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ORIGINAL ARTICLE

The longevity gene *Klotho* and its cerebrospinal fluid protein profiles as a modifier for Parkinson´s disease

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Abstract

Background: Parkinson´s disease (PD) has a large phenotypic variability, which may, at least partly, be genetically driven including alterations of gene products. Candidates might not only be proteins associated with disease risk but also pathways that play a role in aging.

Objective: To evaluate phenotype-modifying effects of genetic variants in *Klotho*, a longevity gene.

Methods: We analyzed two longitudinal cohorts: one local cohort comprising 459 PD patients who underwent genotyping for the KL-VS haplotype in *Klotho* including a subgroup of 125 PD patients and 50 healthy controls who underwent biochemical cerebrospinal fluid (CSF) analyses of Klotho and fibroblast growth factor 23 as well as vitamin D metabolites. The second cohort comprised 297 patients from the Parkinson's Progression Markers Initiative (PPMI) for validation of genetic–clinical findings.

Results: PD patients carrying the KL-VS haplotype demonstrated a shorter interval between PD onset and onset of cognitive impairment (both cohorts) and higher Unified Parkinson´s Disease Rating Scale part III (UPDRS III) scores (PPMI). CSF protein levels of Klotho and fibroblast growth factor 23 were lower in PD patients irrespective of gender compared to controls. Moreover, low CSF levels of Klotho were associated with higher scores in the UPDRS III and Hoehn and Yahr Scale.

Conclusions: Our results indicate that genetic variants in *Klotho* together with its corresponding CSF protein profiles are associated with aspects of disease severity in PD. These findings suggest that pathways associated with aging might be targets for future biomarker research in PD.

KEYWORDS
aging, genetic modifier, *Klotho*, longevity genes, Parkinson´s disease

INTRODUCTION

Parkinson´s disease (PD) is marked by a large phenotypic variability including not only differences in the manifestation of motor and non-motor symptoms but also differences in progression and the timeline for reaching disease milestones such as cognitive impairment. One factor accounting for these differences may be additional disease-modifying factors such as genetic variation and corresponding protein profiles.[1,2] Genome-wide association studies (GWAS) for PD identified several genetic variants which have been associated with an increased risk of developing PD.[3,4] Consequently, most studies evaluating the impact of genetics on phenotypical characteristics have focused on known disease-associated risk variants. Another approach to better understand diseased aging is to investigate alterations of potentially protective factors, for example, genes and proteins that are associated with longevity and healthy aging. Our study focused on this approach. A prime candidate in this context is Klotho, a protein that was first described in a mouse model. Knockout mice with loss-of-function mutations in the *Klotho* gene showed a phenotype with premature-aging symptoms such as arteriosclerosis, osteoporosis, various ectopic calcifications, skin and muscle atrophy, neuronal degeneration, a short lifespan and infertility.[5,6] Overexpression of *Klotho* extended the lifespan of mice.[7] The Klotho protein is a 130 kDa single-pass transmembrane protein that is expressed especially in the kidney [5] and the brain.[8] Klotho acts as co-receptor for fibroblast growth factor 23 (FGF23).[9] The complex of FGF23 and Klotho inhibits 1-alpha-hydroxylase, thereby reducing the production of 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) (calcitriol).

Mice with Klotho deficiency showed nigrostriatal dopaminergic degeneration which was rescued by a vitamin D restriction diet.[10,11] Moreover, Klotho plays a role in the metabolism of reactive oxygen species (ROS), a pathway that is frequently discussed in the pathogenesis of neurodegenerative disorders.[12] The complex of Klotho and FGF23 leads to a higher expression of SOD2 and CAT via FOXO3a, leading to detoxification of ROS. In cases of Klotho deficiency this balance is shifted to an overproduction of ROS.[13]

Based on these previous observations, we aimed to investigate the influence of genetic variations in *Klotho* on phenotypical characteristics such as motor performance and cognitive impairment longitudinally in two cohorts: the local Tuebingen Parkinson cohort (TUEPAC) comprising 459 PD patients and the Parkinson's Progression Markers Initiative cohort (PPMI) for validation. In order to evaluate findings biochemically, we performed analysis of CSF protein levels of Klotho, FGF23 and vitamin D metabolites in a subgroup of the TUEPAC patients (125 PD patients and 50 healthy control participants).

METHODS

Participants and clinical investigations

Two longitudinal cohorts were analyzed in this study.

The *TUEPAC cohort (Tuebingen Parkinson Cohort)* comprised: (1A) 459 PD patients with *Klotho* genotype status as well as standardized longitudinal clinical data and (1B) a subgroup of 125 PD patients as well as 50 healthy elderly control participants without evidence of neurodegenerative diseases (CON) with *Klotho* genotype status as well as cerebrospinal fluid (CSF) protein levels of Klotho, FGF23 and vitamin D metabolites.

All PD patients were recruited and examined between 2001 and 2018 from the ward and outpatient clinic for PD at the University of Tuebingen; some of the individuals are participants in PD-related observational prospective longitudinal studies of our Department.[14-16] All patients were examined by a movement disorder specialist. Diagnosis of PD was defined according to the UK Brain Bank criteria.[17] The following demographic and clinical data were obtained: age, gender, age at onset of parkinsonism, and disease duration. We assessed severity of motor symptoms using part III of the Unified Parkinson's Disease Rating Scale (UPDRS-III); from 2000 to 2008 the old version, and from 2009 the MDS-UPDRS, were applied.[18] Disease stage was categorized by the modified Hoehn and Yahr Scale (H&Y).[19] Cognitive function was tested using the Montreal Cognitive Assessment (MoCA)[20] or the Mini Mental Status Examination (MMSE).[21] Since the MoCA was available only from 2009 onwards, all MMSE scores were converted into MoCA equivalent scores according to the algorithm by Steenoven et al.[22] A MoCA cut off ≤25 indicated cognitive impairment (point of maximum combined sensitivity and specificity[23]). Mood disturbances were assessed using Beck's Depression Inventory (BDI).[24] In the TUEPAC cohort, disease duration differed for patients when standardized assessments were done for the first time (baseline).

Of the 459 PD patients, 283 completed 2 years of follow-up, 238 completed 4 years of follow-up, 187 completed 6 years of follow-up, 106 completed 8 years of follow-up and 32 completed 10 years of follow-up together with standardized clinical assessments as described above.

The *Parkinson's Progression Markers Initiative (PPMI) cohort* comprising 297 PD patients with *Klotho* genotype status and longitudinal clinical data was used for validation. In PPMI the first standardized assessment was done from the diagnosis onwards (de novo status). Data were obtained from the PPMI database (www.ppmi-info.org/data).

Genetic analysis

The SNP rs9527024 in the *Klotho* gene was assessed using the NeuroChip array in the TUEPAC cohort whereas the SNP rs9536314 using the NeuroX array was assessed in the PPMI cohort. Both SNPs are part of the six variants that define the *Klotho* KL-VS haplotype, all of which are in high linkage disequilibrium.

We only included patients with heterozygous KL-VS haplotypes although the homozygous haplotypes would be a valuable source for proof of concept. However, in TUEPAC we identified two homozygous haplotype carriers that have been excluded from the analysis due to the small sample size. In PPMI no homozygous haplotype carrier was identified.

Sanger polymerase chain reaction (PCR) validation would be valuable to have estimates of true/false positives/negatives with the arrays but this was not done. However, we have in-house data applying both the NeuroChip and NeuroX array in different cohorts (controls, PD, atypical PD, etc.) in 1355 subjects. Comparing these, we have an overlap of 1354 of 1355 correctly classified genotypes which translates into a concordance rate of 99.93%.

Patients of the local TUEPAC cohort with known pathogenic mutations in *LRRK2* (p.G2019S, p.I2020T) and *GBA* (L444P; N370S, E326K, R398X) were excluded. Moreover, patients were screened and controlled for not carrying mutations in *Parkin*, *PINK1* and *DJ1* if the following criteria were fulfilled: age of onset ≤40 years or a positive family history compatible with a recessive mode of inheritance.

Measurement of CSF levels of Klotho, FGF23 and vitamin D metabolites

The quantitative determination of Klotho was performed using human soluble α-Klotho assay kit (enzyme-linked immunosorbent assay, ELISA) with an assay sensitivity of 6.15 pg/mL (IBL America). The determination of FGF23 (C-terminal) concentration was quantified using a second-generation human FGF23 (C-term) two-site ELISA kit with an assay sensitivity of 1.5 RU/mL (Immutopics, Inc.). The concentration of 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol in CSF were quantified using an enzyme immunoassay (EIA) kit (Immunodiagnosticsystems, IDS). The assay sensitivity for $25(OH)D_3$ is 2.7 ng/mL and for $1,25(OH)_2D_3$ 2.5 pg/mL, respectively. For data acquisition, an iMark microplate absorbance reader was used (Bio-Rad).

Statistics

Statistical analysis was performed using IBM SPSS 22.0 software for Windows (SPSS Inc., Chicago, IL, USA).

Phenotypical effect of *Klotho* haplotype (TUEPAC 1A and PPMI cohorts)

- Group comparisons of PD patients stratified by *Klotho* KL-VS haplotype focusing on demographic and clinical characteristics were calculated using univariate analysis of variance (ANOVA).
- Kaplan–Meier survival curves and Cox proportional hazard models stratified by *Klotho* KL-VS haplotype together with age at onset, age, sex and UPDRS III as covariates were used to estimate disease duration free from cognitive impairment (MoCA ≤25). Risk for cognitive impairment was calculated with Cox proportional hazards models to estimate hazard ratios (HRs) with 95% confidence intervals (95% CIs) and *p* values for pairwise comparisons.

CSF profiles of Klotho, FGF23 and vitamin D metabolites (TUEPAC 1B cohort)

- Group comparisons of CSF profiles of Klotho, FGF23 and vitamin D metabolites in PD patients versus CON were calculated using univariate analysis of variance with age and sex as covariates (ANCOVA).
- Associations between CSF profiles of Klotho, FGF23 and vitamin D metabolites with clinical characteristics were assessed using Pearson's correlation.

Ethics

The study was approved by the Ethics Committee of the Faculty of Medicine at the University of Tuebingen, and all participants gave written informed consent.

RESULTS

TUEPAC 1A cohort

PD patients stratified by Klotho *haplotype*

Of the 459 PD patients, 351 patients carried the *Klotho* wildtype haplotype (male: 63%) and 108 patients the *Klotho* KL-VS haplotype (male: 58%). There were no significant differences in age at examination, age at PD onset and disease duration between the two genotypes.

There were no significant differences in terms of disease stage (H&Y), motor performance (UPDRS III), cognitive function (MoCA) and mood disturbances (BDI) at baseline examination. For more details see Table 1.

Kaplan–Meier survival curves and Cox proportional hazard models focusing on cognitive impairment stratified by Klotho *haplotype*

Female PD patients carrying the *Klotho* KL-VS haplotype developed cognitive impairment earlier in the disease course compared to those with the wildtype haplotype (8.8 vs. 13.4 years; HR 1.721, 95% CI 1.084–2.732; $p = 0.019$) (Fig. 1). Age and age at onset were associated with the outcome cognitive impairment ($p \leq 0.001$, respectively) whereas sex and UPDRS III were not associated ($p > 0.05$).

PPMI cohort

PD patients stratified by Klotho *haplotype*

Of the 297 PD patients, 218 patients carried the *Klotho* wildtype haplotype (male: 67%) and 79 patients (male: 70%) the *Klotho* KL-VS haplotype.

TABLE 1 Group comparisons of the TUEPAC cohort stratified by *Klotho* haplotype

	Female			Male			Overall		
Parameter	Wildtype (*n* = 131)	KL-VS (*n* = 45)	*P* value	Wildtype (*n* = 220)	KL-VS (*n* = 63)	*P* value	Wildtype (n = 351)	KL-VS (n = 108)	*P*-value
Sex (% male)							63%	58%	0.430
AAO (years)	60 (11)	63 (9)	0.093	60 (11)	59 (10)	0.465	60 (11)	61 (10)	0.579
Age (years)	65 (10)	67 (9)	0.232	66 (10)	65 (9)	0.874	65 (10)	66 (9)	0.514
Disease duration (years)	5 (5)	4 (5)	0.210	5 (5)	6 (5)	0.212	5 (5)	5 (5)	0.918
MoCA	24 (5)	24 (4)	0.978	23 (5)	24 (4)	0.290	24 (5)	24 (4)	0.422
H&Y	2.0 (0.7)	2.0 (0.6)	0.426	2.0 (0.7)	2.0 (0.7)	0.766	2 (0.7)	2 (0.7)	0.486
UPDRS III	21 (10)	23(12)	0.534	23 (12)	25 (13)	0.401	22 (119	24 (13)	0.279
Cognitive impairment during study	49%	58%	0.195	62%	62%	0.556	57%	60%	0.579
Estimated interval PD onset to PD CI (Kaplan–Meier) (years)	13	9	0.019	11	11	0.507	12	10	0.448

Clinical data refer to the first standardized assessment in the TUEPAC cohort. All the data are given as mean and standard deviation as well as percentage for prevalence. Univariate analysis of variance was performed for comparison of means and chi-squared test for comparison of sex and prevalence of dementia.

AAO, age at onset; CI, cognitive impairment; H&Y, Hoehn and Yahr stage; MoCA, Montreal Cognitive Assessment; PD, Parkinson's disease; UPDRS, Unified Parkinson Disease Rating Scale.

There were no significant differences in age at last examination, age at PD onset and disease duration between the two genotypes.

Patients with the *Klotho* KL-VS haplotype showed a higher UPDRS III score at last visit compared to patients with *Klotho* wildtype (overall: KL-VS: 27.7, wildtype: 22.4, $p = 0.006$; male: KL-VS: 29.0, wildtype: 23.9, $p = 0.024$, female: KL-VS: 24.4, wildtype: 19.1, $p = 0.153$). There were no significant differences in terms of disease stage (H&Y) and MoCA at last examination. For more details see Table 2.

Kaplan–Meier survival curves and Cox proportional hazard models focusing on cognitive impairment stratified by Klotho *haplotype*

Male PD patients carrying the *Klotho* KL-VS haplotype developed cognitive impairment earlier in the disease course compared to those with the *Klotho* wildtype (7.7 vs. 5.4 years; HR 1.463, 95% CI 1.004–2.132; $p = 0.046$) (Fig. 1). Age ($p = 0.014$), age at onset ($p = 0.002$) and UPDRS III (0.012) were associated with cognitive impairment. Sex showed a trend to be associated with cognitive impairment ($p = 0.062$) but did not reached statistical significance.

TUEPAC 1B cohort

CSF profiles of Klotho, FGF23 and vitamin D metabolites in PD patients versus CON

PD patients were older at age at examination (PD 67.5 vs. CON 60.0 years; $p \leq 0.001$) and more often male (PD male: n = 86 (68%) vs. CON male: n = 24 (48%); $p = 0.009$).

PD patients showed significant lower mean CSF levels of Klotho (PD 832.7 pg/mL, CON 980.9 pg/mL; $p \leq 0.001$) and of FGF23 (PD 14.8 RU/mL, CON 31.3 RU/mL; $p \leq 0.001$) in comparison to CON. Stratification for sex revealed similar results with lower CSF levels of FGF23 (female: PD 16.5 RU/mL, CON 34.6 RU/mL; $p = 0.002$; male: PD: 14.0 RU/mL, CON 26.8 RU/mL; $p \leq 0.001$) and Klotho in PD (female: PD 928.9 pg/mL, CON 1022.1 pg/mL; $p = 0.337$; male: PD 788.5 pg/mL, CON 925.0 pg/mL; $p = 0.056$), though the latter did not reach statistical significance. For more details see Table 3.

Associations between CSF profiles of Klotho, FGF23 and vitamin D metabolites with clinical characteristics

Lower CSF levels of Klotho were associated with higher scores in UPDRS III (coefficient: −0.231; $p = 0.013$) and H&Y stage (coefficient: −0.308; $p = 0.001$). There was no significant correlation between CSF Klotho and age at examination or age at onset of PD.

Stratification for sex revealed similar results: lower CSF levels of Klotho were associated with higher scores in H&Y stage in female and male patients (female: coefficient: −0.326; $p = 0.049$; male: coefficient: −0.303; $p = 0.008$) and with higher scores in UPDRS III in male patients (coefficient: −0.317; $p = 0.004$). Higher CSF levels of 1,25-dihydroxycholecalciferol were associated with higher UPDRS III scores in males (coefficient: 0.315; $p = 0.037$). For more details see Table 4.

CSF data of $Abeta_{1\_42}$, t-Tau and p-Tau were available in 12 healthy controls and in 117 PD patients from this cohort. No

associations were found with CSF Klotho pathway levels ($p > 0.05$, respectively).

Klotho *haplotype-specific effects on CSF profiles of Klotho, FGF23 and vitamin D metabolites*

PD patients with the *Klotho* KL-VS haplotype showed higher concentrations of CSF Klotho (*Klotho* KL-VS haplotype: 968.0 pg/mL, wildtype: 791.4 pg/mL; $p = 0.009$) and of CSF 1,25-dihydroxycholecalciferol (*Klotho* KL-VS haplotype: 4.2 pg/mL, wildtype: 3.4 pg/mL; $p = 0.049$), which could be confirmed after matching for "age at examination" and "sex" (CSF Klotho: *Klotho* KL-VS haplotype: 914.2 pg/mL, wildtype: 778.4 pg/mL; $p = 0.087$; CSF 1,25-dihydroxycholecalciferol: *Klotho* KL-VS haplotype: 4.6 pg/mL, wildtype: 2.4 pg/mL; $p = 0.002$) (n = 21). For more details see Table 3.

DISCUSSION

By evaluating the impact of genetic variation in the longevity gene *Klotho* and its corresponding CSF protein profiles we could show that: (1) the genetic haplotype KL-VS in *Klotho* predisposes to an earlier onset of dementia in the disease course of PD patients in both analyzed cohorts and to higher scores in UPDRS III in the PPMI cohort and (2) PD patients show lower CSF protein levels of Klotho and FGF23 when compared to healthy control participants. Moreover, lower concentrations of Klotho are associated with higher UPDRS III scores and higher H&Y stages. These findings could also be confirmed in the gender-specific subanalysis. Taken together, findings from both cohorts support the link of the klotho pathway with motor and cognitive impairment in PD.

The Klotho pathway is of special mechanistic interest in terms of accelerated aging and neurodegeneration by two pathways. One pathway involves vitamin D metabolism in which Klotho acts as co-receptor for FGF23.[9] The complex of Klotho and FGF23 inhibits 1-alpha-hydroxylase resulting in the reduced production of 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) (calcitriol). Interestingly, mice with Klotho deficiency showed nigrostriatal dopaminergic degeneration which was rescued by vitamin D restriction diet.[10,11] In this line of evidence, we could show that in a large cohort of PD patients, CSF protein levels of Klotho and FGF23 are reduced and associated with disease severity. Similar results have been described in a small study with Alzheimer's disease (AD) patients who also presented with lower CSF levels of Klotho compared to control participants.[25] Since PD patients carrying the KL-VS variant presented with higher CSF protein levels of Klotho, but also higher levels of 1,25-dihydroxycholecalciferol compared to wildtype carriers, one might argue for a compensatory effect. Specifically, alterations of Klotho protein function due to the KL-VS haplotype might result in a lower ability to activate the FGF23–receptor complex. This in turn weakens the inhibition of 1-alpha-hydroxylase leading to higher 1,25-dihydroxycholecalciferol-levels. Consequently,

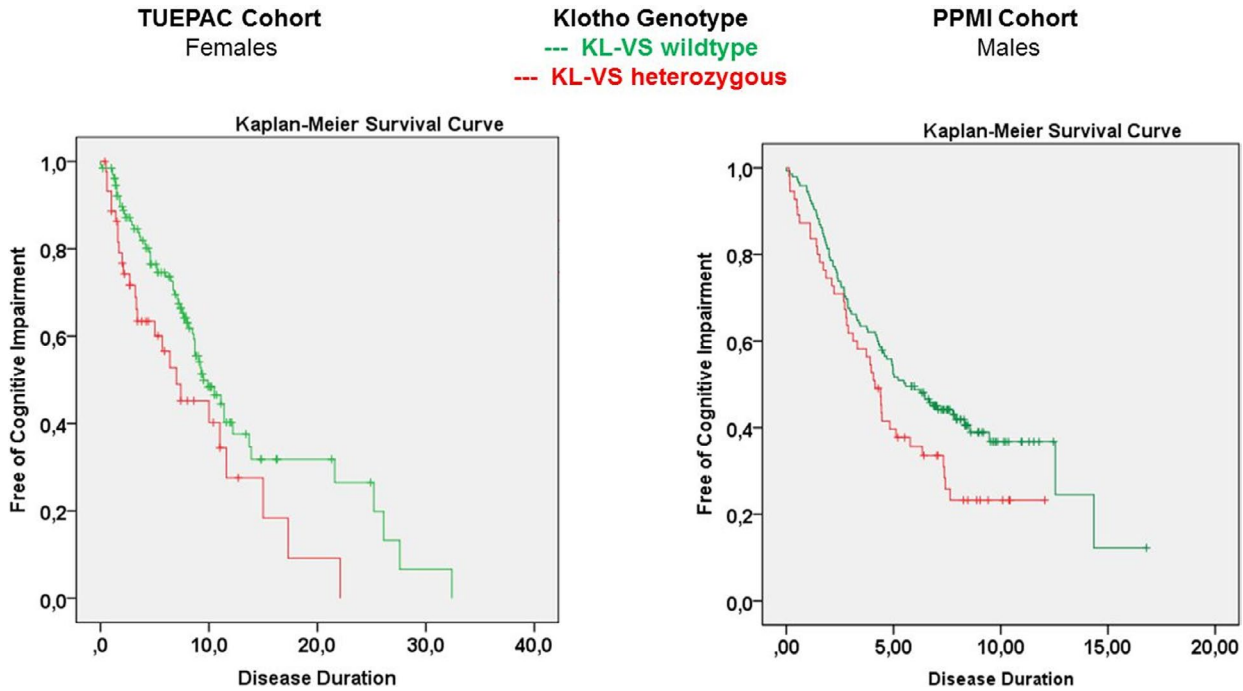

FIGURE 1 Kaplan–Meier survival curves depicting the effect of KL-VS on cognitive decline. In the TUEPAC cohort, female Parkinson's disease (PD) patients carrying the *Klotho* KL-VS genotype developed cognitive impairment earlier in the disease course compared to those with the *Klotho* wildtype genotype (8.8 vs. 13.4 years; hazard ratio [HR] 1.721, 95% confidence interval [95% CI] 1.084–2.732; $p = 0.019$). In the Parkinson's Progression Markers Initiative (PPMI) cohort, male PD patients carrying the *Klotho* KL-VS genotype developed cognitive impairment earlier in the disease course compared to those with the *Klotho* wildtype genotype (7.7 vs. 5.4 years; HR 1.463, 95% CI 1.004–2.132; $p = 0.046$). [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Group comparisons of the Parkinson's Progression Markers Initiative (PPMI) cohort stratified by *Klotho* haplotype

	Female			Male			Overall		
Parameter	**Wildtype (*n* = 73)**	**KL-VS (*n* = 24)**	***P* value**	**Wildtype (*n* = 145)**	**KL-VS (*n* = 55)**	***P* value**	**Wildtype (n = 218)**	**KL-VS (n = 79)**	***P*-value**
Sex (% male)	-	-	-	-	-	-	67%	70%	0.360
AAO (years)	58 (9)	57 (9)	0.490	60 (10)	62 (8)	0.213	59 (10)	60 (8)	0.464
Age (years)	66 (9)	64 (8)	0.317	68 (10)	70 (8)	0.318	68 (10)	68 (8)	0.704
Disease duration (years)	9 (3)	8 (2)	0.331	8 (2)	8 (2)	0.211	8 (2)	8 (2)	0.115
MoCA	27 (4)	27 (3)	0.960	26 (4)	25 (5)	0.171	26 (4)	26 (4)	0.220
H&Y	2 (0.6)	2 (0.3)	0.339	2 (0.5)	2 (0.2)	0.674	2 (0.6)	2 (0.2)	0.344
UPDRS III	19 (12)	24 (12)	0.153	24 (12)	29 (12)	0.024	22 (12)	28 (12)	0.006
Cognitive impairment during study	49%	33%	0.129	60%	73%	0.065	56%	61%	0.297
Estimated interval PD onset to PD CI (Kaplan-Meier) (years)	8	8	0.273	8	5	0.046	8	6	0.287

Clinical data refer to the last assessment available in the PPMI cohort. All data are given as mean and standard deviation as well as percentage for prevalence. Univariate analysis of variance was performed for comparison of means and chi-squared test for comparison of sex and prevalence of cognitive impairment during study.

AAO, age at onset; CI, cognitive impairment; H&Y, Hoehn and Yahr stage; MoCA, Montreal Cognitive Assessment; UPDRS III, Unified Parkinson Disease Rating Scale part III..

TABLE 3 Comparisons of cerebrospinal fluid profiles of Klotho, fibroblast growth factor 23 and vitamin D metabolites in Parkinson's disease (PD) patients versus controls and in PD patients stratified by *Klotho* haplotype

Parameter	**CON (*n* = 50)**	**PD (*n* = 125)**	***P* value PD vs. CON**	**PD (*n* = 96)**	**PD (*n* = 29)**	***P* value PD KL-VS vs. wildtype**
Klotho genotype				Wildtype	KL-VS	
Sex (male)	24 (48%)	86 (68%)	0.009	65 (68%)	21 (72%)	0.407
AAO (years)	-	61 (9)	-	60.9 (9.5)	60.5 (8.6)	0.704
Age (years)	60 (14)	68 (9)	≤ 0.001	67.4 (8.5)	67.9 (8.6)	0.748
Disease duration (years)	-	7 (5)	-	6.4 (5.4)	7.4 (4.6)	0.215
MoCA	-	25 (3)	-	24.8 (3.6)	24.7 (2.7)	0.679
BDI	-	10 (7)	-	9.3 (6.6)	12.5 (7.8)	0.052
H&Y	-	2 (0.7)	-	2.2 (0.7)	2.1 (0.6)	0.357
UPDRS III	-	25 (11)	-	25 (11)	24 (11)	0.299
CSF Klotho (pg/mL)	981 (352)	833 (272)	≤ 0.001	791 (227)	968 (356)	0.009
CSF FGF23 (RU/mL)	31 (22)	15 (8)	≤ 0.000	14.4 (8.5)	16.1 (8.3)	0.131
CSF 1,25-dihydroxycholecalciferol (pg/mL)	3.3 (1.2)	3.6 (2.0)	0.138	3.4 (2.0)	4.2 (1.8)	0.049
CSF 25-hydroxycholecalciferol (ng/mL)	8.2 (1.7)	8.6 (1.5)	0.333	8.7 (1.5)	8.4 (1.5)	0.559

Data are given as mean and standard deviation as well as percentage for prevalence. Univariate analysis of variance with the covariates age at examination and sex was performed for group comparison between PD and CON. Mann–Whitney *U* test was performed for comparison between *Klotho* genotypes in PD. Chi-squared test was used for comparison of sex.

AAO, age at onset; CON; control; CSF, cerebrospinal fluid; FGF23, fibroblast growth factor 23; H&Y, Hoehn and Yahr stage; PD, Parkinson's disease; UPDRS, Unified Parkinson Disease Rating Scale; MoCA, Montreal Cognitive Assessment; BDI, Beck's Depression Inventory.

a positive feedback with increasing levels of Klotho, as previously described for FGF23, might set in.[26]

The second pathway involves Klotho as a factor in the metabolism of reactive oxygen specifies (ROS).[12] The complex of Klotho and FGF23 leads to a higher expression of SOD2 and CAT via FOXO3a, leading to detoxification of ROS. In cases of Klotho deficiency this balance is shifted to an overproduction of ROS,[13] which further promotes neurodegeneration.

These findings raise the question whether dysfunction in the Klotho pathway promotes accelerated aging, thereby lowering the

TABLE 4 Association between cerebrospinal fluid protein levels of Klotho, fibroblast growth factor 23 and vitamin D levels with clinical characteristics in Parkinson's disease

Parameter	Age CON	Age PD	Disease duration PD	UPDRS-III PD	H&Y PD	MoCA PD
CSF Klotho (pg/mL)	–	–	−0.227**	−0.231**	−0.308***	–
CSF FGF23 (RU/mL)	–	–	–	–	–	–
CSF 25-hydroxycholecalciferol (ng/mL)	–	–	–	–	–	–
CSF 1,25- dihydroxycholecalciferol (pg/mL)	–	−0.309**	–	–	–	0.302**

Data are given as correlation coefficients (Pearson).

CON, controls; CSF, cerebrospinal fluid; FGF23; fibroblast growth factor 23; H&Y, Hoehn and Yahr stage; MoCA, Montreal Cognitive Assessment; PD, Parkinson's disease; UPDRS, Unified Parkinson Disease Rating Scale..

* $p < 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. – , no significant p value.

burden for the development of neurodegenerative diseases such as PD or AD depending on an individual's vulnerability. Importantly, Klotho-associated findings in AD seem controversial. A recent study reported that the KL-VS haplotype may protect against the effect of the *APOE* ε4 genotype in cognitively normal elderly individuals with an average age of 61 years. Whereas in KL-VS non-carriers, the presence of at least one *APOE* ε4 allele resulted in increased amyloid burden (represented by either CSF β-amyloid$_{1\text{-}42}$ profiles or carbon 11-labeled Pittsburg Compound B (^{11}C-PIB) binding), this effect was markedly reduced in KL-VS heterozygotes.[27]Conversely, a similar study from Australia investigating 581 cognitively normal elderly individuals found no association between *Klotho* KL-VS, neocortical amyloid-β (Aβ) burden and the presence of an *APOE* ε4 allele.[28] In PD it is well known that next to the typical alpha-synuclein pathology a concomitant AD pathology as well as vascular risk profiles are associated with cognitive decline. However, we did not observe an association between CSF levels of Klotho and Abeta$_{1_42}$, t-Tau or p-Tau.

Another point of discussion is the interaction of Klotho with sex as also seen in our cohorts. While the KL-VS haplotype was associated with an earlier cognitive decline only in females in the TUEPAC cohort, we saw an opposite effect in the the PPMI cohort with males being more prone to show earlier cognitive decline in the presence of KL-VS.

An interesting study examined the relationship between *Klotho* KL-VS haplotype, cognition and brain structure in childhood and adolescence in a cohort of 1387 children and adolescents aged 3–21 years. KL-VS heterozygotes had better cognition than non-carriers before age 11 years, but lower cognition after age 11 years. Heterozygotes had smaller brains than non-carriers did in early childhood. However, sex moderated the association between KL-VS and white matter volume. Among girls, KL-VS heterozygotes had smaller white matter volumes than non-carriers. Among boys, heterozygotes had greater white matter volumes than non-carriers.[29]

All these findings suggest that the effect of the *Klotho* KL-VS haplotype may depend on several aspects which warrants more complex investigations in the future that also take into account sex and lifestyle factors as well as a concomitant AD pathology and vascular risk factors.

The following limitations are to be discussed: (a) the variable longitudinal follow-up might pose a bias to clinical findings; (b) validation of findings on CSF profiles of Klotho and FGF23 in the PPMI needs to be addressed in future studies and (c) the biochemical evaluation of the interaction between Klotho and ROS metabolism should be further explored.

Taken together, we were able to show that genetic variants in the longevity gene *Klotho* together with its corresponding CSF protein profiles are associated with aspects of disease severity in PD. These findings suggest that pathways which are associated with aging are worthy of further investigation in order to better understand the phenotype variability of PD. In this context, evaluating the interaction of pathways with accelerated aging and neurodegeneration on a mechanistic level as well as in relation to phenotypical characteristics is of particular interest. This in turn might offer new entry points for targeted biomarker research for patient stratification.

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AUTHOR CONTRIBUTION

Milan Zimmerman: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Project administration (lead); Writing-original draft (lead). **Leonie Köhler:** Data curation (supporting); Formal analysis (supporting); Writing-original draft (supporting). **Marketa Kovarova:** Conceptualization (supporting); Investigation (supporting). **Erwin Schleicher:** Conceptualization (supporting); Investigation (supporting); Methodology (supporting); Resources (supporting); Writing-original draft (supporting).

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Prof. Dr. Thomas Gasser serves on the editorial boards of *Parkinsonism and Related Disorders*, *Movement Disorders*, and *Journal of Neurology*; holds a patent re: KASPP (LRRK2) Gene, its Production and Use for the Detection and Treatment of Neurodegenerative Diseases; serves as a consultant for Cephalon, Inc. and Merck Serono; serves on speakers' bureaus of Novartis, Merck Serono, SCHWARZ PHARMA, Boehringer Ingelheim and Valeant Pharmaceuticals International; and receives research support from Novartis, the European Union, BMBF (the Federal Ministry of Education and Research) and Helmholtz Association.

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DATA AVAILABILITY

The data that support the findings of this study are available upon reasonable request.

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