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Original article

Long non-coding RNAs H19 and NKILA are associated with the risk of death and lacunar stroke in the elderly population

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ABSTRACT

Introduction: Differential expression of long non-coding RNAs (lncRNAs) is a hallmark of cardiovascular aging, cerebrovascular diseases, and neurodegenerative disorders. This research article investigates the association between a panel of lncRNAs and the risk of death and ischemic stroke in a cohort of non-institutionalized elderly subjects.

Method: A total of 361 healthy individuals aged 75 years old, prospectively recruited in the Vienna Transdanube Aging (VITA) cohort, were included. Expression of lncRNAs at baseline was assessed using quantitative polymerase chain reaction PCR with pre-amplification reaction, using 18S for normalization. The primary endpoint was all-cause mortality; the secondary endpoint was the incidence of new ischemic brain lesions. Death was assessed over a 14-year follow-up, and ischemic brain lesions were evaluated by magnetic resonance imaging (MRI) over a 90-month follow-up. Ischemic brain lesions were divided into large brain infarcts (Ø≥ 1.5 cm) or lacunes (Ø< 1.5 cm)

Results: The primary endpoint occurred in 53.5 % of the study population. The incidence of the secondary endpoint was 16 %, with a 3.3 % being large brain infarcts, and a 12.7 % lacunes.

After adjustment for potential confounders, the lncRNA H19 predicted the incidence of the primary endpoint (HR 1.194, 95 % C.I. 1.012–1.409, $p = 0.036$), whereas the lncRNA NKILA was associated with lacunar stroke (HR 0.571, 95 % C.I. 0.375–0.868, $p = 0.006$).

Conclusion: In a prospective cohort of non-institutionalized elderly subjects, high levels of lncRNA H19 are associated with a higher risk of death, while low levels of lncRNA NKILA predict an increased risk of lacunar stroke.

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1. Introduction

Aging is a multifaceted biological process characterized by a progressive decline in physiological function and increased vulnerability to various diseases [1]. It is associated with multiple cellular and molecular changes, including genomic instability, cellular senescence, mitochondrial dysfunction, and impaired repair mechanisms [2].

In the cardiovascular system, aging is characterized by a large variety of detrimental processes, such as arterial stiffening, accelerated atherosclerosis, endothelial dysfunction, pro-thrombotic state, heart failure with preserved ejection fraction, and calcific aortic valve degeneration [3–6]. As a result, aging is an independent risk factor for the development of cardiovascular diseases (CVD), and CVD are the main cause of death and disability among the elderly [7].

Besides CVD, cognitive decline is another hallmark of aging [8]. Vascular dementia is the second most common cause of dementia after Alzheimer's disease, covering around 15 % of cases. Pathological findings in vascular dementia are various and include white matter lesions, large cortical ischemic lesions, micro-bleedings and lacunar stroke [9]. Lacunar strokes are defined as small sub-cortical ischemic lesions in the territory of the perforating arterioles, having a diameter of 3–15 mm, which are usually found upon neuroimaging in elderly people above 75 years old [10]. Multiple factors link aging to vascular dementia, including reduced autoregulation of the brain vasculature, atherosclerosis of the supra-aortic trunks, microvascular thrombosis, and hypercoagulability [11–13]. Compared to other forms of dementia, no specific pharmacological treatment is currently available for vascular dementia.

Long non-coding RNAs (lncRNAs) have emerged as crucial regulators of aging [14,15]. lncRNAs are a heterogeneous class of non-coding RNAs larger than 200 nucleotides, which participate in diverse biological functions, such as chromatin remodeling, transcriptional regulation, and post-transcriptional modification of proteins [16]. Over the past decade, they were associated with multiple physiological and pathological processes within the cardiovascular system [17] and the central nervous system [18]. In particular, the differential expression of lncRNAs seems to be a hallmark of cardiovascular aging, cerebrovascular diseases, and neurodegenerative disorders [19–21]. LncRNAs may drive the aging process by acting on multiple cellular processes. In this field, Grammatikakis et al. classified six different mechanisms of action for lncRNAs: control of telomere length, epigenetic modification of chromatin, control of proteostasis, modulation of stem cells function, regulation of senescence and proliferation, and regulation of intercellular communication [22].

Thus, accumulating evidence suggests that lncRNAs hold potential as predictive biomarkers and therapeutic targets for preventing and treating aging-related diseases, yet clinical evidence is still scarce. In particular, longitudinal cohort studies investigating the predictive role of lncRNAs are needed.

This study investigates the association between a panel of lncRNAs and the risk of death and ischemic stroke in a prospective longitudinal cohort of non-institutionalized subjects aged 75 years-old.

2. Methods

2.1. Study design and outcomes

The present study is a sub-analysis of the Vienna Transdanube Aging Study (VITA), a population-based cohort study involving non-institutionalized subjects aged 75 years old at the time of enrolment [23–26], and resident in the 21st and 22nd district of Vienna, Austria. Briefly, 1745 individuals were potentially eligible for inclusion; of them, 606 were actually included in the study. All included subjects gave written informed consent at the time of enrolment. All procedures have been approved by the local Ethics Committee of the Medical University of Vienna, Vienna, Austria. Later, subjects underwent a comprehensive clinical assessment at the Danube Hospital in Vienna, including medical history, blood collection, cognitive function assessment (Mini-mental state examination – MMSE) and brain magnetic resonance imaging (MRI) [27]. Serum samples for the measurement of circulating lncRNAs were available for 361 subjects (Fig. 1A).

The primary outcome of the study is death from any cause occurred during the 14 years follow up, from 2000 until 2013. Data were retrieved from the registry office of the city of Vienna (Fig. 1B).

The secondary outcome of the study was the incidence of new brain ischemic lesions detected by MRI. Subjects underwent scheduled MRI scans of the head at baseline and then every 30 months (2.5 years) over a time-lapse of 90 months (7.5 years) for a total of four MRI scans [23]. Adherence of subjects to the scheduled MRI plan is reported in Fig. 1C. The secondary outcome was further classified as large brain infarcts, indicating lesions with a diameter larger than 1.5 cm, and lacunes having a smaller size (Fig. 1B).

2.2. Selection of long non-coding RNAs

A systematic review of the existing literature was performed on pubmed.gov, entering the MeSH terms [noncoding RNA] AND one of the following MeSH terms: [arterial inflammation], [myocardial infarction], [anoxia ischemia, brain], [atherosclerosis]. The search retrieved 2454 results. Articles were then screened by two independent investigators: articles other than original research articles and articles only focusing on micro-RNAs were excluded. Finally, after the exclusion of duplicate results, a total of 28 lncRNAs were selected for outcome profiling (Supplementary Table 1).

Amplification of these lncRNAs was tested in a subset of 55 randomly chosen samples. A total of 22 lncRNAs were detected sporadically at levels below the PCR detection threshold. An acceptable level of amplification ($\leq$30 Ct) was observed for six lncRNAs (H19, MALAT1, GAS5, NEAT1, PACER, and NKILA), which were then measured in the whole cohort. To avoid any missing data bias, only those detectable in more than 40 % of all samples were then selected for statistical analysis (H19, GAS5, and NKILA – Supplementary Fig. 1).

2.3. Long non-coding RNAs measurement

Serum samples were stored at -80 °C until RNA extraction. Total RNA was isolated from 50 μL of serum using miRNeasy Serum/Plasma kit (Qiagen, Hombrechtikon, Switzerland) according to the manufacturer's instructions. Quality control of RNA samples was performed using a microplate reader Infinite® 200 PRO (TECAN, Tecan Trading AG, Männedorf, Switzerland).

Extracted RNA was reverse transcribed into cDNA and pre-amplified using the RT2 PreAMP cDNA Synthesis Kit (Qiagen), according to the manufacturer's instructions.

This includes an initial genomic DNA elimination step, isothermal cDNA conversion, and a pre-amplification program consisting of 14 cycles on a device using a GeneAmp® PCR System 2700 (Applied Biosystems, USA). We mixed RT2 PreAmp PCR master mix and RT2 lncRNA PreAmp mix of specific primers (TaqMan Assays); then, we added 2.5 μl of cDNA to improve the detection of low copy number lncRNAs [24]. The pre-amplified cDNA was then used to assess the predefined lncRNAs.

LncRNA-specific RT2qPCR Primers (TaqMan Assays) were employed (Supplementary Table 2). Quantitative real-time polymerase chain reaction (RT-qPCR) was carried out at 95 °C for 10 min and in 40 cycles at 95 °C for 15 s and 60 °C for 60 s on an Applied Biosystems 7500 fast real-time PCR device (Applied Biosystems, Darmstadt, Germany). The threshold cycle value (Ct) for each amplification product was determined. The relative expression levels of each lncRNAs were calculated using the $2-\Delta CT$ method and normalized using the 18S as the reference gene.

2.4. Statistical analysis

The statistical analysis was conducted using the SPSS software package, version 29 (IBM, Armonk, NY), GraphPad Prism 8 (GraphPad Software Inc, Boston, MA) and R 4.1.2 (URL http://www. R- project. Org). Discrete variables are summarized as counts (percentages), and continuous variables are shown as medians (interquartile ranges [IQR]). Student's *t*-test, Fisher's exact test and Mann–Whitney U test were used for two-group comparisons, as appropriate. Comparison of repeated measures was performed using 2-way ANOVA with Sidak's correction for multiple comparisons. Data distribution was visually assessed using Q-Q plots. Data points of lncRNAs below the 5th percentile or above the 95th were considered outliers and excluded from the analysis. Data were log-transformed (base 2) to normalize variables with skewed distributions. A two-tailed *p*-value <0.05 under the assumption of the null hypothesis was considered statistically significant. Optimal out-of-sample cut-offs were derived using Youden`s index and applied to Kaplan-Meier survival curves with Log-rank test.

Proportional hazards assumption was tested using Schoenfeld residuals. Results of univariable and multivariable Cox proportional regression models are reported as hazard ratios (HR), along with their 95 % confidence interval (95 % C.I.) and p-value. Multivariable models were built to include parameters significantly associated with the study endpoints. In case of multiple correlated parameters significantly associated with the study endpoint, the variable with the tightest association was chosen to prevent co-linearity effect. Goodness-of-fit was tested as likelihood ratio and internal validity was tested by bootstrapping on 5′000 random samples. Potential determinants of circulating lncRNAs were analyzed by Spearman's rank correlation. A single-mediator mediation model was built using the "mediation" package in R. The effect of the independent variable on the mediator and the effect of the mediator on the outcome were estimated by generalized linear model. Average Causal Mediated Effect (ACME) and Average Direct Effect (ADE) were estimated by a Monte Carlo simulation on 1′000 random samples [28].

3. Results

3.1. Cohort characteristics

The baseline characteristics of the VITA study participants have already been described in previous publications [23–26]. The current sub-analysis was performed in 361 subjects (59.6 % of the total population initially enrolled), who were not significantly different from the overall study population in terms of baseline characteristics and endpoint incidence, except for baseline glucose profile, which translates into a higher prevalence of diabetes and pre-diabetes in the sub-group where measurements were performed (Supplementary Table 3).

3.2. H19 predicts all-cause mortality

One-hundred ninety-three subjects (53.5 %) died along the 14-year follow-up. Subjects meeting the primary endpoint had higher levels of H19; besides, they were more often males, had higher prevalence of diabetes mellitus (DM), atherosclerotic cardiovascular diseases (coronary artery disease and peripheral artery disease), and ischemic brain lesions at baseline MRI scan (Table 1). No significant difference was observed in the circulating levels of GAS5 and NKILA.

The estimated cut-off for H19 was 1.3×10^{-4}, approximately corresponding to the 44th percentile. After dividing the population by this cut-off, the Kaplan-Meier plot showed that subjects with higher circulating levels of H19 had a significantly reduced survival (median survival: 10.3 years, IQR [6.5–12.3] vs 11.7 years, IQR [8.9–12.9]; Fig. 2A). Overall, subjects with higher levels of H19 had a 36.3 % increased risk of dying over the 14 years of follow-up.

The Cox proportional regression model showed that H19 is an independent predictor of mortality (Fig. 2B). The model included sex, DM, coronary artery disease and the MMSE at baseline. After bootstrapping, the model had a significant internal validity (Supplementary Table 4).

3.3. NKILA predicts the incidence of lacunar ischemic lesions

The secondary endpoint was met by 57 subjects (16 %). None of the analyzed lncRNAs was significantly associated with this endpoint. Forty-six subjects (12.7 %) reported a new lacunar ischemic lesion upon MRI scan along the 7.5-year follow-up and displayed significantly lower levels of NKILA (Table 2). No significant difference was observed in the circulating levels of GAS5 and H19.

The estimated cut-off for NKILA was 4.1×10^{-5}, approximately corresponding to the 23rd percentile. After dividing the population by this cut-off, the Kaplan-Meier plot showed that subjects with lower circulating levels of NKILA had a significantly increased risk of lacunar stroke (Fig. 3A). Overall, subjects with lower levels of NKILA had a 28.5 % increased risk of lacunar stroke over the 7.5 years of follow-up.

The Cox proportional regression model confirmed that NKILA is a protective factor towards the incidence of new lacunar lesions, independent of the presence of lacunes at the baseline MRI scan (Fig. 3B), which remained significant following a resampling approach (Supplementary Table 3). Consistently, the subjects with lower levels of NKILA had also a faster decline in cognitive function, with a significant difference after 7.5 years of follow-up (Supplementary Figure 2).

3.4. Potential determinants of lncRNAs

Potential determinants of lncRNAs are reported in Supplementary Table 5. Both NKILA and H19 significantly correlate with sex. None of the other analyzed variables was significantly correlated to these lncRNAs. The mediation analysis confirmed the mediator role of H19 between sex and mortality (Fig. 4).

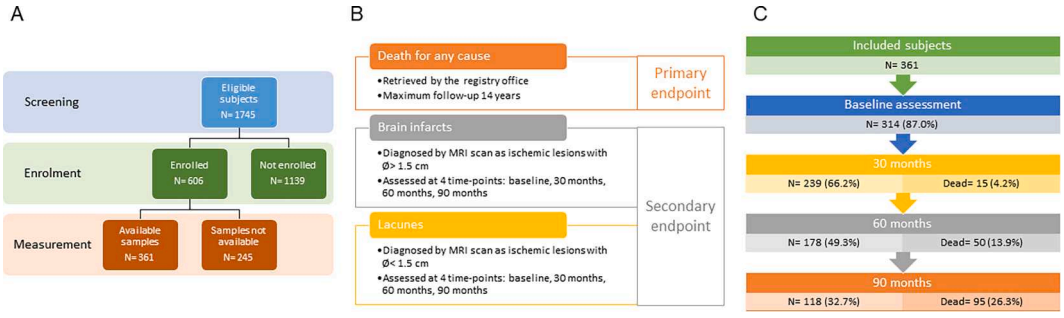

Fig. 1. Design of the study. A. Flow-chart of the enrolment. B. Definition of the study endpoints. C. Adherence of the enrolled subjects to the schedule of magnetic resonance imaging (MRI) scans. Each block reports the number of subjects undergoing MRI scan in a specific time point. In the right side of each block, the number of dead subjects is reported.

Table 1
Baseline characteristics of enrolled subjects, meeting and not meeting the primary study endpoint (death for any cause).

Parameter	No ($n = 168$)	Yes ($n = 193$)	p
Female sex	**114 (67.9)**	**107 (55.4)**	**0.017**
Body mass index (kg/m^2)	26.8 [25.0 - 29.3]	26.5 [24.2 – 29.2]	0.224
Smokers (previous or current)	103 (62.8)	106 (55.2)	0.161
Medical history			
Diabetes mellitus	**21 (12.8)**	**51 (26.6)**	**0.001**
Systemic arterial hypertension	120 (73.6)	127 (69.0)	0.406
Myocardial infarction	**8 (4.9)**	**32 (16.7)**	**<0.001**
Coronary artery disease	**34 (20.7)**	**65 (33.9)**	**0.008**
Peripheral artery disease	**14 (8.6)**	**31 (16.2)**	**0.037**
Symptomatic stroke	14 (8.5)	25 (13.0)	0.233
MMSE	**28.1 (1.5)**	**27.6 (2.1)**	**0.005**
Medications			
Insulin	4 (2.4)	10 (5.2)	0.274
Oral anti diabetic	**13 (7.9)**	**33 (17.2)**	**0.021**
Anti-hypertensive	127 (77.4)	148 (77.1)	0.999
Lipid-lowering	42 (25.6)	45 (23.4)	0.711
Anti-platelets	57 (34.8)	74 (38.7)	0.443
Anticoagulants	10 (6.1)	18 (9.4)	0.324
Biochemical parameters			
FBG (mg/dL)	**101 [93 - 116]**	**106 [96 - 130]**	**0.002**
HbA1c (%)	5.7 [5.3 - 6.1]	5.7 [5.3 - 6.4]	0.519
Total cholesterol (mg/dL)	239 [199 - 269]	228 [195 - 263]	0.126
LDL-cholesterol (mg/dL)	148 [116 - 182]	144 [112 - 168]	0.217
HDL-cholesterol (mg/dL)	59 [50 - 69]	56 [46 - 66]	0.227
Triglycerides (mg/dL)	125 [91 - 160]	117 [86 - 161]	0.262
Lp(a) (g/L)	0.09 [0.09 - 0.17]	0.09 [0.09 - 0.19]	0.973
Fibrinogen (mg/dL)	371 [325 - 432]	386 [331 - 451]	0.211
C-reactive protein (mg/dL)	2.0 [1.0 - 6.0]	3.0 [1.0 - 5.0]	0.999
Creatinine (mg/dL)	1.1 [1.0 - 1.2]	1.1 [1.0 - 1.2]	0.986
MRI findings			
Brain ischemic lesions at baseline	**26 (17.3)**	**46 (27.7)**	**0.032**
Lacunes at baseline	21 (14.1)	36 (21.8)	0.081
Infarcts at baseline	**6 (4.0)**	**17 (10.2)**	**0.049**
lncRNAs			
NKILA (ΔΔCt)	7.7E-5 [3.7E-5 - 1.4E-4]	1.2E-4 [5.4E-5 - 1.9E-4]	0.064
GAS5 (ΔΔCt)	1.6E-5 [8.2E-6 - 3.1E-5]	1.4E-5 [7.8E-6 - 2.2E-5]	0.108
H19 (ΔΔCt)	**1.2E-4 [8.0E-5 - 2.1E-4]**	**1.5E-4 [1.0E-4 - 2.4E-4]**	**0.028**

FBG: fasting blood glucose; HbA1c: glycated hemoglobin; lncRNAs: long non-coding RNAs; MMSE: Mini-Mental State Examination; MRI: magnetic resonance imaging.

4. Discussion

Results from the present sub-analysis of a well-characterized cohort of non-institutionalized elderly subjects with long-term follow-up show for the first time that high circulating levels of the lncRNA H19 are associated with a higher all-cause mortality, whereas low circulating levels of the lncRNA NKILA are associated with a higher risk of lacunar stroke.

The current knowledge about the biological role of H19 largely originates from cancer research. In this field, H19 was found to promote carcinogenesis and cell growth in multiple cancer-derived cell lines [29]. Interestingly, it was also shown to confer resistance to hypoxia and cellular senescence [30]. H19 is an epigenetic regulator which represses gene expression by interacting with the methyl-CpG–binding domain protein 1 (MBD1). Together, they form a ribonucleoprotein complex (H19-MBD1) which recruits histone lysine methyltransferases and promotes chromatin condensation [31]. The cluster of genes down-regulated by H19 contains H19 itself and the insulin-like growth factor 2 (IGF2), therefore, high circulating levels of H19 can be interpreted as a loss of imprinting on this genetic cluster. Indeed, loss of imprinting within the IGF2-H19 locus is observed during aging, leading to enhanced expression of IGF2 and H19, and other genes in this locus which are associated with age-related diseases, including cancer [22, 32–34].

Experimental evidence suggests that H19 regulates glucose homeostasis, β cell function and body weight [35]. Low levels of H19 were associated with insulin resistance and type 2 diabetes mellitus [36,37]. Interestingly, according to the present study, diabetes mellitus is a strong predictor of mortality in the elderly. Still, it is independent of H19, suggesting that the association between H19 and lifespan is not mediated by glucose metabolism. These results are consistent with the results of a previous sub-analysis of the VITA study [26], showing no association between H19 and the risk of incident diabetes.

Finally, contradicting evidence was so far collected about the association between H19 and cardiovascular disorders, including atherosclerosis, coronary artery disease, myocardial infarction, cardiac hypertrophy, and ischemic stroke [38]. This heterogeneity of results can be explained by the cell-type specific effects of H19 in the cardiovascular system. In endothelial and vascular smooth muscle cells, H19 prevents apoptosis and promotes cell viability and proliferation [39,40]. In vascular smooth muscle cells, it also promotes migration and the switch towards a synthetic phenotype, two pivotal processes in age-related vascular remodeling and atherosclerotic plaque formation [40]. Consistently, H19 promotes also the pro-inflammatory phenotype in the macrophages, another pivotal player of atherosclerotic disease progression [41]. Upon an ischemic heart injury, H19 is quickly upregulated in myocardial fibroblasts: this is likely a compensatory mechanism

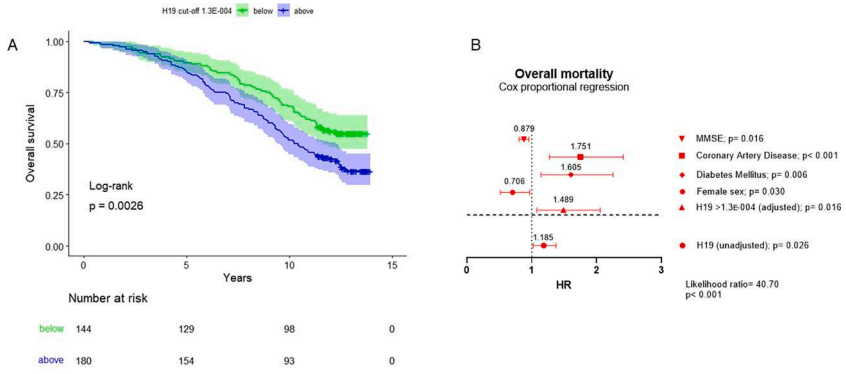

Fig. 2. H19 predicts all-cause mortality. A. Kaplan-Meier plot. Subjects are classified according to the cut-off of H19 derived by the Youden's index. B. Forest-plot of the Cox proportional regression. Hazard ratio in the unadjusted regression analysis is intended for doubling of H19. Hazard ratio of the MMSE in the adjusted model is intended for unit score increase. Significant predictors are highlighted with red bars. MMSE= Mini-Mental State Examination.

Table 2
Baseline characteristics of enrolled subjects, meeting and not meeting the secondary study endpoints (ischemic brain lesions at magnetic resonance imaging).

	Any MRI lesion			Lacunes			Infarcts		
Parameter	No ($n = 195$)	Yes ($n = 57$)	p	No ($n = 206$)	Yes ($n = 46$)	p	No ($n = 241$)	Yes ($n = 12$)	p
Female sex	121 (62.1)	37 (64.9)	0.757	127 (61.7)	31 (67.4)	0.504	152 (63.1)	7 (58.3)	0.766
Body mass index (kg/m^2)	26.9 [24.8–29.2]	27.1 [24.7–29.0]	0.952	27.0 [25.0–29.3]	26.4 [23.9–28.7]	0.197	**26.8 [24.6–29.1]**	**28.2 [26.7–32.5]**	**0.025**
Smokers (previous or current)	76 (40.0)	26 (45.6)	0.540	81 (40.3)	21 (45.7)	0.512	98 (41.5)	5 (41.7)	0.999
Medical history									
Diabetes mellitus	33 (17.4)	10 (17.5)	0.999	36 (17.9)	7 (15.2)	0.830	40 (16.9)	3 (25.0)	0.442
Systemic arterial hypertension	52 (28.3)	18 (31.6)	0.621	140 (71.8)	31 (67.4)	0.590	67 (29.1)	4 (33.3)	0.751
Myocardial infarction	20 (10.5)	5 (8.8)	0.806	21 (10.6)	4 (8.7)	0.999	24 (10.3)	1 (8.3)	0.999
Coronary artery disease	52 (28.6)	12 (21.4)	0.389	55 (28.5)	9 (20.0)	0.270	61 (26.8)	3 (27.3)	0.999
Peripheral artery disease	23 (12.1)	8 (14.0)	0.655	24 (11.9)	7 (15.2)	0.621	30 (12.7)	1 (8.3)	0.999
Symptomatic stroke	17 (8.9)	7 (12.3)	0.451	19 (9.5)	5 (10.9)	0.784	22 (9.3)	2 (19.7)	0.327
MMSE	27.8 (1.7)	28.3 (1.6)	0.057	27.9 (1.7)	28.1 (1.6)	0.456	**27.9 (1.7)**	**29.2 (0.9)**	**0.010**
Medications									
Insulin	9 (4.7)	2 (3.5)	0.999	9 (4.5)	2 (4.3)	0.999	11 (4.7)	0 (0.0)	0.999
Oral anti diabetic	23 (12.1)	6 (10.5)	0.999	25 (12.4)	4 (8.7)	0.616	27 (11.4)	2 (16.7)	0.637
Anti-hypertensive	141 (74.2)	42 (73.7)	0.999	150 (74.6)	33 (71.7)	0.711	174 (73.9)	9 (75.0)	0.999
Lipid-lowering	52 (27.4)	14 (24.6)	0.735	53 (26.4)	13 (28.3)	0.854	66 (28.0)	1 (8.3)	0.189
Anti-platelets	67 (35.3)	22 (38.6)	0.641	70 (34.8)	19 (41.3)	0.496	86 (36.4)	3 (25.0)	0.546
Anticoagulants	15 (7.6)	6 (10.5)	0.588	18 (9.0)	3 (6.5)	0.773	18 (7.6)	3 (25.0)	0.070
Biochemical parameters									
FBG (mg/dL)	103 [94–117]	101 [94–122]	0.862	103 [94–119]	101 [94–121]	0.491	102 [94–118]	105 [97–132]	0.381
HbA1c (%)	5.7 [5.3–6.2]	5.8 [5.4–6.2]	0.638	5.7 [5.3–6.2]	5.6 [5.4–6.1]	0.967	5.7 [5.3–6.2]	5.8 [5.3–6.8]	0.509
Total cholesterol (mg/dL)	235 [198–267]	232 [195–268]	0.980	235 [199–267]	227 [192–276]	0.643	233 [197–268]	240 [209–285]	0.473
LDL-cholesterol (mg/dL)	146 [115–175]	139 [112–186]	0.970	147 [117–178]	136 [108–184]	0.493	146 [113–179]	152 [120–204]	0.354
HDL-cholesterol (mg/dL)	57 [47–69]	57 [45–67]	0.699	57 [47–69]	61 [47–68]	0.619	57 [47–69]	56 [40–63]	0.176
Triglycerides (mg/dL)	116 [88–154]	134 [92–171]	0.210	117 [90–159]	84 [134–167]	0.597	117 [88–159]	137 [100–183]	0.241
Lp(a) (g/L)	0.09 [0.09–0.19]	0.09 [0.09–0.16]	0.374	0.09 [0.09–0.19]	0.09 [0.09–0.14]	0.225	0.09 [0.09–0.18]	0.12 [0.09–0.39]	0.343
Fibrinogen (mg/dL)	385 [330–438]	354 [322–413]	0.051	380 [330–438]	353 [322–414]	0.073	379 [328–437]	365 [325–423]	0.813
C-reactive protein (mg/dL)	3.0 [1.0–6.0]	2.0 [0.0–4.0]	0.194	3.0 [1.0–6.0]	2.0 [0.0–4.0]	0.317	3.0 [1.0–6.0]	2.0 [0.0–6.0]	0.483
Creatinine (mg/dL)	1.1 [0.9–1.2]	1.1 [1.0–1.2]	0.060	1.1 [1.0–1.2]	1.1 [1.0–1.2]	0.113	1.1 [1.0–1.2]	1.1 [0.9–1.4]	0.627
MRI findings									
Brain ischemic lesion at baseline	41 (21.4)	17 (30.4)	0.208	44 (21.7)	14 (31.1)	0.178	54 (22.9)	4 (33.3)	0.483
Lacunes at baseline	31 (16.3)	15 (26.8)	0.083	43 (18.4)	3 (25.0)	0.702	33 (16.4)	13 (28.9)	0.059
Infarcts at baseline	12 (6.3)	5 (8.9)	0.548	13 (6.4)	4 (8.9)	0.522	16 (6.8)	1 (8.3)	0.584
lncRNAs									
NKILA (ΔΔCt)	8.9E-5 [5.1E-5 - 1.6E-4]	6.5E-5 [2.9E-5 - 1.7E-4]	0.094	**8.9E-5 [5.1E-5 - 1.6E-4]**	**5.4E-5 [2.4E-5 - 1.4E-4]**	**0.006**	8.0E-5 [4.3E-5 - 1.5E-4]	1.5E-4 [5.8E-5 – 4.0E-4]	0.212
GAS5 (ΔΔCt)	1.6E-5 [8.3E-6 - 2.5E-5]	1.5E-5 [7.3E-6 - 2.5E-5]	0.568	1.6E-5 [83.3E-6 - 2.5E-5]	1.5E-5 [6.9E-6 - 2.5E-5]	0.498	1.6E-5 [7.9E-6 - 2.6E-5]	1.5E-5 [9.2E-6 – 3.0E-5]	0.832
H19 (ΔΔCt)	1.4E-4 [8.3E-5 - 2.4E-4]	1.5E-4 [1.0E-4 - 1.9E-4]	0.818	1.5E-4 [9.6E-5 – 2.4E-4]	1.4E-4 [1.0E-4 - 1.8E-4]	0.176	1.4E-4 [8.6E-5 - 2.3E-4]	1.5E-4 [1.2E-4 - 2.2E-4]	0.318

FBG: fasting blood glucose; HbA1c: glycated hemoglobin; lncRNAs: long non-coding RNAs; MMSE: Mini-Mental State Examination; MRI: magnetic resonance imaging.

to prevent the enlargement of the ischemic area, but it leads to later fibrosis [42]. As described above, the upregulation of H19 promotes a pro-inflammatory environment too, mainly led by resident macrophages [43]. Expression of H19 is reduced after reperfusion and in chronic ischemia, and this drop associates to a deterioration in cardiac function [36,44]. So, H19 seems to have a protective effect in myocardial ischemia, however it may also associate with a stronger progression to fibrosis. In this regard, different animal models of chronic heart injury displayed different roles for H19 in myocardial fibrosis. H19 is upregulated in pressure overload and adriamycin toxicity, whereas it is downregulated in metabolic cardiomyopathy, and it expression seems to be protective in pressure overload, whereas it is deleterious in adriamycin toxicity and metabolic cardiomyopathy [45–47]. In the present study, no association was observed between circulating levels H19 and the history of arterial hypertension or cardiovascular diseases. Concerning ischemic stroke, previous studies in murine models showed a reduction of the ischemic damage after H19 knock-down, likely due to a blunted inflammatory state [41,48]. Conversely, H19 seems to also have a pivotal role in neuronal regeneration after hypoxic damage [49]. In the present study, no association was observed between H19 circulating levels and the risk of incident ischemic brain lesions, suggesting a neutral effect of H19 on this clinically relevant endpoint.

Based on the above-mentioned evidence, we can speculate that the increased mortality associated to the higher levels of H19 could be attributed to an increased risk of developing cancer and cardiovascular disorders, whereas a relevant role for glucose metabolism disorders seems unlikely.

Interestingly, H19 is differentially expressed in the two sexes, with a higher expression in males, consistent with the shorter lifespan of male subjects. The mediator effect of H19 was confirmed by the mediation analysis model. Biological sex is known to affect lifespan in mammals, likely through multiple pathophysiological mechanisms (e.g., sex hormones, lipid metabolism, and systemic inflammation) [50]. According to our results, we hypothesize that the differential expression of H19 might be one of the several mechanisms by which sex affects lifespan, at least in the elderly.

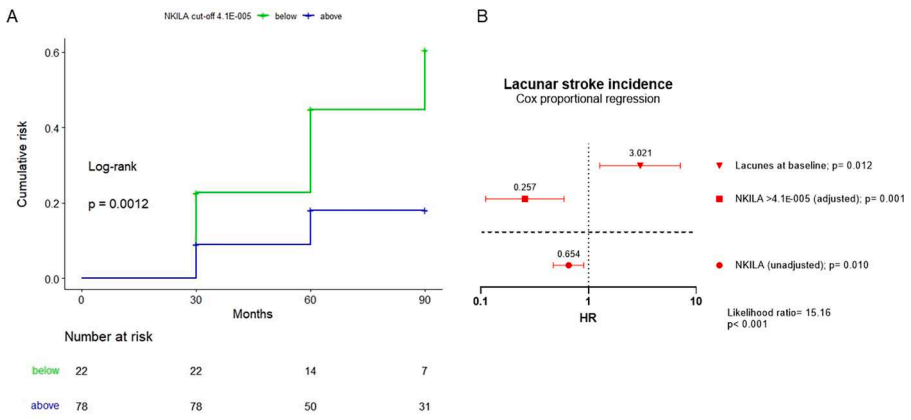

Fig. 3. NKILA predicts the incidence of lacunar ischemic lesions. A. Kaplan-Meier plot. Subjects are classified according to the cut-off of NKILA derived by the Youden's index. B. Forest-plot of the Cox proportional regression. Hazard Ratio in the unadjusted regression analysis is intended for doubling of NKILA. Significant predictors are highlighted with red bars.

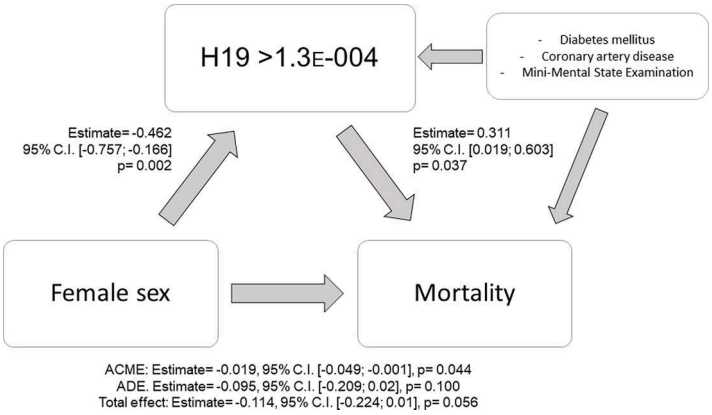

Fig. 4. Mediation model analysis. Sex is the independent variable, H19 is the mediator, and mortality is the outcome. ACME= Average Causal Mediated Effect; ADE= Average Direct Effect. Variables included in the model are all dichotomized.

Results from the present study also suggest an inverse association between the lncRNA NKILA and incident lacunar stroke. This observation is largely in line with a previous pre-clinical study, showing that NKILA is a protective factor towards brain ischemia, by alleviating the inflammatory response and reducing oxidative stress [51]. NKILA (i.e. NF-κB Interacting lncRNA) is a recently identified lncRNA which interacts with the pro-inflammatory transcription factor NF-κB and inhibits its transcriptional action [52]. In details, NKILA binds NF-κB at the p65 subunit in the cytoplasm, thus stabilizing the complex with its inhibitor IκB, and ultimately preventing the nuclear translocation of NF-κB [53]. Furthermore, the NKILA promoter region contains an NF-κB binding motif, suggesting a self-regulating feedback [54]. In epidemiological and basic research studies, NKILA was associated with coronary artery disease and ischemic myocardial injury [55]. Moreover, it exerts a protective function towards myocardial ischemia, by promoting cardiomyocytes survival [56]. However, its role in neuronal survival is still controversial since NKILA was reported to promote both neuronal death in the setting of Alzheimer dementia, as well as reducing neuroinflammation and oxidative stress in the setting of acute ischemic stroke [51,57].

The main strength points of the present study are the granular characterization of the cohort, with a long-term follow-up and the inclusion of brain MRI data, which allowed to detect asymptomatic ischemic lesions. At the same time, results should be interpreted with some caution. First, the predictive performance of H19 and NKILA as potential biomarkers for longevity and lacunar stroke, respectively, was not validated in an independent external cohort. Then, as in any observational study, the risk of residual confounding persists. However, the independent association using different model building approaches, which we confirmed internally by using resampling approaches, and its biological plausibility suggest high external validity. Furthermore, a sensitivity analysis by causes of death could not be performed, as the cause of death was not consistently documented. Finally, in the mediation analysis model, the total effect estimate was not significant: this is likely due to the small size of the effects and to possible additional mediators, not included in the final model. However, the proposed model is biologically plausible and a specifically designed, basic-science, study is warranted to confirm the differential expression of H19 between sexes, on a molecular level.As regards the association between NKILA and lacunar stroke, the number of events was relatively low, thus reducing the overall generalizability of the multivariable model.

In summary, the present study reports the association between high circulating levels of H19 and a higher risk of death. This is in line with previous reports of an association between H19 and accelerated aging [22,32–34]. Finally, H19 is differentially expressed between males and females, thus H19 could be considered as a molecular mediator of sexual dimorphism in lifespan. Whether H19 expression is genetically determined, or it is an acquired hallmark, is an intriguing question which warrants additional research. The present study also confirms the pre-clinical evidence about the protective effect of NKILA towards brain ischemia. This opens exciting research avenues to assess a potential therapeutic or diagnostic role of NKILA in vascular dementia. This latter aspect is particularly relevant, because vascular dementia is a common clinical entity; this notwithstanding, compared to other forms of

dementia, no specific treatment is available at present. The overall results highlight the role of lnc RNAs as early determinants of aging-related diseases, with a potentially causative role. In particular, the lncRNAs here investigated are both associated with chronic inflammation, which is a pathogenic feature of multiple age-related conditions, including cardiovascular diseases, sarcopenia and frailty [58]. Since lncRNAs are nowadays regarded as promising pharmacological targets, these results could be employed to investigate new preventive interventions in subjects with increased risk of age-related diseases.

Author contributions

TLB and SM prepared the draft; TLB, YMP, SAM, SC and AA performed laboratory measurements; SM and SK performed the statistical analysis; TLB and MK performed the systematic review of the literature; WK, PR, MH, PF, and EG collected and curated data; FP, SB and LL revised the manuscript; TFL, EG, AA and GGC conceptualized and supervised the project.

Data availability

Original database is available on motivated request to the corresponding author.

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Declaration of Competing Interest

Prof. Camici and Prof. Liberale are coinventors on the International Patent WO/2020/226,993 filed in April 2020; the patent relates to the use of antibodies which specifically bind interleukin-1a to reduce various sequelae of ischemia-reperfusion injury to the central nervous system. Prof. Camici is the recipient of an H.H. Sheikh Khalifa bin Hamad Al Thani Foundation Assistant Professorship at the Faculty of Medicine, University of Zurich and is a consultant to Sovida solutions limited. Dr. Lapikova-Bryhinska is the recipient of a “Scholars at risk” grant from the Swiss National Science Foundation. Dr. Ministrini has received financial support from the Swiss Heart Foundation. Dr. Puspitasari is the recipient of a Forschungskredit Candoc grant from the University of Zurich and a grant from Swiss Life Foundation for Public Health and Medical Research. Dr. Kraler received research grants to the institution from the Jubiläumsstiftung SwissLife, the Lindenhof Foundation, the Novartis Foundation for Medical-biological Research, the Swiss Heart Foundation, the Swiss Society of Cardiology, and the Theodor-Ida-Herzog-Egli Foundation, and equipment and materials from Roche Diagnostics outside the submitted work. Further, he has received travel support from the European Atherosclerosis Society, the European Society of Cardiology, the European Society of Clinical Investigation, Sphingotec GmbH, the 4TEEN4 Pharmaceuticals GmbH, and PAM Theragnostics GmbH. Prof. Liberale has received financial support from the Swiss Heart Foundation and the Novartis Foundation for Medical-Biological Research. Prof. Lüscher has, outside this work, received research and educational grants and in part honoraria from Abbott, Amgen, AstraZeneca, Boehringer Ingelheim, Daiichi Sankyo, Novartis, Sanofi, Servier, and CSL Vifor. Dr. Mohamed is the recipient of a Forschungskredit Candoc grant from the University of Zurich. Prof. Paneni is the recipient of an H.H. Sheikh Khalifa bin Hamad Al Thani Foundation Assistant Professorship at the Faculty of Medicine, University of Zurich; a grant by the Swiss National Science Foundation (no. 310,030_197,557), the Swiss Heart Foundation (no. FF19045), the Stiftung für wissenschaftliche Forschung, the Olga Mayenfisch Foundation, the Swiss Life Foundation for Public Health and Medical Research, the Kurt und Senta-Hermann Stiftung, the EMDO Stiftung and the Schweizerische Diabetes-Stiftung. Dr. Costantino received financial support from the Holcim Foundation, the Swiss Heart Foundation, and the Swiss Life Foundation for Public Health and Medical Research and the Gebauer Stiftung. Prof. Montecucco is the recipient of the RCR-2022–23,682,288 - Rete CARDIOLOGICA. The remaining Authors have no competing interest to disclose.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejim.2023.11.013.

References

[1] López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell 2013;153:1194–217. https://doi.org/10.1016/j.cell.2013.05.039.
[2] Camici GG, Savarese G, Akhmedov A, Lüscher TF. Molecular mechanism of endothelial and vascular aging: implications for cardiovascular disease. Eur. Heart J. 2015;36:3392–403. https://doi.org/10.1093/eurheartj/ehv587.
[3] Camici GG, Sudano I, Noll G, Tanner FC, Lüscher TF. Molecular pathways of aging and hypertension. Curr. Opin. Nephrol. Hypertens. 2009;18:134–7. https://doi.org/10.1097/MNH.0b013e328326093f.
[4] Liberale L, Camici GG. The role of vascular aging in atherosclerotic plaque development and vulnerability. Curr. Pharm. Des. 2019;25:3098–111. https://doi.org/10.2174/1381612825666190830175424.
[5] Paneni F, Diaz Cañestro C, Libby P, Lüscher TF, Camici GG. The aging cardiovascular system: understanding it at the cellular and clinical levels. J. Am. Coll. Cardiol. 2017;69:1952–67. https://doi.org/10.1016/j.jacc.2017.01.064.
[6] Kraler S, Blaser MC, Aikawa E, Camici GG, Lüscher TF. Calcific aortic valve disease: from molecular and cellular mechanisms to medical therapy. Eur. Heart J. 2022;43:683–97. https://doi.org/10.1093/eurheartj/ehab757.
[7] Camici GG, Liberale L. Aging: the next cardiovascular disease? Eur. Heart J. 2017;38:1621–3. https://doi.org/10.1093/eurheartj/ehx239.
[8] Cohen RA, Marsiske MM, Smith GE. Neuropsychology of aging. Handb. Clin. Neurol. 2019;167:149–80. https://doi.org/10.1016/B978-0-12-804766-8.00010-8.
[9] O’Brien JT, Thomas A. Vascular dementia. Lancet 2015;386:1698–706. https://doi.org/10.1016/S0140-6736(15)00463-8.
[10] Regenhardt RW, Das AS, Lo EH, Caplan LR. Advances in understanding the pathophysiology of lacunar stroke: a review. JAMA Neurol. 2018;75:1273–81. https://doi.org/10.1001/jamaneurol.2018.1073.
[11] Kalaria RN. The pathology and pathophysiology of vascular dementia. Neuropharmacology 2018;134:226–39. https://doi.org/10.1016/j.neuropharm.2017.12.030.
[12] Mari D, Parnetti L, Coppola R, Bottasso B, Reboldi GP, Senin U, et al. Hemostasis abnormalities in patients with vascular dementia and Alzheimer’s disease. Thromb. Haemost. 1996;75:216–8.
[13] Lee CS, Lee ML, Gibbons LE, Yanagihara RT, Blazes M, Kam JP, et al. Associations between retinal artery/vein occlusions and risk of vascular dementia. J. Alzheimers Dis. 2021;81:245–53. https://doi.org/10.3233/JAD-201492.
[14] Kour S, Rath PC. Long noncoding RNAs in aging and age-related diseases. Ageing Res. Rev. 2016;26:1–21. https://doi.org/10.1016/j.arr.2015.12.001.
[15] Montes M, Lund AH. Emerging roles of lncRNAs in senescence. FEBS J. 2016;283:2414–26. https://doi.org/10.1111/febs.13679.
[16] Boon RA, Jaé N, Holdt L, Dimmeler S. Long noncoding RNAs from clinical genetics to therapeutic targets? J. Am. Coll. Cardiol. 2016;67:1214–26. https://doi.org/10.1016/j.jacc.2015.12.051.
[17] Bär C, Chatterjee S, Thum T. Long noncoding RNAs in cardiovascular pathology, diagnosis, and therapy. Circulation 2016;134:1484–99. https://doi.org/10.1161/CIRCULATIONAHA.116.023686.
[18] Andersen RE, Lim DA. Forging our understanding of lncRNAs in the brain. Cell Tissue Res. 2018;371:55–71. https://doi.org/10.1007/s00441-017-2711-z.
[19] Bink DI, Pauli J, Maegdefessel L, Boon RA. Endothelial microRNAs and long noncoding RNAs in cardiovascular ageing. Atherosclerosis 2023. https://doi.org/10.1016/j.atherosclerosis.2023.03.019.
[20] Chen J, Liu P, Dong X, Jin J, Xu Y. The role of lncRNAs in ischemic stroke. Neurochem. Int. 2021;147:105019. https://doi.org/10.1016/j.neuint.2021.105019.
[21] Riva P, Ratti A, Venturin M. The long non-coding RNAs in neurodegenerative diseases: novel mechanisms of pathogenesis. Curr. Alzheimer Res. 2016;13:1219–31. https://doi.org/10.2174/1567205013666160622112234.
[22] Grammatikakis I, Panda AC, Abdelmohsen K, Gorospe M. Long noncoding RNAs (lncRNAs) and the molecular hallmarks of aging. Aging 2014;6:992–1009. https://doi.org/10.18632/aging.100710 (Albany NY).

[23] Fischer P, Jungwirth S, Krampla W, Weissgram S, Kirchmeyr W, Schreiber W, et al. Vienna transdanube aging "VITA": study design, recruitment strategies and level of participation. J. Neural Transm. Suppl. 2002:105–16. https://doi.org/10.1007/978-3-7091-6139-5_11.
[24] Jungwirth S, Zehetmayer S, Bauer P, Weissgram S, Tragl KH, Fischer P. Screening for Alzheimer's dementia at age 78 with short psychometric instruments. Int. Psychogeriatr. 2009;21:548–59. https://doi.org/10.1017/S1041610209008904.
[25] Mostafaie N, Rossmanith W, Hombauer H, Dechat T, Raffelsberger T, Bauer K, et al. Mitochondrial genotype and risk for Alzheimer's disease: cross-sectional data from the Vienna-Transdanube-Aging "VITA" study. J. Neural Transm. 2004;111:1155–65. https://doi.org/10.1007/s00702-004-0161-8 (Vienna).
[26] Wenzl FA, Mengozzi A, Mohammed SA, Pugliese NR, Mongelli A, Gorica E, et al. Circulating long noncoding RNA signatures associate with incident diabetes in older adults: a prospective analysis from the VITA cohort study. Diabetes Care 2023;46:1239–44. https://doi.org/10.2337/dc23-0012.
[27] Kautzky A, Seiger R, Hahn A, Fischer P, Krampla W, Kasper S, et al. Prediction of autopsy verified neuropathological change of Alzheimer's disease using machine learning and MRI. Front. Aging Neurosci. 2018;10.
[28] Vo TT, Superchi C, Boutron I, Vansteelandt S. The conduct and reporting of mediation analysis in recently published randomized controlled trials: results from a methodological systematic review. J. Clin. Epidemiol. 2020;117:78–88. https://doi.org/10.1016/j.jclinepi.2019.10.001.
[29] Barsyte-Lovejoy D, Lau SK, Boutros PC, Khosravi F, Jurisica I, Andrulis IL, et al. The c-Myc oncogene directly induces the H19 noncoding RNA by allele-specific binding to potentiate tumorigenesis. Cancer Res. 2006;66:5330–7. https://doi.org/10.1158/0008-5472.CAN-06-0037.
[30] Matouk IJ, DeGroot N, Mezan S, Ayesh S, Abu-lail R, Hochberg A, et al. The H19 non-coding RNA is essential for human tumor growth. PLoS One 2007;2:e845. https://doi.org/10.1371/journal.pone.0000845.
[31] Monnier P, Martinet C, Pontis J, Stancheva I, Ait-Si-Ali S, Dandolo L. H19 lncRNA controls gene expression of the imprinted gene network by recruiting MBD1. Proc. Natl. Acad. Sci. U. S. A. 2013;110:20693–8. https://doi.org/10.1073/pnas.1310201110.
[32] Thorvaldsen JL, Duran KL, Bartolomei MS. Deletion of the H19 differentially methylated domain results in loss of imprinted expression of H19 and Igf2. Genes Dev. 1998;12:3693–702. https://doi.org/10.1101/gad.12.23.3693.
[33] Issa JP, Vertino PM, Boehm CD, Newsham IF, Baylin SB. Switch from monoallelic to biallelic human IGF2 promoter methylation during aging and carcinogenesis. Proc. Natl. Acad. Sci. U. S. A. 1996;93:11757–62. https://doi.org/10.1073/pnas.93.21.11757.
[34] Christofori G, Naik P, Hanahan D. A second signal supplied by insulin-like growth factor II in oncogene-induced tumorigenesis. Nature 1994;369:414–8. https://doi.org/10.1038/369414a0.
[35] Bi Y, Wang Y, Sun X. Recent advances of LncRNA H19 in diabetes LncRNA H19 in diabetes. Horm. Metab. Res. 2022;54:212–9. https://doi.org/10.1055/a-1769-5032.
[36] Gao Y, Wu F, Zhou J, Yan L, Jurczak MJ, Lee HY, et al. The H19/let-7 double-negative feedback loop contributes to glucose metabolism in muscle cells. Nucleic Acids Res. 2014;42:13799–811. https://doi.org/10.1093/nar/gku1160.
[37] Alfaifi M, Verma AK, Alshahrani MY, Joshi PC, Alkhathami AG, Ahmad I, et al. Assessment of cell-free long non-coding RNA-H19 and miRNA-29a, miRNA-29b expression and severity of diabetes. Diabetes Metab. Syndr. Obes. 2020;13:3727–37. https://doi.org/10.2147/DMSO.S273586.
[38] Busscher D, Boon RA, Juni RP. The multifaceted actions of the lncRNA H19 in cardiovascular biology and diseases. Clin. Sci. 2022;136:1157–78. https://doi.org/10.1042/CS20210994 (Lond).
[39] Hofmann P, Sommer J, Theodorou K, Kirchhof L, Fischer A, Li Y, et al. Long non-coding RNA H19 regulates endothelial cell aging via inhibition of STAT3 signalling. Cardiovasc. Res. 2019;115:230–42. https://doi.org/10.1093/cvr/cvy206.
[40] Gomez D, Owens GK. Smooth muscle cell phenotypic switching in atherosclerosis. Cardiovasc. Res. 2012;95:156–64. https://doi.org/10.1093/cvr/cvs115.
[41] Wang J, Zhao H, Fan Z, Li G, Ma Q, Tao Z, et al. Long noncoding RNA H19 promotes neuroinflammation in ischemic stroke by driving histone deacetylase 1-dependent M1 microglial polarization. Stroke 2017;48:2211–21. https://doi.org/10.1161/STROKEAHA.117.017387.
[42] Hobuß L, Foinquinos A, Jung M, Kenneweg F, Xiao K, Wang Y, et al. Pleiotropic cardiac functions controlled by ischemia-induced lncRNA H19. J. Mol. Cell. Cardiol. 2020;146:43–59. https://doi.org/10.1016/j.yjmcc.2020.07.001.
[43] Elia E, Ministrini S, Carbone F, Montecucco F. Diabetic cardiomyopathy and inflammation: development of hostile microenvironment resulting in cardiac damage. Minerva Cardiol. Angiol. 2022;70:357–69. https://doi.org/10.23736/S2724-5683.20.05454-7.
[44] Li X, Luo S, Zhang J, Yuan Y, Jiang W, Zhu H, et al. lncRNA H19 Alleviated Myocardial I/RI via Suppressing miR-877-3p/Bcl-2-Mediated Mitochondrial Apoptosis. Mol. Ther. Nucleic Acids 2019;17:297–309. https://doi.org/10.1016/j.omtn.2019.05.031.
[45] Liu L, An X, Li Z, Song Y, Li L, Zuo S, et al. The H19 long noncoding RNA is a novel negative regulator of cardiomyocyte hypertrophy. Cardiovasc. Res. 2016;111:56–65. https://doi.org/10.1093/cvr/cvw078.
[46] Liu Y, Xu XY, Shen Y, Ye CF, Hu N, Yao Q, et al. Ghrelin protects against obesity-induced myocardial injury by regulating the lncRNA H19/miR-29a/IGF-1 signalling axis. Exp. Mol. Pathol. 2020;114:104405. https://doi.org/10.1016/j.yexmp.2020.104405.
[47] Zhang Y, Zhang M, Xu W, Chen J, Zhou X. The long non-coding RNA H19 promotes cardiomyocyte apoptosis in dilated cardiomyopathy. Oncotarget 2017;8:28588–94. https://doi.org/10.18632/oncotarget.15544.
[48] Gao N, Tang H, Gao L, Tu GL, Luo H, Xia Y. LncRNA H19 aggravates cerebral ischemia/reperfusion injury by functioning as a ceRNA for miR-19a-3p to target PTEN. Neuroscience 2020;437:117–29. https://doi.org/10.1016/j.neuroscience.2020.04.020.
[49] Fan B, Pan W, Wang X, Wei M, He A, Zhao A, et al. Long noncoding RNA mediates stroke-induced neurogenesis. Stem Cells 2020;38:973–85. https://doi.org/10.1002/stem.3189.
[50] Austad SN, Fischer KE. Sex Differences in Lifespan. Cell Metab. 2016;23:1022–33. https://doi.org/10.1016/j.cmet.2016.05.019.
[51] Gao W, Ning Y, Peng Y, Tang X, Zhong S, Zeng H. LncRNA NKILA relieves astrocyte inflammation and neuronal oxidative stress after cerebral ischemia/reperfusion by inhibiting the NF-κB pathway. Mol. Immunol. 2021;139:32–41. https://doi.org/10.1016/j.molimm.2021.08.002.
[52] Liu B, Sun L, Liu Q, Gong C, Yao Y, Lv X, et al. A cytoplasmic NF-κB interacting long noncoding RNA blocks IκB phosphorylation and suppresses breast cancer metastasis. Cancer Cell 2015;27:370–81. https://doi.org/10.1016/j.ccell.2015.02.004.
[53] Wu W, Chen F, Cui X, Yang L, Chen J, Zhao J, et al. LncRNA NKILA suppresses TGF-β-induced epithelial–mesenchymal transition by blocking NF-κB signaling in breast cancer. Int. J. Cancer 2018;143:2213–24. https://doi.org/10.1002/ijc.31605.
[54] Huang D, Chen J, Yang L, Ouyang Q, Li J, Lao L, et al. NKILA lncRNA promotes tumor immune evasion by sensitizing T cells to activation-induced cell death. Nat. Immunol. 2018;19:1112–25. https://doi.org/10.1038/s41590-018-0207-y.
[55] Hussen BM, Azimi T, Hidayat HJ, Taheri M, Ghafouri-Fard S. NF-KappaB interacting LncRNA: review of its roles in neoplastic and non-neoplastic conditions. Biomed. Pharmacother. 2021;139:111604. https://doi.org/10.1016/j.biopha.2021.111604.
[56] Liu Q, Liu Z, Zhou LJ, Cui YL, Xu JM. The long noncoding RNA NKILA protects against myocardial ischaemic injury by enhancing myocardin expression via suppressing the NF-κB signalling pathway. Exp. Cell Res. 2020;387:111774. https://doi.org/10.1016/j.yexcr.2019.111774.
[57] Zhou Y, Wang Y, Wang Y, Chen L, Wang N, Su Y, et al. LncRNA NKILA exacerbates Alzheimer's disease progression by regulating the FOXA1-mediated transcription of TNFAIP1. Neurochem. Res. 2023. https://doi.org/10.1007/s11064-023-03944-6.
[58] Liberale L, Montecucco F, Tardif JC, Libby P, Camici GG. Inflamm-ageing: the role of inflammation in age-dependent cardiovascular disease. Eur. Heart J. 2020;41:2974–82. https://doi.org/10.1093/eurheartj/ehz961.