Mechanisms of Ageing and Development 178 (2019) 33–40

Contents lists available at ScienceDirect

Mechanisms of Ageing and Development

journal homepage: www.elsevier.com/locate/mechagedev

Klotho gene polymorphisms are associated with healthy aging and longevity: Evidence from a meta-analysis

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ARTICLE INFO

Keywords:
Klotho
Gene polymorphisms
Aging
Meta-Analysis

ABSTRACT

Klotho gene polymorphisms have been implicated in healthy aging, but inconsistences in findings from previous case-control studies have raised concerns regarding the associations between KLOTHO gene polymorphisms and susceptibility to aging-related diseases and longevity. Hence, this meta-analysis was performed. We assessed the associations between two polymorphisms (G-395 A/rs1207568 and F352 V/rs9536314) and five parameters (urolithiasis, cognitive impairment, cardiovascular disease, cancer, and longevity) by calculating pooled odds ratios with 95% confidence intervals. According to the pooled results, the G allele of the G-395 A polymorphism conferred a significantly higher risk of urolithiasis; G-395 A was related to the susceptibility to cardiovascular disease under allele, dominant, and recessive models. There was no significant association between the G-395 A polymorphism and cognitive impairment among the elderly. The F allele of the F352 V polymorphism protected against breast and ovarian cancer susceptibility. Interestingly, based on the results of the subgroup analysis, the F352 V polymorphism was associated with the overall risk of neoplasms in BRCA1 mutation carriers but not in BRCA2 mutation carriers. Moreover, the F allele played a protective role in determining human longevity. In conclusion, Klotho G-395 A polymorphisms were associated with urolithiasis and cardiovascular disease but not with cognitive impairment. Additionally, Klotho F352 V polymorphisms were associated with cancers and longevity.

1. Introduction

The Klotho gene, which is located on human chromosome 13, contains five exons and was characterized as an anti-aging gene by Kuro-o et al. in 1997 (Kuro-o et al., 1997). Mice with a deficiency in the KLOTHO gene developed a syndrome including a short lifespan, arteriosclerosis, hypoglycemia, osteoporosis, emphysema, skin atrophy and gonadal dysplasia (Kuro-o et al., 1997; Kurosu et al., 2005), which resembles human aging. Conversely, overexpression of Klotho attenuates the aging process and prolongs the life span of mice (Kuro-o et al., 1997; Masuda et al., 2005). It has attracted increasing attention from a broad range of international researchers. Although the molecular mechanism underlying the anti-aging function of Klotho remains incompletely understood, recent studies have identified potentially different functions of transmembrane Klotho and circulating secreted Klotho (Matsumura et al., 1998). Transmembrane Klotho is primarily expressed in renal distal tubule cells and choroid plexus cells in the brain. Circulating secreted Klotho is generated from the alternative splicing of the mRNA and may function as a hormone to regulate similar physiological and pathological processes in tissues with low or even no expression of Klotho, such as artery walls (Matsumura et al., 1998; Wang and Sun, 2009), which may explain why Klotho gene mutations lead to multiple aging phenotypes. As research has progressed, Klotho has been reported to regulate Ca^{2+} and phosphate homeostasis by affecting Ca^{2+} reabsorption channels in the renal epithelial cell membrane, such as the transient receptor potential cation channel subfamily V member 5 (TRPV5) (Chang et al., 2005). Klotho also attenuates reactive oxygen species (ROS)-related oxidative stress by increasing the phosphorylation of Forkhead transcription factor (FOXO) 3a (Emerling et al., 2008; Yamamoto et al., 2005). Furthermore, Klotho plays a fundamental role in regulating intracellular insulin/IGF1 signaling 2 pathway, which is closely related to diabetes (Lin and Sun, 2015). Additionally, recent in vitro and in vivo studies have indicated that Klotho is involved in the mechanism inhibiting tumorigenesis by regulating multiple signaling pathways, including transforming growth factor (TGF) β1, wingless-related integration site (Wnt), IGF and fibroblast growth factor (FGF) 23 signaling pathways (Kurosu et al., 2006; Erben, 2016; Mencke et al., 2017; Xu and Sun, 2015). Thus, Klotho is involved in a series of pathophysiological processes, including but not limited to calcium and phosphate homeostasis,

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https://doi.org/10.1016/j.mad.2018.12.003
Received 17 October 2018; Received in revised form 10 December 2018; Accepted 30 December 2018
Available online 10 January 2019

inflammatory reactions, glycometabolism, and carcinogenesis (Xu and Sun, 2015; Kuro, 2018; Chen et al., 2013).

Because the Klotho gene plays a vital role in the healthy aging process and the Klotho gene and protein were highly conserved across species, such as humans, mice and rats (Xu and Sun, 2015), an increasing number of epidemiological studies have been performed to evaluate the associations between allelic variations in KLOTHO gene and the etiology of aging-related diseases. Greater than 10 single nucleotide polymorphisms (SNPs) have been identified in the human KLOTHO gene, and studies have evaluated whether KLOTHO gene mutations, such as G-395 A/rs1207568, F352 V/rs9536314 and C1818 T, are associated with arterial cardiovascular diseases (CVDs) (Donate-Correa et al., 2016; Jo et al., 2009; Kim et al., 2006), glucose metabolism (Slominski et al., 2018; Shimoyama et al., 2009a; Rhee et al., 2006a), bone mineral density (Shimoyama et al., 2009a; Riancho et al., 2007; Mullin et al., 2005), renal stones (Gurel et al., 2016; Ali et al., 2017; Telci et al., 2011), various cancers (Fathi et al., 2018; Liu et al., 2015; Kim et al., 2014; Laitman et al., 2012; Wolf et al., 2010), cognitive impairment among the elderly (Abulizi et al., 2017; Hao et al., 2016) and longevity (Invidia et al., 2010; Novelli et al., 2008; Arking et al., 2002a). However, inconsistences in findings from previous case-control studies raised concerns regarding the close associations between KLOTHO gene polymorphisms and the susceptibility to aging-related diseases and longevity. The controversial results from those individual studies may be related to the limited sample size or ethnic differences in the population being investigated. Therefore, the current meta-analysis was performed to quantify the potential between-study heterogeneity and provide pooled results from all eligible studies.

2. Materials and methods

2.1. Data sources and search

PubMed, Cochrane Library, Web of Science and EMBASE were searched to identify relevant studies published up to September 2018. The search was conducted independently by two authors with the following search terms: Klotho AND polymorphism/SNPs/variants/mutation AND gene. We also screened the reference lists of the included studies to identify potential genetic studies.

2.2. Study selection, quality assessment and data extraction

Articles were selected according to the criteria based on Preferred Reporting Items for Systematic Reviews and Meta-analysis criteria (PRISMA) guidelines (Liberati et al., 2009). Inclusion criteria for the genetic studies that were ultimately selected were: (1) the language of full-text article was limited to English; (2) case–control studies; (3) genotype frequencies or distributions were reported in detail; and (4) a sufficient number of homogenous studies (a minimum of three studies) was pooled for the meta-analysis. Scores of the Newcastle-Ottawa Scale (NOS) ranging from 0 to 9 points were employed to assess the methodological quality of case-control studies (Stang, 2010). A score of ≤ 3 points was judged as low quality, 4–5 points as moderate quality and ≥ 6 points as high quality. The following data were extracted from each included study: the first author's name, year of publication, ethnicity, source of controls, age and gender of patients and controls, and genotype of subjects. Article selection, quality assessment and data extraction were performed independently by two authors, and any discrepancies were resolved by discussion or by consulting a third author.

2.3. Data analysis

The effect of KLOTHO gene polymorphisms was estimated using the allele model, homozygote model, dominant model and recessive model. The χ^2 test was used to assess the HWE (Hardy-Weinberg equilibrium) of the genotype in the control group. Odds ratios (ORs) with 95% confidence intervals (CIs) were used to evaluate the associations between KLOTHO gene polymorphisms and various parameters. Heterogeneity among studies was assessed using the inconsistency (I^2) and χ^2 statistic (Higgins and Thompson, 2002). An I^2 value > 50% or P value < 0.10 was considered significant heterogeneity among the included studies. When significant heterogeneity was not detected, pooled estimates were calculated with the fixed-effect model (Mantel-Haenszel method) (Mantel and Haenszel, 1959). Otherwise, the random-effect model (DerSimonian-Laird method) (DerSimonian and Laird, 1986) was used to address the significant heterogeneity and obtain a relatively conservative result, and a sensitivity analysis was employed to explore the reliability of the results by omitting a specific study from each calculation. The significance of pooled ORs was determined using the Z test (Zaykin, 2011), and a p value less than 0.05 was considered statistically significant. Potential publication bias was explored by constructing Begg's funnel plot and performing Egger's linear regression test (Egger et al., 1997). All data were analyzed using Review Manager software (RevMan v.5.3, Cochrane Collaboration, Oxford, UK) and STATA 12.0 software (Stata Corp LP, College Station, TX, USA).

3. Results

As illustrated in the diagram of the selection processes (Fig. 1), 14 publications of a total of 29 case-control studies were eligible for the meta-analysis. We assessed the associations between two polymorphisms in KLOTHO gene (G-395 A/rs1207568 and F352 V/ rs9536314) and five parameters (urolithiasis, cognitive impairment, cardiovascular disease, cancer, and longevity) in the current meta-analysis. The main characteristics of included studies are presented in Tables 1 and 2. The methodological quality of all included studies was judged to be high quality (Supplementary Table 1). Additionally, 2 studies were not consistent with HWE (Telci et al., 2011; Imamura et al., 2006).

3.1. Quantitative synthesis

3.1.1. G-395A-Urolithiasis

Three case-control studies with 339 patients (males/females: 209/130) and 247 controls (males/females: 141/106) were eligible for the quantitative synthesis (Gurel et al., 2016; Ali et al., 2017; Telci et al.,

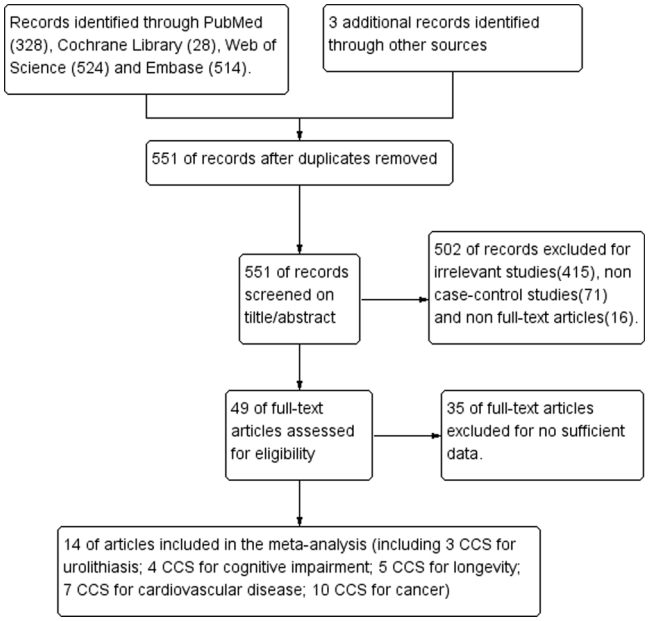

Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-analysis flow chart of the study selection process.

Table 1
Baseline characteristics of included studies for G395 A polymorphisms.

Diseases	References	Ethnicity	SC	Case					Control					HWE
				Age**	Gender(M/F)	Genotype			Age**	Gender(M/F)	Genotype			
						GG	GA	AA			GG	GA	AA	
Diseases Urolithiasis	Telci 2011	Turkish	HB	39.6 ± 8	74/34	63	41	4	37.7 ± 9	24/27	19	31	1	N
	Gurel 2016	Turkish	HB	48	52/51	54	45	4	52	51/51	32	68	2	Y
	Ali 2017	Chinese(Uygur)	PB	43 ± 10	83/45	74	46	8	46 ± 9	66/28	61	23	10	Y
Cognitive impairment	Kim 2006	Korean	HB	67.4	114/93	140	61	6	68.3	237/218	325	118	12	Y
	Hao 2016	Chinese	PB	93.6 ± 3.1	113/363	330	140	6	93.2 ± 3.3	119/117	148	80	8	Y
	Abulizi(A) 2017*	Chinese(Uygur)	PB	75.1 ± 6.9	162/161	97	62	7	74.7 ± 6.2	166/177	120	49	3	Y
	Abulizi(B) 2017*	Chinese(Han)	PB			108	43	6			126	43	2	
Cardiovascular diseases	Kim(S) 2006	Korean	HB	64.6	165/128	193	87	13	68.3	237/218	325	118	12	Y
	Kim(LAA)2006	Korean	HB	66.5	54/42	70	23	3		237/218	325	118	12	
	Kim(SVO) 2006	Korean	HB	65.0	67/45	72	38	2		237/218	325	118	12	
	Imamura(CAD)2006	Japanese	PB	63.5 ± 9.8	150/47	138	59	0	53.6 ± 11.4	238/93	268	62	1	N
	Imamura(VSA) 2006	Japanese	PB	58.5 ± 11.2	50/27	59	18	0		238/93	268	62	1	
	Jo(CAD) 2006	Korean	HB	64.1 ± 11.5	139/86	157	60	8	59.3 ± 12.1	103/106	164	39	6	Y
	Rhee(CAD) 2006	Korean	PB	61.0	NA	116	33	6	55.3	NA	87	28	4	Y

CAD = coronary heart disease; HB = hospital based; HWE = Hardy Weinberg equilibrium; LAA = large artery atherosclerosis; N = no; NA = not available; PB = population based; S = stroke; SC = source of control; VSA = vasospastic angina; Y = Yes.
* Two different studies were marked with 'A' and 'B' respectively.
** age was presented as means and standard deviation.

2011). Briefly, all studies were performed in Asian countries, and the age was comparable between the case and control groups (Table 1). The control groups of two studies were HB (hospital-based); one was PB (population-based). Based on the pooled results, the G allele of the G-395 A polymorphism conferred a significantly higher risk of urolithiasis (G vs. A; OR = 1.31, 95% CI = 1.01–1.71; P = 0.04), but no significant association was noted in the homozygote, dominant and recessive models (Table 3 and supplementary Fig. 1).

3.1.2. G-395A-Cognitive impairment

Four case-control studies with 1006 patients (males/females: 389/617) and 1034 controls (males/females: 582/452) were included in the quantitative synthesis (Table 1) (Kim et al., 2006; Abulizi et al., 2017; Hao et al., 2016). All studies were performed in Asian countries, and the ages of patients and controls were similar. Additionally, 3 studies were HB and one study was PB. Overall, no significant association between the G-395 A polymorphism and cognitive impairment among the elderly was observed (Table 3 and Supplementary Fig. 2).

3.1.3. G-395A-Cardiovascular diseases

Seven case-control studies with 1155 patients and 2355 controls were eligible for the quantitative synthesis (Table 1) (Jo et al., 2009; Kim et al., 2006; Imamura et al., 2006; Rhee et al., 2006b). Because of data availability, the cardiovascular diseases investigated in this study only included coronary heart disease, large artery atherosclerosis, stroke, and vasospastic angina, and we are unable to compare the genders and ages of patients and controls. Additionally, all studies were performed in Asian countries; 4 studies were HB and 3 studies were PB. According to the results of the meta-analysis, the G-395 A polymorphism was related to the susceptibility to CVDs under the allele model (G vs. A; OR = 0.78, 95% CI = 0.68-0.90; P < 0.001), dominant model (GG + GA vs. AA; OR = 0.55, 95% CI = 0.36-0.85; P = 0.007) and recessive model (GG vs. GA + AA; OR = 0.75, 95% CI = 0.64-0.89; P < 0.001). Thus, individuals with the G allele had a

Table 2
Baseline characteristics of included studies for F352 V polymorphisms.

Diseases/ Parameters	References	BRCA mutation	Ethnicity	SC	Case					Control					HWE
					Age** (year)	Gender (M/F)	Genotype			Age** (year)	Gender (M/F)	Genotype			
							FF	FV	VV			FF	FV	VV	
Breast cancer	Wolf(A) 2010*	None	Jewish	HB	50	–	56	31	7	51	–	61	42	6	Y
	Wolf(B) 2010*	BRCA1	Jewish	HB	45	–	158	72	16	38	–	195	80	15	
	Wolf(C) 2010*	BRCA2	Jewish	HB	46	–	9	82	25	43	–	16	64	21	
	Laitman(A) 2012*	BRCA1	Mixed	PB	39	–	2160	775	85	42	–	1941	701	79	Y
	Laitman(B) 2012*	BRCA2	Mixed	PB	43	–	909	504	79	43	–	1201	589	60	
Ovarian cancer	Wolf(D) 2010*	None	Jewish	HB	57	–	21	9	3	51	–	61	42	6	Y
	Wolf(E) 2010*	BRCA1	Jewish	HB	51	–	60	28	7	38	–	195	80	15	
	Wolf(F) 2010*	BRCA2	Jewish	HB	62	–	1	24	6	43	–	16	64	21	
	Laitman(C) 2012*	BRCA1	Mixed	PB	39	–	512	170	23	42	–	1447	536	36	Y
	Laitman(D) 2012*	BRCA2	Mixed	PB	43	–	125	69	13	43	–	787	435	64	
Longevity	Arking(A) 2002*	–	Bohemian Czech	HB	> 75	NA	308	103	4	newborn	NA	307	73	10	Y
	Arking(B) 2002*	–	Baltimore Caucasian	HB	> 75	NA	530	185	8	newborn	NA	309	100	11	
	Arking(C) 2002*	–	Baltimore African American	HB	> 75	NA	169	68	5	newborn	NA	156	58	12	
	Novelli 2008	–	US Caucasian	HB	93-111	273/435	517	170	21	0-35	164/168	241	85	6	Y
	Invidia 2010	–	Italian	HB	> 66	229/297	439	174	13	< 66	191/272	348	103	12	Y

HB = hospital based; HWE = Hardy Weinberg equilibrium; NA = not available; PB = population based; SC = source of control; Y = Yes.
* Different studies were marked with Arabic alphabet respectively.
** age was presented as means and standard deviation or ranges.

Table 3
Pooled results of associations between Klotho polymorphisms and parameters.

SNP-Parameters	Comparison	No of studies	Heterogeneity (P)	Statistical method	OR (95% CI)	P Value
G395A-Urolithiasis	G/A	3	0.16	OR (M-H, Fixed)	1.31 [1.01, 1.71]	0.04
	GG/AA	3	0.79	OR (M-H, Fixed)	1.23 [0.56, 2.72]	0.60
	GG + GA/AA	3	0.33	OR (M-H, Fixed)	1.13 [0.53, 2.41]	0.76
	GG/GA + AA	3	0.005	OR (IV, Random)	1.60 [0.72, 3.54]	0.25
G395A-Cognitive impairment	G/A	4	0.01	OR (IV, Random)	0.88 [0.63, 1.23]	0.46
	GG/AA	4	0.05	OR (IV, Random)	0.85 [0.32, 2.20]	0.73
	GG + GA/AA	4	0.06	OR (IV, Random)	0.83 [0.32, 2.16]	0.70
	GG/GA + AA	4	0.08	OR (IV, Random)	0.86 [0.64, 1.16]	0.32
G395A-Cardiovascular diseases	G/A	7	0.50	OR (M-H, Fixed)	0.78 [0.68, 0.90]	< 0.001
	GG/AA	7	0.96	OR (M-H, Fixed)	0.75 [0.47, 1.21]	0.24
	GG + GA/AA	7	0.38	OR (M-H, Fixed)	0.55 [0.36, 0.85]	0.007
	GG/GA + AA	7	0.34	OR (M-H, Fixed)	0.75 [0.64, 0.89]	< 0.001
F352V-Neoplasm	F/V	10	0.53	OR (M-H, Fixed)	0.93 [0.88, 0.99]	0.03
	FF/VV	10	0.38	OR (M-H, Fixed)	0.73 [0.61, 0.88]	< 0.001
	FF + FV/VV	10	0.50	OR (M-H, Fixed)	0.78 [0.65, 0.93]	0.005
	FF/FV + VV	10	0.21	OR (M-H, Fixed)	0.95 [0.88, 1.02]	0.16
F352V-Breast cancer	F/V	5	0.15	OR (M-H, Fixed)	0.93 [0.86, 1.00]	0.04
	FF/VV	5	0.13	OR (M-H, Fixed)	0.77 [0.63, 0.95]	0.02
	FF + FV/VV	5	0.23	OR (M-H, Fixed)	0.82 [0.67, 1.00]	0.05
	FF/FV + VV	5	0.12	OR (M-H, Fixed)	0.93 [0.86, 1.02]	0.11
F352V-Ovarian cancer	F/V	5	0.88	OR (M-H, Fixed)	0.95 [0.84, 1.08]	0.82
	FF/VV	5	0.81	OR (M-H, Fixed)	0.62 [0.44, 0.88]	< 0.001
	FF + FV/VV	5	0.77	OR (M-H, Fixed)	0.68 [0.49, 0.95]	0.02
	FF/FV + VV	5	0.97	OR (M-H, Fixed)	1.00 [0.86, 1.16]	0.97
F352V-Neoplasm (BRCA1)	F/V	4	0.65	OR (M-H, Fixed)	0.98 [0.91, 1.07]	0.38
	FF/VV	4	0.22	OR (M-H, Fixed)	0.86 [0.67, 1.10]	0.22
	FF + FV/VV	4	0.20	OR (M-H, Fixed)	0.86 [0.67, 1.10]	0.22
	FF/FV + VV	4	0.72	OR (M-H, Fixed)	1.00 [0.91, 1.10]	0.97
F352V-Neoplasm (BRCA2)	F/V	4	0.50	OR (M-H, Fixed)	0.85 [0.77, 0.94]	< 0.001
	FF/VV	4	0.96	OR (M-H, Fixed)	0.59 [0.44, 0.78]	0.24
	FF + FV/VV	4	0.47	OR (M-H, Fixed)	0.71 [0.54, 0.92]	< 0.001
	FF/FV + VV	4	0.17	OR (M-H, Fixed)	0.84 [0.74, 0.95]	0.007
F352V-Longevity	F/V	5	0.51	OR (M-H, Fixed)	0.96 [0.85, 1.08]	0.48
	FF/VV	5	0.15	OR (M-H, Fixed)	1.47 [0.98, 2.20]	0.06
	FF + FV/VV	5	0.13	OR (M-H, Fixed)	1.51 [1.01, 2.26]	0.04
	FF/FV + VV	5	0.47	OR (M-H, Fixed)	0.90 [0.79, 1.04]	0.14

CI = Confidence interval; M-H = Mantel-Haenszel method; IV = Inverse variance; OR = Odds Ratio; SNP = Single-nucleotide polymorphism.

significantly lower CVD susceptibility. No significant association was noted in the homozygote model (Table 3 and Supplementary Fig. 3).

3.1.4. F352V-Neoplasms

Ten case-control studies with 6038 patients and 8876 controls were eligible for the meta-analysis, including 5 studies of breast cancer and 5 studies of ovarian cancer (Table 1) (Laitman et al., 2012; Wolf et al., 2010). Six studies were performed in a Jewish population and 4 studies in a population with mixed ethnicities. Additionally, the median age of the case group was slightly older than the control group. Compared to the control group, the pooled OR for overall neoplasms (breast and ovarian cancers) was 0.93 (95% CI = 0.88-0.99; P = 0.03) under the allele model, 0.73 (95% CI = 0.61-0.88; P < 0.001) under the homozygote model, 0.78 (95% CI = 0.65-0.93; P = 0.005) under the dominant model, and 0.95 (95% CI = 0.88–1.02; P = 0.16) under the recessive model (Supplementary Fig. 4). Based on the pooled results, healthy individuals with the F allele exhibited a significantly lower overall risk of developing neoplasms. Similarly, the analysis of subgroups stratified by neoplasm type revealed a significant association between the F352 V polymorphism and the susceptibility to breast cancer (Supplementary Fig. 5) under the homozygote model (FF vs. VV; OR = 0.77, 95% CI = 0.63-0.95; P = 0.02) and ovarian cancer (Supplementary Fig. 6) under the homozygote (FF vs. VV; OR = 0.62, 95% CI = 0.44-0.88; P < 0.001) and dominant models (FF vs. FV + VV; OR = 0.68, 95% CI = 0.49-0.95; P = 0.02). Interestingly, in the subgroup analysis stratified by BRCA1/2 mutation carriers, no significant association was observed between the F352 V polymorphism and the overall risk of developing neoplasms among individuals carrying the BRCA1 mutation using any of the models (Supplementary Fig. 7), while significant associations between the F352 V polymorphism and the overall risk of developing neoplasms were observed in carriers of the BRCA2 mutation using the allele, dominant and recessive models (F vs. V; OR = 0.85, 95% CI = 0.77-0.94; P < 0.001; FF + FV vs. VV; OR = 0.71, 95% CI = 0.54-0.92; P < 0.001; FF vs. FV + VV; OR = 0.84, 95% CI = 0.74-0.95; P = 0.007; Supplementary Fig. 8).

3.1.5. F352V-Longevity

Five case-control studies with 2714 patients and 1831 controls were eligible for the quantitative synthesis (Table 1) (Invidia et al., 2010; Novelli et al., 2008; Arking et al., 2002a). The control groups of all studies were HB. The ethnicity of included studies varied and included Bohemian Czech, Baltimore Caucasian, Baltimore African American, US Caucasian and Italian. Inconsistencies in the definition of the longevity group and the control group were noted in the included studies. The longevity group was defined as people over the age of 75 and 93 years in the studies by Arking et al. (2002a) and Novelli et al. (2008), respectively, and the control group was defined as newborns and people under the age of 35 in the studies by Arking et al. (2002a) and Novelli et al. (2008), respectively. Meanwhile, Invidia et al., 2010) relaxed the definition of longevity (aged greater than 66 years) and the control group (aged less than 66 years). We were unable to analyze the ages of the case and control groups because the data were unavailable. The pooled results did not detect statistically significant heterogeneity (P = 0.13) and showed that the F352 V polymorphism was associated with longevity under the dominant model (FF + FV vs. VV; OR = 1.51, 95% CI = 1.01–2.26; P = 0.04); no association was noted in the allele,

homozygote and recessive models (Table 3 and Supplementary Fig. 9).

3.2. Sensitivity analysis

A sensitivity analysis was performed by consecutively omitting each included study to evaluate the influence of each study on the pooled ORs, and statistical robustness was noted in all comparisons, except the associations between the G-395 A polymorphism and urolithiasis risk under the allele model (Supplementary Fig. 10D).

3.3. Publication Bias

No evidence of asymmetry was observed in funnel plots of all comparisons, indicating that all included studies had little publication bias (Supplementary Fig. 11).

4. Discussion

4.1. Summary of the findings

The current meta-analysis only systematically evaluated the associations of several aging-related diseases (urolithiasis, cognitive impairment, cardiovascular disease, and cancer) and longevity with the G-395 A and F352 V polymorphisms due to the limited number of studies focusing on the same SNP in the Klotho gene. The pooled results showed positive associations of G-395 A polymorphisms with urolithiasis and cardiovascular disease, positive associations of F352 V polymorphisms with cancers (breast and ovarian cancer) and longevity, and negative associations of G-395 A polymorphisms with cognitive impairment. According to the subgroup analyses, F352 V polymorphisms were associated with the overall risk of developing neoplasms among individuals carrying BRCA1 mutations, but not with individuals carrying BRCA2 mutations. According to the results of the sensitivity analysis, the associations between the G-395 A polymorphism and urolithiasis risk were unstable.

4.2. Interpretation of the findings

The G-395 A polymorphism is located in the promoter of the Klotho gene, and the allelic mutation results in the downregulation of Klotho expression (Nguyen Thi and Nong Van, 2018). Additionally, the F352 V polymorphism is one gene mutation associated with “KL-VS”, which is composed of 6 SNPs in perfect linkage disequilibrium, spanning from exon 2 to the flanking sequence, and the gene mutation alters protein function by influencing the trafficking and catalytic activity of Klotho (Arking et al., 2002b). Given the importance of the G-395 A and F352 V polymorphisms, the pooled results are expected to clarify the associations between Klotho polymorphisms and aging-related diseases in humans.

Urolithiasis is a common global health problem, and the incidence increases with age (Shoag et al., 2015; Issler et al., 2017). Based on the pooled results, urolithiasis was related to the G-395 A polymorphism. This finding may be explained by the mechanism by which Klotho regulates renal calcium reabsorption through PI3K signaling and the activation of the Ca^{2+} channels (Chang et al., 2005; Nguyen Thi and Nong Van, 2018), and hypercalciuria has been established as a risk factor for calcium-based stone formation. Among the included studies, Ali et al. did not report a significant association of the G-395 A polymorphism with renal stones (Ali et al., 2017), while significant associations were noted in other similar studies (Gurel et al., 2016; Telci et al., 2011). The inconsistency may be attributed to the ethnic differences in the population and limited sample sizes. Additionally, the meta-analysis also suggested that the susceptibility to CVDs was also closely associated with the G-395 A polymorphism, consistent with the results of most published case-control studies. Several mechanisms have been proposed to explain the protective effect of Klotho on the vascular system, including nitric oxide synthesis, the stimulation of calcitriol and the attenuation of oxidative stress (Liu et al., 2007). Furthermore, the pooled results suggested that individuals with the allele A had a significantly higher CVD susceptibility, consistent with the finding that carriers of the mutant A allele exhibited lower Klotho expression in the vasculature (Donate-Correa et al., 2016). Interestingly, a previous meta-analysis based on cohort studies suggested that renal stones increase the cardiovascular risk (Liu et al., 2014). The current meta-analysis also showed close associations between both urolithiasis and CVDs with the Klotho G-395 A polymorphism, suggesting that Klotho may be the potential link between kidney stones and CVDs.

Klotho is also identified as an anti-cancer protein. Klotho functions as a circulating hormone that plays an important role in inhibiting the growth, migration and proliferation of cancer cells by regulating various signal transduction pathways, such as the IGF-1, FGF, and Wnt/β-catenin signaling pathways (Mencke et al., 2017; Xu and Sun, 2015; Lee et al., 2010; Wolf et al., 2008). Klotho is also involved in immune evasion to inhibit tumor cell migration (Shu et al., 2013). Additionally, in contrast to the protective role of suicidal death in normal cells, Klotho induces the apoptosis of cancer cells (Nguyen Thi and Nong Van, 2018). Therefore, based on accumulating evidence, alterations in Klotho expression are one of the major risk factors for cancers. Genetic mutations in the Klotho gene have been reported to be involved in the pathogenesis of various cancers, such as cervical (Lee et al., 2010), hepatocellular (Shu et al., 2013), colorectal (Liu et al., 2015), breast, ovarian (Wolf et al., 2010) and prostate (Kim et al., 2014) cancers, but inconsistent conclusions regarding the association between klotho gene polymorphisms and cancer risk have been reported. For example, Kim et al. analyzed 25 SNPs in the Klotho gene and found that the C1548 T polymorphism was a risk factor for the development of prostate cancer (Kim et al., 2014). Liu et al. reported significant associations between the AA and GA genotypes of the G-395 A polymorphism with a higher risk of colorectal cancer (Liu et al., 2015), while other similar studies reported null associations between colorectal cancer risk and the G-395 A and 1818 T polymorphisms (Fathi et al., 2018). Similarly, Wolf et al. reported associations between KL−VS and increased susceptibility to breast and ovarian cancer, while null associations were reported in another similar study (Laitman et al., 2012). Due to limited number of published studies investigating the same SNP, the current meta-analysis only included studies investigating the associations between the F352 V polymorphism and breast and ovarian cancer. According to the pooled results, healthy individuals carrying the F allele exhibited a lower overall risk of cancer. Interestingly, the subgroup analysis revealed and association between the F352 V polymorphism with the overall risk of neoplasms in BRCA1 mutation carriers, but not in BRCA2 mutation carriers. The results indicated a potential combination and interaction with other nucleotide polymorphisms that together exert an overall effect on the susceptibility to cancer. Further research is still needed, and Klotho may be a potential therapeutic target for the treatment of various neoplasms.

Klotho was also identified as a pleiotropic protein that enhances cognitive functions (Dubal et al., 2015; Yokoyama et al., 2015). The serum klotho levels were reported to be inversely correlated with cognitive impairment (Shardell et al., 2016), and a higher serum concentration of Klotho correlated with measures of greater intrinsic connectivity in the front parietal and default mode networks (Yokoyama et al., 2017). Moreover, the level of secreted Klotho decreased with the increasing age, and a noticeable reduction was observed in older adults with Alzheimer’s disease (Semba et al., 2014). KL-VS heterozygosity was shown to be associated with increased serum Klotho levels and conferred a lower susceptibility to dementia (Arking et al., 2002a). However, inconsistences were noted in studies evaluating the association between the G-395 A polymorphism and cognitive impairment. The G-395 A polymorphism is associated with cognitive impairment in the Uygur population, but not in the Han population (Abulizi et al., 2017). As shown in the study by Hao et al., Chinese individuals with the

GA + AA genotype had a significantly lower risk of cognitive impairment (Hao et al., 2016), while no association was noted in Korean individuals (Kim et al., 2006). According to the pooled results, we did not identify an association between the G-395 A polymorphism and cognitive impairment in the elderly, and further studies are still required.

Klotho gene polymorphisms have also been reported to be associated with other features of the aging process in human. For example, lipid metabolism, glucose metabolism and bone mineral density are associated with Klotho gene polymorphisms such as G-395 A, F352 V, and C1818 T (Shimoyama et al., 2009a; Rhee et al., 2006a; Riancho et al., 2007; Kawano et al., 2002; Ogata et al., 2002; Shimoyama et al., 2009b; Freathy et al., 2006). Based on accumulating evidence that Klotho gene polymorphisms are strongly correlated with the healthy aging process, case-control studies have also been performed to determine whether gene polymorphisms correlate with human longevity, but inconsistencies were noted among those studies (Invidia et al., 2010; Novelli et al., 2008; Arking et al., 2002a; Majumdar et al., 2010). The current meta-analysis revealed an association between the F352 V polymorphism and longevity under the dominant model. Nonetheless, the data should be interpreted with caution due to the risks of pooling the association between gene polymorphisms and longevity (Sebastiani et al., 2017). First, the clarification of the real associations is challenging due to the rarity of individuals displaying extreme longevity and the requirement of huge sample sizes to achieve a forceful statistical power. Second, although no significant heterogeneity was noted in the current meta-analysis, the lack of a consistent definition for the longevity group and the control group may have biased estimates of the positive correlation between gene polymorphisms and longevity. Third, the current study included populations of mixed ethnicities, which is a non-negligible genetic confounder that may affect the prevalence of the allele, but we were unable to perform a subgroup analysis stratified by ethnicity because the data were not available. Fourth, the optimal source of controls should be matched with newborns to decrease the secular effect of unmeasurable factors, such as the gene-environment interaction.

4.3. Study strengths and limitations

To our knowledge, the current meta-analysis represents the most comprehensive evaluation of the associations between previously investigated Klotho gene variants and aging-related diseases. Nevertheless, to a certain extent, some limitations should be emphasized when interpreting the data. (1) The prevalence of allele may be diverse in groups with different ethnicities, and most populations included in this meta-analysis were Asian; therefore, the generalization of those positive associations, such as G-395 A and urolithiasis and G-395 A and CVDs, are difficult and premature, and further studies using populations of various ethnicities are needed. (2) The number of included studies in several groups was relatively small, with limited statistical power to evaluate the real correlation. (3) We were unable to calculate adjusted estimates in the current meta-analysis because insufficient data were available for adjustment for other covariates, such as ethnicity, age, gene-gene interactions, and gene-environment interactions, among others. (4) We were unable to quantitatively synthesize other Klotho SNPs due to the limited number of published studies.

4.4. Perspectives

The current study reinforced the evidence that Klotho gene polymorphisms play a vital role in healthy aging in humans. Motivated by this observation, the identification of alleles of Klotho gene that confer an inherited risk of developing multiple aging-related diseases may yield critical insights into their roles in healthy aging. Further studies of the underlying molecular mechanisms of Klotho-mediated pathways will enable the development of strategies based on Klotho to become a promising treatment for various aging-related disorders. The meta-analysis also showed that a mutation in the same allele of Klotho led to multiple aging-related disorders, such as urolithiasis and CVDs. Combined with the findings other studies, our data may strengthen the findings that circulating Klotho may function as a hormone to play an extensive role in tissues with little or no expression of Klotho. However, the binding sites or the receptors for Klotho have not been identified (Mencke et al., 2017; Kuro, 2018). Therefore, receptors for circulating Klotho must be identified and characterized, and its downstream signaling pathway must be clarified.

5. Conclusions

In conjunction with other studies, the current meta-analysis indicated that Klotho G-395 A polymorphisms were associated with urolithiasis and cardiovascular disease, but not with cognitive impairment. Additionally, Klotho F352 V polymorphisms were associated with cancers and longevity. Considering the limitations of this study, additional well-designed studies are warranted to strengthen those findings; further investigations using the genetic and environmental interaction model that are designed to determine how Klotho contributes to multiple aging-related disorders in humans are expected.

Funding

This study was supported by grants from the National Natural Science Foundation of China (81770705), the Nature Science Foundation of Hunan province, China (2017JJ2395), and Key Research and Development Projects of Hunan Science and Technology Department (2016JC2041).

Conflict of interest

The authors have no conflicts of interest to declare.

Acknowledgements

We are grateful for the funding provided by the National Natural Science Foundation of China 81770705 (to Hequn Chen), the Nature Science Foundation of Hunan province, China 2017JJ2395 (to Yang Li), and Key Research and Development Projects of Hunan Science and Technology Department 2016JC2041 (to Hequn Chen).

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.mad.2018.12.003.

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