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Research Article

The Role of Klotho in Pediatric Diseases and Aging

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Abstract

Klotho seems to play an important role in different pediatric diseases. We focus on the role of Klotho and different pediatric diseases. Any correlations were found in chronic kidney disease, sickle cell disease, depression in childhood, Non-Alcoholic Fatty Liver Disease (NAFLD), surgery for Congenital Heart Defects (CHD), beta thalassemia major, diabetic pediatric nephropathy, pediatric hematopoietic stem cell development and erythropoiesis, sevoflurane induced pediatric neuronal injury and nevertheless, aging in children. Klotho plays a crucial role in aging processes, even in children. Especially in children with Hutchinson-Gilford syndrome, in which the children undergo a premature aging process, Klotho could play a crucial role. Investigating this would be a desirable approach to evaluate more precisely the role of Klotho on the child organism.

Introduction

In greek mythology, Klotho (Greek Κλωθώ Klōthṓ, German 'Spinner ') was the youngest of the three Moirs. Her task was to spin the thread of life, which is measured by Lachesis and cut by Atropos. According to Hesiod, Klotho was a daughter of Zeus and Themis. Elsewhere in Theogony, however, the goddesses of fate (Moirs) were called children of Nyx ("Night"). Their Roman equivalent was Nona (the "Ninth"), originally a goddess invoked in the 9th month of pregnancy. She was supposed to decide the fate of the birth. Klotho is a proteohormone that can prolong life in mice by approximately 20 to 30%. It is a membrane-bound protein with a size of 130 kDa. In addition, there is a soluble form of the protein called Solube Klotho (SKL). Proteolytic cleavage upstream (α-cut) or downstream (β-cut) of the KL2 domain of the protein, results in either a 130-kDa sKL, or 60-70-kDa klotho fragments. In 1997, Makoto Kuro'o at the National Institute of Neuroscience in Tokyo identified a new gene that extends lifespan in mice when expressed in increased amounts [1-25,26]. A defect in this gene leads to a syndrome similar to human aging. Affected animals have a reduced life expectancy, are infertile and develop typical age-related diseases such as arteriosclerosis, skin atrophy, osteoporosis and emphysema. The Klotho gene encodes a transmembrane protein with a transmembrane domain. The Klotho protein binds as a co-receptor to several fibroblast growth factor receptors 22. fibroblast growth factors are known, but only four different receptors. It is therefore thought that the specificity of the receptor proteins is mediated by co-receptors. Only the binding of Klotho to fibroblast growth factor receptor 1, FGFR1 subtype IIIc, leads to the specific binding of fibroblast growth factor 23. FGF23 is a bone-derived hormone that inhibits phosphate reabsorption and vitamin D synthesis in the kidney. Mice treated with a monoclonal antibody against Klotho no longer respond to FGF [23]. The Klotho protein is expressed primarily in the kidney, but also in the placenta, prostate, lung, spleen, and parathyroid gland 6. Current knowledge suggests that the kidney is the major source of soluble Klotho, as no sKL circulates in kidney-specific Klotho knockout mice. In the kidney inhibits reabsorption of phosphate in proximal tubule cells by direct binding to the FGF receptor, regulates calcium reabsorption by stabilizing the TRPV5 calcium channel in the cell membrane. Klotho inhibits 1α-hydroxylase and thus the activation of 25(OH)vitamin D3 to calcitriol. The effect on calcium and on phosphate transport in the kidney is synergistic with the effects of parathyroid hormone, whereas the effect on calcitriol synthesis is antagonistic. The extracellular domain of Klotho

is cleaved off and becomes a humoral factor. The secreted Klotho protein regulates several signaling pathways, including the insulin/IGF-1 pathway and the Wnt signaling pathway, as well as the activity of many ion channels.

Klotho

Klotho is a proteohormone that can prolong life in mice by approximately 20 to 30% [1-5]. It is a membrane-bound protein with a size of 130 kDa [1,4,13]. In addition, there is a soluble form of the protein called Solube Klotho (sKL). Proteolytic cleavage upstream (α-cut) or downstream (β-cut) of the KL2 domain of the protein, results in either a 130-kDa sKL, or 60-70-kDa klotho fragments. A defect in this gene leads to a syndrome similar to human aging. Affected animals have a reduced life expectancy, are infertile and develop typical age-related diseases such as arteriosclerosis, skin atrophy, osteoporosis, and emphysema. The Klotho gene encodes a transmembrane protein with a transmembrane domain [1-38]. The Klotho protein binds as a co-receptor to several fibroblast growth factor receptors [36]. To date, 22 fibroblast growth factors are known, but only four different receptors. Therefore, it is thought that the specificity of the receptor proteins is mediated by co-receptors [36]. Only the binding of Klotho to fibroblast growth factor receptor 1(FGFR1 subtype IIIc) leads to the specific binding of fibroblast growth factor 23 (FGF23) [36]. FGF23 is a bone-derived hormone that inhibits phosphate reabsorption and vitamin D synthesis in the kidney. Mice treated with a monoclonal antibody against Klotho no longer respond to FGF23. The Klotho protein is expressed primarily in the kidney, but also in the placenta, prostate, lung, spleen, and parathyroid gland [1-38]. According to current knowledge, the kidney is the main source of soluble Klotho, since no sKL circulates in kidney-specific Klotho knockout mice. In the kidney, Klotho inhibits phosphate reabsorption in proximal tubule cells by direct binding to the FGF receptor, Klotho regulates calcium reabsorption by stabilizing the TRPV5 calcium channel in the cell membrane, Klotho inhibits 1α-hydroxylase and thus activation of 25 (OH)vitamin D3 to calcitriol. The effect on calcium and on phosphate transport in the kidney is synergistic with the effects of parathyroid hormone, whereas the effect on calcitriol synthesis is antagonistic. The extracellular domain of Klotho is cleaved off, becoming a humoral factor. The secreted Klotho protein regulates several signaling pathways, including the insulin/IGF-1 pathway and the Wnt signaling pathway, as well as the activity of many ion channels. Klotho protein protects cells and tissues from oxidative stress, but the exact mechanism has not yet been elucidated. Mice with a defect in the gene for FGF23 have a close resemblance to mice with a defect in the Klotho gene. Knockout mice for FGF23 and Klotho have multiple defects: decreased lifespan, decreased sexual maturity with infertility, kyphosis, Arteriosclerosis, extensive soft tissue calcifications, atrophy of the skin, muscle atrophy, impaired T cell function, pulmonary emphysema, osteopenia, impaired mineral balance, especially calcium-phosphate balance, impaired vitamin D metabolism. Because of these changes, there is currently much speculation about a close link between aging of the vascular system, vitamin D and phosphate balance.

In 2007, a homozygous mutation of the Klotho gene was described for the first time in humans. It affected a 13-year-old girl who developed severe calcinosis (Teutschländer's disease) with calcifications of the common carotid artery and dura mater. There were disturbances in mineral metabolism with increased serum phosphate, increased serum calcium, increased parathyroid hormone, and increased FGF23. Expression and secretion of Klotho were markedly reduced, resulting in impaired signal transduction of FGF23 via the FGF23 receptor. Patients with chronic kidney disease have decreased Klotho levels compared to healthy individuals. Especially in the late stage of the disease, Klotho can be used as an indicator for prognosis. A reduction of the protein can be triggered, for example, by oxidative stress, inflammatory mediators or hyperglycemia. Deficiency of Klotho leads to increased FGF23 expression, which in turn leads to reduction of vitamin D3 synthesis. As a consequence, the renin-angiotensin system is activated, which also leads to Klotho suppression. Through multiple feedback mechanisms, CKD patients experience low levels of klothos, which is associated with poorer disease outcome. While every human has the klotho gene, one in five also has the KL-VS variant, which is more highly expressed. These people have both a longer life expectancy and greater intelligence, as recent tests involving more than 700 participants between the ages of 52 and 85 showed. Complementing this, experiments in transgenic mice have confirmed that increased levels of Klotho increase learning ability and memory, regardless of the age of the mice. The mechanisms of action of Klotho are very diverse and complex. For its action in the central nervous system, it is important that it increases GluN2B subunits at the NMDA receptor. These subunits are critical for stimulus transmission at synapses and for brain plasticity. However, high levels of Klotho do not protect against degenerative processes in the brain or dementia [37,38]. The advantage for dementia patients who possess the KLVS gene is that mental decline begins at a higher brain performance level and lasts correspondingly longer [37,26].

Klotho and its Role in Different Pediatric Diseases and Aging

Any correlations were found in chronic kidney disease [5,13-15,17,21], sickle cell disease [10,12], depression [16], Non-Alcoholic Fatty Liver Disease (NAFLD) [2,18], surgery for congenital heart defects (CHD) [3,19,20], beta thalassemia major [11], diabetic pediatric nephropathy [8,9,22] pediatric hematopoietic stem cell development and erythropoiesis [23], sevoflurane induced pediatric neuronal injury [6] and nevertheless, aging in children [4,24,25].

Chronic Kidney Disease in Children

The change in concentration of soluble α-klotho during the 1.5-year follow-up was an indicator of CKD progression [5]. Renal damage associated with a reduction of α-klotho may involve the

upregulation of plasma aldosterone [5]. Future studies are needed to validate our findings, and to investigate the underlying mechanism by which α-klotho and aldosterone may cause renal damage. Circulating α-Klotho seems to be related to plasma aldosterone and its follow-up change predicts chronic kidney disease progression in childhood [13-15, 17, 21].

Sickle Cell Disease

Klotho serum levels and Ca2+ may be a useful marker for detection of low BMD related to SCD with high sensitivity and specificity; however, klotho may be a better indicator as it is less affected by the nutritional and endocrinal status of patients or by intake of Ca supplements [12]. Klotho seems to be a marker of low bone mineral density [10,12].

Depression in Childhood

Peripheral sKlotho levels in a clinical sample cannot confirm a global Klotho dysregulation in depression as it has been already suggested by others. Nonetheless, further preclinical, and clinical studies on the involvement of Klotho in affective disorders should be carried out. The role of Klotho in depressive disorders is not completely ruled out [16].

Non-Alcoholic Fatty Liver Disease (NAFLD) In Children

β-Klotho gene variation is associated with liver damage in children with NAFLD [2,18]. Genetic and environmental factors strongly impact on the pathogenesis and progression of non-alcoholic fatty liver disease [2]. The FGF19/FGFR4/KLB pathway plays a pivotal role in the pathogenesis of NAFLD. KLB protein seems to play a protective role against lip toxicity and inflammation in hepatocytes [18].

Surgery for Congenital Heart Defects (CHD)

The Klotho protein family plays important roles in several metabolic pathways [3]. Soluble Klotho has been recently found showing cardiovascular protective traits [3]. Cardiopulmonary Bypass CPB) support during cardiac surgery has been implicated in several adverse outcomes in pediatric and adult patients. Cardiac surgery with CPB results in a significant decrease of serum Klotho levels 2h after surgery in pediatric patients with CHDs, which can be used to predict development of postoperative complications in this patient population [19,20].

Beta-Thalassemia Major

Klotho seems to be a biomarker for muscle strength and osteoporosis in beta thalassemia major [11].

Diabetic Pediatric Nephropathy

Diabetic Nephropathy (DN) is associated with renal mitochondrial injury and decreased renal klotho expression [8,9]. Klotho is known as an aging suppressor, and mitochondrial dysfunction is the hallmark of aging. Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) is a master regulator of mitochondrial biogenesis, and Adenosine Monophosphate-Activated Protein Kinase (AMPK) is known as a guardian of mitochondria. rKL treatment ameliorated major disorders from diabetes, such as obesity, hyperglycemia, and intrarenal Reactive Oxygen Species (ROS) generation, in db/db mice. rKL also diminished albuminuria, recovered renal proximal tubular mitochondria, increased renal p-AMPK and PGC1α, and down-regulated mTOR/TGF-β in db/db mice. In S1 mouse proximal tubular cells, rKL treatment ameliorated HG-mediated cellular and mitochondrial damage and enhanced oxidative phosphorylation, with an increase in PGC1α-AMPK-induced mitochondrial recovery. Klotho seems to exert a mitochondrial protective effect in diabetic kidney disease by inducing AMPK-PGC1α expression [8,22].

Klotho and Pediatric Hematopoietic Stem Cell Development and Erythropoiesis

Klotho deficiency is a characteristic feature of chronic kidney disease in which anemia and cardiovascular complications are prevalent. Disruption of the Klotho gene in mice results in hypervitaminosis D and a syndrome resembling accelerated aging that includes osteopenia and vascular calcifications. Injection of klotho protein results in hematopoietic changes opposite to the ones observed in Klotho (-/-) mice. These observations unveil a novel role for the antiaging hormone klotho in the regulation of prenatal and postnatal hematopoiesis and provide new insights for the development of therapeutic strategies targeting klotho to treat hematopoietic disorders associated with aging. These aspects could play a role in childhood [23].

Sevoflurane Induced Pediatric Neuronal Injury

Kloth seems to have a protective role in surgical narcotics like sevoflurane and seems to protect from neuronal injury [6].

Aging in Children, Enpp1 and Klotho

Klotho levels were significantly lower among children with organic GHD compared to both GH sufficient participants and those with idiopathic GHD. The difference between GHS children and children with idiopathic GHD was not significant. Klotho levels positively correlated with IGF-1-standard deviation scores, but were not associated with gender, pubertal status, age or anthropometric measurements. An association between low serum klotho levels and organic GHD could be found [25]. Enpp 1 is an anti-aging factor that regulates Klotho under phosphate overload conditions: Control of phosphate metabolism is crucial to regulate aging in mammals. Klotho is a well-known anti-aging factor that regulates phosphate metabolism: mice mutant or deficient in Klotho exhibit phenotypes resembling human aging. Ectonucleotide pyrophosphatase/phosphodiesterase [1] (Enpp1) is required for Klotho expression under phosphate overload conditions. Loss-of-function Enpp1 ttw/ttw mice under phosphate overload conditions exhibited phenotypes resembling human aging and Klotho mutants, such as short life span, arteriosclerosis and osteoporosis, with elevated serum [1,25] (OH)2D3 levels. Enpp1 ttw/ttw mice also exhibited

significantly reduced renal Klotho expression under phosphate overload conditions, and aging phenotypes in these mice were rescued by Klotho overexpression, a low vitamin D diet or vitamin D receptor knockout. These findings indicate that Enpp1 plays a crucial role in regulating aging via Klotho expression under phosphate overload conditions [4,24]. One review aims to address the early life determinants of EVA in children and adolescents with a particular focus on racial or ethnic differences [39].

Discussion

Klotho was discovered in 1997 as a gene responsible for life expectancy, and therefore named after the Greek goddess who spins the thread of life [1-25,26]. Overexpression of Klotho prolongs lifespan, and Klotho-deficient mice show accelerated atherosclerosis, osteoporosis, ectopic calcification, and atrophy of the skin and other organs. Polymorphisms of the Klotho gene are also associated with shorter lifespan in humans. α-Klotho was discovered completely independently of FGF23. Due to the phenotype of Klotho-deficient mice, which exhibits many characteristics of human aging - such as shortened lifespan, organ atrophy, vascular calcification, and reduced bone mass - it was initially assumed that α-Klotho is an "anti-ging gene". However, it was later recognized that FGF23 and α-Klotho are parts of the same signaling pathway and that the aging-like phenotype of Klotho-deficient mice essentially results from unleashed production of the vitamin D hormone (1,25-dihydroxyvitamin D) in these mice, leading to hypercalcemia and hyperphosphatemia.

α-Klotho is a transmembrane protein that functions with FGF receptor 1 as a receptor complex for FGF23. α-Klotho is expressed mainly in the kidney, parathyroid gland, and choroid plexus of the cerebral ventricles. The extracellular portion of α-Klotho has sequence homologies to glycosidases and can be cleaved by cell surface-anchored proteases. The cleaved form is called soluble klotho (sKL). sKL circulates in the blood, the most essential source being the kidney. Although a variety of effects have been described for sKL, it remains controversial whether sKL has a physiological function. A specific receptor for sKL has never been found. In this regard, further publications have taken the discussion in a new direction by showing that sKL does not have FGF23-independent functions but acts as a co-receptor for FGF23 as does the transmembrane form. However, whether the physiological concentrations of sKL present in blood are sufficient for it to function as a coreceptor in target cells remains unclear.

The main products of the Klotho gene are a transmembrane protein (membrane-bound Klotho) and a secreted form (circulating Klotho), which have receptor, enzyme, and hormone properties [1-25]. Membrane-bound Klotho is expressed in several tissues [1-25]. In the renal distal tubule, membrane-bound Klotho serves as a co-receptor for FGF23 and regulates phosphate metabolism or increases FGF23-induced phosphate secretion [1-25,34]. Beta-Klotho, a member of the Klotho protein family, acts as a co-receptor for FGF19 in adipocytes and hepatocytes and regulates adipogenesis and glucose metabolism. The extracellular domain of the membrane-bound proteins can be separated and secreted into the circulation. In vitro, this domain exerts a glucuronidase effect and activates the TRPV5 cation channel in the distal tubule. The secreted klotho has endocrine effects [1-25]. The most important of these is inhibition of the insulin and IGF1 receptor signal transduction pathway and thereby glucose uptake in the cell. This is the main mechanism for the anti-aging effect of Klotho. Klotho also increases glucose-induced insulin secretion in vitro, has antidiabetogenic effects in animal studies, and is decreased in patients with type 2 diabetes. Klotho-deficient mice show growth retardation. In humans, an inverse correlation is found between circulating Klotho and age: serum Klotho is higher in children and then decreases with age. Furthermore, Klotho is lower in children with growth hormone deficiency and increased in growth hormone-secreting pituitary adenomas. FGF23 is secreted by osteocytes, acts only on Klotho-expressing tissues, and controls phosphate homeostasis or increases phosphaturia and has a phosphate-lowering effect. Deficiency of Klotho leads to hyperphosphatemia, resulting in extraosseous or vascular calcifications. In translocation associated with increased Klotho expression, phosphate deficiency rickets and hyperparathyroidism have been described. Klotho expression is extremely reduced in patients with chronic renal failure regardless of etiology. Due to Matsubara, 2013, reduced Klotho can be positively influenced by sporting activity [27]. In addition, an anti-inflammatory diet is recommended, e.g., by means of a Mediterranean diet. Nuts, fresh fruits and vegetables, fish, and healthy fats can lead to an increase of Klotho levels. Alcohol and nicotine abusus in teenagers have a negative influence, this is well known [27,28]. Klotho inhibits angiotensin II-induced cardiac hypertrophy, fibrosis, and dysfunction in mice through suppression of transforming growth factor-β1 signaling pathway [29]. High levels of Klotho seem to have a protective role in cardiac function [29,32]. One study showed that continuous infusion of angiotensin II downregulated renal klotho gene expression, which was an AT1 receptor-dependent [30].

Mitani and colleagues showed in vivo klotho gene transfer ameliorates angiotensin II-induced renal damage [31]. Moreover, Klotho seems to play an important role in human brain function and baroflex functioning in adults but was never evaluated in children [26,34,36,37]. Klotho is best known for its role in delaying age-related pathologies in various organ systems, including skeletal muscle [38,39]. The pro-longevity effects of Klotho have been partially attributed to modulation of fibroblast growth factor [23], Wnt, and mTOR pathways [40], several of which have also been targets in sarcopenia research [41]. With age, circulating Klotho levels gradually decline, and epidemiological studies have revealed that decreased circulating Klotho levels are associated with an accelerated loss of skeletal muscle mass and strength [42]. Similarly, mice deficient for Klotho display significant muscle wasting, which is hypothesized to be caused by increased mitochondrial reactive oxygen species (ROS) [43]. These findings are consistent with the observation that mitochondrial accumulation of ROS leads to a de-

cline in muscle mass and decreased regenerative potential [43]. Taken together, these studies suggest an unexplored mechanistic link between sarcopenia and a decline of Klotho with increasing age. It is known that low levels of Klotho promote the aging process, while high levels protect against premature aging [1-37,44]. Studies exist to determine potential associations between common ageing-related outcomes/mortality and KLOTHO polymorphisms. The 1818T allele was a risk factor for myocardial infarction-related death in elder people. The 395A and 370C alleles were protective factors for stroke-related death in elderly from community; this was never explored in children to date supposing a potential risk for these diseases in later life [43]. It is also known that certain Klotho variants can influence life and aging differently [35-37].

In children, very few studies have been done on Klotho levels in the blood [18,39-44]. In Hutchinson-Gilford syndrome, an pre-aging syndrome in children, low Klotho levels in blood should be expected and this idea could be part of a study concerning aging and the levels and influence of Klotho in this aspect [38,45-59]. Hutchinson-Gilford progeria syndrome (HGPS or progeria) is typically caused by a dominant-negative C•G-to-T•A mutation (c.1824 C>T; p.G608G) in LMNA, the gene that encodes nuclear lamin A. This mutation causes RNA mis-splicing that produces progerin, a toxic protein that induces rapid ageing and shortens the lifespan of children with progeria to approximately 14 years [60]. Recent research on gene editing in progeric mice, published in Nature in 2022, showed that a single injection of ABE-expressing AAV9 at postnatal day 14 improved vitality and greatly extended the median lifespan of the mice from 215 to 510 days [60]. These findings demonstrate the potential of in vivo base editing as a possible treatment for HGPS and other genetic diseases by directly correcting their root cause [60]. Children with progeria would be the best population to work also on further studies towards Klotho levels and the role of Klotho levels, membrane bounded and soluble, in aging in children [39]. Klotho is, overall, very interesting. Klotho plays a crucial role in aging processes, even in children [54-56]. Especially in children with Hutchinson-Gilford syndrome, in which the children undergo a premature aging process, Klotho could play a crucial role [38, 56-60]. Investigating this would be a desirable approach to evaluate more precisely the role of Klotho on the child organism.

Acknowledgement

None.

Conflict of Interest

No conflict of interest.

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