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Klotho at the Edge of Alzheimer's Disease and Senile Depression

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Abstract
Klotho, encoded by the *KL* gene, is a single-pass transmembrane protein and a circulating factor that plays a key role in cellular metabolism and body homeostasis and has been associated with age-related diseases. Alterations of this protein seem to influence the development of serotonergic neurons and could play a role in major depression in the elderly. Pretreatment of neurons with Klotho protein can avoid neuronal injury related to the toxic amyloid-β and glutamate, centrally related to the pathogenesis of Alzheimer's disease (AD), in order that Klotho protein could play a neuroprotective role in AD patients. Late-life depression, mild cognitive impairment, and dementia are different nosological entities but share common neurobiological facets and could represent a clinical continuum. Enhancement of Klotho levels in the early stages of the disease could represent a therapeutic strategy to prevent further deterioration and to ameliorate the outcome of elderly AD patients with or without major depression.

Keywords Klotho · KL · Alzheimer's disease · Late-life major depressive disorder · MDD · Depression · Aging

Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder worldwide and the most frequent cause of dementia associated with aging, especially considering the form with no specific family history of dementia, called sporadic AD (SAD). According to the Global Burden of Disease from the Institute for Health Metrics and Evaluation (IHME), University of Washington [http://www.healthdata.org/gbd], SAD is expected to exponentially increase with aging of the general population. About 5.3 million Americans are diagnosed with SAD, accounting for 60–80% of all dementia cases in US and in European countries. Currently, one out of every five elderly persons aged 65 years and over is predicted to develop SAD in his lifetime [1]. SAD patients may also show depression, one of the most common mental disorders in late life [2]. Late-life depression (LLD) is a heterogeneous disorder with a major public health impact, given the progressive aging of the general population [3]. LLD refers to depressive syndromes arising in adults older than 65 years [3], encompassing both late-onset cases as well the cases with onset before the age of 60 years that recur or continue into later years of life [4]. Furthermore, there is a general consensus on a syndromic approach to LLD, to identify symptom clusters, such as major depressive disorder (MDD) [3]. Estimates of the prevalence of clinically significant LLD vary widely from 4.5 to 37.4%, according to the population studied, sample size and non-psychiatric co-morbidity [5, 6], with a pooled prevalence of 7.2% [6] and an incidence rate of 0.2–14.1 per 100 person years of late-life MDD [7]. In a community-based older sample, the rate of clinically significant LLD was 14.7% and one-quarter of these patients had late-life MDD (3.7%) [8]. More recent data indicates a prevalence of LLD of 54.14% and a prevalence of late-life MDD of 17.85% [9]; however, LLD prevalence will probably increase with world population aging, becoming a major public health concern for the future [10, 11].

A huge amount of studies over the last several years have revealed that depression and AD have in common neurobiological and clinical features. Depression represents a risk factor for AD development and the progression of mild cognitive impairment (MCI) into AD is bolstered by the occurrence of

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depressive symptoms [12]. Depression and AD share analogous pathophysiological processes, comprising neuroinflammation with an anomalous tumor necrosis factor-α (TNF-α) signaling and a weakened brain-derived neurotrophic factor (BDNF) and transforming-growth-factor-β1 (TGF-β1) signaling [12]. TGF-β1 is a cytokine with anti-inflammatory capacity and neuroprotective action opposed to neurodegeneration provoked by amyloid-β (Aβ). In MDD patients, TGF-β1 plasma levels are decreased and correlate with depression severity and treatment resistance in MDD [12]. An initial process in AD pathogenesis is the deficiency of Smad-dependent TGF-β1 signaling, which takes part in inflammaging and cognitive decline in AD [12]. Antidepressants such as selective serotonin reuptake inhibitors (SSRIs) augment TGF-β1 release from astrocytes playing a significant neuroprotective role in experiments performed in animal models of AD and decrease the risk of AD in patients with depression upon long-standing therapy [12].

Recently, a family of proteins with anti-aging property encoded by the Klotho (*KL*) gene at locus 13q12 have been evidenced to protect neurons from degeneration [13]. Pretreatment of neurons with KL can prevent neuron death in the presence of the toxic Aβ protein and glutamate, so that therapeutic approaches increasing Klotho levels might prevent further deterioration if started at the beginning of the disease and advance the outcome for AD patients [14–16].

KL encodes a circulating and a type I membrane protein related to beta-glucosidases, which controls the insulin/insulin-like growth factor (IGF)-1 signaling (IIS) pathway, among other functions. In the past, alterations in its levels were associated in mice to premature aging and in men to increased risk of diseases, such as osteoporosis, atherosclerosis, and cognitive decline, including behavioral disorders such as depression [16–18]. This gene is now known as one of the key players to significantly extend life expectancy [19]. In addition, animal models and patients with polymorphisms of the KL gene show reduced cognitive abilities [20, 21], as well as reduced life duration [22, 23].

The Klotho Proteins

The KL gene encodes a family of transmembrane proteins (α-Klotho, β-Klotho, and KL-related protein, Klrp) expressed in various organs, including the brain and involved in multiple biological functions [24–27]. KL proteins play a key role as co-receptors, binding their related endocrine fibroblast growth factor (FGF) receptors (FGFR) to cooperate for their biological activities. FGFs are renowned as hormones that control different metabolic processes. FGF19 is secreted from intestine upon feeding and restrains hepatic bile acid synthesis. FGF21 is secreted from liver upon fasting and props up lipolysis in adipose tissue. FGF23 is secreted from bone and holds back phosphate reabsorption and vitamin D synthesis in kidney [28]. α-Klotho is described as an anti-aging protein as well as a soluble factor in serum and a co-receptor for FGFR3 to respond to FGF23, involved in the control of mineral ion and vitamin D homeostasis [24, 29]. α-Klotho is also expressed in the choroid plexus, where it is released into the cerebrospinal fluid [30]. The β-Klotho protein is a type I membrane protein related to β-glucuronidases, whose overexpression is associated with life extension and up until now no secreted form has been revealed [18, 19, 24]. β-Klotho is mainly expressed in liver and adipose tissue and is entailed in metabolic regulation, glucose uptake, bile acid synthesis, and fatty acid metabolism [24].

The mechanisms of KL action are not entirely clear, even if it has been described that α-Klotho increases synaptic and cognitive functions, and it may also influence various structures and functions during central nervous system maturation and aging [25]. The mouse α-Klotho gene has two transcripts, one encoding a transmembrane isoform (m-KL), and the other a putative secreted isoform (s-KL) [24]. Unfortunately, little is known about the secreted α-Klotho isoform, since available antibodies cannot discriminate s-KL from the KL1 domain cleaved from the transmembrane isoform. The KL transcript produced by alternative splicing generates a stable protein (70 kDa), which differently from the transmembrane Klotho isoform is ten times more abundant in the brain than in the kidney, suggesting that the two isoforms may have different functions [24]. Klotho expression in the central nervous system is influenced by aging, AD, or a healthy lifestyle, such as voluntary moderate continuous exercise: experiments performed in wild-type and 3xTg-AD model of AD evidenced a strong correlation between high expression levels of the two KL transcripts and the healthy status of the animals. In particular, KL expression in brain areas declined more rapidly in the 3xTg-AD model of AD than in healthy animals, while moderate continuous exercise in adulthood prevented the waning in both KL transcripts expression [31].

The single-pass transmembrane protein consists of 1014 amino acids; it contains a presumed signal sequence to the N-terminal and a single transmembrane domain near the C-terminal portion; these features identify KL as type I membrane protein [13]. The extracellular domain of the protein consists of two internal repetitions, KL1 and KL2, each of 450 amino acids that share a 20–40% homology of amino acid sequence with the enzyme β-glucosidase of bacteria, with the enzyme lactase of archaea and with the enzyme glucosilceramidase of mammals [13, 32]. However, the glucosidase activity is absent in the recombinant protein KL, and a glutamate residue, which is essential in the active center of the enzyme β-glucosidase, is replaced with asparagine and alanine, respectively, in KL1 and KL2. The analysis of the cDNA revealed that the KL gene also encodes a secreted form of 549 amino acids, lacking the repetition KL2, and the

transmembrane and intracellular domains, due to alternative RNA splicing [13].

Possible Biological Roles of Klotho Protein

KL protein is implicated in different biological mechanisms, so there are many hypotheses about the biological role played by KL in the regulation of the process of aging and in neurodegeneration.

Klotho: Regulator of Calcium and Phosphorus

The pathophysiological mechanisms underlying neurodegenerative diseases and in particular AD remain hard to pin down. Progress in molecular biology and live imaging techniques highlighted a significant role for calcium (Ca2+), an omnipresent second messenger managing a huge assortment of cellular processes, including neuronal excitation and communication [33, 34]. The concentrations of serum calcium is tightly regulated by the coordinated actions of parathyroid hormone (PTH), calcitonin (CT), calcium, phosphorus, and vitamin D (1,25-(OH)2D) [35–37]. PTH and CT positively regulate the synthesis of 1,25-(OH)2D through transcriptional activation of *CYP27B1*, the gene encoding 1α-hydroxylase [38–40]. On the other hand, 1,25-(OH)2D inhibits its synthesis through a negative feedback regulation on the activity of 1α-hydroxylase and through a positive setting on the activity of 24-hydroxylase [41, 42]. The KL mutant ($kl^{-/-}$) mouse showed an altered homeostasis of calcium and phosphorus and an increase in serum concentrations of 1,25-(OH)2D, while serum concentrations of PTH and CT were normally regulated in response to calcium levels [43]. The serum levels of other vitamin D derivatives were found to be lower than those in WT mice, suggesting that in $kl^{-/-}$ mice, the precursor is preferentially converted to an active form of vitamin D. This is an unexpected response in the presence of high levels of calcium in serum, as the synthesis of 1,25-(OH)2D is normally inhibited when calcium levels are increased in the serum [44]. The discrepancy in the serum levels of 1,25-(OH)2D between WT mice and $kl^{-/-}$ mice is the first discovery that could justify an alteration in the metabolism of calcium and phosphorus [43]. Serum levels of 1,25-(OH)2D are regulated by the balance of the expression of 1α-hydroxylase and 24-hydroxylase, the rate limiting enzyme in the metabolism of vitamin D. The expression of *CYP27B1* was significantly increased in $kl^{-/-}$ mice, and this is probably the cause of elevated serum 1,25-(OH)2D levels. As previously stated, PTH and CT positively regulate the expression of the 1α-hydroxylase gene [38–40], while 1,25-(OH)2D exerts a negative control [41, 42]. In normal conditions, the negative regulatory signals predominate over the positive control. In $kl^{-/-}$ mice, the expression of *CYP27B1* is still positively regulated by PTH and CT, and even in this case, 1,25-(OH)2 can dominantly inhibit the positive signals from PTH and CT [43]. This shows that the regulatory functions performed by PTH, CT, and 1,25-(OH)2D are not altered in $kl^{-/-}$ mice. Therefore, the overexpression of *CYP27B1* indicates that KL is part of a new route of negative regulation of the expression of this gene and thus of calcium homeostasis [43]. The alteration of vitamin D endocrine system can participate in many of the phenotypes reported in $kl^{-/-}$ mice because of toxicity due to increased levels of calcium, phosphorus, and 1,25-(OH)2D [43]. The increased activation of vitamin D, due to altered regulation of 1α-hydroxylase, may therefore, be the main cause responsible for the multiple abnormalities in $kl^{-/-}$ mice [43]. There may be two kinds of phenotypes: those due solely to the toxic action of increased calcium, phosphorus, and 1,25-(OH)2D, and those that require additional loss of KL. In the latter case, KL would play another role as well as that of regulator of calcium homeostasis. The lack of KL protein, in fact, may result in morphological and functional cell deterioration causing severe tissue damage along the toxic action of the increase in the concentration of calcium, phosphorus, and 1,25-(OH)2D in serum. To explain how KL regulates *CYP27B1*expression, it must be assumed that the function of KL protein is, at least in part, mediated by intercellular or paracrine mechanisms. In fact, *CYP27B1* is expressed in a wide region of the renal tubule cells [45], while the expression of *KL* is restricted to cells of the distal convoluted tubule in the kidney [13]. As aforementioned, there are two types of KL protein, a membrane form, with a single transmembrane domain, and a secreted form, so that KL protein might potentially operate on the membrane and/or as a secreted factor. In this regard, many possible molecular actions of KL protein have been proposed. In the secreted or membrane form, KL might interact with a presumed receptor as a ligand and induce signal transduction required for *CYP27B1* regulation [46]. Alternatively, KL could serve as a receptor complex to mediate the signals required for the synthesis and/or secretion of humoral factors that critically control *CYP27B1* expression [46]. Furthermore, KL could convert an inactive precursor molecule into an active one that participates in the regulation of 1α-hydroxylase [46]. To determine the ultimate role of KL protein, further studies will be required, considering that recent data pinpoint its involvement in the regulation of phosphorus balance, as well as hormone secretion and circadian behavior.

The Role of Klotho in the Regulation of Insulin/IGF-1 Signaling

AD is characterized by progressive neurodegeneration leading to loss of cognitive abilities and ultimately to death. In an animal model of AD, weakened insulin-like growth factor (IGF)-1/insulin receptor substrate (IRS)-2 signaling averted premature death and deferred amyloid accumulation [47].

Besides, clinical and experimental data propose that augmented serum insulin and IGF-1 levels do not unavoidably associate with neuronal insulin/IGF-1 receptor signaling upregulation [47, 48]. IGF-1 receptor (IGF-1R) haploinsufficiency as well as neuronal deficiency of the insulin receptor (IR) or IGF-1R in mice crossed with mouse models for AD causes deferred amyloid (A)β accumulation [48]. In addition, IRS-2 knockout mice show reduced Aβ levels in an Alzheimer background. Moreover, postmortem investigations showed reduced expression of cerebral IGF-1R and IRS proteins in patients with AD and brains of AD patients were insulin and IGF-1 resistant, suggesting potential therapeutic implications of modulating cerebral insulin/IGF-1 signaling [47, 48]. Overall, these data imply beneficial effects of decreased neuronal insulin/IGF-1 signaling on AD pathology.

KL overexpression slows down the process of aging, extending life in mice by a surprising mechanism, the induction of insulin resistance [49]. In particular, it was observed that male mice genetically modified to overexpress KL, have a survival greater than average (20–30.8%) compared to controls, while the survival of transgenic females shows smaller increase (18.8–19.0%) [49]. Many genetic data demonstrate that a negative regulation of insulin and IGF-1 signaling extends lifespan of different animal models (*Caenorhabditis elegans*, *Drosophila melanogaster*, *Mus musculus*) [50]. It has been shown that mice lacking expression of KL gene have reduced levels of glucose and insulin in the blood coupled with increased insulin sensitivity [13, 51]. Transgenic male mice that overexpress KL, however, have higher levels of insulin in the blood compared to wild-type male mice, suggesting that they are insulin resistant; transgenic female mice, moreover, are significantly resistant to IGF-1 [49]. This confirms that KL overexpression induces resistance to insulin and IGF-1 signaling. KL protein, in its soluble form, functions as a circulating hormone that binds to a cell membrane receptor and inhibits the intracellular signaling of insulin and IGF-1 [24–27]. In particular, it has been observed that KL in vitro does not inhibit insulin or IGF-1 binding but suppresses ligand-stimulated autophosphorylation of the insulin and IGF-1 receptor. In addition, KL reduces the activation of signal transduction downstream of the activation of the receptor, including the phosphorylation of IRS-1 and IRS-2 and their association with the phosphatidylinositol 3-kinase (PI3-K) p85 regulatory subunit [52]. Since the inhibitory effect of KL on insulin signaling is almost immediate, it is unlikely that the reduction of the signal, which starts from the insulin and IGF-1 receptor is due simply to receptor loss. KL protein could inactivate the active insulin receptors that had previously been phosphorylated on tyrosine under the stimulus of insulin itself. In general, KL may inhibit the activation of insulin and IGF-1 receptors and may suppress the activated insulin and IGF-1 receptors. A possible role of KL might be to prevent cellular lipid overload by reducing the availability of glucose as lipogenic substrate, which occurs in response to insulin. In addition, lipid-rich cells are vulnerable to programmed cell death induced by lipids (lipoapoptosis), so that a reduction of KL-mediated intracellular lipid content could increase the apoptotic threshold and extend cell lifespan [53]. Indeed, lipoapoptosis is prevented by eliminating lipid excess or blocking the formation of potentially harmful fatty acid derivatives, such as ceramide and reactive oxygen species (ROS). The "healthy" type of insulin resistance mediated by KL is in contrast with the "unhealthy" insulin resistance found in obesity, a component of the metabolic syndrome, which is rather a consequence of lipid overload [54]. The lipid-rich cells are vulnerable not only to lipoapoptosis, but also to other apoptotic insults. This is probably due to an increased production of ROS and of ceramide and a reduced antiapoptotic protection by antioxidants superoxide dismutase and catalase. The cells devoid of lipids, in contrast, are remarkably resistant to damage induced by cytokines, toxic, and immune-mediated events. If KL extends lifespan by regulating cellular lipid homeostasis, it is clear that other longevity genes may reduce fat overload, and that other liporegulatory hormones, preventing fat overload, may extend the life span. It was also demonstrated that exercise and calorie restriction lower the intracellular lipid content and extend the life span [55, 56]. The hormones leptin and adiponectin lower tissue lipid content by stimulating the oxidation of fatty acids through the activation of AMPK, and could therefore extend life span [55, 56]. The effects on lipid lowering and life extension induced by exercise and caloric restriction have been confirmed in various species, and it is believed to be mediated by members of the sirtuin family, which also reduce the intracellular lipid content [55, 56]. Hyper-nutrition increases the activity of insulin and IGF-1, leading to an increase in cellular lipid content probably by phosphorylating and then expelling from the nucleus the FOX (Forkhead box) transcription factors that reduce lipid content. KL may prevent their expulsion blocking their phosphorylation, through the suppression of signals located downstream of insulin and IGF-1 [55, 56]. Another cause of lipid lowering due to blocking of insulin signaling induced by KL is the reduced translocation of GLUT4 to the plasma membrane. This excludes glucose by certain cells, thus limiting the formation of malonyl-CoA, the first intermediate of the lipogenic pathway and a potent inhibitor of fatty acid oxidation [57]. If the ability of KL to inhibit insulin and IGF-1 signaling extends survival by delaying senescence, manipulations that inhibit insulin and IGF-1 signaling can improve some of the phenotypes similar to aging in $KL^{-/-}$ mice. To assess this hypothesis, a loss of function mutation of IRS-1 has been transported in $KL^{-/-}$ mice [49]. Survival was improved in $KL^{-/-}$ mice heterozygous for the IRS-1 null allele ($KL^{-/-}$ $IRS\text{-}1^{\pm}$) compared to control $KL^{-/-}$ mice. In addition, $KL^{-/-}$ $IRS\text{-}1^{\pm}$ mice showed improvements in many pathological signs of age, typical of $KL^{-/-}$, including atherosclerosis,

ectopic calcification, atrophy of the skin, pulmonary emphysema, and hypogonadism [49].

β-Klotho, encoded by *klb* gene, is a divergent structural member of the glycosidase I superfamily, is primarily expressed in the liver and pancreas, with lower expression in adipose tissue, and assists fibroblast growth factor 21 (FGF21). FGF21 is a hepatokine that acts as a regulator of insulin-independent glucose transport in adipocytes, plays a role in the regulation of body weight, and regulates ketogenesis and adaptive responses to starvation to modulate fuel partitioning and metabolism and repress growth [58]. FGF21 complexes with β-Klotho in the suprachiasmatic nucleus of the hypothalamus and the dorsal vagal complex of the hindbrain and signals through a heteromeric cell-surface receptor composed of one of three FGF receptors (FGFR1c, FGFR2c, or FGFR3c) [59, 60]. Over and above its recognized action on peripheral metabolism, FGF21 augments systemic glucocorticoid levels, restrains physical activity, and changes circadian behavior. Mice deficient in *Klb* in the abovementioned regions are resistant to these effects, as well as those on metabolism, insulin, and growth [59, 60].

Klotho as a Humoral Factor in the Circulation

Higher plasma KL concentrations were found associated with lower risk of significant reduction and smaller average decline in mini-mental state examination (MMSE) [61]. Accordingly, cerebrospinal fluid KL concentrations were lower in patients affected by AD, and in particular in females compared with males, as well as in older versus younger adults [15].

Since a defect in the KL gene leads to systemic degeneration that depends on age, it could be argued that KL protein functions as a humoral factor in the circulation, regulating the natural process of aging [13]. This has been hypothesized on the basis of the observation that, in spite of the evidence that KL mutant mice exhibit a phenotype of systemic aging, only some organs express the gene KL endogenously [62]. On these premises, how may KL be involved in major depression in the elderly? Depression is the most common mental disorder in community settings. In fact, the Global Burden of Disease Study suggests that depression will be second only to cardiovascular disease as a cause of disability by 2020 [63]. A well-known factor that is influenced by the treatment with SSRIs, usually the first-choice medication for depression, is brain-derived neurotrophic factor (BDNF). Furthermore, the inflammatory system, the serotonin system, the dopamine system, and the hypothalamic–pituitary–adrenal (HPA) axis interact causing indirect effects. The downregulation of proinflammatory cytokines plays a key role in mediating the antidepressant effects of SSRIs treatment by inhibition of the HPA axis and influencing the synthesis and reuptake of serotonin (5-HT) and dopamine (DA). For years, research on depression focused on extracellular mediators of the stress response, such as corticotrophin-releasing hormone (CRH), cortisol, monoamine neurotransmitters [64, 65], and vascular endothelial growth factor (VEGF) [66, 67]. Recent data have expanded considerably our knowledge in the fields of research on stress, depression, and AD also considering epigenetic factors as inducers of aging-related changes [68], in addition to inflammatory and oxidative stress at the cellular level as associated with structural changes at the level of the central nervous system. During stress and depression, metabolic changes are activated to provide "fuel" for the brain. These adjustments consist of the development of insulin resistance in association with the activation of the HPA axis and the sympathetic nervous system [69–71]. Besides, there is an activation of the immune system, perhaps as a response to the threat of a possible adverse event. Neurogenesis in the adult has been described several decades ago [72, 73] and KL directly contributes in the regulation of adult hippocampal neurogenesis and hippocampal dependent cognition [74]. A clear cellular effect of different antidepressants is the induction of neurogenesis in the dentate gyrus of adult rodents and neurogenesis in response to antidepressants has been reported in several animal models [75–79]. The mechanisms by which neurogenesis might promote antidepressant effects are unknown. Although various forms of stress reduce neurogenesis in the hippocampus [75–79], decrease of neurogenesis by itself does not induce anxiety or depression-like behaviors [80]. Until now, the role of two closely interdependent stress-related processes in LLD, parainflammation and endoplasmic reticulum (ER) stress, as well as the role of three potential modulators of these processes, i.e., insulin at the level of the central nervous system, peroxisome proliferator-activated receptor-γ (PPAR-γ), and KL, is unknown or at least underestimated [81]. CNS insulin, PPAR-γ, and KL are not only closely related to para-inflammation and ER stress, but also firmly interdependent. If possible, they should contribute to oppose stress-induced parainflammation and ER stress and reduce the harmful effects of inflammatory and metabolic alterations linked with depression to support an adaptive response to treatment [81].

KL is highly expressed in the choroid plexus and neurons, especially in the hippocampus, as well as in the reproductive organs and kidney [13], but its function in the brain is unclear. As already said, KL is a type I transmembrane protein adherent to cell membrane ADAM10 and ADAM17 [82, 83] and is detectable in serum and cerebrospinal fluid (CSF) [84].

Among the well-known functions of KL are included the regulation of fibroblast growth factor (FGF) 23 signaling, the suppression of the insulin/IGF-1 signaling pathways, and the regulation of calcium and phosphate homeostasis [85]. On the contrary, none of the downstream effects of KL in CNS has been studied and little is known about the mechanism of signal transduction by which KL operates in the CNS. Considering the distribution of KL expression in the CNS and its

abovementioned effects at the cognitive level, KL is perfectly positioned to influence a wide variety of structures and functions involved in brain development and aging. KL knockout mice have a reduction in synaptic density in the hippocampus, reflecting an increase in oxidative stress in the hippocampus itself [86], and important memory deficits that can be corrected by the administration of KL [20]. KL knockout mice also show a strong deficiency of nitric oxide system with a consequent widespread endothelial disease that can, however, be reversed by administration of KL [87]. Furthermore, KL knockout mice have a generalized increase in the global burden of oxidative stress in the CNS [49]. KL exerts antioxidant and anti-inflammatory effects in the brain [20, 86]. KL decreases parainflammation, improves the efficiency of the ER stress response and modulates the metabolism of calcium at the level of the CNS, in part through the mediation of calcium transport through the blood-brain barrier [88]. KL also acts on the transient voltage potential receptors of the brain involved in the formation of CSF [89]. In the CNS, KL is produced exclusively from the apical ependymal cells that are adjacent to the subventricular zone [90], which is one of two sites that produce neural stem cells. Finally, KL is secreted directly into the CSF, which is the communication path for its biological actions in the brain. Interestingly, ROS production causing oxidative damage and neuronal cell death is crucial in AD and other neurodegenerative disorders pathogenesis [91]. KL protein works out neuroprotection opposing neuronal damage related to neurodegeneration and oxidative stress [91]. Pretreatment of rat primary hippocampal neurons and mouse hippocampal neuronal cell line HT22 with recombinant KL shielded these cells from glutamate and oligomeric amyloid-β (oAβ)-induced cytotoxicity [91]. Moreover, primary hippocampal neurons derived from KL-overexpressing mouse embryos were less susceptible to both cytotoxic insults, glutamate and oAβ, respect to neurons obtained from wild-type littermates [91]. An antioxidative stress array analysis of neurons treated with KL showed that KL significantly augments the expression of the thioredoxin/peroxiredoxin (Trx/Prx) system with the maximum achievement on the stimulation of Prx-2, an antioxidant enzyme, whose augment was corroborated at the mRNA and protein levels [91]. KL-induced phosphorylation of the PI3K/Akt pathway, playing a key role in apoptosis and longevity, was coupled with unrelenting inhibitory phosphorylation of FOXO3A and was indispensable for Prx-2 induction [91]. Prx-2 expression downregulation roughly totally eliminated the capacity of KL to salvage neurons from glutamate-induced death and significantly, but not entirely, hindered cell death induced by Aβ, hinting Prx-2 as vital modulator of neuroprotection [91]. These data revealed, for the first time, the neuroprotective role of KL and disclosed a novel mechanism underlying this effect [91].

Accordingly, increase of KL expression reduces premature mortality and alleviates network dysfunction in human amyloid precursor protein (hAPP) transgenic mice, which reproduces crucial features of AD [16]. KL levels amplification avoided diminution of N-methyl-D-aspartate (NMDA) receptor (NMDAR) subunits in the hippocampus and improved spatial learning and memory in hAPP mice [16]. Besides, KL increase in hAPP mice boosted the quantity of the glutamate [NMDA] receptor subunit epsilon-2 (GluN2B) subunit of NMDAR in postsynaptic densities and NMDAR-dependent long-term potentiation, which is decisive for learning and memory, suggesting that augmenting wild-type KL levels or activities advances synaptic and cognitive functions, and may be of therapeutic benefit in neurodegenerative diseases, such as AD and other cognitive disorders [16].

Besides, peripheral delivery of an α-klotho protein fragment (αKL-F) in young, aging, and transgenic α-synuclein mice provoked cleavage of the GluN2B subunit of NMDAR increasing NMDAR-dependent synaptic plasticity and stimulated cognitive improvement as well as neural resilience notwithstanding impermeability to the blood-brain barrier [92].

As well, experiments performed in 10-month-old senescence-accelerated mouse prone-8 (SAMP8) mice showed that KL increased the therapeutic effect of ligustilide against AD-like neuropathologies and memory impairment in aging [14]. Ligustilide treatment decreased memory deficits, amyloid-β (1)-42 accumulation, tau phosphorylation, and neuron loss, augmented mitochondrial manganese-superoxide dismutase and catalase expression and activity, and reduced malondialdehyde, protein carbonyl, and 8-hydroxydesoxyguanosine levels in the brain [14]. Ligustilide upregulated KL expression in the cerebral choroid plexus and serum, reduced Akt and FOXO1 phosphorylation. Besides, ligustilide restrained the IGF-1 signaling pathway and induced FOXO1 activation in 293T cells in conjunction with KL upregulation [14]. An inverse correlation was found between KL expression and the AD phenotype, suggesting that KL might be a novel therapeutic target for age-related AD, and KL upregulation might contribute to the neuroprotective effect of ligustilide against AD [14].

Interestingly, KL interacts with the p16(INK4a) and the expression levels of α-klotho and p16(INK4a) are oppositely correlated during aging [93]. The tumor suppressor p16(INK4a) inhibits α-klotho promoter activity by blocking the functions of E2Fs and plays a well-known role in the realization of cellular senescence in stem/progenitor cells, a process that takes part in organismal aging [93]. Differently from what happens in α-klotho knockout mice ($kl^{-/-}$), in *kl*(*kl*/*kl*) mice, mutant animal models homozygous for a hypomorphic allele of the α-klotho gene, p16(INK4a) ablation recovers the accelerated aging phenotypes, implying that p16(INK4a) is an upstream regulator of α-klotho expression (Fig. 1).

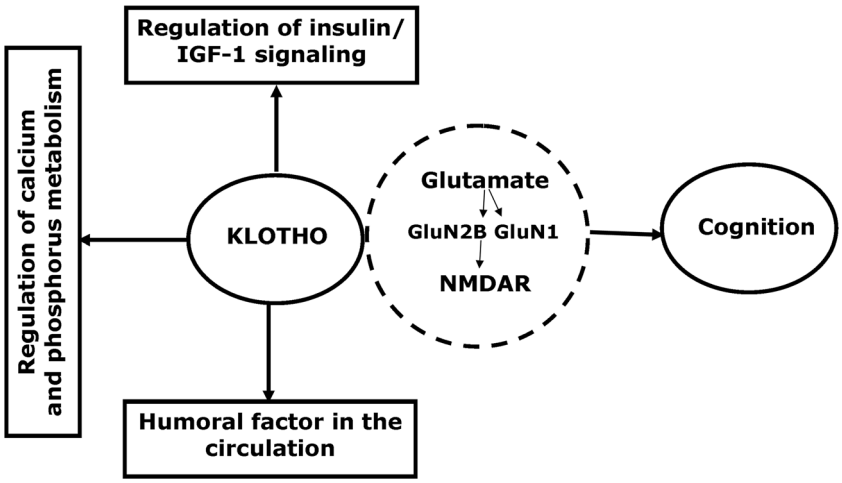

Fig. 1 Scheme illustrating the signaling pathways impacted by Klotho. Continuous lines ending with arrows render positive interactions. NMDAR, N-methyl-D-aspartate (NMDA) receptor (NMDAR); GluN, glutamate [NMDA] receptor subunit

Functional Variants of the Human *KL* Gene

To isolate a human homolog of the *KL* gene, a human kidney cDNA library was screened using a mouse restriction fragment. The cDNA of the isolated human *KL* coded for a protein of 1012 amino acids and showed 86% amino acid identity with the mouse KL protein. In addition, as the mouse KL gene, human KL gene encodes both the membrane form and the secreted form; however, the secreted form predominates in humans [94, 95]. The human *KL* gene was found to be localized in a band of the long arm of chromosome 13 (13q12) [94], where genes responsible for the syndrome of premature aging or related disorders have not yet been identified. However, in the human KL locus, seven single nucleotide polymorphisms (SNPs) were detected, and it has been hypothesized that these SNPs are associated with reduced bone mineral density and other disorders associated with aging [46]. Then, to verify that indeed the KL gene was involved in human aging, a population-based association study was performed [18], which allowed to establish that the KL locus is associated with human survival, defined as the hope of postnatal life, and then is connected to longevity, defined as life expectancy after 75 years of age. It was therefore identified an allele, called KL-VS, containing six SNPs in complete linkage disequilibrium, that when homozygous is associated with a decrease in survival. Subsequent studies have shown that this functional variant of KL is also associated with coronary artery disease [22]. However, the risk of onset of illness imposed by KL-VS is influenced by other risk factors, including hypertension, smoking status, and levels of HDL cholesterol. The most recent studies on the functional KL variant KL-VS allowed to determine that the KL-VS allele is associated with an increased risk of cardiovascular disease and premature mortality [23]. Another study, however, showed that the KL-VS variant is associated with higher cognitive function in heterozygous carriers. Transgenic mice with systemic overexpression of KL showed better performance in tests of learning and memory, and had the best long-term potentiation [19]. A role for KL in human aging and in cardiovascular disease was initially postulated based on the observation that mice deficient in KL exhibit phenotypes resembling human premature aging [13]. In addition, they exhibit impaired endothelium-dependent vasodilation [87] and impaired angiogenesis [96], suggesting that KL protein can protect the cardiovascular system through the production of NO by endothelial cells.

This topic is particularly relevant considering that numerous studies have documented the key role played by vascular pathology in AD and that cardiovascular disease is ever-increasingly acknowledged as a fundamental risk factor for AD [97].

Late-Life Major Depressive Disorders

MDD is a common disease that involves complex interactions between genes and environment, and is now the largest social burden of non-fatal diseases in different countries [98]. In developed countries, depression is on the second place among the global diseases, and the fourth worldwide. As a result of the impact of widespread depression, antidepressants are the second class of drugs prescribed in the USA, second only to analgesics including non-prescription drugs, such as aspirin. Depression found in the elderly, defined LLD, may cause an increased risk of hospitalization, an increased burden of assistance, an increased use of medical care and greater physical disability [99]. However, despite these negative consequences, only a minority of depressed elderly are treated properly for this condition. Estimates suggest that less than 10% of patients with depression in old age have an official evaluation and specific treatment for this condition [100]. Moreover, the relationship between age of onset and variability of clinical response and treatment of LLD is not fully understood. Only recently there has been increased attention on the course and outcome of depression in the elderly, with the hope that

putting more emphasis on the study of this disease may lead to an increase in the percentage of old patients who eventually undergo appropriate treatment. The MDD is an independent factor that doubles the risk of coronary heart disease [101], and increases of 60% or more the risk of developing type 2 diabetes [102]. The overall mortality rate in patients with major depression at any age is doubled [103], leading to a loss of 7–11 years of life [104]. The causes that lead to depression are still unclear. Initially, there were two opposing currents of thought, one which attributed greater importance to biological and genetic causes, the other to environmental and psychological causes. At present, the available data suggest that depression is a complex combination of genetic, biological, environmental, and psychological factors [105]. Family factors and genetic components are involved. Studies performed on monozygotic and dizygotic twins and on adopted subjects have shown some heritability of depressive disorder, although in a less consistent way compared to bipolar disorder. The rate of heritability for depressive symptoms is around 76%. Depression, then, like many other psychiatric illnesses, does not follow a model of direct transmission, but a model where multiple genes are involved. Inheritance is, however, less likely for mild forms of depression, and seems to play a role more strongly in early-onset depression: 70% of depressed children have, in fact, at least one parent who has a mood disorder [105]. This may be due in part to the fact that a depressed parent establishes a not favorable relationship with their child, already genetically vulnerable, which increases the chance for the child to develop a mood disorder [105]. Many experimental evidences have demonstrated that depressive symptoms are associated with a functional impairment of the serotonin (5-hydroxytryptamine, 5-HT) system, which is involved in regulating mood in humans. For example, many antidepressants in current use are direct inhibitors of receptors and enzymes of this system [106], a delay of serotonin neurotransmission from tryptophan depletion induces a rapid worsening of depressive symptoms [107, 108], and in the brains of depressed patients abnormalities in the serotonin transporter and receptor function have been found [109, 110]. As a result, the genes involved in the synthesis, transport, and degradation of serotonin have gained much attention in the effort to explain the genetic basis of depression.

Pharmacological Treatment of LLD

Depression medications are administered to normalize the altered balance of neurotransmitters. Their effectiveness, however, is minimal in patients with mild or moderate depression but significant in patients with very severe disease. A meta-analysis showed a weak correlation with genetic variations that would help to determine individual differences in response to antidepressants. In children and adolescents, the Cochrane survey of 2012 did not show the superiority of the "relative effectiveness" of psychotherapy respect to drug therapy or a combination of the two types of intervention [111]. In the elderly population, the long-term treatment benefits of antidepressants for the prevention of depressive relapse and recurrence are not clear, and recommendations for this kind of treatment cannot be easily made, as suggested by a recent meta-analysis [112]. Depression medications include monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants (TCAs), tetracyclic antidepressants (TeCAs), the SSRIs, and serotonin-norepinephrine reuptake inhibitors (SNRIs). The guidelines of the National Institute for Health and Clinical Excellence (NICE) recommend SSRIs as first-line drug treatment for moderate and severe depression. The main goal of SSRI treatment is the serotonin transporter. The blockade of this transporter causes an increase in extracellular levels of serotonin and subsequently increased activation of serotonin receptors and their downstream signaling pathways. SSRIs, however, can also activate other neurotransmitter systems through a low-affinity binding with other carriers and may influence neurogenesis [113] and the inflammatory system by means of a downregulation of cytokines [114]. In addition, the activity of the HPA axis, which is involved in the control of the stress response, is downregulated and other processes, such as the regulation of hunger and reproduction, are affected by treatment with SSRIs [114]. To sum up, SSRIs positively affect neuroplasticity and exert anti-inflammatory and antioxidative effect, possible additional mechanisms of action of these antidepressants [113, 114].

Klotho Single Nucleotide Polymorphisms, Cognitive Impairment, and Response to SSRIs

A most important problem in aging is represented by cognitive abilities diminution and the *KL* gene could play an important role for the functioning of the central nervous system in late adulthood. Besides, the role of *KL* gene in the response to SSRIs treatment in LLD is not well defined. In a study conducted on Danish people (1.480 subjects aged between 92 and 100 years), 19 tagging *KL* gene variants were studied in relation to 2 measures of cognitive function, a composite cognitive test score (summing up 5 test items targeting several different cognitive domains) and the MMSE. Heterozygotes for the KL-VS showed lower cognitive function compared to noncarriers [115]. Two other variants, rs398655 and rs562020, positioned at the 5′ end of the gene, were associated with superior cognitive function independently of KL-VS, with the common AG haplotype associated with inferior cognition, consistently across two cognitive measures in two cohort strata [115]. The haplotype effect was stronger than that of KL-VS. Two variants, rs2283368 and rs9526984, were significantly associated with cognitive decline over 7 years,

hinting an age-dependent effect of KL and the possibility that multiple gene variants in *KL* gene are important for cognitive function among the oldest old participants [115]. The likely association between G-395A polymorphism in the promoter region of the *KL* gene and cognitive impairment was evaluated among Chinese nonagenarians and centenarians as a secondary analysis of the Project of Longevity and Aging in Dujiangyan (PLAD) study, including 706 community-dwelling Chinese people aged 90 years or older (68.0% female; mean age 93.5 ± 3.6 years) [116]. G-395A (rs1207568) genotyping in the promoter region of the *KL* gene was performed using the TaqMan allelic discrimination assay and cognitive function was assessed with the MMSE. The KL G-395A polymorphism genotype frequencies for the whole sample were 2.0% AA, 30.3% GA, and 67.7% GG, respectively. The GG genotype frequencies for the cognitive impairment and control groups were 70.2 and 62.7%, respectively [116]. Cognitive impairment prevalence was significantly lower in the GA + AA group than in the GG genotype group and GA + AA genotype subjects had a significantly lower risk of cognitive impairment respect to GG genotype subjects after adjusting for age, gender, and other relevant risk factors, suggesting that KL G-395A polymorphism associates with reduced cognitive impairment in a sample of Chinese nonagenarians and centenarians [116]. In consideration of the importance of the functions and the role of the signaling pathways in which KL is involved, the hypothesis that *KL* gene variants could influence the response to SSRIs in elderly patients with depressive disorder deserved evaluation. Three SNPs of KL were genotyped in 329 older patients with diagnosis of late-life MDD, treated with SSRIs and evaluated with the Hamilton Rating Scale for Depression 21-items (HRSD-21) at baseline and after 6 months [117]. The statistical analysis showed a significant difference in the distribution of KL genotypes between responding patients, low responders and non-responders, in particular for the SNP rs9536314, with a statistically nonsignificant trend for the SNP rs1207568 [117]. Patients with at least one mutated allele in rs1207568 showed a significantly higher response to treatment with SSRIs compared to carriers of both wild-type alleles, whereas patients homozygous for the mutation in rs9536314 showed a significantly worse response to treatment with SSRIs compared to carriers of at least one wild-type allele [117]. Consequently, the SNP rs1207568 follows a pattern of dominant inheritance, while the SNP rs9536314 follows a recessive pattern of inheritance. These results were confirmed by considering the pre-post differences of Hamilton scores as a continuous variable, and checking how it behaves in different patterns of genetic inheritance for the three SNPs [117]. A significant improvement after treatment was evidenced in patients carrying at least one mutated allele in rs1207568 and a worse response in patients homozygous for the mutant allele in rs9536314 [117]. In addition, following the dominant model of inheritance, there was a statistically significant greater reduction in the HRSD-21 score from baseline to end of SSRIs treatment for the patients who showed at least one mutated allele in rs1207568 compared to carriers of both wild-type alleles [117]. As well, following the model of recessive inheritance, there was a statistically significant lower reduction in the HRSD-21 score for patients homozygous for the mutation in rs9536314 compared to those with at least one wild-type allele. These results suggest that the T allele of the SNP rs1207568 may be a protective factor that induces a greater response to treatment with SSRIs, while the G allele for the SNP rs9536314 may be a risk factor that hinders the response to treatment with SSRIs [117]. So, the patients carrying the double mutant allele in rs1207568 (T/T) and noncarriers of the double mutation in rs9536314 (not G/G) would respond better to treatment with SSRIs. In contrast, patients without double mutation in rs1207568 (non-T/T) and carriers of the double mutant allele in rs9536314 (G/G), can be considered a class of patients who will respond worse to treatment with SSRIs [117].

Conclusions

Late-life depression, mild cognitive impairment, and dementia are different nosological entities but share common neurobiological facets and could represent a clinical continuum. AD is the most frequent cause of dementia in people over 65 years of age [118, 119]. A number of preclinical and epidemiological studies associated AD pathogenesis to metabolic risk factors, but at this time, therapeutic strategies for AD are merely symptomatic and do not avoid or hold up disease evolution [118, 119]. Klotho proteins, encoded by the *KL* gene, play a key role in cellular and tissue homeostasis and have been associated with age-related diseases, such as AD and major depression in the elderly. In recent times, the neuroprotective role of KL has been identified and therapeutic interventions impacting on KL levels may prevent further deterioration if started early on to hold back disease evolution in AD patients [120–122]. A possible role for the KL gene in the complex path of the response to SSRIs in elderly patients with major depression has been suggested. Although there is a lack of reliable evidence in the literature about specific predictors of response to antidepressants, a method for optimizing drug prescription to patients based on their genetic characteristics would improve therapy effectiveness. Pharmacogenomics and personalized medicine would allow physicians to plan AD and depression treatments more effectively and KL could represent a valuable and reliable genetic profiler and biomarker.

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Compliance with Ethical Standards

Conflict of Interest Statement The authors declare that they have no conflict of interest.

References

1. Takizawa C, Thompson PL, van Walsem A, Faure C, Maier WC (2015) Epidemiological and economic burden of Alzheimer's disease: a systematic literature review of data across Europe and the United States of America. J Alzheimers Dis 43(4):1271–1284. https://doi.org/10.3233/JAD-141134.
2. Naismith SL, Norrie LM, Mowszowski L, Hickie IB (2012) The neurobiology of depression in later-life: clinical, neuropsychological, neuroimaging and pathophysiological features. Prog Neurobiol 98(1):99–143. https://doi.org/10.1016/j.pneurobio.2012.05.009
3. Alexopoulos GS, Canuso CM, Gharabawi GM, Bossie CA, Greenspan A, Turkoz I, Reynolds C 3rd (2008) Placebo-controlled study of relapse prevention with risperidone augmentation in older patients with resistant depression. Am J Geriatr Psychiatry 16:21–30
4. Panza F, Frisardi V, Capurso C, D'Introno A, Colacicco AM, Imbimbo BP, Santamato A, Vendemiale G et al (2010) Late-life depression, mild cognitive impairment, and dementia: possible continuum? Am J Geriatr Psychiatry 18(2):98–116. https://doi.org/10.1097/JGP.0b013e3181b0fa13
5. Blazer DG (2003) Depression in late life: review and commentary. J Gerontol A Biol Sci Med Sci 58(3):249–265
6. Luppa M, Luck T, König HH, Angermeyer MC, Riedel-Heller SG (2012) Natural course of depressive symptoms in late life. An 8-year population-based prospective study. J Affect Disord 142(1–3):166–171
7. Büchtemann D, Luppa M, Bramesfeld A, Riedel-Heller S (2012) Incidence of late-life depression: a systematic review. J Affect Disord 142(1–3):172–179. https://doi.org/10.1016/j.jad.2012.05.010
8. Blazer D, Williams CD (1980) Epidemiology of dysphoria and depression in an elderly population. Am J Psychiatry 137(4):439–444
9. Seripa D, Panza F, D'Onofrio G, Paroni G, Bizzarro A, Fontana A, Paris F, Cascavilla L et al (2013) The serotonin transporter gene locus in late-life major depressive disorder. Am J Geriatr Psychiatry 21(1):67–77. https://doi.org/10.1016/j.jagp.2012.10.012
10. Ellison JM, Kyomen HH, Harper DG (2012) Depression in later life: an overview with treatment recommendations. Psychiatr Clin North Am 35(1):203–229. https://doi.org/10.1016/j.psc.2012.01.003
11. Katon WJ (2003) Clinical and health services relationships between major depression, depressive symptoms, and general medical illness. Biol Psychiatry 54(3):216–226
12. Caraci F, Spampinato SF, Morgese MG, Tascedda F, Salluzzo MG, Giambirtone MC, Caruso G, Munafò A, Torrisi SA, Leggio GM, Trabace L, Nicoletti F, Drago F, Sortino MA, Copani A. (2018). Neurobiological links between depression and AD: the role of TGF-β1 signaling as a new pharmacological target. Pharmacol Res. doi: https://doi.org/10.1016/j.phrs.2018.02.007. pii: S1043-6618(17)31600–6
13. Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, Ohyama Y, Kurabayashi M et al (1997) Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature 390(6655):45–51
14. Kuang X, Chen YS, Wang LF, Li YJ, Liu K, Zhang MX, Li LJ, Chen C et al (2014) Klotho upregulation contributes to the neuroprotection of ligustilide in an Alzheimer's disease mouse model. Neurobiol Aging 35(1):169–178. https://doi.org/10.1016/j.neurobiolaging.2013.07.019
15. Semba RD, Moghekar AR, Hu J, Sun K, Turner R, Ferrucci L, O'Brien R (2014) Klotho in the cerebrospinal fluid of adults with and without Alzheimer's disease. Neurosci Lett 558:37–40. https://doi.org/10.1016/j.neulet.2013.10.058
16. Dubal DB, Zhu L, Sanchez PE, Worden K, Broestl L, Johnson E, Ho K, Yu GQ et al (2015) Life extension factor klotho prevents mortality and enhances cognition in hAPP transgenic mice. J Neurosci 35(6):2358–2371. https://doi.org/10.1523/JNEUROSCI.5791-12.2015
17. Yokoyama JS, Marx G, Brown JA, Bonham LW, Wang D, Coppola G, Seeley WW, Rosen HJ et al (2017) Systemic klotho is associated with KLOTHO variation and predicts intrinsic cortical connectivity in healthy human aging. Brain Imaging Behav 11(2):391–400. https://doi.org/10.1007/s11682-016-9598-2
18. Arking DE, Krebsova A, Macek M Sr, Macek M Jr, Arking A, Mian IS, Fried L, Hamosh A et al (2002) Association of human aging with a functional variant of klotho. Proc Natl Acad Sci U S A 99(2):856–861
19. Dubal DB, Yokoyama JS, Zhu L, Broestl L, Worden K, Wang D, Sturm VE, Kim D et al (2014) Life extension factor klotho enhances cognition. Cell Rep 7(4):1065–1076. https://doi.org/10.1016/j.celrep.2014.03.076
20. Nagai T, Yamada K, Kim HC, Kim YS, Noda Y, Imura A, Nabeshima Y, Nabeshima T (2003) Cognition impairment in the genetic model of aging klotho gene mutant mice: a role of oxidative stress. FASEB J 17(1):50–52
21. Deary IJ, Harris SE, Fox HC, Hayward C, Wright AF, Starr JM, Whalley LJ (2005) KLOTHO genotype and cognitive ability in childhood and old age in the same individuals. Neurosci Lett 378(1):22–27
22. Arking DE, Becker DM, Yanek LR, Fallin D, Judge DP, Moy TF, Becker LC, Dietz HC (2003) KLOTHO allele status and the risk of early-onset occult coronary artery disease. Am J Hum Genet 72(5):1154–1161
23. Arking DE, Atzmon G, Arking A, Barzilai N, Dietz HC (2005) Association between a functional variant of the KLOTHO gene and high-density lipoprotein cholesterol, blood pressure, stroke, and longevity. Circ Res 96(4):412–418
24. Xu Y, Sun Z (2015) Molecular basis of klotho: from gene to function in aging. Endocr Rev 36(2):174–193. https://doi.org/10.1210/er.2013-1079
25. Kim JH, Hwang KH, Park KS, Kong ID, Cha SK (2015) Biological role of anti-aging protein klotho. J Lifestyle Med 5(1):1–6. https://doi.org/10.15280/jlm.2015.5.1.1
26. Lim K, Groen A, Molostvov G, Lu T, Lilley KS, Snead D, James S, Wilkinson IB et al (2015) α-Klotho expression in human tissues. J Clin Endocrinol Metab 100(10):E1308–E1318
27. Hayashi Y, Ito M (2016) Klotho-related protein KLrP: structure and functions. Vitam Horm 101:1–16. https://doi.org/10.1016/bs.vh.2016.02.011.
28. Kuro-o M (2012) Klotho and βKlotho. Adv Exp Med Biol 728:25–40. https://doi.org/10.1007/978-1-4614-0887-1_2

29. Chen G, Liu Y, Goetz R, Fu L, Jayaraman S, Hu MC, Moe OW, Liang G et al (2018) α-Klotho is a non-enzymatic molecular scaffold for FGF23 hormone signalling. Nature 553(7689):461–466. https://doi.org/10.1038/nature25451
30. Cararo-Lopes MM, Mazucanti CHY, Scavone C, Kawamoto EM, Berwick DC (2017) The relevance of α-KLOTHO to the central nervous system: some key questions. Ageing Res Rev 36:137–148. https://doi.org/10.1016/j.arr.2017.03.003
31. Massó A, Sánchez A, Gimenez-Llort L, Lizcano JM, Cañete M, García B, Torres-Lista V, Puig M et al (2015) Secreted and transmembrane αKlotho isoforms have different spatio-temporal profiles in the brain during aging and Alzheimer's disease progression. PLoS One 10(11):e0143623
32. Mian IS (1998) Sequence, structural, functional, and phylogenetic analyses of three glycosidase families. Blood Cells Mol Dis 24: 83–100
33. Agostini M, Fasolato C (2016) When, where and how? Focus on neuronal calcium dysfunctions in Alzheimer's disease. Cell Calcium 60(5):289–298. https://doi.org/10.1016/j.ceca.2016.06.008
34. Alzheimer's Association Calcium Hypothesis Workgroup (2017) Calcium hypothesis of Alzheimer's disease and brain aging: a framework for integrating new evidence into a comprehensive theory of pathogenesis. Alzheimers Dement 13(2):178–182.e17. https://doi.org/10.1016/j.jalz.2016.12.006
35. Hurwitz S (1996) Homeostatic control of plasma calcium concentration. Crit Rev Biochem Mol Biol 31:41–100
36. Jones G, Strugnell SA, DeLuca HF (1998) Current understanding of the molecular actions of Vitamin D. Physiol Rev 78:1193–1231
37. Bushinsky DA, Monk RD (1998) Calcium. Lancet 352:306–311
38. Shinki T, Shimada H, Wakino S, Anazawa H, Hayashi M, Saruta T, DeLuca HF, Suda T (1997) Cloning and expression of rat 25-hydroxyvitamin D3 1α-hydroxylase cDNA. Proc Natl Acad Sci U S A 94:12920–12925
39. Shinki T, Ueno Y, DeLuca HF, Suda T (1999) Calcitonin is a major regulator for the expression of renal 25-hydroxyvitamin D3 1α-hydroxylase gene in normocalcemic rats. Proc Natl Acad Sci U S A 6:8253–8258
40. Murayama A, Takeyama K, Kitanaka S, Kodera Y, Hosoya T, Kato S (1998) The promoter of the human 25-hydroxyvitamin D3 1α-hydroxylase gene confers positive and negative responsiveness to PTH, calcitonin, and 1α,25-(OH)2D3. Biochem Biophys Res Commun 249:11–16
41. Iida K, Shinki T, Yamaguchi A, DeLuca HF, Kurokawa K, Suda T (1995) Possible role of Vitamin D receptors in regulating Vitamin D activation in the kidney. Proc Natl Acad Sci U S A 92:6112–6116
42. Kato S (2000) The function of Vitamin D receptor in Vitamin D action. J Biochem 127:717–2267
43. Yoshida T, Fujimori T, Nabeshima Y (2002) Mediation of unusually high concentrations of 1.25-hydroxyvitamin D3 in homozygous klotho mutant mice by increased expression of renal 1α-hydroxylase gene. Endocrinology 143:683–689
44. Kumar R (1984) Metabolism of 1,25-hydroxyvitamin D3. Physiol Rev 64:478–504
45. Zehner D, Hewison M (1999) The renal function of 25-hydroxivitamin D3 1α-hydroxylase. Mol Cell Endocrinol 151: 213–220
46. Nabeshima Y (2002) Klotho: a fundamental regulator of aging. Ageing research Rev 1:627–638
47. Freude S, Hettich MM, Schumann C, Stöhr O, Koch L, Köhler C, Udelhoven M, Leeser U et al (2009) Neuronal IGF-1 resistance reduces Abeta accumulation and protects against premature death in a model of Alzheimer's disease. FASEB J 23(10):3315–3324. https://doi.org/10.1096/fj.09-132043
48. Zemva J, Schubert M (2014) The role of neuronal insulin/insulin-like growth factor-1 signaling for the pathogenesis of Alzheimer's disease: possible therapeutic implications. CNS Neurol Disord Drug Targets 13(2):322–337
49. Kurosu H, Yamamoto M, Clark JD, Pastor JV, Nandi A, Gurnani P, McGuinness OP, Chikuda H et al (2005) Suppression of aging in mice by the hormone Klotho. Science 309:1829–1833
50. Guarente L, Kenyon C (2000) Genetic pathways that regulate ageing in model organisms. Nature 408(6809):255–262
51. Kuro-o M (2001) Disease model: human aging. Trends Mol Med 7(4):179–181
52. Jimenez C, Hernandez C, Pimentel B, Carrera AC (2002) The p85 regulatory subunit controls sequential activation of phosphoinositide 3-kinase by Tyr kinases and Ras. J Biol Chem 277(44):41556–41562
53. Unger RH (2006) Klotho-induced insulin resistance: a blessing in disguise? Nat Med 12(1):56–57
54. Boden G, Shulman GI (2002) Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and β-cell dysfunction. Eur J Clin Investig 32(Suppl. 3):14–23
55. Brunet A, Sweeney LB, Sturgill JF, Chua KF, Greer PL, Lin Y, Tran H, Ross SE et al (2004) Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. Science 303(5666):2011–2015
56. Picard F, Kurtev M, Chung N, Topark-Ngarm A, Senawong T, Machado de Oliveira R, Leid M, McBurney MW et al (2004) Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-γ. Nature 429:771–776
57. McGarry JD, Mannaerts GP, Foster DW (1997) A possible role for malonyl-CoA in the regulation of hepatic fatty acid oxidation and ketogenesis. J Clin Invest 60:265–270
58. Staiger H, Keuper M, Berti L, Hrabe de Angelis M, Häring HU (2017) Fibroblast growth factor 21-metabolic role in mice and men. Endocr Rev 38(5):468–488. https://doi.org/10.1210/er.2017-00016
59. Suzuki M, Uehara Y, Motomura-Matsuzaka K, Oki J, Koyama Y, Kimura M, Asada M, Komi-Kuramochi A et al (2008) betaKlotho is required for fibroblast growth factor (FGF) 21 signaling through FGF receptor (FGFR) 1c and FGFR3c. Mol Endocrinol 22(4): 1006–1014
60. Bookout AL, de Groot MH, Owen BM, Lee S, Gautron L, Lawrence HL, Ding X, Elmquist JK et al (2013) FGF21 regulates metabolism and circadian behavior by acting on the nervous system. Nat Med 19(9):1147–1152
61. Shardell M, Semba RD, Rosano C, Kalyani RR, Bandinelli S, Chia CW, Ferrucci L. (2015). Plasma klotho and cognitive decline in older adults: findings from the InCHIANTI study. J Gerontol A Biol Sci Med Sci. pii: glv140.
62. Takahashi Y, Kuro-o M, Ishikawa F. (2000). Aging mechanisms. From the Academy. 97(23):12407–12408.
63. Murray CJ, Lopez AD (1997) Alternative projections of mortality and disability by cause 1990–2020: Global Burden of Disease Study. Lancet 349(9064):1498–1504
64. Gold PW, Chrousos G, Kellner C, Post R, Roy A, Augerinos P, Schulte H, Oldfield E et al (1984) Psychiatric implications of basic and clinical studies with corticotropin-releasing factor. Am J Psychiatry 141:619–627
65. Gold PW, Loriaux DL, Roy A, Kling MA, Calabrese JR, Kellner CH, Nieman LK, Post RM et al (1986) Responses to corticotropin-releasing hormone in the hypercortisolism of depression and Cushing's disease. Pathophysiologic and diagnostic implications. N Engl J Med 314:1329–1335
66. Thakker-Varia S, Krol JJ, Nettleton J, Bilimoria PM, Bangasser DA, Shors TJ, Black IB, Alder J (2007) The neuropeptide VGF

produces antidepressant-like behavioral effects and enhances proliferation in the hippocampus. J Neurosci 7:12156–12167
67. Hunsberger JG, Newton SS, Bennett AH, Duman CH, Russell DS, Salton SR, Duman RS (2007) Antidepressant actions of the exercise-regulated gene VGF. Nat Med 13:1476–1482
68. Nativio R, Donahue G, Berson A, Lan Y, Amlie-Wolf A, Tuzer F, Toledo JB, Gosai SJ et al (2018) Dysregulation of the epigenetic landscape of normal aging in Alzheimer's disease. Nat Neurosci 21(4):497–505. https://doi.org/10.1038/s41593-018-0101-9
69. Gold PW, Wong ML, Goldstein DS, Gold HK, Ronsaville DS, Esler M, Alesci S, Masood A et al (2005) Cardiac implications of increased arterial entry and reversible 24-h central and peripheral norepinephrine levels in melancholia. Proc Natl Acad Sci U S A 102:8303–8308
70. Brandi LS, Santoro D, Natali A, Altomonte F, Baldi S, Frascerra S, Ferrannini E (1993) Insulin resistance of stress: sites and mechanisms. Clin Sci 85:525–535
71. Wong ML, Kling MA, Munson PJ, Listwak S, Licinio J, Prolo P, Karp B, McCutcheon IE et al (2000) Pronounced and sustained central hypernoradrenergic function in major depression with melancholic features: relation to hypercortisolism and corticotropin-releasing hormone. Proc Natl Acad Sci U S A 97:325–330
72. Kaplan MS, Hinds JW (1977) Neurogenesis in the adult rat: electron microscopic analysis of light radioautographs. Science 197: 1092–1094
73. Altman J, Das GD (1965) Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. J Comp Neurol 124:319–335
74. Salech F, Varela-Nallar L, Arredondo SB, Bustamante DB, Andaur GA, Cisneros R, Ponce DP, Ayala P, Inestrosa NC, Valdés JL, Behrens MI, Couve A. (2017) Local Klotho enhances neuronal progenitor proliferation in the adult hippocampus. J Gerontol A Biol Sci Med Sci. 30. doi: https://doi.org/10.1093/gerona/glx248.
75. Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J et al (2003) Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 301:805–809
76. Sahay A, Hen R (2007) Adult hippocampal neurogenesis in depression. Nat Neurosci 10:1110–1115
77. Surget A, Saxe M, Leman S, Ibarguen-Vargas Y, Chalon S, Griebel G, Hen R, Belzung C (2008) Drug dependent requirement of hippocampal neurogenesis in a model of depression and of antidepressant reversal. Biol Psychiatry 64:293–301
78. Surget A, Tanti A, Leonardo ED, Laugeray A, Rainer Q, Touma C, Palme R, Griebel G et al (2011) Antidepressants recruit new neurons to improve stress response regulation. Mol Psychiatry 16: 1177–1188
79. Malberg JE, Eisch AJ, Nestler EJ, Duman RS (2000) Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci 20:9104–9110
80. Zhao C, Deng W, Gage FH (2008) Mechanisms and functional implications of adult neurogenesis. Cell 132:645–660
81. Gold PW, Licinio J, Pavlatou MG (2013) Pathological parainflammation and endoplasmic reticulum stress in depression: potential translational targets through the CNS insulin, klotho and PPAR-γ systems. Mol Psychiatry 18(2):154–165. https://doi.org/10.1038/mp.2012.167
82. Chen CD, Podvin S, Gillespie E, Leeman SE, Abraham CR (2007) Insulin stimulates the cleavage and release of the extracellular domain of Klotho by ADAM10 and ADAM17. Proc Natl Acad Sci U S A 104:19796–19801
83. Bloch L, Sineshchekova O, Reichenbach D, Reiss K, Saftig P, Kuro-o M (2009) Klotho is a substrate for α-, β- and γ-secretase. FEBS Lett 583:3221–3224
84. Imura A, Iwano A, Tohyama O, Tsuji Y, Nozaki K, Hashimoto N (2004) Secreted Klotho protein in sera and CSF: implication for post-translational cleavage in release of Klotho protein from cell membrane. FEBS Lett 565:143–147
85. Kuro-o M (2010) Klotho. Pflugers Arch 459:333–343
86. Kuro-o M (2008) Klotho as a regulator of oxidative stress and senescence. Biol Chem 389:233–241
87. Saito Y, Yamagishi T, Nakamura T, Ohyama Y, Aizawa H, Suga T, Matsumura Y, Masuda H et al (1998) Klotho protein protects against endothelial dysfunction. Biochem Biophys Res Commun 248:324–329
88. Imura A, Tsuji Y, Murata M, Maeda R, Kubota K, Iwano A, Obuse C, Togashi K et al (2007) alpha-Klotho as a regulator of calcium homeostasis. Science 316:1615–1618
89. de Groot T, Bindels RJ, Hoenderop JG (2008) TRPV5: an ingeniously controlled calcium channel. Kidney Int 74:1241–1246
90. Li SA, Watanabe M, Yamada H, Nagai A, Kinuta M, Takei K (2004) Immunohistochemical localization of Klotho protein in brain, kidney, and reproductive organs of mice. Cell Struct Funct 29:91–99
91. Zeldich E, Chen CD, Colvin TA, Bove-Fenderson EA, Liang J, Tucker Zhou TB, Harris DA, Abraham CR (2014) The neuroprotective effect of Klotho is mediated via regulation of members of the redox system. J Biol Chem 289(35):24700–24715
92. Leon J, Moreno AJ, Garay BI, Chalkley RJ, Burlingame AL, Wang D, Dubal DB (2017) Peripheral elevation of a klotho fragment enhances brain function and resilience in young, aging, and α-synuclein transgenic mice. Cell Rep 20(6):1360–1371. https://doi.org/10.1016/j.celrep.2017.07.024
93. Sato S, Kawamata Y, Takahashi A, Imai Y, Hanyu A, Okuma A, Takasugi M, Yamakoshi K et al (2015) Ablation of the p16(INK4a) tumour suppressor reverses ageing phenotypes of klotho mice. Nat Commun 6:7035. https://doi.org/10.1038/ncomms8035
94. Matsumura Y, Aizawa H, Shiraki-Iida T, Nagai R, Kuro-o M, Nabeshima Y (1998) Identification of the human klotho gene and its two transcripts encoding membrane and secreted klotho protein. Biochem Biophys Res Commun 242:626–630
95. Shiraki-Iida T, Aizawa H, Matsumura Y, Sekine S, Iida A, Anazawa H, Nagai R, Kuro-o M et al (1998) Structure of the mouse klotho gene and its two transcripts encoding membrane and secreted protein. FEBS Lett 424:6–10
96. Fukino K, Suzuki T, Saito Y, Shindo T, Amaki T, Kurabayashi M, Nagai R (2002) Regulation of angiogenesis by the aging suppressor gene klotho. Biochem Biophys Res Commun 293:332–337
97. de Bruijn RF, Ikram MA (2014) Cardiovascular risk factors and future risk of Alzheimer's disease. BMC Med 12:130. https://doi.org/10.1186/s12916-014-0130-5
98. World Health Organization. http://www.who.int/mental_health/management/ depression/wfmh_paper_depression_wmhd_2012.pdf. Accessed 3.4.2018
99. Charney DS, Nemeroff CB, Lewis L, Laden SK, Gorman JM, Laska EM, Borenstein M, Bowden CL et al (2002) National Depressive and Manic-Depressive Association consensus statement on the use of placebo in clinical trials of mood disorders. Arch Gen Psychiatry 59:262–270
100. Weissman MM, Bruce ML, Leaf PJ, Florio LP, Holzer CE (1991) Affective disorders. In: Robins LN, Regier DA (eds) Psychiatric disorders in America. The Free Press, New York, pp. 53–80
101. Wulsin LR, Evans JC, Vasan RS, Murabito JM, Kelly-Hayes M, Benjamin EJ (2005) Depressive symptoms, coronary heart disease, and overall mortality in the Framingham Heart Study. Psychosom Med 67:697–702
102. Eaton WW, Armenian H, Gallo J, Pratt L, Ford DE (1996) Depression and risk for onset of type II diabetes: a prospective population-based study. Diabetes Care 19:1097–1102

103. Penninx BW, Beekman AT, Honig A, Deeg DJ, Schoevers RA, van Eijk JT, van Tilburg W (2001) Depression and cardiac mortality: results from a community-based longitudinal study. Arch Gen Psychiatry 58:221–227
104. Chang CK, Hayes RD, Perera G, Broadbent MT, Fernandes AC, Lee WE, Hotopf M, Stewart R (2011) Life expectancy at birth for people with serious mental illness and other major disorders from a secondary mental health care case register in London. PLoS One 6(5):e19590
105. Harrington R (2002) Affective disorders. In: Rutter M, Taylor E (eds) Child and adolescent psychiatry, Fourth edn. Blackwell, Oxford, pp. 463–485
106. Tamminga CA, Nemeroff CB, Blakely RD, Brady L, Carter CS, Davis KL, Dingledine R, Gorman JM et al (2002) Developing novel treatments for mood disorders: accelerating discovery. Biol Psychiatry 52:589–609
107. Quintin P, Benkelfat C, Launay JM, Arnulf I, Pointereau-Bellenger A, Barbault S, Alvarez JC, Varoquaux O et al (2001) Clinical and neurochemical effect of acute tryptophan depletion in unaffected relatives of patients with bipolar affective disorder. Biol Psychiatry 50:184–190
108. Smith KA, Fairburn CG, Cowen PJ (1997) Relapse of depression after rapid depletion of tryptophan. Lancet 349:915–919
109. Bhagwagar Z, Rabiner EA, Sargent PA, Grasby PM, Cowen PJ (2004) Persistent reduction in brain serotonin1A receptor binding in recovered depressed men measured by positron emission tomography with [11C]WAY–100635. Mol Psychiatry 9:386–392
110. Malison RT, Price LH, Berman R, van Dyck CH, Pelton GH, Carpenter L, Sanacora G, Owens MJ et al (1998) Reduced brain serotonin transporter availability in major depression as measured by [123I]–2 beta-carbomethoxy–3 beta-(4-iodophenyl) tropane and single photon emission computed tomography. Biol Psychiatry 44:1090–1098
111. Cox GR, Callahan P, Churchill R, Hunot V, Merry SN, Parker AG, Hetrick SE (2012) Psychological therapies versus antidepressant medication, alone and in combination for depression in children and adolescents. Cochrane Database Syst Rev 11:CD008324
112. Wilkinson P, Izmeth Z (2012) Continuation and maintenance treatments for depression in older people. Cochrane Database Syst Rev 11:CD006727
113. Kraus C, Castrén E, Kasper S, Lanzenberger R (2017) Serotonin and neuroplasticity—links between molecular, functional and structural pathophysiology in depression. Neurosci Biobehav Rev 77:317–326. https://doi.org/10.1016/j.neubiorev.2017.03.007
114. Gałecki P, Mossakowska-Wójcik J, Talarowska M (2018) The anti-inflammatory mechanism of antidepressants—SSRIs, SNRIs. Prog Neuropsychopharmacol Biol Psychiatry 80(Pt C): 291–294. https://doi.org/10.1016/j.pnpbp.2017.03.016
115. Mengel-From J, Soerensen M, Nygaard M, McGue M, Christensen K, Christiansen L. (2015). Genetic variants in KLOTHO associate with cognitive function in the oldest old group. J Gerontol A Biol Sci Med Sci. pii: glv163.
116. Hao Q, Ding X, Gao L, Yang M, Dong B (2016) G-395A polymorphism in the promoter region of the KLOTHO gene associates with reduced cognitive impairment among the oldest old. Age (Dordr) 38(1):7. https://doi.org/10.1007/s11357-015-9869-7
117. Paroni G, Seripa D, Fontana A, D'Onofrio G, Gravina C, Urbano M, Addante F, Lozupone M et al (2017) Klotho gene and selective serotonin reuptake inhibitors: response to treatment in late-life major depressive disorder. Mol Neurobiol 54(2):1340–1351. https://doi.org/10.1007/s12035-016-9711-y
118. Wang Q, Yuan J, Yu Z, Lin L, Jiang Y, Cao Z, Zhuang P, Whalen MJ, Song B, Wang XJ, Li X, Lo EH, Xu Y, Wang X. (2017). FGF21 attenuates high-fat diet-induced cognitive impairment via metabolic regulation and anti-inflammation of obese mice. Mol Neurobiol. doi: https://doi.org/10.1007/s12035-017-0663-7.
119. Codocedo JF, Ríos JA, Godoy JA, Inestrosa NC (2016) Are microRNAs the molecular link between metabolic syndrome and Alzheimer's disease. Mol Neurobiol 53(4):2320–2338. https://doi.org/10.1007/s12035-015-9201-7
120. Kuriyama N, Ozaki E, Mizuno T, Ihara M, Mizuno S, Koyama T, Matsui D, Watanabe I et al (2018) Association between α-Klotho and deep white matter lesions in the brain: a pilot case control study using brain MRI. J Alzheimers Dis 61(1):145–155. https://doi.org/10.3233/JAD-170466
121. Almeida OP, Morar B, Hankey GJ, Yeap BB, Golledge J, Jablensky A, Flicker L (2017) Longevity Klotho gene polymorphism and the risk of dementia in older men. Maturitas 101:1–5. https://doi.org/10.1016/j.maturitas.2017.04.005
122. Abraham CR, Mullen PC, Tucker-Zhou T, Chen CD, Zeldich E (2016) Klotho is a neuroprotective and cognition-enhancing protein. Vitam Horm 101:215–238. https://doi.org/10.1016/bs.vh.2016.02.004