The inflamed uremic phenotype - a mediator of premature aging
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Disclosure of Interest
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The details of each Disclosure of Interest are available at the Invited Speakers’ desk (located in the Registration Area).
Good morning. It's a pleasure for me to be able to address you about the inflamed uremic phenotype as a mediator of premature aging.

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Advanced Chronic Kidney Disease
- Characterized by Many Features of Aging

- Atherosclerosis
- Osteoporosis
- Sarcopenia
- Oxidative stress
- Inflammation
- Poor wound healing
- Insulin resistance
- Infertility
- Hypogonadism
- Skin atrophy
- Cognitive dysfunction
- Depression
- Frailty

We know that CKD is characterised by many features of aging. Our patients are atherosclerotic, osteoporotic, sarcopenic; they often have oxidative stress and inflammation, infertility, hypogonadism, skin atrophy, depression and frailty.

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So when a colleague and I recently researched the literature, we really found that CKD could be regarded an excellent model of premature aging.

Senescent cells change their morphological characteristics and these changes are accompanied by alterations in nuclear structure, gene expression, protein processing and metabolism.

The key feature of the aging process are these senescence cells, you can see them here depicted, the change of morphological characteristics and these changes are accompanied by alterations in nuclear structure, gene expression, protein processing and metabolism.
Senescent cells change their morphological characteristics and these changes are accompanied by alterations in nuclear structure, gene expression, protein processing and metabolism.

In the non-renal literature, it has been suggested that the senescent cells could be used as a novel therapeutic target for aging and age-related diseases.
We know that there are many both intrinsic and extrinsic factors that induce cellular senescence. We have neutral deficiency, mitochondrial dysfunction, telomere shortening, genetic modulators, oxidative stress and inflammation. Partners in crime that play a very important role here.

More Than 300 Theories Have Been Proposed to Explain the Aging Process

- Evolutionary theory
  (based on Darwin’s natural selection theory)

 Supporting this hypothesis is the evidence that animals living in a protected environment (such as a zoo) live longer.

 Some animal species do not appear to undergo aging or senescence at all in the wild. They maintain body composition throughout life.

 Improved understanding of the process that have evolved in these species to increase healthy life span provides unique opportunities to develop novel treatment strategies against human aging.

- Rougheye rockfish
  205 yrs!

- Turtles

- Naked mole rat
There have been so far more than 300 theories proposed to explain the aging process in humans. The first one is the evolutionary theory which is based on Darwin’s Natural Selection Theory and there is much support for this theory. One theory that supports this is the evidence that animals living in a protected environment such as a zoo or a captive state live much longer. But I found even more interesting that some animal species do not undergo aging or senescence at all in the wild. These animals maintain body composition throughout life. These are three examples of such animals: the Rougheye rockfish can become more than 200 years old. Of course, we have turtles. The naked mole rat is another example and a little later in my talk, I will discuss one of these creatures, which I think is especially interesting because improved understanding of the processes that have evolved in these specific species to increase health life span provides unique opportunities to develop novel treatment strategies against human aging.

More Than 300 Theories Have Been Proposed to Explain the Aging Process

- Evolutionary theory
- Gene regulation theory ("Churchill genes")
- Free radical theory
- Mitochondrial theory (extension of the free radical theory)
- Inflammation hypothesis

We have other theories to explain the aging process. The Gene Regulation Theory. Also, those of you who have the so-called 'Churchill genes' may expect to live longer. We have the Free Radical Theory, the Mitochondrial Theory and then we have the Inflammation Hypothesis becoming so interesting now that it has got its own neologism 'Inflamm-aging'.
Persistent Inflammation and Cellular Senescence

- IL-6 regulates oncogene-induced senescence (*Cell*. 2008; 133:1019-1031)
- Cellular senescence impairs the successful reprogramming of pluripotent cells (*Genes Dev*. 2009;23:2134-2139.)
- In the inflamed uremic milieu stem cells differentiate to aggressive monocytes at the expense of reduced production of endothelial repair cells (*Exp Gerontol*. 2010;45:797-800.)

Persistent inflammation and cellular senescence are closely correlated. IL-6 has been shown to regulate oncogene-induced senescence. Senescence cell secrete pro-inflammatory cytokines and chemokines. Cellular senescence is linked with endothelial dysfunction and the inflammatory process of atherosclerosis. Also cellular senescence impairs the successful reprogramming of pluripotent cells. As I think maybe discussed by Gunnar Heine later, the inflamed uremic milieu stem cells differentiate to aggressive monocytes at the expense of reduced production of endothelial repair cells.

The beneficial effects of inflammation devoted to the neutralization of dangerous/harmful agents **early in life** become detrimental **late in life** (a period not foreseen by evolution).

Unregulated inflammation shortens human longevity and has an important role in the cause, progression and shortened life span of patients with presenile dementia, osteoporosis, diabetes and atherosclerosis.

We know that inflammation is associated with progression of a shortened lifespan in many patient groups including pre-senile dementia, osteoporosis, diabetes and atherosclerosis. So upregulation of inflammation seems to shorten human longevity. I think this is a very important point that the beneficial effects of inflammation devoted to the neutralization of dangerous or harmful agents early in life may become detrimental late in life, which is actually a period not foreseen by evolution.
This leads me to an extremely interesting hypothesis that I've never heard discussed among nephrologists and that's the Antagonistic Pleiotropy Theory. In short, that is that alleles displaying beneficial effects in youth by conferring a fitness advantage would be strongly selected for in nature even if these same alleles had a detrimental effect on survival late in life.

First proposed by George Williams 1957 as an explanation for senescence
The Antagonistic Pleiotropy Hypothesis

I think this is probably the best example of this Antagonistic Pleiotropy Hypothesis. Here mTOR,

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The Antagonistic Pleiotrophy Hypothesis

the mammalian target of rapamycin plays a very central important role because this transcription factor is stimulated by insulin, insulin growth factor, growth hormones and nutrients.

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This is very good early in life because this will lead to beneficial muscle growth effects, a period of life when physical strength is needed for both survival and reproduction.

However, when you get older, over activation of mTOR with increased signalling of insulin-IGF-1 will increase risk of accelerated aging.
Of course, there are numerous animal studies that show that disrupting the signalling pathways for insulin or IGF-1 exerts anti-aging properties.

So it's no surprise that remedies or interventions that prolong aging in animal models are those that inhibit the mTOR system: Resveratrol, metformin, rapamycin and caloric restriction are four examples.
A recent Science paper showed that mTOR is also involved in the hyper secretory senescent phenotype.

more than 300 theories have been proposed to explain the aging process

- Evolutionary theory
- Gene regulation theory ("Churchill genes")
- Free radical theory
- Mitochondrial theory (extension of the free radical theory)
- Inflammation hypothesis ("inflamma-aging")
- Immune theory (links to inflammation and free radicals)
- Neuroendocrine theory (hypothalamo-pituitary adrenal axis regulates onset and termination of each stage of life)
- Neuroendocrine-immuno theory (combination theory)
- Telomere theory

So let's discuss another of these 300 aging processes and that is the Telomere theory. You're all aware of this biological clock which is the home of repair systems, has anti-cancer effects, numerous patient groups, shorter telomeres are predictive for mortality.
and you could say that shortening of telomeres reflects cellular senescence, stem cell exhaustion, cellular hyperactivity and the hyper-secretory phenotype.

A couple of years ago we studied Telomere attrition in haemodialysis patients and we could confirm that like in other patient groups males have shorter telomeres. We could also show that like in other patient groups shorter telomeres predict poor outcome.
We also found associations to inflammation. There was a correlation although not very strong to IL-6, so the shorter the telomeres the higher the IL-6 levels. But in this study the strongest association was found between telomeres and fetuin,

which I think is of interest because we all know that fetuin plays an important role in the calcification process. This is a picture of the fetuin knockout mice, with extensive calcification at early age.
In humans, senescent vascular smooth muscle cells exhibit increased osteogenic differentiation and calcification potential.

Calciprotein Particles (CPPs) – a Novel Concept of Crystal Clearance

- Formation and inhibition of crystallization are fetuin-A dependent
- Decreasing fetuin-A causes reduces the stability of CCPs
This leads us to a novel concept of crystal clearance, the Calciprotein Particles, CPPs because in analogy to the LDL particle we also have this CPP particle containing phosphate. This is what these particles look like in the circulation and these particles are cytotoxic, they induce cytokine expression and trigger the innate immune response. So one can hypothesise that high phosphate via formation of the high CPPs create inflammation and aging. In this scenario, fetuin plays a very important role because the formation and inhibition of crystallisation are fetuin-dependent and decreased fetuin, like you will have in inflammation, causes reduced stability of CPPs.

There are emerging links between phosphate and inflammation. This is just one paper showing that phosphate binders have anti-inflammatory effects in dialysis patients.

I think one important and very interesting indirect link of phosphate to longevity is this very
There is a strong correlation between longevity and phosphate levels in different mammals.

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Relation Between Longevity and Serum Phosphate in Mammals

At this far end, we have the klotho mice which have an extremely short lifespan and extremely high phosphate levels.

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Relation Between Longevity and Serum Phosphate in Mammals

In contrast here we have humans who can become more than 100 years old and this is the mammal with the lowest phosphate levels.

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In between, we have different animals such as the elephant, rhinoceros, bears and rodents and the higher their phosphate, the shorter their longevity.

Can Reduction of the Phosphate Burden Delay Aging?

So I think a very important question is if reduction of the phosphate burden will delay the aging process. We don't know that but there are indeed some studies that support this. This is one study showing that the progression of aging in the klotho mutant mice can be modified by dietary phosphate intake.
So in our review soon to be published in AJKD we have been reviewing what interventions can delay the aging process. Men have been looking for this fountain of youth since the 16th century when explorers were trying to find this mythical land of Bimini where this fountain of youth was supposed to be found.

**The Fountain of Youth – can Nutritional and Pharmacological Interventions Extend Age?**

**Nutritional and lifestyle interventions**
- Caloric restriction (96)
- Red wine (121)
- Fish oil (omega 3) (106)
- Phosphate restriction (152)
- Physical exercise (104)

**Pharmacological interventions**
- SIRT activation; resveratrol (101)
- Increased Klotho expression; drugs that alter DNA hypermethylation (154), inhibition of NF-kB (81), PPAR-γ agonists (146), thyroid hormones (147), ACE-inhibition (148), D vitamin (155)
- mTOR inhibition: rapamycin (108), metformin (110), resveratrol (111)
- Stabilizing telomeres: statins (130), estrogens (131), telomerase reactivation (129), D-vitamin (132)
- Limit DNA damage; inhibition of NF-kB (135), anti-oxidants (136)
- Phosphate-lowering: phosphate binders (156), blocking of the intestinal phosphate transporter Npt2b (153)

This is the list of interventions and pharmacological and nutritional interventions that in the literature have been shown to increase aging in various models.
Probably, the most interesting is caloric restriction. In mice, caloric restriction leads to a 40% increased lifespan. This is a review in which the effects of caloric restriction on markers of health and longevity are discussed. They find in summary that caloric restriction reduces oxidative stress, improves the cardiometabolic risk factor profile and last but not least, is a very strong deactivator of the mTOR system. Interestingly, fasting has for thousands of years been included in various religions used for spiritual reasons.

but I think there are now merging evidence that intermittent fasting is a very good way to prevent diabetes and cardiovascular disease.
Due to the role of klotho in the aging process, there has been a lot of discussion about drugs that increase klotho expression and its impact on aging. We have for example, PPAR-γ, agonist thyroid hormones, ACE inhibitors, Vitamin D.

Probably those anti-inflammatory interventions also affect klotho expression because this Spanish paper showed that the inflammatory cytokines TWEAK and MMF reduce klotho expression through NF-κB.
The effects of stabilizing telomeres have also been discussed.

This is one study, not in renal patients showing that high serum vitamin D concentrations are associated with longer telomere length in women.
So it indirectly suggests a potential benefit of vitamin D on the aging process.

Statin therapy was associated with longer telomers.

but also a number of studies have shown that statins are associated with longer telomeres. The fact that in general females have longer telomeres than males may occur via oestrogens because oestrogens deficiency in post-menopausal women induces telomere shortening.
The Fountain of Youth – can Nutritional and Pharmacological Interventions Extend Age?

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We should limit the innate damage to get old and that you can do by anti-oxidants, drugs, interventions that inhibit NF-κB

MultiPathways Stimulate NF-κB in the Uremic Milieu

because in this very toxic inflamed uremic milieu there are multiple pathways to stimulate the NF-κB:
reactive oxygen species

Multiple Pathways Stimulate NF-κB in the Uremic Milieu

Adapted from McCullough PA, Ali S. Drug Design, Development and Therapy 2012;6:141-49

may be one way, lipopolysaccharides

Adapted from McCullough PA, Ali S. Drug Design, Development and Therapy 2012;6:141-49

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stimulate the TLR4 receptor, TNF stimulates TNFR,

albumin acts via the megalin/cubulin receptor,
angiotensin 2 via the angiotensin 2 receptor.

High blood pressure, mechanical stress may affect this via the integrins.
and of course, we have hyperglycaemia and the production of advanced glycation end products, which will work via RAGE.
These are multiple pathways

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**Multiple Pathways Stimulate NF-κB in the Uremic Milieu**

that will stimulate an increased expression of the transcription factor NF-κB.

**slide 55**

**Multiple Pathways Stimulate NF-κB in the Uremic Milieu**

We all know that if you have increased expression of NF-κB, there will be also increased production of multiple harmful mediators depicted here.

**slide 56**
that will not only have effects on our vessels but also on the glomerular endothelium, mesangial cells, podocytes, vascular endothelium, proximal tubular cells, promoting the aging process. So we need to find one way to downregulate NF-κB.

You probably have heard about this very interesting transcription factor Nrf2
which in its active state upregulates about 250 cytoprotective and antioxidant genes

and this will decrease NF-κB activity.
A very interesting recent paper shows that NF-κB inhibition delays DNA damage-induced senescence and promotes aging in mice.

In our renal patients, Nrf2 activation is suppressed. Unfortunately, the BEACON trial had to be prematurely stopped but I hope that this unfortunate event did not preclude nephrologists from continuing to be interested in Nrf2 activation.
Of course, you should know that there are a number of nutrients that may have effects on Nrf2 activity. This is a recent review I collaborated with Brazilian dieticians under the leadership of Denise Mafra going through the literature finding that a number of things that you eat, hopefully every day, like allicin, curcumin, cinnamon, lycopene, resveratrol, green tea also cause activation of Nrf2.
Like I previously showed you, by affecting antioxidant and phase 2 detoxifying enzymes, this may decrease ROS activity and NF-kB.

The Naked Mole Rat
- an Extraordinarily Long-lived Eusocial Mammal

- Subjected to high levels of oxidative stress
- Extremely resistant to neoplasia
- Minimal changes in age-associated alterations in body composition
- Healthy reproductive status until death
- Biochemical activity of mitochondrial and anti-oxidant enzymes are unchanged
- Superior resistance to toxins and oxidants
- Very tolerant to oxygen deprivation

Despite similar body size - live 8 times longer than mice

- 3-fold higher levels of Nrf2 in fibroblasts of naked mole rats compared to mice
- Highly efficient control of their proteome
This leads me back to this extremely ugly rat, the naked mole rat that lives in burrows in Africa. This is an interesting species because it lives 8 times longer than mice despite its similar size. A recent paper in Nature revealed the genome sequencing of this extraordinarily long-lived social animal. In the burrows it's subjected to high levels of oxidative stress, they are extremely resistant to neoplasia, there are minimal changes in age-associated alterations in body composition, they have a healthy reproductive status until they die, a superior persistence to toxins and oxidants and they are very tolerant to oxygen deprivation. The interesting fact is that they have 3-fold higher levels of Nfr2 compared to mice.

So, let's summarise. How should we prevent premature aging in our patients?

So I'll try to put this together in a complicated scheme. In the uremic milieu,
we have uremic toxins promoting oxidative stress and inflammation. This may decrease klotho
gene expression but also causing shorter telomeres, mitochondrial dysfunction and DNA
damage promote the senescent cells and stem cell exhaustion. We know that in senescent
cells you’ll find altered cell morphology and a loss of ability to undergo cell division that will
cause premature aging.

Also phosphate needs to be integrated because it directly stimulates premature aging but it
can also promote further inflammation via Calciprotein particles.
So what advice could I give to you on how you should get old? I don't think I have any good advice but you might find of interest to learn a little bit more about Jeanne Clément who is the longest documented lifespan in history. When she was born in 1875, she actually met Vincent Van Gogh when she was a small child and when she died in 1997 she could experience internet. Her secret was that she engaged in sports activities, tennis and swimming, she took up fencing when she was 85 years old, she would eat more than 2 pounds of chocolate a week, drink port wine, and pour olive oil on all her food. She quit smoking at the age of 117 years!
Chairman: Thank you Peter for this very interesting talk. We have time for one or two questions. So how do we become more like the naked rat?

Prof. Stenvinkel: We should shave!

Chairman: Start shaving!

Prof. Stenvinkel: In addition, try to promote your Nrf2 activity. I would guess that keeping a healthy lifestyle with the nutrients that I showed you and probably physical activity.

Question: Can you measure Nrf2 activity?

Prof. Stenvinkel: Yes, you can and you can look at the expression you have.

Question: Thank you for an interesting lecture. What would be the drawback? You talked about the development and that certain species have a lot of inflammation activity and because of that, they have less longevity and the other way round. So if you promote longevity, what would be the drawback on the left side so to say?

Prof. Stenvinkel: It’s a very relevant question and actually I’ve been thinking about this and for the naked mole rat there is a specific drawback. This is a paper showing that these African naked mole rats are extremely susceptible to infections. This is a study where they looked at when they were subjected to herpes simplex virus type 1 and they could not resist this probably due to the lack of pro-inflammatory neuropeptides in this milieu. So there is a very delicate balance between pro and anti-inflammation and we should have the ability to both react to infections but not have an overreaction in our inflammatory system like you have when you get older or when you get kidney disease.

Chairman: Ok thank you.