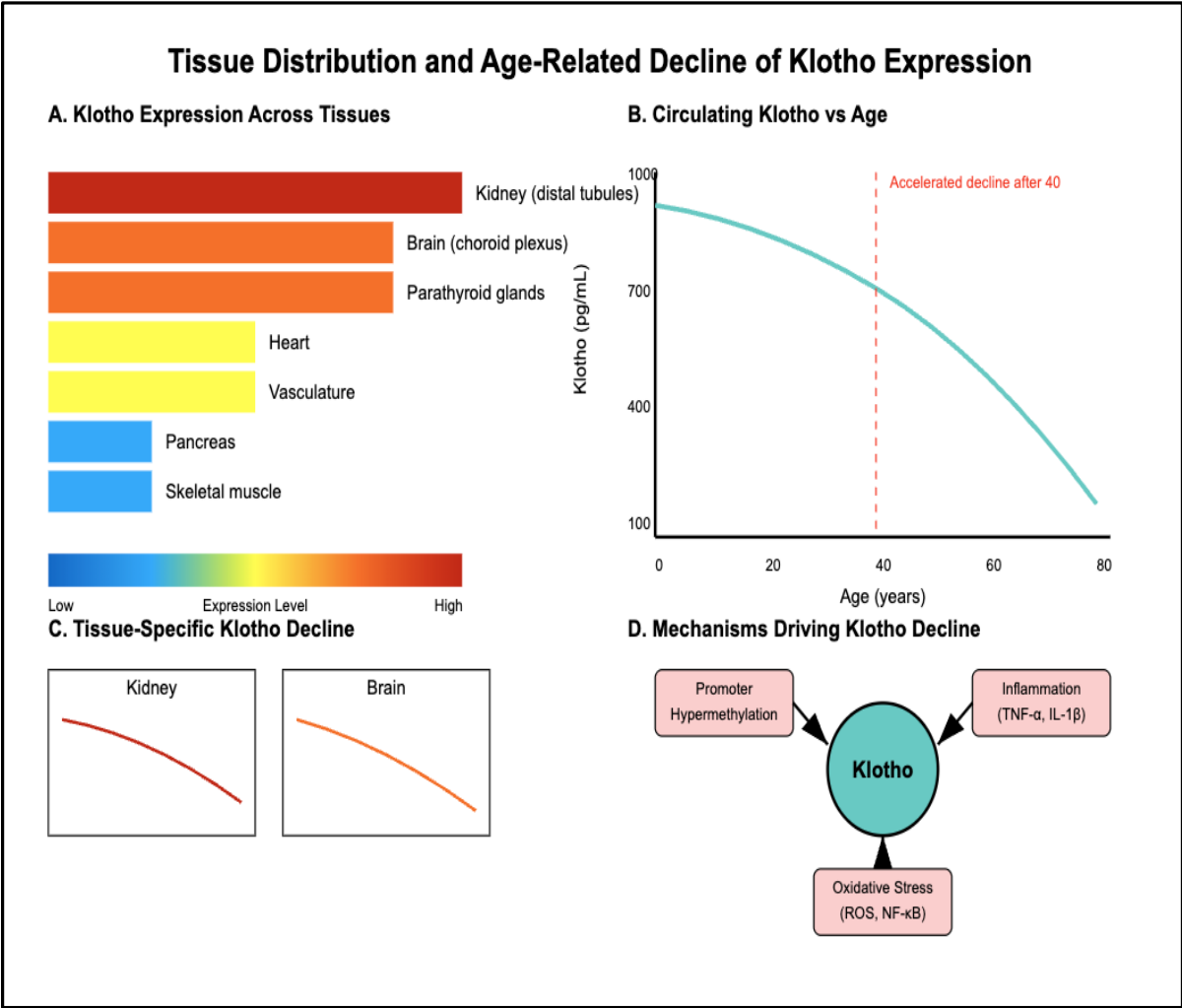


Figure 2: Tissue Distribution and Age-Related Decline of Klotho Expression.

A: Heat map showing relative Klotho expression levels across human tissues, with highest expression in kidney distal tubules, choroid plexus, and parathyroid glands. Color scale represents expression levels from low (blue) to high (red). **B:** Age-related decline in circulating Klotho levels from birth to 80 years, showing accelerated decrease after age 40. **C:** Parallel decline in kidney and brain Klotho mRNA expression with aging. **D:** Mechanisms driving age-related Klotho decline including promoter hypermethylation, inflammatory suppression (TNF- α , IL-1 β), and oxidative stress. Data represent mean $\pm$ SEM from multiple human cohort studies.

Several mechanisms contribute to this age-related decline. Epigenetic changes, particularly hypermethylation of the Klotho promoter region, reduce transcriptional activity with age [12].

Chronic inflammation, a hallmark of aging sometimes termed "inflammaging," suppresses Klotho expression through inflammatory mediators like $\text{TNF-}\alpha$ and $\text{IL-1}\beta$. Additionally, oxidative stress, which increases with age, downregulates Klotho through mechanisms involving $\text{NF-}\kappa\text{B}$ signaling and reactive oxygen species (ROS) [13].

The physiological consequences of declining Klotho levels are profound and multisystemic. Reduced Klotho contributes to phosphate retention and vitamin D dysregulation, accelerating vascular calcification and bone mineral density loss. Diminished neuroprotection leads to increased vulnerability to oxidative stress and inflammation in the brain, potentially contributing to cognitive decline and neurodegenerative diseases. Metabolically, lower Klotho levels correlate with insulin resistance and impaired glucose homeostasis, hallmarks of age-related metabolic disorders [14].

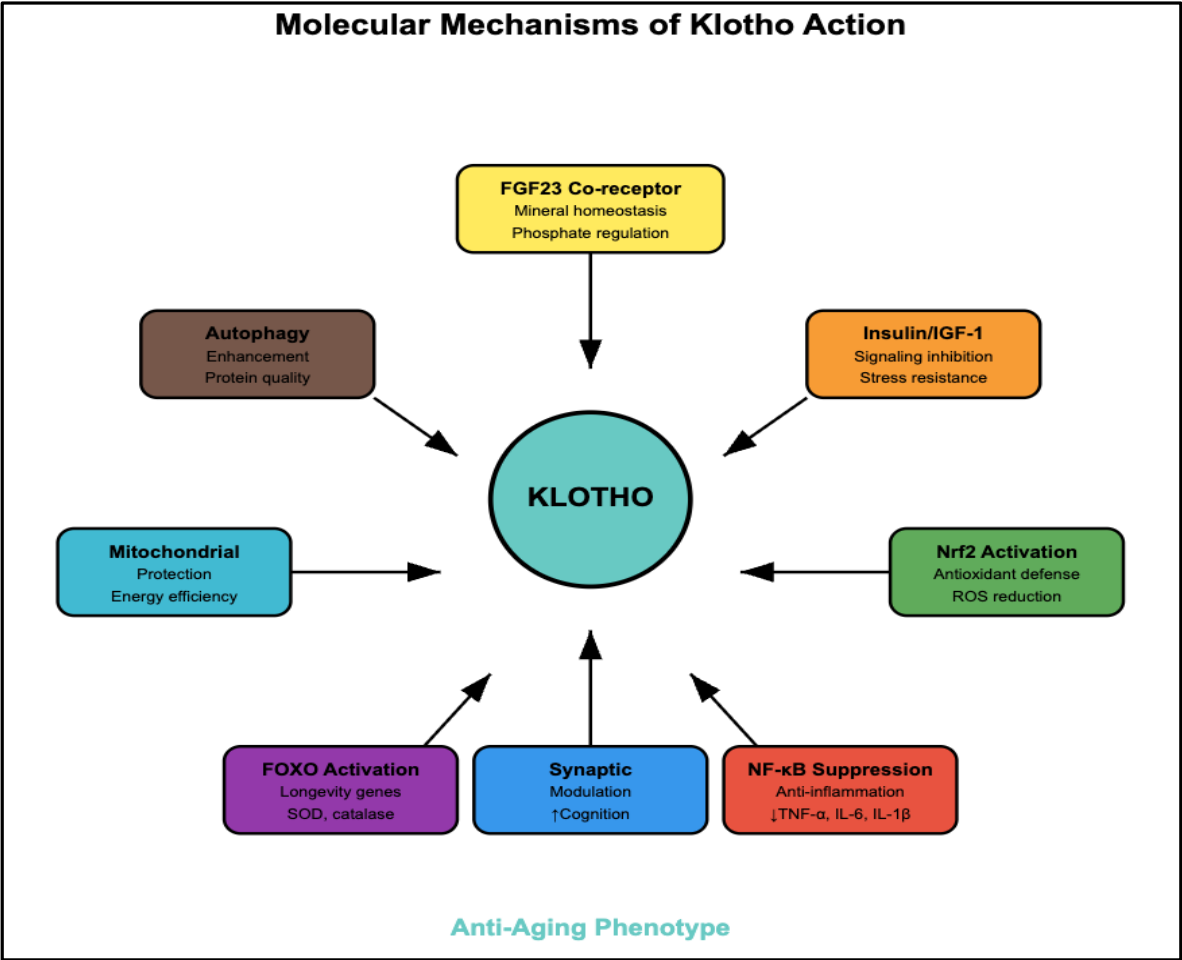


Figure 3: Molecular Mechanisms of Klotho Action.

Overview of Klotho's pleiotropic anti-aging mechanisms. Central node shows Klotho protein with arrows indicating diverse downstream effects: **1:** FGF23 co-receptor function regulating mineral homeostasis; **2:** Insulin/IGF-1 signaling inhibition promoting stress resistance; **3:** Nrf2 activation enhancing antioxidant defenses; **4:** NF- κ B suppression reducing inflammation; **5:** FOXO activation promoting longevity genes; **6:** Mitochondrial protection maintaining cellular energy; **7:** Autophagy enhancement improving protein quality control; **8:** Synaptic modulation enhancing cognition. Each pathway contributes to the overall anti-aging phenotype.

Mineral Homeostasis Regulation

Klotho's most established function involves mineral metabolism regulation as an obligatory co-receptor for FGF23 [15]. The membrane-bound form of Klotho forms a complex with FGFR1, enabling FGF23 binding and signaling [16]. This complex triggers a signaling cascade in kidney tubular cells that inhibits phosphate reabsorption by downregulating sodium-phosphate co-transporters (NaPi-2a and NaPi-

2c) [17]. Additionally, the FGF23-Klotho complex suppresses 1 α -hydroxylase (CYP27B1), the enzyme responsible for converting 25-hydroxyvitamin D to its active form, 1,25-dihydroxyvitamin D (calcitriol). By simultaneously reducing phosphate reabsorption and vitamin D activation, Klotho effectively functions as an anti-aging hormone that prevents mineral imbalances associated with aging [18].

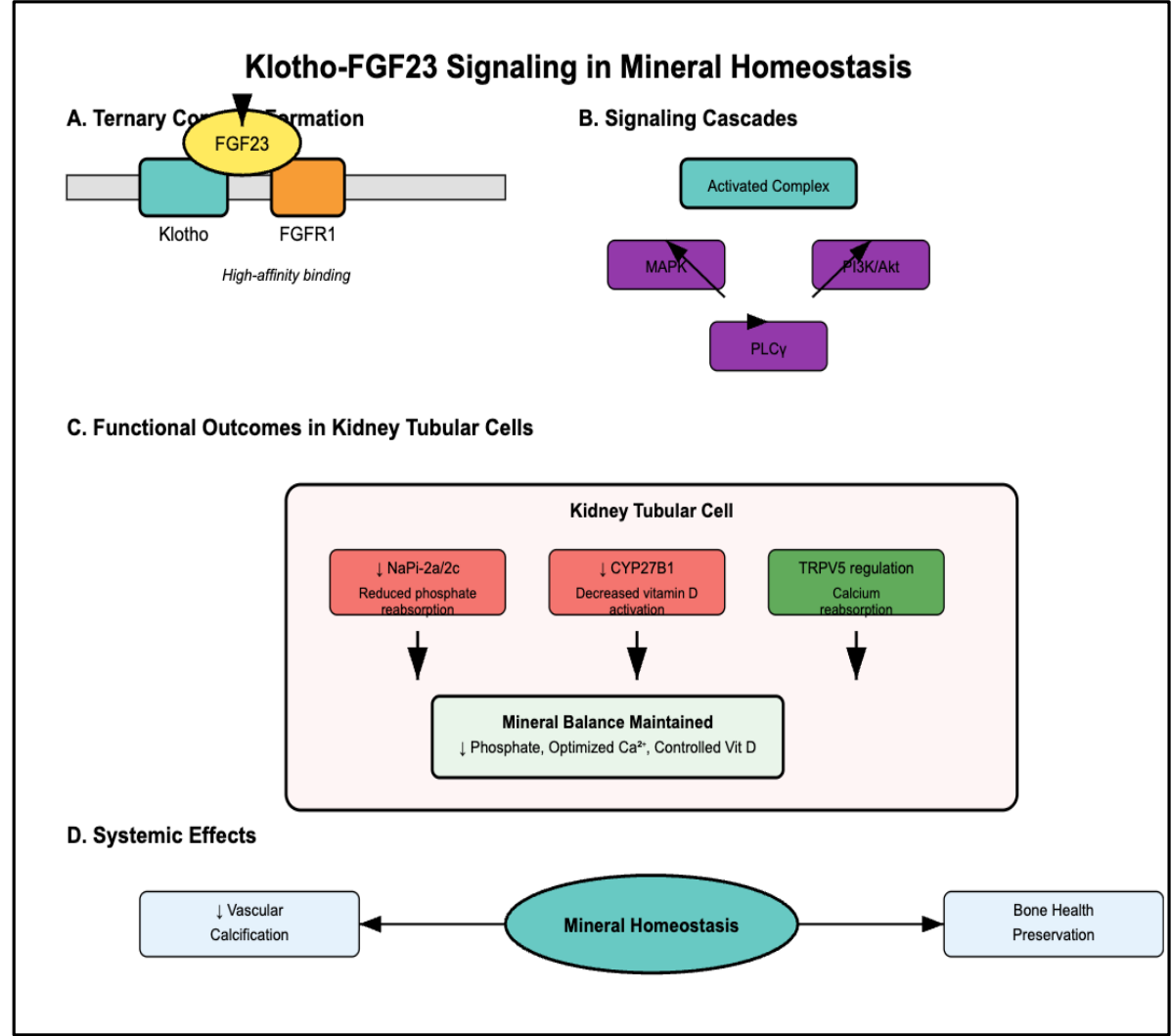


Figure 4: Klotho-FGF23 Signaling in Mineral Homeostasis.

Molecular mechanism of Klotho-FGF23 signaling in kidney tubular cells. **A:** Membrane-bound Klotho forms a ternary complex with FGFR1 and FGF23, enabling high-affinity binding. **B:** Activated complex triggers downstream signaling cascades including MAPK and PI3K pathways. **C:** Signaling outcomes include: downregulation of sodium-phosphate co-transporters (NaPi-2a/2c) reducing phosphate reabsorption; suppression of 1 α -hydroxylase (CYP27B1) decreasing vitamin D activation; regulation of TRPV5 channels modulating calcium reabsorption. **D:** Systemic effects maintain mineral balance and prevent age-related vascular calcification.

Without adequate Klotho, FGF23 resistance develops, leading to hyperphosphatemia, increased vitamin D activity, and elevated calcium levels. These mineral disturbances accelerate tissue calcification, particularly in the vasculature and kidneys, driving age-related dysfunction [19]. Indeed, the dramatic aging phenotype observed in Klotho-deficient mice largely stems from these mineral imbalances, as dietary restriction of vitamin D can substantially rescue many aspects of premature aging in these animals [20].

Beyond its role in phosphate regulation, the Klotho-FGF23 complex also influences calcium homeostasis by regulating both parathyroid hormone (PTH) and transient receptor potential vanilloid-5 (TRPV5) channels. This sophisticated mineral homeostasis system deteriorates with age-related Klotho decline, providing a strong rationale for Klotho-based interventions in age-related diseases characterized by mineral dysregulation [21].

Insulin/IGF-1 Signaling Modulation

Beyond its canonical role in mineral metabolism, Klotho exerts significant influence on aging through modulation of insulin/IGF-1 signaling a highly conserved pathway that regulates lifespan across multiple species. Soluble Klotho acts as an inhibitor of insulin and IGF-1 receptor signaling, creating a state of partial insulin resistance that paradoxically extends lifespan [2]. This function parallels mechanisms observed in other longevity interventions, including caloric restriction and mutations affecting nutrient-sensing pathways.

Mechanistically, Klotho suppresses insulin/IGF-1 signaling through direct inhibition of receptor autophosphorylation, reducing downstream activation of PI3K/Akt pathways [22]. This inhibition enhances cellular stress resistance by multiple means: activating AMPK, which promotes energy conservation and mitochondrial biogenesis

[23]; stimulating autophagy, improving cellular waste disposal and protein quality control [24]; and enhancing nuclear localization of forkhead box O (FOXO) transcription factors, promoting expression of stress resistance genes and antioxidant enzymes like superoxide dismutase and catalase [25].

Oxidative Stress Reduction and Anti-inflammatory Effects

A critical mechanism through which Klotho exerts anti-aging effects is by enhancing cellular defenses against oxidative stress—a major driver of aging characterized by the accumulation of reactive oxygen species (ROS) and subsequent macromolecular damage. Klotho orchestrates multiple pathways that collectively improve the cellular redox state and confer remarkable resistance to oxidative damage [26].

Central to Klotho's antioxidative function is its ability to activate the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, the master regulator of antioxidant responses. Klotho enhances nuclear translocation of Nrf2, increasing its binding to antioxidant response elements (AREs) in the promoter regions of cytoprotective genes [27]. This activation upregulates the expression of key antioxidant enzymes, including superoxide dismutase (SOD), catalase, glutathione peroxidase, and heme oxygenase-1 (HO-1) [28].

Klotho also inhibits NADPH oxidase, a major source of ROS production, particularly in vascular and renal tissues. By suppressing the assembly and activation of NADPH oxidase complexes, Klotho directly reduces ROS generation at its source [29]. At the mitochondrial level, Klotho improves function and reduces ROS leakage, enhancing mitochondrial integrity, respiratory chain efficiency, and membrane potential effects that collectively reduce mitochondrial ROS production while optimizing energy generation [30].

Complementing its antioxidative properties, Klotho exhibits potent anti-inflammatory effects that directly counteract "inflammaging" the chronic low-grade inflammation characteristic of aging. At the molecular level, Klotho suppresses nuclear factor-kappa B (NF- κ B) activation a master regulator of inflammatory responses. By inhibiting NF- κ B translocation to the nucleus, Klotho reduces transcription of pro-inflammatory cytokines including TNF- α , IL-6, and IL-1 β [31]. This anti-inflammatory action extends to inhibition of inflammasome activation, particularly

NLRP3, which drives caspase-1-mediated IL-1 β production [32].

Neuroprotection and Cognitive Enhancement

Klotho's neuroprotective and cognitive-enhancing effects represent particularly promising aspects of its anti-aging profile, with direct relevance to age-related neurological decline. In the brain, both membrane-bound and soluble Klotho contribute to neuronal health and function.

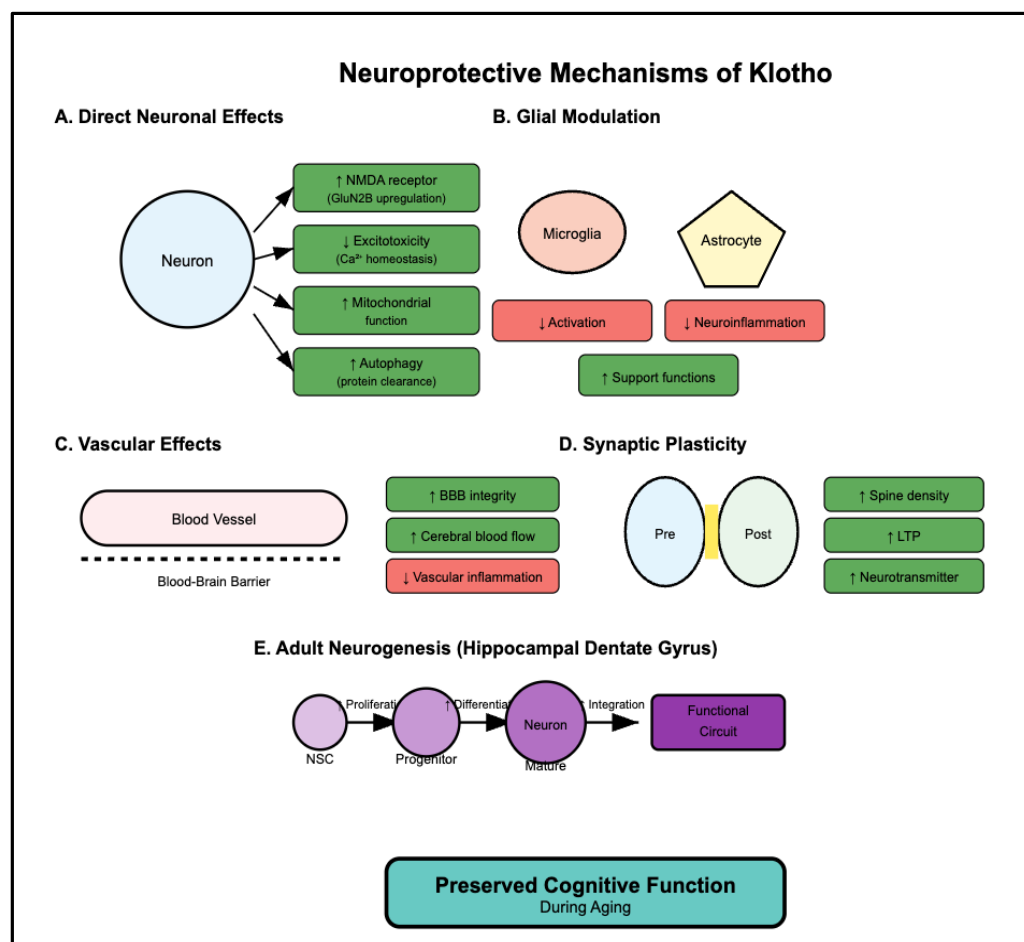


Figure 5: Neuroprotective Mechanisms of Klotho.

Klotho's multifaceted neuroprotection in the aging brain. **A:** Direct neuronal effects: enhanced NMDA receptor function through GluN2B upregulation, reduced excitotoxicity, improved mitochondrial function, and enhanced autophagy. **B:** Glial modulation: suppressed microglial activation, reduced neuroinflammation, enhanced astrocytic support functions. **C:** Vascular effects: improved blood-brain barrier integrity, enhanced cerebral blood flow, reduced vascular inflammation. **D:** Synaptic plasticity: increased spine density, enhanced LTP, improved neurotransmitter release. **E:** Adult neurogenesis: enhanced neural stem cell proliferation and differentiation in the hippocampal dentate gyrus. Together, these mechanisms preserve cognitive function during aging.

Klotho enhances synaptic plasticity the fundamental process underlying learning and memory by modulating N-methyl-D-aspartate (NMDA) receptor function. Specifically, Klotho increases the expression of the GluN2B subunit, which enhances long-term potentiation (LTP) and promotes experience-dependent plasticity [34].

This effect results in measurably improved cognitive performance, with Klotho-overexpressing mice demonstrating superior learning and memory compared to wild-type counterparts across multiple testing paradigms [35].

Beyond NMDA receptor modulation, Klotho influences additional neurotransmitter systems critical for cognitive function. Research indicates that Klotho regulates dopaminergic neuron survival and function, potentially protecting against age-related decline in reward processing and motor control [36]. Similarly, Klotho affects cholinergic signalling, which plays essential roles in attention and memory processing, and which typically deteriorates in Alzheimer's disease and other dementias [37].

Klotho provides remarkable neuroprotection against various insults associated with aging and neurodegeneration. It shields neurons from beta-amyloid toxicity through mechanisms involving enhanced antioxidant defences and reduced inflammatory responses [38].

Similarly, Klotho protects against glutamate excitotoxicity a common pathway in stroke and neurodegenerative diseases by preserving calcium homeostasis and mitochondrial function. These protective effects extend to oligodendrocytes and white matter integrity, potentially preserving neural connectivity with age [39].

At the structural level, Klotho supports adult neurogenesis, the generation of new neurons from neural stem cells which typically declines with age. Klotho enhances neural stem cell

proliferation, differentiation, and integration into functional circuits, particularly in the hippocampus, a brain region critical for memory formation [40]. This preservation of neural regenerative capacity may partially explain Klotho's cognitive benefits and suggests potential applications in conditions characterized by impaired neurogenesis [41].

Preclinical Evidence

Lifespan Extension in Animal Models

Preclinical studies have provided robust evidence for Klotho's longevity-promoting effects across multiple animal models. Transgenic mice overexpressing Klotho demonstrate 20-30% lifespan extension compared to wild-type controls, with benefits more pronounced in males (31%) than females (19%) [2]. These animals maintain better physical function, resistance to stressors, and delayed onset of age-related pathologies throughout their extended lifespan [42].

Importantly, Klotho interventions initiated later in life still yield significant benefits. AAV-mediated Klotho delivery to 18-month-old mice (equivalent to ~60 human years) extended remaining lifespan by 13-20%, accompanied by improved cognitive function and reduced systemic inflammation [43].

This late-life intervention efficacy is a promising finding for potential human applications [44]. Beyond standard laboratory mice, Klotho's longevity effects have been demonstrated in models of accelerated aging. In particular, AAV-Klotho treatment significantly extended lifespan in senescence-accelerated mouse models, including the SAMP8 strain characterized by accelerated aging and cognitive decline [45]. Mechanistic studies indicate that Klotho-induced lifespan extension involves multiple pathways.

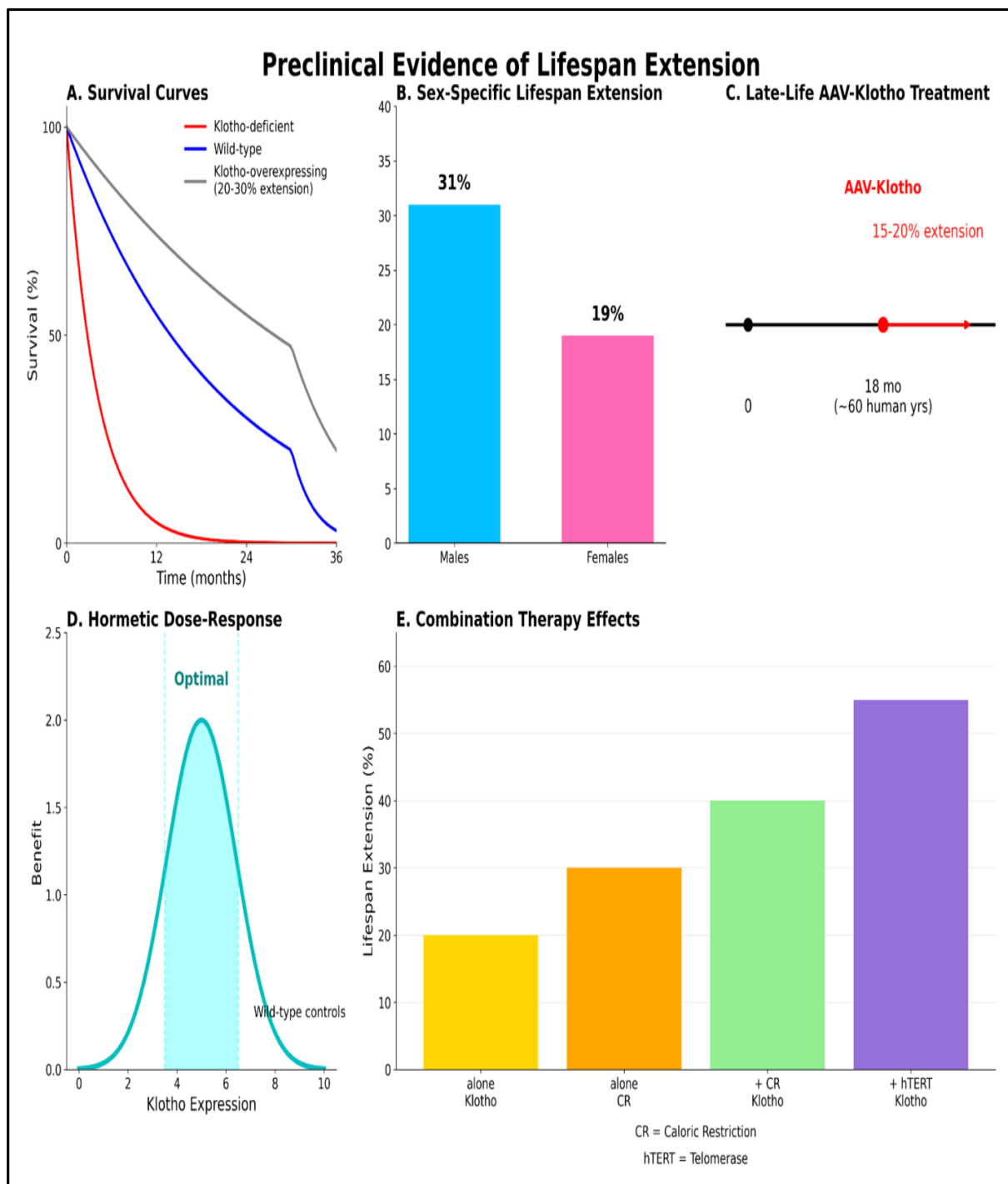


Figure 6: Preclinical Evidence of Lifespan Extension.

Summary of lifespan extension studies with Klotho interventions.

A: Kaplan-Meier survival curves comparing wild-type mice (gray), Klotho-deficient mice (red, dramatically shortened lifespan), and Klotho-overexpressing transgenic mice (blue, 20-30% extension). **B:** Sex-specific lifespan extension showing greater benefit in males (31%) versus females (19%). **C:** Late-life intervention efficacy: AAV-Klotho treatment initiated at 18 months (equivalent to ~60 human years) extends remaining lifespan by 13-20%. **D:** Dose-response relationship demonstrating hormetic curve with optimal benefits at moderate expression levels. **E:** Synergistic effects when combined with caloric restriction or telomerase activation.

Genetic analysis of long-lived Klotho-overexpressing mice revealed changes in the expression of genes related to oxidative stress resistance, inflammatory regulation, and DNA repair all critical factors in determining lifespan [46]. Combination studies examining Klotho alongside other longevity interventions have yielded particularly interesting

results. When combined with caloric restriction, Klotho overexpression produced synergistic effects on lifespan [47].

Similarly, the combination of Klotho with telomerase activation provided greater benefits than either intervention alone, pointing toward potential multi-target approaches for addressing complex aging processes [48].

Study	Model	Intervention	Key Findings	Lifespan Effect	Reference
Kurosu et al. 2005	Transgenic mice	Klotho overexpression	Reduced insulin/IGF-1 signaling	20-30% extension	[2]
Xie et al. 2015	Aged mice	AAV-Klotho at 18 months	Cognitive improvement, reduced inflammation	13-20% extension	[43]
Dubal et al. 2015	hAPP mice	AAV-Klotho	Reduced A β , enhanced cognition	Prevented mortality	[34]
Hu et al. 2015	CKD mice	Recombinant Klotho	Reduced cardiac remodeling	Not assessed	[18]
Castner et al. 2023	Aged primates	AAV-Klotho	Cognitive enhancement	Ongoing	[35]
Sewell et al. 2021	Human pilot	AAV-hTERT/Klotho	Cognitive stabilization	N/A	[48]

Table 3: Preclinical Studies of Klotho Gene Therapy.

Neurocognitive, Cardiovascular, and Renal Benefits

In neurocognitive studies, Klotho overexpression preserves cognitive function well into advanced age. Aged mice with elevated Klotho levels outperform age-matched controls in spatial learning and memory tasks, including Morris water maze and radial arm maze tests [33].

These animals maintain superior working memory, cognitive flexibility, and attention executive functions that typically decline with age. The cognitive preservation correlates with maintained hippocampal and prefrontal

cortical volumes, regions particularly vulnerable to age-related atrophy [49].

Electrophysiological studies demonstrate that Klotho-overexpressing mice maintain more robust long-term potentiation (LTP) in hippocampal slices compared to age-matched controls [41]. This enhanced synaptic strengthening mechanism translates to improved memory acquisition and retention. Additionally, these animals show increased neural stem cell proliferation in the subgranular zone of the dentate gyrus, supporting preserved neurogenesis with age [40].

Cardiovascular benefits of Klotho are equally compelling. In models of atherosclerosis, Klotho overexpression reduces plaque

formation and improves endothelial function through enhanced nitric oxide production and reduced oxidative damage [50]. Vascular calcification, a key feature of cardiovascular aging, is dramatically attenuated by Klotho, which inhibits phosphate-induced calcification in vascular smooth muscle cells [19].

Additionally, Klotho inhibits vascular smooth muscle cell calcification and phenotypic switching, processes that contribute to arterial stiffening with age [28].

Cardiac hypertrophy and fibrosis, hallmarks of age-related heart disease, are substantially attenuated by Klotho. In models of pressure overload, such as transverse aortic constriction, Klotho administration reduces pathological cardiac remodeling, preserves contractile function, and prevents the transition to heart failure [43].

These benefits appear mediated through inhibition of stress-activated signaling pathways, including MAPK cascades and TGF- β /Smad signaling, which typically drive hypertrophic and fibrotic responses [47]. In the kidney, Klotho's protective effects are equally impressive. Klotho-overexpressing mice demonstrate remarkable resistance to acute kidney injury from various insults, including ischemia-reperfusion, nephrotoxins, and inflammatory stimuli [51].

Following injury, these mice show reduced tubular damage, faster functional recovery, and less fibrotic remodeling. Chronic kidney disease progression is significantly slowed by Klotho therapy. In models of diabetic nephropathy, unilateral ureteral obstruction, and other chronic kidney diseases, Klotho administration reduces glomerulosclerosis, tubulointerstitial fibrosis, and proteinuria while preserving kidney function [52].

Human Studies

KL-VS Variant and Human Longevity

Human genetic studies have provided compelling evidence linking Klotho to longevity and healthy aging. The most extensively studied genetic variation is the KL-VS variant, a functional haplotype in the human KLOTHO gene consisting of six single nucleotide polymorphisms (SNPs), two of which result in amino acid substitutions (F352V and C370S) that affect Klotho function [53]. This variant influences both Klotho levels and activity, offering natural insights into how Klotho modulation might affect human aging.

Population studies reveal that heterozygous carriers of the KL-VS variant have longer lifespans compared to non-carriers. A landmark study examining Ashkenazi Jewish centenarians found significant enrichment of the KL-VS allele among long-lived individuals [54].

Subsequent meta-analyses across diverse ethnic groups confirmed this association, with heterozygous carriers showing 1.5-2 years increased median lifespan [55]. The KL-VS variant correlates with better preservation of cognitive function in aging populations, reduced risk of cardiovascular disease, and improved metabolic parameters [33].

The relationship between KL-VS and longevity follows a hormetic pattern. While heterozygous carriers show survival advantages, homozygous carriers experience adverse outcomes, including shorter lifespan and increased disease risk [56]. This pattern suggests an optimal window for Klotho activity, where moderate increases provide benefits, but excessive levels may become counterproductive an important consideration for therapeutic development.

Biomarker Studies and Preliminary Clinical Interventions

Human biomarker studies have revealed important associations between circulating Klotho levels and various age-related conditions. Cardiovascular disease shows consistent negative correlations with serum Klotho levels. Multiple cohort studies have demonstrated that individuals with lower circulating Klotho exhibit increased coronary artery calcification, arterial stiffness, and endothelial dysfunction all predictors of adverse cardiovascular outcomes [14].

Furthermore, prospective studies indicate that baseline Klotho levels independently predict cardiovascular events and mortality [57]. Kidney function demonstrates particularly strong relationships with Klotho levels. As the primary source of circulating Klotho, the kidneys show declining Klotho production with progressive kidney disease. Observational studies reveal that patients with chronic kidney disease (CKD)

exhibit substantially lower serum Klotho compared to age-matched individuals with normal kidney function, with levels declining in parallel with glomerular filtration rate [58]. Lower Klotho independently predicts faster CKD progression and higher risk of end-stage renal disease, suggesting potential utility as a prognostic biomarker [59].

Cognitive function and neurodegenerative diseases also show significant Klotho associations. Multiple cohort studies have found that individuals with higher circulating Klotho demonstrate better cognitive performance across various domains, including executive function, memory, and processing speed [60].

Conversely, patients with Alzheimer's disease and other dementias exhibit reduced Klotho levels compared to cognitively normal age-matched controls, with levels correlating inversely with disease severity [61].

Study Type	Population	Key Finding	Clinical Implication	Reference
Genetic association	Ashkenazi centenarians	KL-VS enrichment in long-lived	Genetic validation of longevity effect	[54]
Biomarker	CVD patients	Low Klotho predicts events	Prognostic marker	[14]
Cohort study	CKD patients	Klotho decline parallels eGFR	Disease progression marker	[58]
Cross-sectional	AD patients	Reduced CSF Klotho	Diagnostic potential	[61]
Meta-analysis	Multiple ethnicities	KL-VS heterozygotes live 1.5-2 years longer	Population health impact	[55]
Intervention	Dementia patients	Combined gene therapy improved cognition	Therapeutic proof-of-concept	[48]

Table 4: Human Studies Linking Klotho to Longevity and Disease.

Preliminary clinical interventions provide encouraging evidence for Klotho-based therapies. A small-scale study of recombinant Klotho administration in CKD patients demonstrated improvements in mineral metabolism and

vascular function without significant adverse effects [62]. More recently, a 2021 safety study of AAV-mediated delivery of combined hTERT and Klotho genes in dementia patients showed telomere elongation and cognitive stabilization over

cognitive stabilization over 6 months, with no serious adverse events [48].

Follow-up assessments of these participants demonstrated improvement on standardized measures of cognitive function, particularly the Folstein exam used to track dementia progression [48].

This cognitive enhancement contrasted with the typical downward trajectory observed in untreated dementia patients, suggesting potential disease-modifying effects.

Gene Therapy Delivery Strategies

Vector Design and Tissue-Specific Targeting

The development of effective gene therapy for Klotho requires careful vector design to ensure optimal expression, safety, and therapeutic efficacy. Several vector systems have been investigated for Klotho delivery, with adeno-associated virus (AAV) vectors emerging as the leading platform due to their favourable safety profile and efficient gene transfer capabilities [63].

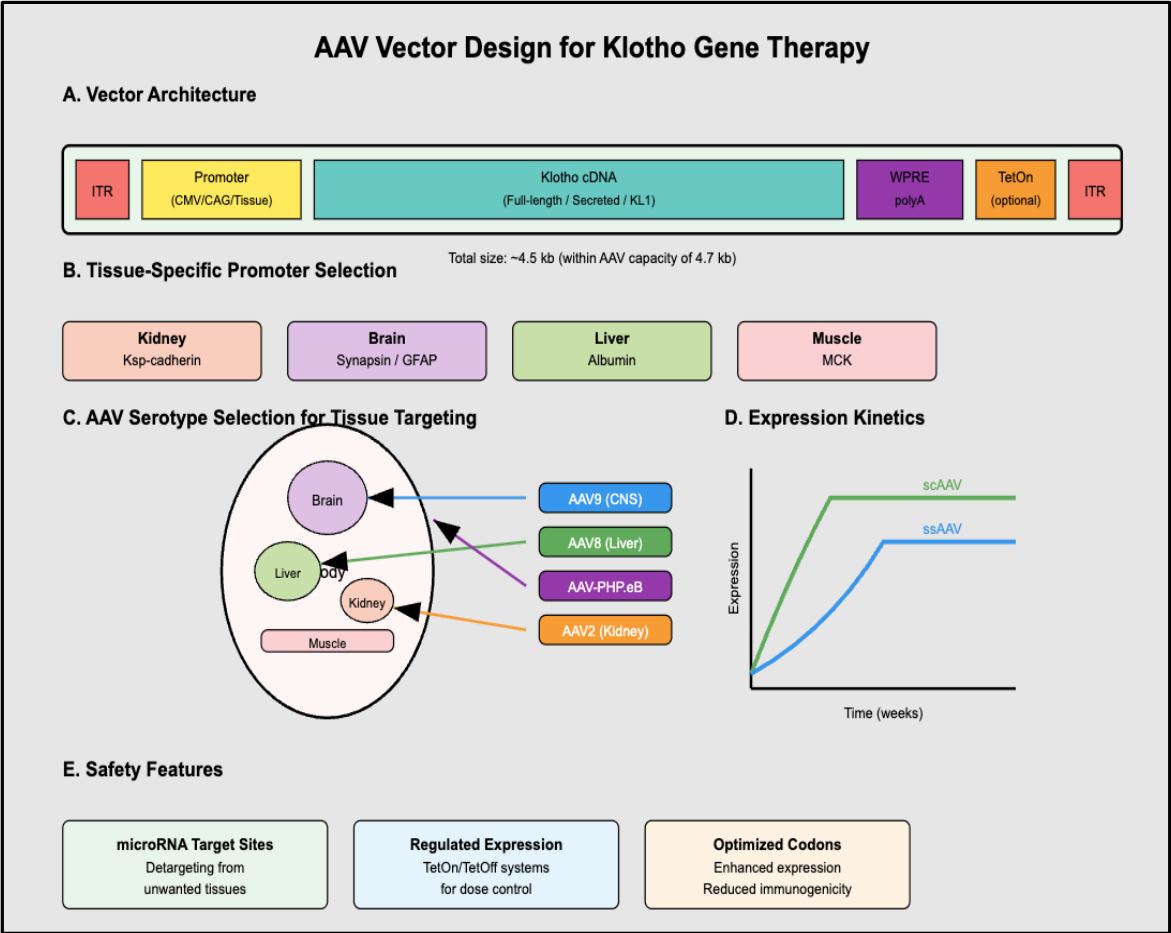


Figure 7: AAV Vector Design for Klotho Gene Therapy.

Optimized AAV vector constructs for Klotho delivery. **A:** Basic vector architecture showing ITRs (inverted terminal repeats), promoter options (CMV, CAG, or tissue-specific), Klotho cDNA variants (full-length, secreted, or KL1 domain), regulatory elements (WPRE, polyA), and optional inducible systems (TetOn). **B:** Tissue-specific promoter selection: kidney (Ksp-cadherin), brain (synapsin, GFAP), liver (albumin), muscle (MCK). **C:** AAV serotype tropism showing preferential targeting: AAV9 (broad CNS), AAV8 (liver/muscle), AAV-PHP.eB (enhanced BBB crossing), AAV2 (kidney). **D:** Expression kinetics comparing self-complementary (scAAV) versus single-stranded AAV vectors. **E:** Safety features including microRNA target sites for detargeting and regulated expression systems.

At approximately 3 kb, the full-length Klotho cDNA (α Klotho) approaches but remains within the packaging capacity of AAV vectors (approximately 4.7 kb), allowing inclusion of regulatory elements while maintaining efficient packaging [64]. For applications where only the soluble form is desired, truncated Klotho constructs encoding just the KL1 domain or the secreted variant can reduce size constraints and potentially improve expression efficiency [7].

Promoter selection critically influences the success of Klotho gene therapy. Constitutive promoters like CMV and CAG provide high-

level expression but lack tissue specificity and may trigger immune responses or silencing over time [65]. For sustained Klotho expression, tissue-specific promoters offer advantages. Kidney-specific promoters (e.g., Ksp-cadherin or sodium-phosphate cotransporter promoters) enable targeted expression in the natural site of Klotho production [58].

Brain-directed Klotho therapy benefits from neuron-specific promoters like synapsin or glial-specific promoters such as GFAP, depending on the target cell population [33].

AAV Serotype	Primary Tropism	Route of Administration	Advantages	Limitations
AAV8	Liver, muscle, heart	Intravenous	Broad distribution, low immunogenicity	Limited CNS penetration
AAV9	CNS, heart, muscle	Intravenous/intrathecal	Crosses BBB, broad tropism	Pre-existing immunity common
AAV-PHP.eB	Enhanced CNS	Intravenous	Superior BBB crossing	Species-specific, requires engineering
AAV2	Kidney, eye	Local injection	Established safety profile	Limited spread, slow expression
AAV5	Lung, CNS	Inhalation/intrathecal	Low seroprevalence	Variable transduction efficiency
AAVrh10	CNS, heart	Intravenous/intrathecal	Good CNS distribution	Moderate immunogenicity

Table 5: AAV Serotypes for Tissue-Specific Klotho Delivery.

BBB: Blood-brain barrier; CNS: Central nervous system

Capsid selection significantly affects tissue tropism and transduction efficiency. For systemic Klotho delivery, AAV8 and AAV9 serotypes offer broad tissue distribution and good expression in liver, muscle, and brain [63]. For kidney-targeted therapy, AAV2 and AAV8 show preferential renal tropism [66]. Central nervous system delivery has advanced with engineered capsids like AAV-PHP.eB, which efficiently cross the blood-brain barrier following systemic administration[67].

Intracerebroventricular or intraparenchymal injection routes can achieve more direct brain targeting when needed [68]. Regulatory

elements enhance therapeutic precision. Self-complementary AAV designs accelerate expression onset, valuable for acute interventions [69].

MicroRNA target sites incorporated into the vector can detarget expression from unwanted tissues, reducing off-target effects [70]. For brain applications, synapsin promoters direct expression to neurons, while glial fibrillary acidic protein (GFAP) promoters target astrocytes [71].

This cellular specificity is particularly relevant for Klotho, as different brain cell types may benefit from either membrane-bound or secreted forms of the protein.

Dosage Considerations and Expression Control

Determining optimal dosing regimens and implementing sophisticated expression control systems represent critical challenges in Klotho gene therapy development. Vector dose-response relationships for Klotho gene therapy demonstrate non-linear characteristics that inform dosing strategies. Preclinical studies indicate a therapeutic threshold effect, where benefits emerge once a minimal level of Klotho expression is achieved [72].

However, evidence from KL-VS genetic studies suggests a hormetic response pattern, where moderate Klotho increases provide benefits while excessive levels may become counterproductive. Heterozygous carriers show longevity benefits while homozygotes experience adverse outcomes [53].

This pattern suggests an optimal therapeutic window rather than a simple "more is better" approach, necessitating careful dose calibration. The different forms of Klotho (membrane-bound versus soluble) require distinct dosing considerations. For membrane-bound Klotho, which functions primarily as an FGF23 co-receptor, expression must reach sufficient levels in appropriate tissues to form functional receptor complexes [6].

Conversely, soluble Klotho acts as a circulating hormone with diverse systemic effects, requiring dosing that achieves therapeutic serum concentrations while avoiding potential off-target effects [7].

Inducible expression systems offer sophisticated control over Klotho therapy. Tetracycline-regulated systems (Tet-On/Tet-Off) allow external regulation through tetracycline analogs like doxycycline, enabling titration of expression levels and discontinuation if adverse effects emerge [73].

These systems can establish intermittent Klotho expression patterns potentially sufficient for therapeutic benefits while reducing any risks associated with continuous high-level expression [25]. Emerging technologies like small molecule-inducible systems provide additional options for fine-tuned control [72].

Route of administration influences dosing requirements. Systemic intravenous delivery requires higher vector doses to achieve therapeutic expression due to distribution across multiple tissues [63]. Localized delivery, such as intrathecal for neurological applications or intra-renal for kidney disorders, allows lower doses while achieving higher local concentrations [68].

Therapeutic Applications

Neurodegenerative Diseases

Klotho gene therapy shows particular promise for neurodegenerative conditions [34]. Alzheimer's disease (AD) stands as a primary target for Klotho intervention. Preclinical studies demonstrate that Klotho reduces multiple AD pathologies, including amyloid-beta accumulation, tau hyperphosphorylation, neuroinflammation, and synaptic loss [74].

Mechanistically, Klotho enhances amyloid clearance through microglial activation and increased expression of amyloid-degrading enzymes like neprilysin. It also suppresses tau phosphorylation by inhibiting GSK3 β activity, a key kinase in tau pathology [75]. Beyond these disease-specific effects, Klotho's general neuroprotective properties reducing oxidative stress, suppressing inflammation, and enhancing mitochondrial function addresses fundamental neurodegenerative processes in AD [72]. Human evidence further supports Klotho's relevance in AD. Patients with Alzheimer's disease show reduced Klotho levels in cerebrospinal fluid and blood compared to age-matched controls, with levels inversely correlating with disease severity [61].

Additionally, the KL-VS variant associated with higher Klotho levels confers reduced AD risk and delayed onset. The preliminary human study of combined Klotho-

hTERT therapy in dementia patients demonstrated cognitive improvements, providing early clinical validation for this approach [48].

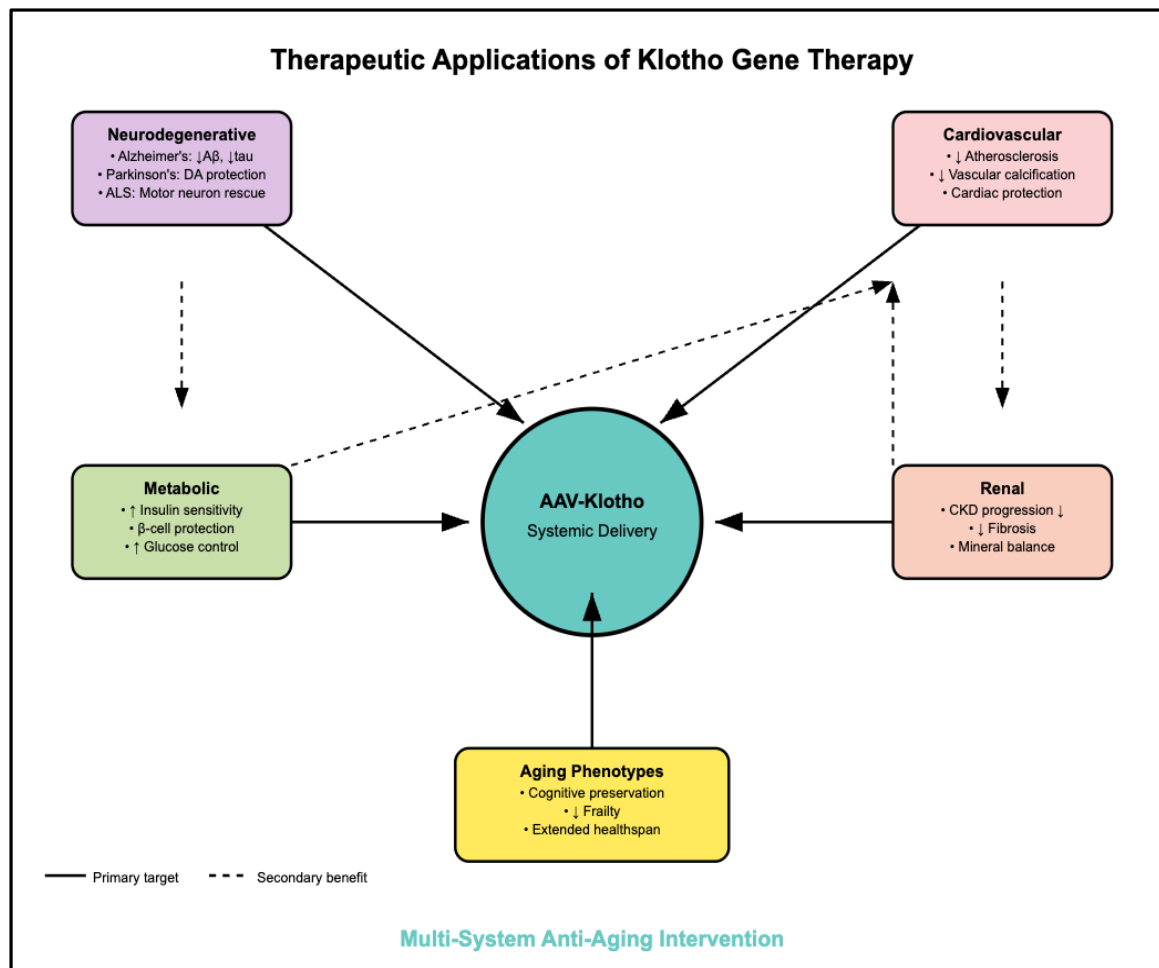


Figure 8: Therapeutic Applications of Klotho Gene Therapy.

Overview of disease targets for Klotho-based interventions. Central image shows systemic AAV-Klotho delivery with tissue-specific effects on major organ systems. **A:** Neurodegenerative diseases: Alzheimer's (reduced Aβ and tau), Parkinson's (dopaminergic protection), ALS (motor neuron preservation). **B:** Cardiovascular: atherosclerosis prevention, reduced vascular calcification, cardiac protection. **C:** Renal: CKD progression slowing, reduced fibrosis, improved mineral balance. **D:** Metabolic: enhanced insulin sensitivity, β-cell protection, improved glucose homeostasis. **E:** Aging phenotypes: cognitive preservation, reduced frailty, extended healthspan. Arrows indicate primary (solid) and secondary (dashed) therapeutic targets.

Parkinson's disease (PD) offers another promising application for Klotho therapy. Preclinical models show that Klotho protects dopaminergic neurons against both toxin-induced and genetic forms of PD [76]. This protection involves multiple mechanisms: reduced α-synuclein aggregation, enhanced mitochondrial function in neurons, suppressed

microglial activation, and increased expression of neurotrophic factors that promote dopaminergic neuron survival [36].

Amyotrophic lateral sclerosis (ALS) and other motor neuron diseases may benefit from Klotho's broad neuroprotective effects. In ALS models, Klotho reduces motor neuron death,

attenuates neuroinflammation, and improves neuromuscular junction integrity [77].

Its ability to enhance autophagy may help clear protein aggregates characteristic of ALS [78]. Recent advancements include Klotho Neurosciences advancing KLTO-202 manufacturing for ALS gene therapy, with FDA orphan drug designation [79].

Metabolic and Cardiovascular Disorders

Metabolic disorders, particularly type 2 diabetes and obesity, represent compelling targets for Klotho therapy. Preclinical evidence demonstrates that Klotho improves insulin sensitivity, enhances glucose uptake in peripheral tissues, and protects pancreatic beta cells from glucotoxicity [80]. In diabetic models, Klotho administration reduces hyperglycemia, ameliorates diabetic nephropathy, and prevents cardiovascular complications [31].

Cardiovascular diseases represent significant potential applications for Klotho gene therapy, supported by strong mechanistic rationale and preclinical evidence. Atherosclerosis, the underlying cause of most heart attacks and strokes, shows particular responsiveness to Klotho in preclinical models [50].

Klotho suppresses multiple atherogenic processes: it improves endothelial function by enhancing nitric oxide production and reducing inflammatory activation; inhibits vascular smooth muscle cell proliferation and migration; reduces foam cell formation by modifying lipid metabolism in macrophages; and decreases platelet adhesion and activation [28].

Vascular calcification, a major contributor to cardiovascular morbidity, represents another compelling target. Klotho powerfully inhibits calcium-phosphate deposition in vascular tissues through multiple mechanisms [19].

As an FGF23 co-receptor, it improves systemic mineral homeostasis, reducing phosphate retention that drives calcification. Directly in vascular cells, Klotho inhibits osteoblastic differentiation of smooth muscle cells by suppressing RUNX2 and other osteogenic transcription factors [81].

Heart failure, both with reduced and preserved ejection fraction, shows responsiveness to Klotho in experimental models [43]. Klotho protects cardiomyocytes from various stressors, including oxidative damage, inflammatory cytokines, and excess aldosterone. It preserves mitochondrial function in cardiac cells, maintaining energetic capacity under stress conditions [18].

Renal Pathologies

Chronic kidney disease (CKD) progression could be substantially altered by Klotho gene therapy. As Klotho deficiency drives many CKD complications, therapeutic restoration could overcome the FGF23 resistance characteristic of CKD, improving phosphate handling and reducing hyperphosphatemia [58].

Safety Profile and Challenges

Potential Adverse Effects and Immune Considerations

While Klotho therapy shows substantial promise, understanding and mitigating potential adverse effects remains essential for safe clinical translation. Evidence from genetic studies suggests a hormetic response to Klotho levels, where excessive elevation could potentially lead to adverse effects similar to those observed in KL-VS homozygotes, including altered mineral metabolism or increased cancer risk [53].

Preclinical studies indicate possible risks of hypophosphatemia or suppressed vitamin D

levels with excessive Klotho activity, potentially affecting bone health if not monitored [20].

Immune responses to gene therapy vectors represent another key consideration. AAV vectors, while generally well-tolerated, can elicit both humoral and cellular immune responses that may affect both safety and efficacy. Vector immunity represents a primary concern, particularly for AAV-based approaches. Pre-existing neutralizing antibodies against AAV capsids are present in a substantial proportion of the human population, with prevalence varying by serotype and geographic region [82].

These antibodies can neutralize the vector before gene delivery, reducing efficacy. Additionally, primary exposure typically generates new antibodies that may prevent successful readministration if needed.

Anti-Klotho immune responses present a more complex consideration. As an endogenous protein expressed in multiple tissues, Klotho typically elicits minimal immune responses when delivered as replacement therapy [65].

However, several factors could potentially increase immunogenicity: supraphysiological expression levels, altered glycosylation or post-translational modifications in non-native production cells, or breakdown of immune tolerance in elderly or autoimmune-prone individuals [83]. Monitoring for anti-Klotho antibodies and T-cell responses should be incorporated into clinical studies, particularly for systemic or high-dose applications [62].

Special considerations apply to brain-targeted Klotho therapy, where neuroinflammation requires particular attention. While the brain's immune privilege reduces some vector-related concerns, microglial and astrocyte activation following

vector administration could influence both safety and efficacy [83].

Preclinical evidence suggests Klotho itself reduces rather than exacerbates neuroinflammation, potentially mitigating these concerns. Nevertheless, initial clinical evaluations should include cerebrospinal fluid analysis for inflammatory markers and detailed neurological monitoring [68].

Conclusion and Research Directions

Klotho Alpha gene therapy represents a promising frontier in addressing aging-related diseases and potentially enhancing human healthspan. The convergence of evidence from molecular biology, animal models, human genetics, and preliminary clinical studies supports Klotho's central role in multiple aging processes and its therapeutic potential [3].

Future research should prioritize optimization of delivery systems, including next-generation AAV capsids with enhanced tropism and reduced immunogenicity [63]. Long-term safety studies in larger animal models, particularly nonhuman primates, will be crucial for clinical translation [35].

Combination therapies integrating Klotho with other interventions may prove particularly effective [48]. Additional combinations—such as Klotho with senolytics, NAD⁺ enhancers, or mitochondrial modulators warrants investigation for their potential to comprehensively address the multifaceted nature of aging [24]. While challenges remain, particularly regarding optimal dosing, long-term safety, and delivery optimization, the substantial therapeutic potential of Klotho gene therapy justifies continued investment in research and development. By targeting a fundamental regulator of aging processes, Klotho-based interventions offer the prospect of addressing multiple age-related diseases simultaneously

rather than treating individual conditions in isolation [84].

In conclusion, Klotho Alpha gene therapy represents a promising approach to addressing the growing global burden of age-related diseases. As research advances our understanding of Klotho biology and refines therapeutic strategies, this intervention may help realize the goal of extending not just lifespan but healthspan, allowing individuals to live longer lives with enhanced physical and cognitive function [85]. The coming years will likely witness significant progress in translating the remarkable potential of this "longevity protein" from basic science to transformative therapies that

reshape our approach to aging and age-related diseases [3, 21].

Conflict of Interest Statement

Dr. Patrick E. Sewell is the founder, CEO, and Chief Scientist of TripleHelix (triplehelixscience.com), a company offering gene therapies mentioned in this review. The author declares that this relationship has not influenced the objectivity of the scientific content presented.

Data Availability Statement

All data discussed in this review are available from the cited sources. No new data were generated for this review.

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