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Applied methodology

Urinary ionomic analysis reveals new relationship between minerals and longevity in a Han Chinese population

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ABSTRACT

Human longevity involves genetic, nutritional, environmental and many other factors playing a key role in healthy aging. Previous studies have shown that mineral metabolism and homeostasis are associated with lifespan extension. However, the majority of them have focused on a limited number of elements and ignored the complex relationship between them. In this study, we carried out a network-based approach to investigate the urinary ionome of nonagenarians and centenarians (longevity group) when compared with their biologically unrelated and younger family members (control group) from a Han Chinese population. Several differentially changed elements were identified, almost all of which showed an elevated level in the longevity group. Correlation analysis of the ionome revealed significant element-element interactions in each group. We then divided each group into distinct subgroups according to age ranges, and built the elemental correlation network for each of them. Significant elemental correlations and correlation changes involving all examined elements were identified within or between different subgroups, implying a highly dynamic and complex crosstalk among the elements during human life. Finally, more similar elemental patterns were observed between extremely old and middle-aged people. Overall, our data reveal new relationship between urinary minerals and human longevity, which may extend our understanding of the mechanism of healthy aging.

1. Introduction

Human longevity is a very interesting but complicated trait which could be influenced by both genetic and environmental factors [1–4]. Among the elder population, nonagenarians and centenarians are exceptional aging paradigms and valuable models exploited to study longevity [5,6]. It is of extreme importance to understand genetic, environmental and other factors, as well as the ways involved in healthy aging and longevity by utilizing these resources. So far, the majority of genome-based studies have focused on the association between longevity and genetic variations including single nucleotide polymorphism and copy number variation. Special emphasis has also been given to dietary, physical activity and psychosocial factors [7,8].

Many trace elements are involved in improving immune functions, metabolic homeostasis and antioxidant defense, which may lead to an escaping of common diseases and contribute to longevity [9–11]. In recent years, ionome has been introduced by analogy with the genome, proteome and metabolome, which refer to the complete set of mineral nutrients and trace elements found in an organism [12–14]. It has been shown that ionomics can provide a powerful approach not only for understanding genes and gene networks that directly or indirectly affect the ionome, but also for complex disease prediction and classification [15–17].

China records the largest population of adults aged 80+ years in the world [18]. Several "longevity counties/towns" have been formally recognized by the government due to the high number of naturally longevous nonagenarians and centenarians living there. Previous studies have been performed either to identify genetic factors associated with longevity [19–21] or to assess various plasma/hair metals of oldest elderly in these areas [22,23]. Considering that many of excessive elements (especially heavy metals) are excreted in the urine, urinary elemental concentrations are often used to evaluate their exposure or

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demand levels. However, it remains largely unclear whether the urinary ionome is related to human longevity.

Here, both nonagenarian/centenarian participants and their biologically unrelated family members from Yongfu County (qualified as a longevity town by the Geriatric Society of China) were recruited. The urinary elemental profiles were measured and further analyzed using network-based approaches. To our knowledge, this is the first effort to systematically study the ionomic patterns that may be associated with human longevity.

2. Materials and methods

2.1. Subjects

A total of 63 volunteers aged 90 + years from Yongfu County, South China, as well as 49 biologically unrelated family members were initially enrolled in this project. Subjects undertaking drug treatment were excluded. The remaining people were healthy and self-reported as Han nationality. We selected 46 paired longevity and control samples (details are shown in Table S1). The longevity group (mean age 96.70 ± 4.03 years; male:10, female:36) was comprised of 38 nonagenarians and 8 centenarians. Ages of the control group varied from 20 to 80 years (male:2, female:44). This study was conducted according to the principles expressed in the Declaration of Helsinki. The Ethics Committee of Beijing Hospital approved the study protocol. All subjects provided written informed consent.

2.2. Urinary sample processing and ionomic analysis

First morning urine samples were collected with clean containers and were frozen at −80 °C until they were analyzed for creatinine and for the elements of interest. Urinary creatinine was measured with spectrophotometric assay.

Ionomic analysis of 31 metal and nonmetal ions (including almost all essential elements and the majority of non-essential and toxic elements detected in humans) was performed as previously described [17,24,25]. In general, urine samples were centrifuged for 10 min at 4 °C (3000 rpm) and 100 u l of clean urine sample was placed in 15 ml centrifuge tube. The samples were mixed with 1.9 ml 2% HNO_3 solution. The ion concentrations of aluminium (Al), arsenic (As), barium (Ba), beryllium (Be), boron (B), cadmium (Cd), calcium (Ca), cesium (Cs), chromium (Cr), cobalt (Co), copper (Cu), gallium (Ga), iron (Fe), lead (Pb, 3 isotopes), magnesium (Mg), manganese (Mn), nickel (Ni), palladium (Pd), potassium (K), rhenium (Re), rubidium (Rb), selenium (Se), silver (Ag), strontium (Sr), thallium (Tl), tungsten (W), uranium (U), vanadium (V) and zinc (Zn), were quantified using an Agilent 7700x collision/reaction cell inductively coupled plasma mass spectrometry (ICP-MS) system (Agilent Technologies, Tokyo, Japan) equipped with an ASX-500 series autosampler at Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. The instrument was tuned to optimal operating conditions (summarized in Table S2) according to sensitivity (Li, Y, Co, and Tl) and CeO/Ce and Ce^{2+}/Ce by using a tuning solution (Agilent Technologies) containing 1 μg/L Li, Y, Tl, Ce, and Co in 2% HNO3 (w/v). The instrument was operated in full quantitative mode. The internal standard containing Ge, ^{6}Li, Lu, Rh, Tb, Sc, In, and Bi (Agilent Technologies) was injected by peristaltic pump into the ion source at an approximate concentration of 500 μg/L in the online mode. The standard curve included 0, 0.1, 1, 10, 100, and 1000 μg/L. Quality control was performed (1 out of 20 samples), and the inter- and intra-assay variations of the instrument were assessed by analyzing concentrations of multiple elements in the standard reference sample (SRM) 2668 prepared by the National Institute for Environmental Studies, all of which were < 5%. To account for urine dilution, original concentration of each element was further adjusted by using urinary creatinine concentrations.

2.3. Statistical analysis

Statistical analysis was performed using *R* language package (version 3.3.0) [26]. For each element, outliers (identified using Dixon's Q-test) and empty values were replaced with the average value of the rest samples. Kolmogorov-Smirnov test was used to check whether the data follow a Gaussian population, and our results suggested that the distribution of most elements could not be well-modeled by normal distribution. Therefore, paired Wilcoxon rank sum test was used to identify differentially changed elements (DCEs) between different groups. Multiple testing adjustment was used to adjust p-value by using the false discovery rate (FDR) approach [27]. Difference was considered significant if p-value (FDR corrected) < 0.05. Binomial logistic regression analysis [28] was carried out to calculate the odds ratio (OR) and 95% confidence interval (CI) for the association between DCEs and longevity (each element was considered as independent variable). In addition, a binomial logistic regression model was established based on each DCE, which was then tested by five-fold cross-validation. The receiver operating characteristic (ROC) curve (generated by the *R* package pROC) was used to evaluate the performance of each model. Hierarchical clustering and heatmap generation were carried out using the pheatmap package from *R*.

In order to investigate urinary ionomic profiles at different stages of longevity and control groups, each group was divided into three subgroups with different age ranges: 20˜40, 40˜60 and 60˜80 years for the control group, and 90˜95, 95˜100 and 100˜110 years for the longevity group. Each subgroup contained more than 10 samples. Kruskal-Wallis test was used to analyze age-related elements within each group.

2.4. Elemental correlation analysis

Spearman's correlation coefficient (SCC) was used to evaluate the relationship between different elements in different groups and subgroups. We used 0.65 as the empirical threshold value to identify significant SCCs. The elemental correlation network for each subgroup was visualized using Cytoscape software [29]. The difference of SCCs between adjacent subgroups (|ΔSCC|) was also calculated. A differentially changed correlation (DCC) was defined if |ΔSCC| was greater than 0.9 (an empirical threshold).

2.5. Comparison of urinary ionomes between different subgroups

To compare the ionomic profiles between different subgroups, we defined a similarity score (*SS*) between subgroup *i* and *j* as follows:

$$SS_{i,j} = S_E + S_C$$

In such an equation, S_E is the similarity of elements between subgroup *i* and *j* and could be calculated as

$$S_E = 1 - \frac{E_{i,j}}{E_{total}}$$

Where $E_{i,j}$ is the number of DCEs between subgroup *i* and *j*, and E_{total} is the total number of elements examined here. S_C is the similarity of significant element-element correlations between subgroup *i* and *j* and could be calculated as

$$S_C = \frac{C_{i,j}}{C_{total}}$$

Where $C_{i,j}$ is the number of significant SCCs present in both subgroups, and C_{total} is the total number of significant SCCs detected in either of the two subgroups.

3. Results

In our study, we analyzed the urinary ionome (including both essential and non-essential elements) of 46 paired longevity and control

Table 1
Binomial logistic regression analysis of DCEs.

Elements	Odds Ratio	95% CI	Significance (p-value)
Cr	3.097	1.243–7.716	0.015
Mn	2.663	1.127–6.293	0.026
Cu	3.439	1.518–7.792	0.003
Zn	3.305	1.416–7.714	0.006
Tl	0.076	0.019–0.296	< 0.001
Constant	0.823		

subjects from the same region, who were thought to be exposed to a similar natural environment and dietary culture. Such a strategy might help to reduce the genetic and environmental effects as few as possible, which in turn, demonstrates intrinsic associations between element homeostasis and longevity. Raw concentrations of all examined elements are shown in Table S1.

3.1. General analysis of the urinary ionome

First, a total of 14 elements, all of which are metals, were identified as DCEs between the longevity and the control groups. The majority of them (12 elements, including Be, Cr, Cs, Cu, Fe, Mn, V, W, Zn and three isotopes of Pb) appeared to be enriched in the urine of the longevity group, whereas concentrations of Tl and U were significantly decreased. Six of the DCEs (Cr, Cu, Fe, Mn, V and Zn) are known to be needed for human health. Binomial logistic regression analysis was used to explore the roles of these DCEs in longevity, and the regression model was statistically significant ($\chi 2(5) = 83.305$, $p < 0.001$). Five metals, including Cr, Mn, Cu, Zn and Tl, were found to be significantly associated with longevity (p-value < 0.05, Table 1). Moreover, we built a binomial logistic regression model based on each DCE, which could be used to classify the longevity and control groups. After five-fold cross-validation, the mean values of AUC for all DCEs were greater than 0.6 (0.615˜0.869, Table S3). This may suggest that, in spite of the limited sample size, the DCEs identified between the longevity and the control groups have good performance to separate the two groups, especially Cu and Cr whose mean AUC was greater than 0.8. It has been known that some of these metals are important for a variety of metabolic pathways involved in aging (such as inflammatory and oxidative processes), overload or imbalance of them may lead to molecular damage to cells and tissues [30]. Therefore, our data suggest that accumulation of a variety of heavy metals might be a potential risk factor for human life extension, and that excessive urinary excretion of these metals might result in low level exposure *in vivo* and contribute to longevity.

It is known that several characteristics such as age might affect elemental concentrations in different human tissues such as hair, blood and urine [23,31]. It has also been suggested that extremely old people (such as nonagenarians and centenarians) may have quite different features when studying the association of biological markers (such as epigenetic clock) with age [32,33]. To further investigate the deviations from the correlation between urinary element levels and ages in both longevity and control groups, each group was divided into three subgroups. Kruskal-Wallis test was adopted to analyze the relationship between elements and age. Only 4 and 3 metals (with an overlap of Ni) were found to be age-related in the longevity and the control groups, respectively (Table 2). It seems that the relationship between urinary levels of elements and age is different between extremely old people and others. Although such a difference might be related to specific mechanisms involved in metal homeostasis in different groups, the possibility that kidney function in the very elderly is different (say, de-optimized performance of aging kidney) from that in relatively young people could not be excluded.

Table 2
Distribution of age-related elements.

Group	Age-related elements
Longevity	Al, Fe, Ni, V
Control	Cs, Ni, Rb

3.2. Clustering analysis

Analysis of ionomic patterns may help to explore functional linkages among the elements. Raw concentration data for each DCE were log10 transformed and used for the calculation of z scores. Hierarchical clustering method was used for clustering analysis. Due to very high correlation between any two of the three Pb isotopes, we combined them as one element in the following part.

The correlation heatmap of all DCEs using the full data is shown in Fig. 1A. The majority of the samples in the longevity group could be separated from those in the control group, implying that urinary ionomic changes observed in extremely old people are somewhat specific. The essential DCEs could be divided into two clusters: one contained Fe and Mn which are known to be functionally related in humans [34], and the other contained V, Cu, Zn and Cr. Tl appeared to be an outgroup which had reduced levels in almost all subjects of the longevity group.

We further carried out clustering analysis of DCEs individually within each group (Fig. 1B). A few clusters of elements with similar distribution patterns were consistently observed, such as Fe and Mn. Zn appeared to be distant from Tl and Cs in the control group while they were clustered together in the longevity group. As Zn is important for immune function, metabolic homeostasis and antioxidant activity [10,35], regulation of Zn homeostasis (e.g., urinary excretion) might be complex for maintaining its proper role during aging. Similarly, a strong interaction between Cr and V was only observed in the longevity group but not in the control group, implying that close relationship between the two essential metals is also associated with longevity.

3.3. Elemental correlation analysis

It has been suggested that crosstalk between different elements may affect the process of healthy aging and is involved in several age-related diseases such as neurodegenerative diseases and cancers [30,36–38]. However, it is unclear whether such a complex interplay is connected with the regulation of human longevity. To this end, we calculated SCCs for all possible element pairs in the longevity and the control groups. Significant SCCs ($|SCC| > 0.65$ as defined in Materials and Methods) are shown in Table 3.

All significant correlations were positive, which were only detected between a small number of metals (mostly non-DCEs). Strong interactions between Ba and Ga, K and Rb as well as Pd and Sr were present in both groups. On the other hand, significant correlations between Ca and Sr as well as Cs and Rb were only observed in the longevity group. Although a close relationship between Fe and Mn was observed in both groups (Fig. 1B), such correlation was only significant in the control group. Thus, our data reveal that, besides individual elements, cross-links between certain elements (especially longevity-specific correlations) might also be related to longer lifespan.

We further calculated SCCs between different elements and built the elemental correlation network for each subgroup. A simplified representation of the network containing significant SCCs is illustrated in Fig. 2 (details are shown in Table S4). Almost all of the correlations in these networks were positive, which involved all elements examined here. Except the 20˜40 subgroup that contained 58 significantly correlated element pairs, the number of such element pairs was quite limited in all subgroups (11–17 pairs). Only a small number of correlations (13.0%) were found to be significant in multiple subgroups, especially Ba-Ga and K-Rb which were present in all subgroups. This suggests that strong interactions between these metals might be stable

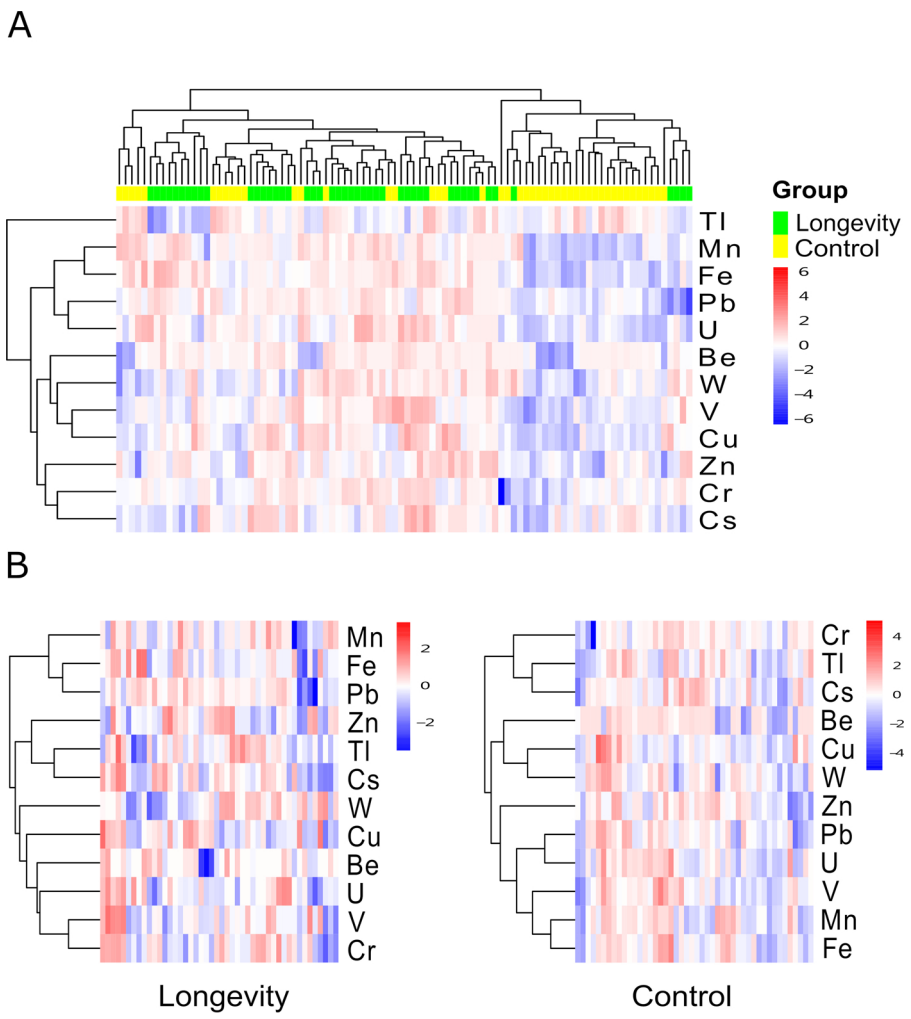

Fig. 1. Clustering analysis of DCEs based on data for all samples and individual groups. DCEs were analyzed by hierarchical clustering approach as described in the text. Red and blue colors represent increased and decreased levels, respectively, when compared with the average of each element. Columns and rows represent samples and DCEs, respectively. (A) All samples: the longevity and the control groups are represented by green and yellow, respectively; (B) individual groups (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 3
Significant element-element correlations in different groups.

Group	Elemental correlation
Longevity	Ba-Ga, Ca-Sr, Cs-Rb, K-Rb, Pd-Sr
Control	Ba-Ga, Ba-V, Ca-Mg, Fe-Mn, K-Rb, Pd-Sr

during the whole life. It is interesting that a significant correlation between Ca and Mg was detected in the 20˜40, 40˜60, 60˜80 and 90˜95 subgroups but was absent in the 95˜100 and 100˜110 subgroups, implying a weakened correlation between the two essential metals in the oldest elderly. In contrast, the Ca-Sr interaction was found to be significant in all subgroups of the longevity group but absent in the majority of subgroups of the control group, indicating that relationship between the two metals might be linked to human longevity. Therefore, our observations suggest a complex and dynamic relationship between these elements (especially non-DCEs) across human lifespan.

Although most of the SCCs between different elements were not significant, difference of SCCs (|ΔSCC|) might be significant (named DCC). Similar situations have been observed when analyzing the relationship between minerals and several complex diseases [17,39,40]. In the present study, we identified all DCCs between adjacent subgroups (Table 4; details are shown in Table S5). The majority of DCCs showed significant changes from positive to negative correlations (ΔSCC < -0.9), especially at relatively younger stages (e.g., 40˜60 vs. 20˜40 and 60˜80 vs. 40˜60). When entering extremely old stages (nonagenarians/centenarians), significant changes from negative to positive correlations were observed (ΔSCC > 0.9), such as Fe-Pb, Se-V and Pb-Zn. It should be noted that half of the element pairs that have DCCs were not significantly correlated in any of the subgroups. Our results suggest that both element-element correlations and changes of such correlations might contribute to healthy aging and longevity.

3.4. Comparison of urinary ionome between different subgroups

Based on the calculation of *SS* value (see Materials and Methods), we compared the elemental profiles between any two subgroups to explore similarities and differences in the urinary ionomes of different age ranges. In general, ionomes of different subgroups of the longevity group appeared to be more similar to each other (Fig. 3), suggesting that the urinary elemental patterns are relatively stable in nonagenarians and centenarians. Interestingly, a strong similarity was observed between the ionome of each subgroup of the longevity group (especially nonagenarians) and that of the 40˜60 subgroup, implying that a relatively younger status (say, middle-aged stage) of the urinary ionome is related to human life extension. However, whether these findings can explain the role of mineral homeostasis in healthy aging and longevity needs thorough investigation in the future.

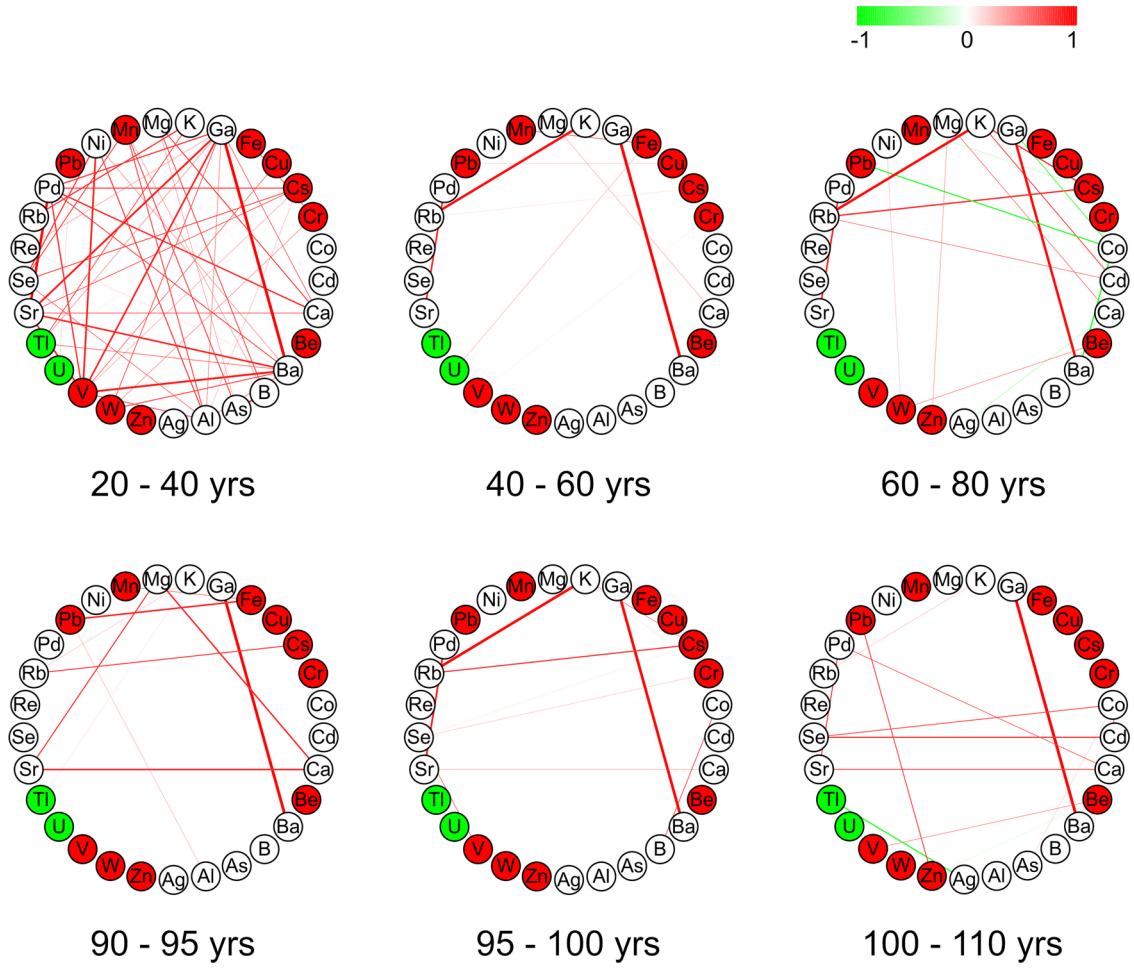

Fig. 2. The elemental correlation network. Only significant SCCs whose values are greater than the threshold (0.65) are shown. Nodes represent different elements. DCEs are highlighted in red (increased) and green (decreased). Red and green lines represent positive and negative correlation, respectively. Different color and width scales represent the magnitude of SCCs (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 4
Differentially changed correlations between adjacent subgroups.

Comparison of subgroups	DCCs	
	positive→negative (ΔSCC < −0.9)	negative→positive (ΔSCC > 0.9)
40~60 vs. 20~40	Cs-Ni, K-W, Ni-Se, Ni-V	–
60~80 vs. 40~60	Ag-Ca, Ba-Co, Ba-Pd, Ba-Mg, Co-Ga, Co-Pb, Ga-Pd, Mn-Sr	–
90~95 vs. 60~80	As-Cr, Se-U	Fe-Pb, Ni-Tl, Tl-W
95~100 vs. 90~95	Al-V, As-Zn, Ga-V	Se-V
100~110 vs. 95~100	Ag-Re, Ag-Tl, As-Cu, Be-Ni, Cu-Sr	Cd-Co, Pb-Zn

4. Discussion

Both essential micronutrients and other minerals are known to be involved in age-altered biological functions. Micronutrients play a key role in maintaining the antioxidant and immune performances and in affecting the complex network of genes involved in a variety of aging-related processes [30,41–43]. Non-essential minerals, especially heavy metals (such as Pb, Cd and As), may also influence lifespan because they can become toxic when overloaded. The absorption, excretion and homeostasis of these elements are fundamental to achieve healthy aging and longevity. Several previous studies have tried to study the relationship between metal concentrations (plasma, hair or local environments) and human longevity in different areas, which showed

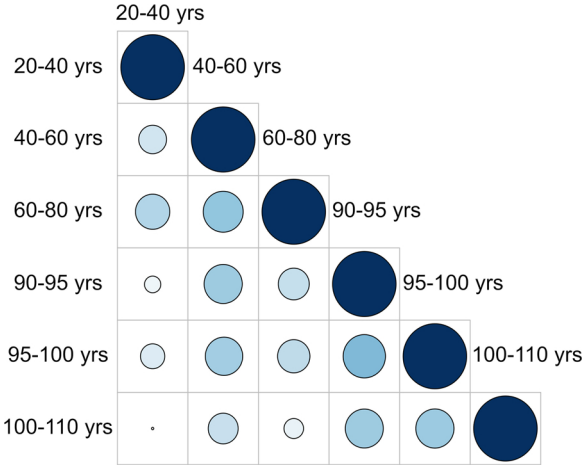

Fig. 3. Comparison of the urinary ionome between different subgroups. The similarity score was calculated based on both DCEs and significant element-element correlations between any two of the subgroups. Different color and circle scales represent the magnitude of the score.

significant correlations between certain elements and longevity [22,23,44–47]. In the recent decade, ionomics has been widely used in the fields of plants and yeasts, which may be helpful to obtain a systems-level understanding of biological complexity in various processes

of these organisms [12–15,48,49]. Very recently, it has begun to be used for the analysis of mineral nutrient and trace element homeostasis in several human diseases and disorders [40,43,50,51]. However, the application of ionomics in the longevity field is still lacking. Therefore, it should be important to systematically investigate the relationship between the ionome and human longevity.

In this study, we reported an extensive ionomic analysis of around 30 elements in the urine samples of paired longevity and control subjects from a Han Chinese population. A total of 14 DCEs were identified, especially Cr, Mn, Cu, Zn and Tl which were significantly associated with longevity via binomial logistic regression analysis. It was reported that overload of Cu and/or Fe may play an important role in age-related neurodegenerative and inflammatory diseases [30,36,52–54]. Although Cr is an essential mineral for carbohydrate and lipid metabolism, high level of Cr is toxic and could be associated with increased risk for different cancers [55,56]. Thus, excretion of these metals might help reduce the risk of metal toxicity and extend lifespan. One previous study examined the amount of different trace elements in the hair of healthy Chinese centenarians, which showed that levels of some elements (such as Cr and Fe) were decreased in the centenarian cohort [23]. Analysis of plasma metal levels in a key area of longevity in Europe also revealed a significant depletion of Fe, Mn and Cu in nonagenarians and centenarians with respect to middle-aged subjects [44]. Our data are generally consistent with previous observations. Zn may improve the inflammatory/immune response and antioxidant defense that are important for healthy aging [10,35]; here, increased level of Zn was observed in the urine samples of the longevity group, suggesting that a tight and complex regulation of Zn homeostasis is required during healthy aging. Other DCEs are not essential and were mostly found to have increased urinary excretion in the longevity group. Elevated levels of some of them (such as Pb and U) were previously reported in different populations of elderly people [57,58]. One possibility is that increased excretion of these non-essential metals might be effective in reducing their toxicity for human health. It is also possible that these metals might be more easily accumulated and thus excreted in higher amounts from the kidney in the oldest elderly. Anyway, it appears that elevated urinary levels of these heavy metals may be related to the regulation of human lifespan.

Clustering analysis of DCE-based ionomic patterns demonstrated that several element clusters were conserved between different groups, suggesting that excretion of these elements might be regulated using similar approaches in human life. Some other interactions were only detected in individual groups, such as Zn, Tl and Cs in the control group as well as V and Cr in the longevity group. Although the mechanism how these metals interact with each other is unknown, a previous study has shown that low concentrations of Cr and V had an increasing effect on the maximum lifespan of fish whereas high levels of Cr and V exhibited a moderate or acute toxicity [59]. Future research is needed to solve these questions.

Only a small number of positively correlated element pairs could be detected in each group. We further identified all significant correlations and built the elemental correlation network for each subgroup, which could offer insights into the dynamic changes of the crosstalk in the urinary ionome (including both DCEs and other elements). The majority of these correlations were subgroup-specific. Interestingly, certain correlations were found to be either stable across the lifespan or specific for all or almost all subgroups of the longevity/control group. For example, positive correlation between K and Rb could be detected in all subgroups, suggesting a potential functional linkage between them. It has been reported that Rb ion can be transported by Na,K-ATPase [60]. In addition, we identified significantly changed correlations (i.e., DCCs) between adjacent subgroups. The majority of DCCs showed significant changes from positive to negative correlations and half of the element pairs that have DCCs were not significantly correlated in any of the subgroups. For the first time, we show that not only the levels of a number of elements in the urine but also a variety of interactions among them are associated with human longevity.

An additional interesting finding is that the urinary ionome of the oldest elderly was more similar to that of middle-aged people based on comparison of both DCEs and element-element interactions between different subgroups, suggesting a correlation between relatively younger status of the urinary ionome and life extension. This may provide a new window for understanding the complex mechanisms of healthy aging and longevity. Further efforts are needed to determine additional factors that may influence the exposure and homeostasis of minerals and their relationship with longevity.

It should be noted that this study may have certain limitations. First, the urinary ionome can be affected by several factors, such as homeostasis of trace elements, environmental metal exposure, increasement of age, and aging kidneys that exhibit slowly developing injury. Here, both naturally longevous people and their biologically unrelated family members from the same region were chosen for our analysis. Thus, changes of the urinary elemental profiles might partially reflect homeostasis of elements as well as their correlation with longevity. Second, due to the cross-sectional nature of our study and insufficient sample size, a causal relationship between urinary excretion of different elements and longevity cannot be established. It is critical to carry out large-scale prospective studies in the future, and our preliminary results may provide clues for further investigation of the behavior of biological trace elements during aging. Third, first morning urine rather than 24-h urine samples were collected in our study, which might be influenced by the biorhythm cycles. In this study, urinary creatinine was used as an independent covariate to account for urine dilution. Finally, it is noteworthy that some other intrinsic and extrinsic factors may affect our results and need to be taken into account in future analyses.

5. Conclusion

In this study, we reported a comprehensive analysis of the urinary ionome of nonagenarians/centenarians in a Han Chinese population. Significant differences between the longevity and the control groups were identified at three different levels: individual elements, elemental correlations and changes of the correlations, which demonstrate a complex and highly dynamic relationship between the urinary ionome and human longevity. Further analysis of the elemental profiles of different subgroups suggests that a relatively younger status of the urinary ionome is related to life extension. Our findings provide insights into how urinary trace elements and minerals change during human life, and may help understand the roles of these elements in healthy aging.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jtemb.2019.02.002.

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