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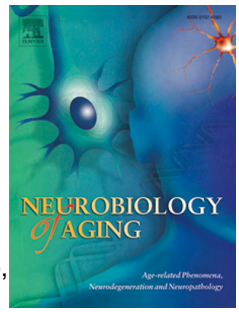

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Klotho, APOEε4, cognitive ability, brain size, atrophy and survival: A study in the Aberdeen Birth Cohort of 1936

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Abstract

A single copy of *klotho* allele *KL-VS* is associated with longevity, better health, increased cognition and bigger regional brain volume. However, its longitudinal effects on cognition and brain volumes, both global and regional, in late life are unclear. In this study we show [1] *KL-VS* heterozygotes had shorter survival and [2] smaller white matter volumes than non-carriers; [3] had slower cognitive decline; and [4] had greater right frontal lobe volumes. The *KL-VS* heterozygote survival and white matter volume disadvantages were unexpected. A possible explanation for these results in the context of the literature is a potential interaction between the environment and/or age of the participants, leading to a heterozygote disadvantage. The longitudinal cognitive trajectories indicate that heterozygotes would have an advantage in very late life. Collectively these results suggest that the genotype-survival advantage of the *KL-VS* allele is age-dependent and possibly mediated through differential cognition and brain volume.

Keywords

Klotho; cognitive aging; brain atrophy; survival; APOEε4

1. Introduction

To live a long, fruitful and independent life is not a matter of chance. Interplay between genes and environment lies at the heart of understanding individual differences in rates of aging. An important requirement for a life free from disability is a healthy brain. The aging brain undergoes structural and functional changes that correlate with cognitive decline (Staff et al., 2006, Waiter et al., 2008). There are marked individual differences in terms of premorbid abilities and the rate of decline (Staff et al., 2016), which are influenced by genetics (Kenyon, 2010). Identifying genetic factors that influence anatomical and functional changes of the aging brain will increase understanding of healthy brain aging. Here, we investigate the effects of an established genetic influence on health in late life– polymorphisms in the *klotho* gene– on cognitive aging, structural changes in the aging brain and survival. If the role of the klotho protein on cognitive aging and possible dementia could be established, klotho's potential function as an anti-aging protein could become a therapeutic target. Compounds that mimic the actions of klotho could emulate its effect in the brain and be beneficial in demyelinating and neurodegenerative diseases (Abraham et al., 2012, Zeldich et al., 2015). **Klotho's potential influence on aging is one of many. The role of *apolipoprotein E-ε4 (APOEε4)* has been extensively investigated, establishing the significant contributions of *apolipoprotein E-ε4 (APOEε4)* to longevity (Soerensen et al., 2013), cognitive aging (Jochemsen et al., 2012), and brain volumes. These associations mandate the analysis of *APOEε4* in this context, in order to establish the independence of klotho's effects from those of *APOEε4*.**

Klotho is a transmembrane protein present in blood and cerebrospinal fluid when cleaved from the cell surface. Circulating klotho affects glycoproteins on brain cell surfaces and multiple organs throughout the body (Imura et al., 2004). Klotho has multiple roles including renal function and growth factor inhibition (Kurosu et al., 2005). These pleiotropic actions may explain how klotho influences aging processes (Dubal et al., 2015). In mouse models of aging, a defect of *klotho* gene expression results in multiple aging-like phenotypes including short lifespan, skin atrophy,

osteoporosis and atherosclerosis (Kuro-o et al., 1997), whereas *klotho* over-expression suppresses aging and extends lifespan (Kurosu et al., 2005). **Moreover, klotho has been shown to causatively enhance cognitive function in mice (Dubal et al., 2014)**. Two variants in humans co-segregate and form a haplotype, *KL-VS* (Yokoyama et al., 2015). The heterozygous polymorphism of *KL-VS* increases secreted forms of klotho, may increase longevity, promotes Fibroblast Growth Factor signaling and decreases risk of heart disease (Arking et al., 2002, Arking et al., 2005). In addition to these anti-aging actions, klotho appears differentially expressed during post-natal neurodevelopment (Clinton et al., 2013), is involved in the maturation of long white matter tracts (Chen et al., 2013), and promotes brain maturation and synaptic plasticity (Dubal et al., 2015).

In human studies *KL-VS* genotype contributes to cognitive differences in early and late-life and to regional brain volumes in the frontal cortex (Deary et al., 2005, Dubal et al., 2014, Houlihan et al., 2009, Yokoyama et al., 2015). Yokoyama et al. 2015 (Yokoyama et al., 2015) suggest one copy of the *KL* allele *KL-VS* increases cognition and grey matter volume within the right dorsolateral prefrontal cortex (rDLPFC) compared to non-carriers, an area critical to learning and memory. In addition, carrying two copies appeared to decrease cognition and rDLPFC volume. Dubal et al. 2014 (Dubal et al., 2014) showed *KL-VS* heterozygosity was associated with increased cognition, and suggested that klotho could increase 'cognitive reserve', slowing aging. Deary et al. 2005 (Deary et al., 2005) showed in the Lothian 1921 Birth Cohort that *KL-VS* homozygosity was associated with lower childhood and late-life cognition. However, Deary et al. 2005 did not find an association between *KL-VS* homozygosity and cognition in the Aberdeen 1936 Birth Cohort examined here, or *KL-VS* heterozygosity and cognition in either cohort. Similarly, Houlihan et al. (Houlihan et al., 2009) did not find a relationship between *KL-VS* and early or late-life cognition in the Lothian 1936 Birth Cohort, **but found that *KL-VS* carriers have lower four choice reaction times**. Transgenic mice with moderately elevated klotho expression performed better than controls in multiple tests of learning and memory (Dubal et al., 2014, Dubal et al., 2015). However, in humans the effects of *KL-VS* genotype on global brain volumes and cognitive performance trajectories within late-life are unclear.

Here, we examine the effects of *KL-VS* genotype on regional and global brain volumes, survival, early-life cognition and late-life longitudinal cognitive change. This holistic approach extends what is known about the effects of *KL-VS* in humans in late life.

Up to two MRI brain exams were collected. Exam 1 was at a mean age of 69, exam 2 at a mean age of 73. The rDLPFC was selected for detailed brain volume analysis, as Yokoyama et al. 2015 found rDLPFC grey matter volume varied with *KL-VS.* The hippocampus was also selected *a priori* for in depth brain analysis because of its importance in healthy aging and Alzheimer's disease (Jack et al., 1999, West et al., 1994).

To measure longitudinal change in cognition, we used the Digit Symbol Substitution (DS) Test. DS was administered on up to five occasions between ages 63 and 78 and measures information processing speed and short term memory. Performance on DS declines with age (Joy et al., 2004). Age-related decline in DS performance is largely independent of participants' duration of education and self-reported health status (Salthouse, 1992). Reports of practice effects are varied possibly because these are modest and small compared to the prominent age effect (M. Wielgos, Walter R. Cunningham, Cynthia, 1999). DS is, therefore, a suitable measure for more complex causal longitudinal modeling of age-related cognitive decline. We used a multilevel linear mixed modeling approach to longitudinal cognitive performance data to test contributions of *KL-VS* and *APOEε4*. Measuring longitudinal decline presents analytical challenges such as data clustering and co-variances between predictors, which may elucidate relationships between the potential predictors of decline. A multilevel linear mixed modeling approach allows such phenomena to be considered.

Here, we examine the longitudinal influence of *KL* allele *KL-VS* on cognition, regional and global brain volumes and survival separately and in combination. In the light of actions of *KL-VS* on survival, brain structure and on cognition, we hypothesize that genetic variation in the *KL-VS* is associated with trajectories of brain aging. Specifically, we hypothesize that variation in *KL-VS* is associated with different trajectories of change in information processing speed (DS test), differences in global and

regional brain volumes (both cross-sectionally and longitudinally), and ultimately in survival rates. A supplementary analysis of *APOEε4*'s influence determined whether *KL-VS*'s effects were independent from those of *APOEε4*.

2. Methods

2.1. Sample

All data were provided by the Aberdeen Birth Cohort of 1936; an extended description of the data set is given in Whalley et al. 2011(Whalley et al., 2011). Instructions on how to request access to the data can be found on the Aberdeen Birth Cohorts website (http://www.abdn.ac.uk/birth-cohorts/1936/for-researchers/data-access). Following approval of the Local Ethics of Research Committee, volunteers gave written informed consent to the study. The whole sample consists of 508 participants.

2.2. Genotyping

*KL*F352V and *APOE* genotypes were determined by TaqMan (Applied Biosystems) analysis, performed at the Wellcome Trust Clinical Research Facility, Western General Hospital Edinburgh. 480 participants provided a relevant *KL* genotyping sample; 478 participants provided a relevant *APOE* genotyping sample. For *KL* allele *KL-VS*, F/F indicates non-carriers, F/V heterozygotes and V/V homozygotes. For *APOEε4*, *–APOEε4* indicates non-carriers and *+APOEε4* carriers.

2.3. Cognitive testing

Childhood cognitive data were provided by the Scottish Council for Research in Education (SCRE) from data archived from the Scottish Mental Survey of 1947. Almost all children born in 1936 and at school in Scotland on the 4th of June 1947 (aged 11 years), sat a group-administered cognitive test (The Moray House Test, MHT) (Deary et al., 2004). A trained psychologist administered the Digit Symbol Substitution test (DS) on up to five occasions between the ages of 63 and 78, which assessed speed of processing and memory. The total number of correct answers was calculated to give the DS score.

2.4. Survival follow-up

On the 5th of January 2015, the vital status of 494 participants was established. No vital status data were available for 14 members of the Aberdeen Birth Cohort of 1936 who had left the Grampian area.

2.5. Education and occupation

Assessment of participants' duration of full-time education in years, their occupation and their father's occupation were recorded at interview on recruitment. Occupational social class (OSC) was coded using the UK Office of Population Statistics Classification (Great Britain: Employment Department Group and Great Britain: Office of Population Censuses and Surveys, 1990). The occupational groups were coded as follows: managerial –1, professional –2, lesser professional –3, secretarial –4, skilled manual –5, semi-skilled ii –6, semi-skilled i –7, unskilled ii –8, and unskilled i –9.

2.6. Brain image acquisition and processing

2.6.1. Acquisition

Up to two MRI Brain images were collected for each participant using a 1.5 Tesla GE NVi system. Three-dimensional brain images were obtained with a T1 SPGR (T1W) MR sequence. Not all participants volunteered and some were ineligible because of contraindications for brain imaging. 245 were imaged between the ages 67 and 72 of whom 244 provided usable images; one image was removed due to excessive movement during acquisition. 156 individuals had a second T1 weighted MRI exam performed, between the ages 71 and 73.

2.6.2. Processing

The MRI data collected at exam 1 and 2 were processed cross-sectionally and longitudinally, using SPM12 version 6225 (http://www.fil.ion.ucl.ac.uk/spm/software/spm12) and FreeSurfer5.3 software (http://freesurfer.net), preceding statistical analysis.

For the cross-sectional processing, SPM12 processed the MRI data prior to voxel-based morphometry (VBM), which compares the images voxel by voxel (Ashburner et al., 2014). Processing consists of segmentation, running Diffeomorphic Anatomical Registration through

Exponential Lie Algebra (DARTEL) (Ashburner, 2007) to create a custom template, and normalizing to Montreal Neurological Institute (MNI) space. The resultant grey matter (GM) and white matter (WM) images were smoothed with an 8 mm kernel. GM and WM volume, total brain volume (TBV), total intracranial volume (TICV) and their rate of change were calculated for each participant. For the longitudinal processing in SPM12 we used Pair wise Longitudinal Registration and DARTEL to generate GM and WM divergence maps that are estimates of the rate of volumetric change (Ashburner et al., 2014).

FreeSurfer was used to carry out automatic volumetric segmentation (Destrieux et al., 2010, Fischl, 2012) to extract the volume of the rDLPFC and hippocampus. A rDLPFC proxy was constructed from the inferior, middle and superior frontal gyri in the right half of the brain (John et al., 2006, Raz et al., 1997). TBV was also extracted. For the second imaging exam, one participant showed an error in the FreeSurfer results, and was excluded from subsequent analyses. To extract rDLPFC/hippocampal volume rate of change, the images were automatically processed with the longitudinal stream (Reuter et al., 2012) in FreeSurfer.

2.7. Statistical analysis

2.7.1. MRI brain volumes

After MRI data processing, statistical analysis was performed. Baseline exams were analyzed cross-sectionally to examine global and regional brain volume differences between *KL-VS* heterozygotes and non-carriers, and *APOEε4* carriers and non-carriers. Longitudinally, baseline and follow up exams were analyzed to examine global and regional brain volume changes differences across groups. *KL-VS* homozygotes were disregarded from this analysis because of their small numbers (n_1=10/n_2=6).

To examine global brain differences, SPM12's baseline whole brain measures TBV, GM and WM volume, and their rates of change per year, were compared across groups. When examining cross-sectional differences, these volumes were divided by total intracranial volume (TICV), resulting in the

measures brain fraction (BF: TBV/TICV), grey matter fraction (GM/TICV) and white matter fraction (WM/TICV).

SPM12 VBM was used to examine regional brain volume differences in GM and WM volume and rates of change: the generated normalized GM and WM volume maps, and GM and WM divergence maps, were compared across *KL-VS* and *APOEε4* groups. Previous work has indicated that differences exist in the right frontal lobe, and in particular the rDLPFC. We applied small volume correction in order to focus on this location of interest. A rDLPFC mask was applied, consisting of the inferior frontal gyrus, the middle frontal gyrus and the superior frontal gyrus in the right half of the brain, which was generated with the SPM12 toolbox WFU PickAtlas (Maldjian et al., 2003). Age at exam, gender and TBV were added as covariates to correct for their effects. Longitudinally, the generated GM and WM divergence maps were compared across groups using VBM. Gender and age at first exam were added as covariates.

FreeSurfer measures of hippocampal and rDLPFC volume were compared across groups employing general linear modeling; FreeSurfer measure of TBV was used to correct for brain size. Longitudinally, rDLPFC and hippocampus rate of change per year were compared across groups.

2.7.2. Cognitive modeling

Cognition at age 11 (MHT) was compared using a one-way analysis of variance to examine differences between *KL-VS* heterozygotes, homozygotes and non-carriers. T-tests examined differences between *APOEε4* carriers and non-carriers.

Longitudinal differences in cognition were examined in participants who provided two or more DS data points. The raw DS scores were first standardized to a mean of 100 and a standard deviation of 15. Age at test was measured as time after the 60th birthday. Multilevel modeling examined the trajectory of decline. $DS_{i,j}$ represents the test score for participant *j* on occasion *i*. *KL-VS* was coded 1 for F/V present and 0 for F/V absent. In Model 1 we assume that current cognitive performance is a linear combination of an intercept ($\beta_{0,j}$), the influence of age ($\beta_{1,j}$), the *KL-VS* variant($\beta_{2,j}$),

gender ($\beta_{3,j}$) and education ($\beta_{4,j}$). The influence of age, *KL* and the intercept were modeled as both fixed and random.

$$DS_{i,j} = \beta_{0,j} + \beta_{1,j}Age + \beta_{2,j}KL(F/V) + \beta_{3,j}Gender + \beta_{4,j}Education + e_{i,j}$$

2.7.3. Survival analysis

In order to assess whether there is a relationship between survival time and the potential predicting parameters such as cognition and *KL-VS* genotype, a Cox regression was performed. Here, we examined each potentially predicting variable separately and then included variables into a final regression model only if it individually predicted survival (p<.05). The final models took two forms: one with and one without brain imaging data.

3. Results

3.1. Cognitive modeling

Our sample is described in Table 1 with early-life cognitive scores (MHT) and late-life cognitive scores (DS) on entry. We found no cross-sectional differences between *KL-VS* groups or between *APOEε4* carriers and non-carriers in terms of childhood ability and DS on entry. Multi-level modeling examined differences in longitudinal decline between groups. Figure 1 shows a spaghetti plot for the DS test for the whole group. As expected, the test shows a decline with age (β_1=-.87 p<.001); being female conveyed an advantage (β_3=4.76 p<.001), as did more education (β_4=1.98 p<.001). The presence of F/V has no effect (β_2=.90 ns) on DS (main effect), confirming the cross-sectional analysis. F/V significantly co-varies with the slope for age ($\rho_{1,2}$= 5.00 p<.01) indicating an association with the trajectory of cognitive decline. When the sample is split into heterozygotes and non-carriers, the slopes with age (β_1) show a decline with age of -.71 and -.90 respectively. No significant findings were observed for homozygotes in a similar analysis, probably attributable to small sample size. Replacing *klotho* with *APOEε4* in model 1 showed that presence of *APOEε4* had no effect on DS and *APOEε4* did not co-vary with the slope with age indicating no influence on the rate of decline.

Including both *KL-VS* and *APOEε4* in the model produced results not different from the separate *KL-VS* and *APOEε4* models.

3.2. Global brain volume analysis

When first imaged and after adjusting for age and gender *KL-VS* heterozygotes had smaller WM fraction (31.6% vs 30.6%, p=.005). No cross-sectional differences were found when the *APOEε4* groups were compared. Longitudinally, after adjusting for age and gender, *APOEε4* carriers lost more TBV and GM than non-carriers (TBV: -10.3 vs -7.5 mm^3 $x10^3$, p=.0031; GM: -7.5 vs -5.3 mm^3 $x10^3$, p=.011. No longitudinal differences were found when *KL-VS* heterozygotes and non-carriers were compared. A combined analysis of *KL-VS* and *APOEε4* showed that *KL*-VS's association with WM is independent of *APOEε4* (p=.004), and that *APOEε4*'s associations with TBV and GM rate of change are independent of *KL-VS* (p=.003 and p=.010 respectively). Table 2 shows GM, WM and TBV, rDLPFC and hippocampal mean volumes per group, and their rates of change.

3.3. Regional brain volume analysis: rDLPFC and hippocampus

When first imaged and after adjusting for age and gender, *APOEε4* non-carriers had significantly more hippocampal volume as percentage of TBV than carriers (.77% vs .75%, p=.026). A combined analysis of *KL-VS* and *APOEε4* showed that the associations were independent of *KL-VS* status: *APOEε4* remained significant for exam 1 hippocampal volume as percentage of TBV (p=.032). No cross-sectional or longitudinal differences were found between *KL-VS* heterozygotes and non-carriers. No longitudinal differences were found between*APOEε4* carriers and non-carriers.

3.4. Regional brain volume analysis: Voxel-based morphometry (VBM)

Using the images acquired at exam 1 and examining F/V>F/F, at uncorrected p<.001 a GM region was found shown (Figure 2a) in the right inferior frontal gyrus. Correcting for multiple comparisons using a Family Wise Error (FWE) approach, with p_{FWE}<.05, no significant voxels were identified. Applying small volume correction (rDLPFC) a significant corrected area was identified (p_{FWE}<.05) at the location indicated in Figure 2.No significant locations were observed for the reverse contrast

F/V<F/F. No significant WM locations (p_{FWE}<.05) were observed. Longitudinal VBM found no significant differences between *KL-VS* heterozygotes and non-carriers in GM or WM divergence maps.

The *APOEε4* VBM analysis found no significant GM or WM differences for exam 1. Longitudinally, carriers showed smaller increases and larger decreases in regional brain volume than non-carriers (Figure 2b shows the most significant region). Differences in GM with an extent>100mm^3 were found in the right inferior temporal gyrus, and in the middle temporal gyrus. Differences in WM with an extent>100mm^3 were found in the right temporal lobe and in the left fusiform gyrus. A combined analysis of *KL-VS* and *APOEε4* showed that *KL-VS* heterozygotes have a larger rDLPFC, independent of *APOEε4*, and *APOEε4* carriers have a larger decrease in GM and WM, independent of *KL-VS*.

3.5. Survival analysis

The Kaplan-Meier survival curves Figure3a show that *KL-VS* heterozygotes (F/V) have a shorter survival than non-carriers (-VF/F). We have previously shown that brain fraction is a significant predictor of survival. Figure 3b shows the *KL-VS* groups split by brain fraction above and below the mean (.754). The figure indicates that greater brain fraction is associated with longer survival.

Cox regressions separately examined the effect of the non-imaging parameters *KL-VS* (comparing heterozygotes with non-carriers), *APOEε4*, gender, DS test score at occasion 1, MHT score, years of education, and early and late-life OSC on survival. Gender, *KL-VS*, DS score, MHT score and early-life OSC were predictors of survival (p<.05): *KL-VS* heterozygotes survived shorter than non-carriers (hazard ratio or HR: 1.64, p=.014); a higher DS and MHT score were associated with survival (DS HR: .97, p<.001; MHT HR: .98, p=.021); a lower early-life OSC was associated with shorter survival (HR: 1.12, p=.011); males had shorter survival than women (HR: 1.50, p=.027). The effects of *APOEε4*, years of education and late-life OSC on survival were non-significant.

We added the significant predictors of survival into one Cox regression, but removed *APOEε4*, years of education and late-life OSC because they were previously non-significant. We found that *KL-VS*

genotype, late-life cognition and childhood socioeconomic circumstance are significant predictors of survival. We repeated the Cox regression analysis using only those who had been imaged, and introduced BF as an additional covariate (*KL-VS* genotype, late life cognition and childhood socioeconomic circumstance remained significant predictors of survival in the imaged subset). Early life cognition and BF are now significant predictors of survival. Table 2 shows the hazard ratios for the two models.

4. Discussion

We present a comprehensive analysis of associations between *klotho*, *APOEε4*, cognition, brain size and survival in a well characterized cohort of elderly people in the North East of Scotland. Previous studies have investigated the effects of *KL-VS* genotype on cognition, local brain volume and survival. However, the role of *KL-VS* on global brain volume and rate of cognitive decline **had not been explored**. Our results show that the *klotho* allele *KL-VS* influences the rate of cognitive decline and global brain volumes, particularly in the white matter.

Limitations of the study include the higher likelihood that individuals in better health return for follow-up cognitive tests and MRI scans. Furthermore, our sample size was relatively small. Locality-specific effects attributable to the homogeneity of the sample cannot be excluded. There may be a Scotland or even Aberdeen specific circumstance or interaction with an environmental factor that influenced our results. However, examining between-cohort differences could improve understanding of *klotho*'s effect on aging.

The absence of an *APOEε4* association with cognition is inconsistent with some published findings. This may be in part due to the cohort only recruiting at baseline unimpaired individuals and our impression that declining individuals were less likely to return for repeat evaluation. In addition, Small et al. 1998 have found that *APOEε4* status may not influence cognitive performance in adults without dementia, including processing speed (Small et al., 1998).

Our cross-sectional cognition results support Deary et al.'s findings in our cohort (Deary et al., 2005), which found no difference between *KL-VS* heterozygotes and non-carriers. However, these data do not support other studies that indicate heterozygotes have higher cognition than non-carriers (Dubal et al., 2014, Yokoyama et al., 2015). Sources of this discrepancy are unclear. Studies that found differences were conducted in older individuals who received more education and who are from higher socioeconomic backgrounds. Longitudinally, *KL-VS* heterozygotes have a **slower cognitive decline than non-carriers**. This indicates that in an older sample heterozygotes would have higher cognition. This supports a heterozygous advantage for cohorts that examined older participants and/or participants within a larger age range (Dubal et al., 2014, Yokoyama et al., 2015). In addition, these differences in decline are independent of *APOEε4* status. There may also be a klotho/socioeconomic interaction. Prather et al. 2015 (Prather et al., 2015) suggested that klotho protein concentrations are sensitive to psychosocial stressors which are associated with socioeconomic position (Cohen et al., 2006). In addition, the advantage of *KL-VS* heterozygosity may only be seen in very late-life and non-carriers may have advantages at different times in the life course. These two explanations are not mutually exclusive and previous human studies may have sampled from populations where the non-carrier advantage was less important given the environment and the age range of the study. Similarly, the controlled environment of the animal studies may not have exposed the sample to an environment where the non-carrier advantage is realized.

Regional imaging analysis, when using no assumptions about the potential location of differences (i.e. whole brain analysis) found no differences between *KL-VS* groups. However, when guided by previous work in the area (Yokoyama et al. 2015) we found a region in the rDLPFC where *KL-VS* heterozygotes had more grey matter than non-carriers. This difference was independent of *APOEε4* status. Although this generally agrees with the earlier study (Yokoyama et al., 2015), our region is more posterior than identified previously. This might be because of methodological differences between the two analyses: first, as discussed above, the samples are probably different in terms of

recruitment; and second the image analysis used different templates which will result in differences in the reported coordinates.

The global brain volume analysis showed that *KL-VS* heterozygotes had smaller white matter volume relative to head size at the first MRI exam. This finding was independent of *APOEε4* status. Greater brain volumes are associated with longevity, with individuals with more white matter surviving longer (Staff et al., 2010, Van Elderen et al., 2016). Longitudinally, there were no differences in brain volume changes, indicating that *KL-VS* is not associated with rate of atrophy.

The survival analysis found that *KL-VS* heterozygotes have a survival disadvantage compared to non-carriers, which was unexpected. As anticipated from our earlier work in an older sample (Staff et al. 2010), a greater brain fraction is associated with longer survival (Staff et al., 2010).The effect of *KL-VS* on survival remained after adjusting for cognition. This suggests that klotho influences survival not (only) through a possible effect on cognition, but also through another mechanism, perhaps influencing differential brain atrophy or differential development. This is supported by our finding that *KL-VS* is no longer significant when brain fraction is included in the Cox regression model.

We have found that *KL-VS* heterozygosity is associated with both positive and negative effects in the Aberdeen Birth Cohort of 1936. *KL-VS* heterozygotes showed increased rDLPFC volume and a slower rate of cognitive decline, but also smaller white matter volumes and decreased rates of survival. These results paint a broad picture of the effects of *KL-VS* in late life and contradict a purely positive effect of *KL-VS* heterozygosity. Projecting forward, the longitudinal cognition result suggests that in an older cohort, heterozygotes would have higher cognition than non-carriers, and possibly subsequent higher survival. This would be consistent with previous work that found a survival advantage for heterozygotes in older groups than the one studied here (Arking et al., 2002, Arking et al., 2005), and supports work that suggested that *KL-VS*'s effect on cognition and survival is age-dependent (Invidia et al., 2010, Mengel-From et al., 2016).

5. Conclusion

Our study demonstrates that *KL-VS* heterozygosity is associated with slower cognitive decline in old age. This may in part explain discrepancies between our findings and other studies that examined cross-sectional cognitive estimates. The regional volumetric results, which show a difference in the rDLPFC, are consistent with what was previously shown (Yokoyama et al., 2015). The global brain volume results demonstrate a relationship between *KL-VS* and white matter volume in late life. The survival results do not support the hypothesis that *KL-VS* heterozygotes possess a survival advantage, as heterozygotes died earlier. Collectively these results are independent of *APOEε4* status suggesting that their mechanisms are separate. Taken together, we show a possible environmental influence on the action of *KL-VS* and that action may represent a gene-by-age effect and differ across the life course.

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Disclosure statement

The authors declare no financial conflicts of interest.

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Figure Legends

Figure 1: Spaghetti plot for the Digit Symbol test for the whole group. The red lines indicate the non-carriers and the black lines the heterozygotes. The two bold lines represent the respective trend lines for each group.

Figure 2: Surface renders of grey matter volume differences. Figure 2a shows the most significant grey matter region when comparing KL-VS heterozygotes with non-carriers in a whole brain analysis, at uncorrected p<.001 (MNI coordinates: 50, 6, 39; extent: 1802mm^3). After small volume correction (rDLPFC) a significant corrected area was identified (p_{FWE}<.05) at the same location (extent: 118mm^3). The arrow roughly indicates the location of the rDLPFC area found by Yokoyama et al. 2015 (MNI coordinates discovery cohort Yokoyama et al.: 39, 26, 36). Figure 2b shows the most significant region for which APOEε4 non-carriers had either a larger positive change or a smaller negative change in brain volume than carriers, at corrected p<.05. bspmview, an SPM12 toolbox, generated the renders. See Supplementary Table 2 for full results of the VBM analyses (groups compared, significance level, and peak location in MNI coordinates, cluster size and probabilities).

Figure 3: Kaplan-Meier survival curves split by: KL-VS heterozygotes and non-carriers (3a), and both KL-VS genotype and brain fraction (3b). The plus signs indicate ages of participants alive at the latest census date (6th of January 2015). MRI exams were performed when participants were between ages 68 and 72 years. Genotyping was performed when the participants were 64.

Tables

	Whole sample (SE)	*F/F (N=331)*	*F/V (N=134)*	*V/V (N=15)*	*−APOEε4 (N=352)*	*+APOEε4 (N=126)*
Sex-Number Male (N=508)	246, 48.4%	154, 46.5%	72, 53.7%	10, 66.7%	177, 50.3%	59, 46.8%
OSC_{adult} (N=472)	4.72 (.10)	4.73 (.12)	4.72 (.20)	4.33 (.41)	4.75 (.12)	4.63 (.19)
$OSC_{childhood}$ (N=473)	6.21 (.11)	6.15 (.14)	6.25 (.20)	7.13 (.49)	6.15 (.13)	6.39 (.22)
Education years (N=477)	11.16 (.10)	11.20 (.12)	11.03 (.19)	11.47 (.68)	11.24 (.12)	10.97 (.16)
Moray House test (N=506)	41.92 (.60)	42.76 (.74)	41.32 (1.16)	44.87 (3.19)	41.78 (.75)	42.40 (1.08)
DS occasion 1 (N=478)	43.15 (.52)	43.59 (.63)	42.32 (1.06)	44.60 (2.21)	43.18 (.62)	43.56 (1.09)

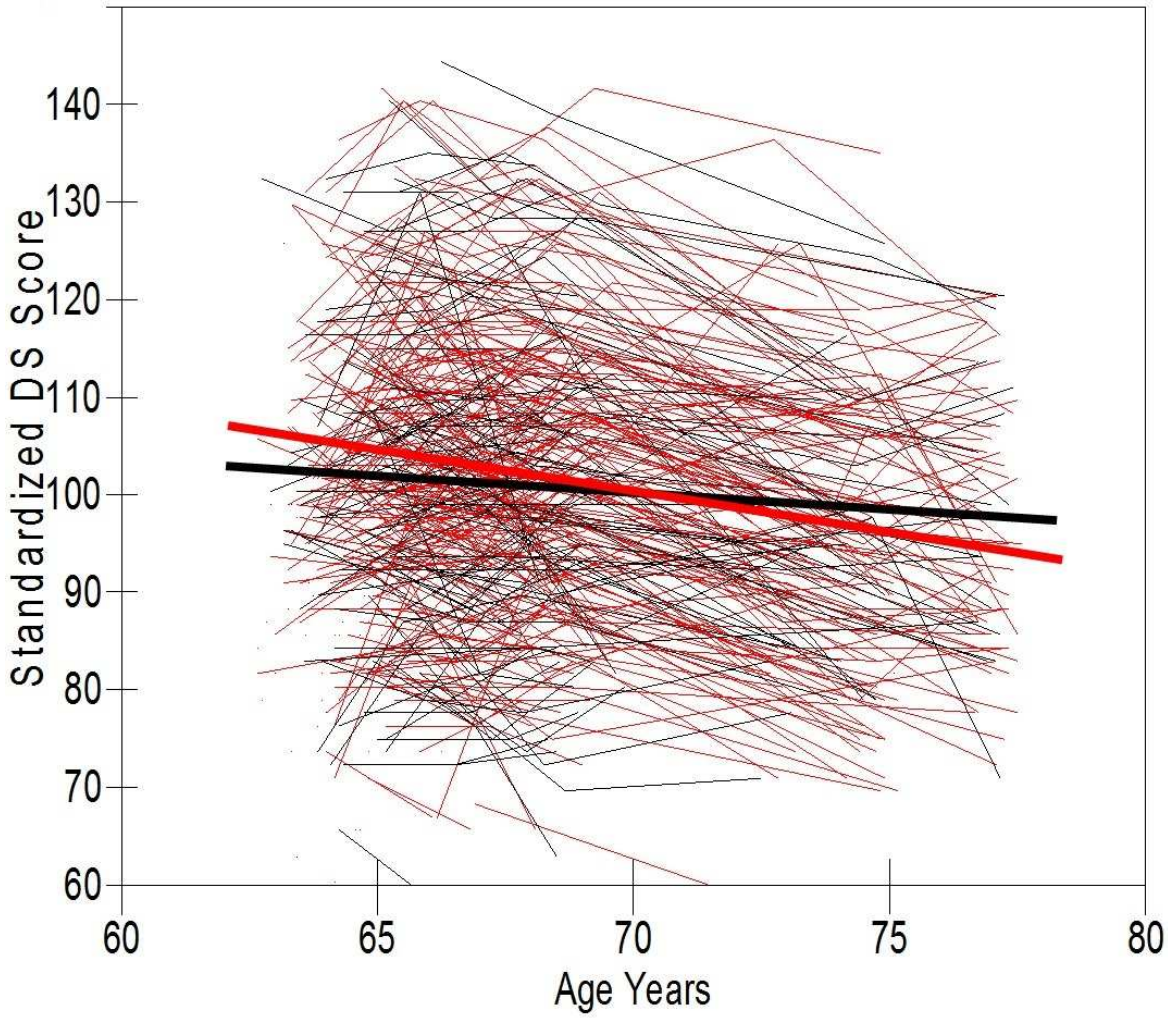
Table 1: Demographic and cognitive description of the sample (N=508). Mean values are given. The values inside the brackets indicate the standard error (SE). F/F: KL-VS non-carriers, F/V: KL-VS heterozygotes, V/V: KL-VS homozygotes, −APOEε4: APOEε4 non-carriers, +APOEε4: APOEε4 carriers, OSC: occupational social class. No significant differences were found (p<.05). See Supplementary Table 1 for mean DS cognitive scores at occasions 1 to 5.

	Imaged (SE)	*F/F*	*F/V*	*V/V*	*−APOEε4*	*+APOEε4*
Exam 1	**N=244**	**N=164**	**N=64**	**N=10**	**N=169**	**N=66**
Sex-Number Male	127, 52.0%	82, 50.0%	36, 56.3%	7, 70.0%	90, 53.3%	35, 53.0%
Age at MRI years	68.69 (.04)	68.68 (.05)	68.72 (.08)	68.49 (.22)	68.68 (.05)	68.67 (.10)
TBV mm³ x10³	1063.6 (6.8)	1068.7 (8.1)	1048.9 (14.3)	1103.6 (24.8)	1065.5 (8.0)	1076.5 (12.8)
TICV mm³ x10³	1412.2 (9.0)	1413.0 (10.8)	1408.0 (18.2)	1463.4 (41.3)	1413.6 (10.6)	1429.5 (17.7)
rDLPFC mm³ x10³	27.63 (.21)	27.68 (.24)	27.36 (.44)	29.53 (.56)	27.70 (.25)	27.82 (.37)
Hippocampus mm³ x10³	7.84 (.06)	7.85 (.07)	7.79 (.12)	8.27 (.27)	7.90† (.07)	7.75 (.11)
Brain Fraction	.754 (.002)	.757 (.003)	.746 (.006)	.756 (.010)	.755 (.003)	.754 (.004)
GM Fraction	.441 (.002)	.441 (.002)	.440 (.003)	.441 (.009)	.441 (.002)	.441 (.003)
WM Fraction	.313 (.001)	.316* (.002)	.306 (.004)	.315 (.006)	.314 (.002)	.313 (.003)
Longitudinal	**N=156/155**	**N=112/111**	**N=36**	**N=6**	**N=110/109**	**N=40**
ΔrDLPFC mm³ x10³/year	-.25 (.02)	-.25 (.03)	-.27 (.04}	-.17 (.07)	-.24 (.02)	-.30 (.04)
ΔHippocampus mm³x10³/year	-.079 (.007)	-.083 (.009)	.072 (.011)	.031 (.012)	-.070 (.006)	-0.103 (.021)
ΔTBV mm³ x10³/year	-8.18 (.38)	-7.96 (.45)	-8.55 (.80)	-9.08 (1.80)	-7.50† (.38)	-10.30 (.91)
ΔGM mm³ x10³/year	-5.82 (.34)	-5.73 (.41)	-5.79 (.68)	-6.70 (1.73)	-5.27† (.35)	-7.48 (.87)
ΔWM mm³ x10³/year	-2.36 (.19)	-2.23 (.22)	-2.28 (.41)	-2.39 (.19)	-2.23 (.22)	-2.82 (.37)

Table 2: Volumetric description of the sample (N=244). Mean values are given. The values inside the brackets indicate the standard error (SE). F/F: KL-VS non-carriers, F/V: KL-VS heterozygotes, V/V: KL-VS homozygotes, −APOEε4: APOEε4 non-carriers, +APOEε4: APOEε4 carriers, TBV: total brain volume, TICV: total intracranial volume, rDLPFC: right dorsolateral prefrontal cortex, brain/GM/WM fraction: TBV/grey matter/white matter divided by TICV, Δ denotes the difference between exam 1 and exam 2. The asterisk (*) indicates a significant difference (corrected for gender, age) between KL-VS heterozygotes and non-carriers. The dagger (†) indicates a significant difference (corrected for gender, age) between APOEε4 carriers and non-carriers (p<.05).

covariates	*N*	*Sex*	*KL-VS*	*MHT*	*DS*	$OSC_{childhood}$	*BF*
Non-Imaging	428	1.20 (.80, 1.81)	1.57* (1.04, 2.36)	1.00 (.98, 1.02)	.97* (.95, .99)	1.10* (1.00, 1.21)	
Imaging and Non-Imaging	221	.77 (.40, 1.46)	1.32 (.67, 2.58)	.96* (.93, .99)	.98 (.95, 1.01)	1.13 (.99, 1.30)	.89* (.83, .96)

Table 3: Hazard ratios for the Cox regressions. The asterisk (*) indicates significant results (p<.05). Values within the brackets indicate the 95% confidence interval. N: number of participants, MHT: Moray House Test, DS: Digit Symbol Substitution test, BF: brain fraction, $OSC_{childhood}$*: occupational social class during childhood.*


Standardized DS Score
140
130
120
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90
80
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60
65
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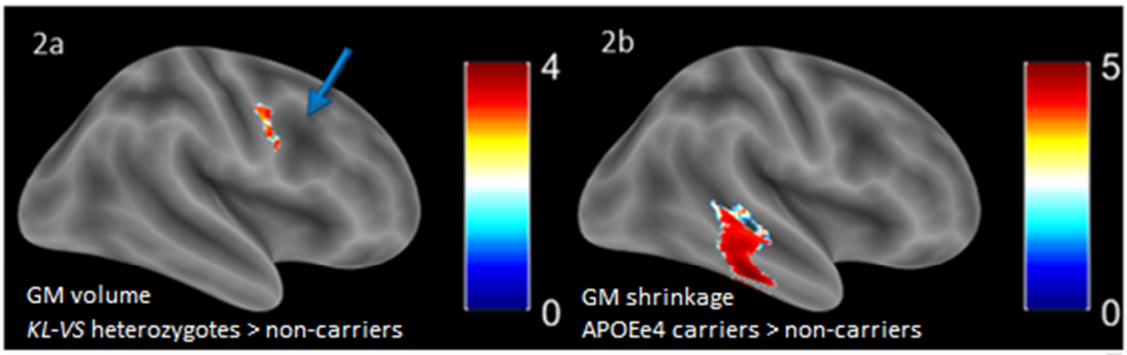
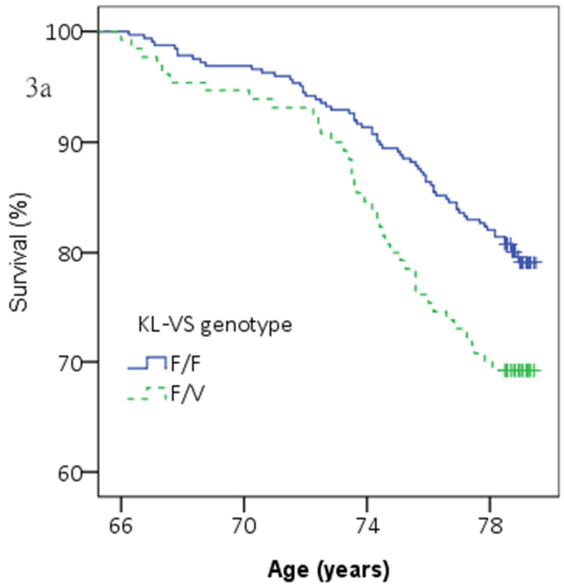
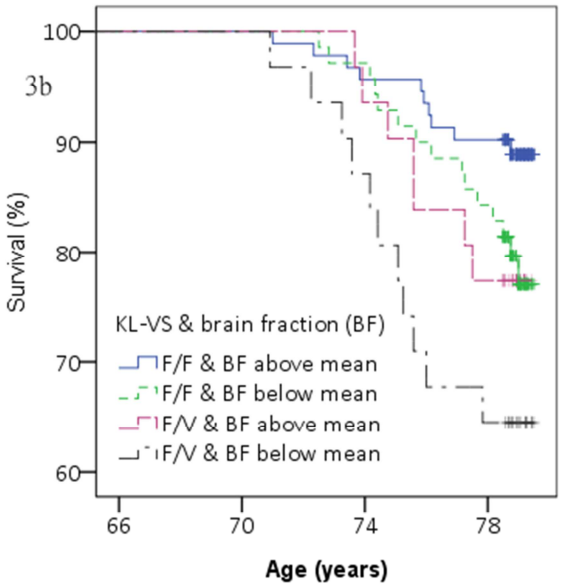
75
80
Age Years







Highlights

- Unexpectedly, *klotho KL-VS* heterozygotes survived shorter
- *KL-VS* heterozygotes had larger right frontal lobe volumes
- Cognitive decline was slower in *KL-VS* heterozygotes' than non-carriers
- Results indicate heterozygotes would have a cognitive advantage in very late life