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# ALU POLYMORPHISMS OF AUTOPHAGY AND APOPTOSIS REGULATORY GENES AS HUMAN LIFESPAN FACTORS

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**Abstract.** To assess the contribution to survival of Alu-insertions in the *ACE, PLAT, COL13A1, LAMA2, CDH4, SEMA6A, PKHD1L1, STK38L, HECW1,* and *TEAD1* genes, which are candidates of aging and longevity, amid the senile physiological and pathological phenotype, was carried out the association analysis with life expectancy. Survival and mortality data were obtained for 1,382 elderly people, who were selected from the sample of Tatars residing in the Republic of Bashkortostan (total 1,790 people from 18 to 109 years). Mortality risk was higher among carriers of the *STK38L* Alu-insertion genotype (Ya5ac2145*II, $HR = 2.07$, $P = 0.02$). Alu insertion in the *HECW1* and *TEAD1* genes has demonstrated a survival protection effect (Ya5NBC182*II, $HR = 0.71$, $P = 0.038$ and Ya5ac2013*II, $HR = 0.74$, $P = 0.035$ respectively). The survival amid the persons with various clinical phenotypes was associated with the Alu polymorphism of the *SEMA6A* (Yb8NBC597*ID, $HR = 0.54$, $P = 0.016$ for the cerebrovascular diseases), *TEAD1* (Ya5ac2013*II, $HR = 0.57$, $P = 0.016$ for the cardiovascular pathologies) and *LAMA2* (Ya5-MLS19*ID, $HR = 0.36$, $P = 0.03$ for multimorbidity status) genes. Thus, the genes involved in the regulation of autophagy and apoptosis were associated with survival and longevity.

**Keywords:** *aging, longevity, Alu polymorphism, TEAD1, HECW1, STK38L, LAMA2, SEMA6A genes, survival analysis*

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## INTRODUCTION

Human lifespan is determined by a complex of environmental, behavioral, and hereditary factors. The dynamic nature of endogenous mechanisms regulating the rate of aging of the organism causes specific features of the later stages of ontogenesis. The absolute and relative number of changes in the body caused by damage to molecules, cells, organs and their systems, collectively lead to a decrease in functional activity and disruption of homeostasis of both the entire organism and its parts, without the possibility of complete recovery. Accordingly, the aging process is characterized by an increased risk of developing a large number of diseases and an increase in the probability of death from all causes [1, 2]. It is important to note that the proportion of age-related pathologies, among which the leading ones are diseases of the circulatory system, respiratory system, and oncological diseases, varies in different age groups [3].

The study of the genome polymorphism role in the formation of age-associated diseases is one of the key areas of molecular genetic research on the determination of lifespan. In particular, the polymorphic state of DNA sites containing mobile genetic elements is associated with increased genome instability. Transposon insertions can cause various mutations, chromosomal rearrangements, affect the epigenetic landscape of the eukaryotic genome,

processes of transcriptional regulation, and gene expression. These effects of insertion events are largely found at the later stages of ontogenesis [4]. The most common family of human transposons are Alu repeats. Alu polymorphisms located in genes of key signaling pathways are involved in physiological and pathological intracellular processes [5, 6]. Previously, we established associations with longevity of Alu polymorphic loci in genes encoding protein kinase STK38L, protein ligase HECW1, calcium channel protein PKHD1L1, cellular receptor SEMA6A, transcription factor TEAD1, plasma enzymes ACE and PLAT, as well as adhesion proteins CDH4 and extracellular matrix proteins COL13A1 and LAMA2 [7–9]. The involvement of these genes in the development of age-dependent diseases, including cardiovascular, neurodegenerative, oncological, as well as metabolic diseases, has been established in a number of studies [10–20]. At the same time, their contribution to survival, including in various clinical phenotypes and, moreover, at an age exceeding the average, has not been previously studied. Alu transposons are one of the key endogenous factors of evolutionary adaptation and development of the human species [21, 22]. Thus, this type of genome polymorphism can be considered as an important molecular genetic predictor of survival in conditions of senile physiological background and, moreover, pathological comorbid state.

In this work, we analyzed the contribution of Alu polymorphic variants of genes encoding structural components of the cell, intercellular interactions, as well as those involved in key signaling pathways of cellular activity, to survival among elderly individuals and long-livers.

## MATERIALS AND METHODS

The sample from the residents of the Republic of Bashkortostan was formed during 2001–2015 and included 1,790 people aged 18 to 109 years, Tatars by ethnicity. The inclusion criteria for middle-aged individuals (18–59 years) in the study were the absence of diabetes mellitus, history of myocardial infarction and/or stroke, autoimmune and oncological diseases in their medical history. For people in the older age cohort (60–89 years), the presence of atherosclerosis, cardiosclerosis, and cerebrosclerosis in their medical history was permitted. The group of long-livers included all individuals who reached 90 years of age.

DNA samples were obtained by phenol-chloroform extraction from 8 ml of whole venous blood. Alu polymorphic loci in *ACE, PLAT*, *COL13A1, LAMA2*, *CDH4, SEMA6A*, *PKHD1L1, STK38L*, *HECW1*, and *TEAD1* genes were selected as genetic predictors of survival based on information about their functional significance and previously discovered association with age-related diseases and longevity [7–20]. Genotyping was performed by PCR followed by separation of amplified fragments in 1% agarose gel. The conditions for identifying Alu polymorphic loci are shown in Table 1.

Information on the survival and mortality of all people over 60 years of age and 115 people over 45 years of age who were previously included in the study group (a total of 1,382 people) was collected from 2022 to 2023 by interviewing their relatives. At the completion of this stage of work (December 30, 2023), information was obtained on survival status, as well as date and causes of death for 1,069 people (response rate was 77.35%), of whom 944 people died, 125 were alive. The studied sample was differentiated by age into a control group, a group of elderly people, and long-livers. The upper limit for the control group corresponded to the average life expectancy of the population of the Republic of Bashkortostan, which was established based on official data from the Federal State Statistics Service (https://rosstat.gov.ru/, accessed January 25, 2024). The characteristics of the formed groups are presented in Table 2.

The research data were processed on the IBM SPSS V22.0 platform (Chicago, Illinois, USA), as well as in the Python programming environment [23]. The frequencies of all Alu polymorphic gene variants were checked for compliance with the Hardy-Weinberg equilibrium in the control group. Changes in the distribution of genotype frequencies with age for each selected Alu polymorphic locus were assessed by pairwise comparison of age groups using Pearson's $\chi 2$ test. The association between Alu polymorphic variants and all-cause mortality was established using Cox proportional hazards regression. Additionally, a stratified survival analysis was conducted considering gender and causes of mortality from various age-related pathologies. The curves of the relative risk of death (Hazard ratio – *HR*) were constructed using lifelines and mathplotlib packages.

## RESULTS

In the ethnic group of Tatars living in the Republic of Bashkortostan, the distribution of allele and genotype frequencies for Alu polymorphic loci in the introns of *ACE*, *COL13A1*, *LAMA2, TEAD1*, *PLAT*, *PKHD1L1*, *STK38L*, *CDH4*, *HECW1*, and *SEMA6A* genes was characterized, both in the general sample and taking into account age. The observed distribution of genotype frequencies for all studied polymorphic markers in the middle-aged group corresponded to the theoretically expected

**Table 1.** Alu polymorphic loci included in the study, their localization, amplification conditions and length of amplified fragments

| Alu element | Gene, localization* | Primer sequences | Annealing temperature, °C | Alleles (fragment length, bp) |
|---|---|---|---|---|
| Ya5ACE | *ACE* 17q23.3 | F 5'-ctg gag acc act ccc atc ctt tct-3' R 5'-gat gtg gcc atc aca ttc gtc aga t-3' | 68 | I (490) D (190) |
| Ya5NBC182 | *HECW1* 7p13 | F 5'-gaa gga cta tgt agt tgc aga agc-3' R 5'-aac cca gtg gaa aca gaa gat g-3' | 64 | I (563) D (287) |
| Yb8NBC597 | *SEMA6A* 5q23.1 | F 5'-tga ggt gtt gca gac gat gt-3' R 5'-cgc atg ctt tag aga ata ccc-3' | 63 | I (429) D (108) |
| Yb8NBC516 | *CDH4* 20q13.33 | F 5'-ggg ctc agg gat act atg ctc-3' R 5'-gcc tag gcc tac cac tca ga-3' | 60 | I (445) D (124) |
| Ya5ac2145 | *STK38L* 12p11.23 | F 5'-tgt tct aat gac cat gcc tac tt-3' R 5'-tgc ctt tag gaa gct aca gat tta-3' | 60 | I (465) D (135) |
| Yb8AC702 | *PKHD1L1* 8q23.2 | F 5'-tgt ttg gaa ata agc caa aca at-3' R 5'-ggg tag caa cct ttt tca tct tt-3' | 60 | I (482) D (161) |
| Ya5ac2013 | *TEAD1* 11p15.2 | F 5'-tgg cag att ctg act ggc ta-3' R 5'-cac gta agg tga aaa ggg ga-3' | 60 | I (489) D (212) |
| TPA25 | *PLAT* 8p11.21 | F 5'-caa cca atg aaa acc act ga-3' R 5'-gtt ctc ctg aca tct tta ttg-3' | 60 | I (518) D (217) |
| Ya5ac1986 | *COL13A1* 10q22.1 | F 5'-tct agt ggg atg agg ata ac-3' R 5'-tgt gcc atg ggg taa gaa ac-3' | 60 | I (431) D (134) |
| Ya5-MLS19 | *LAMA2* 6q22.33 | F 5'-cta tga cgg agt aaa aag aag t-3' R 5'-gaa aga gtg cca acc ctg tcc-3' | 63 (7 cycles) 60 (22 cycles) | I (401) D (106) |

Note. F – forward primer; R – reverse primer; bp – base pairs; * – data from UCSC database

Hardy-Weinberg equilibrium ($P_{HW} > 0.05$, Table 3). Changes in the spectrum of genotype frequencies with age were assessed by pairwise comparison of age groups for each selected Alu polymorphic locus using Pearson's $\chi 2$ test (Table 3). The most pronounced differences in genotype frequencies between age groups were observed for the polymorphic locus *HECW1**Ya5NBC182 ($P < 0.001$). For polymorphic loci *LAMA2**Ya5-MLS19, *TEAD1**Ya5ac2013 and *SEMA6A**Yb8NBC597, statistically significant changes in the distribution of genotype frequencies were observed for individuals who reached longevity ($P < 0.05$). Among elderly individuals (in the groups of senile age and long-livers), a deviation in the distribution of genotype frequencies was observed for the genetic marker *STK38L**Ya5ac2145 ($P < 0.01$). In addition, the distribution of genotype frequencies in the senile age group for the polymorphic locus *COL13A1**Ya5ac1986 and among long-livers for the polymorphic locus *PKHD1L1**Yb8AC702 differed from those in the control group of middle-aged individuals ($P < 0.05$).

To assess the role of the established Alu polymorphic markers of aging and longevity in determining life expectancy and achieving longevity, a survival analysis

**Table 2.** Characteristics of the study group

| Groups | *n* (% of all cases) | Age range | $M \pm \sigma$ |
|---|---|---|---|
| Total | 1,790 (100) | 18–109* | 67.85 ± 21.22 |
| males<br>females | 809 (45.2)<br>981 (54.8) | | |
| Age groups | | | |
| Middle age | 631 | 18–65 (for males)<br>18–74 (for females) | 43.93 ± 15.86 |
| Old age | 724 | 66–89 (for males)<br>75–89 (for females) | 84.11 ± 10.45 |
| Long-livers | 435 | 90–114 | 94.5 ± 3.61 |
| Survival status | | | |
| Total | 1,069 | 45–114 | 83.3 ± 10.8 |
| Alive | 125 (11.7) | 45–96 | 70.82 ± 11.8 |
| Deceased | 944 (88.3) | 45–114 | 85.59 ± 9.35 |
| Causes of death | | | |
| Old age | 352 (37.29) | 70–114 | 88.96 ± 6.21 |
| CVD | 241 (25.53) | 46–104 | 84.67 ± 8.74 |
| CVA | 166 (17.59) | 53–105 | 87.09 ± 8.63 |
| COPD history | 26 (2.75) | 53–98 | 83.52 ± 8.19 |
| T2DM history | 10 (1.06) | 59–97 | 83.45 ± 8.19 |
| Cancer | 41 (4.34) | 45–99 | 78.82 ± 11.83 |
| Other causes | 42 (4.45) | 49–97 | 78.98 ± 13.35 |
| Polymorbidity | 66 (6.99) | 59–100 | 84.22 ± 8.54 |

Note. CVD – cardiovascular diseases; CVA – cerebrovascular accidents; COPD – chronic obstructive pulmonary disease; T2DM – type 2 diabetes mellitus; n – sample size (group); M – mean age; σ – standard deviation; * – age information at the time of material collection

was conducted. According to the results, the relative risk of death from all causes is statistically significantly increased in carriers of insertions in the Alu polymorphic locus of the *STK38L* gene; carriers of insertions in the *TEAD1* and *HECW1* genes showed a decrease in relative risk (Table 4).

Detailed analysis of the obtained results showed that the carriage of the homozygous insertion genotype of the *STK38L* gene doubles the risk of death from all causes in the general group of studied individuals (Ya5ac2145*II, $HR = 2.07$, $P = 0.02$, Fig. *1a*). Alu insertions in the TEAD1 and HECW1 genes are associated with a reduced risk of death ($HR = 0.74$, $P = 0.035$ for the genotype Ya5ac2013*II of the TEAD1 $HR = 0.71$, $P = 0.038$, and $HR = 0.67$, $P = 0.015$ for genotypes Ya5NBC182*II and Ya5NBC182*ID of the HECW1 gene respectively, Fig. *1b–d*).

Survival analysis by gender revealed a similar trend in relative risk of death separately for men and women (Table 4). In men, the homozygous Alu

**Table 3.** Distribution of genotype frequencies for Alu polymorphism of genes in three age groups

| Gene Alu polymorphism | Genotype | Middle age | | | Old age | | | Long-livers | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *n* | *p*, % | $P_{HW}$ | *n* | *p*, % | $P\chi^{2*}$ | *n* | *p*, % | $P\chi^{2*}$ | $P\chi^{2**}$ |
| *ACE* Ya5ACE | *II* | 104 | 23.53 | 0.85 | 176 | 25.43 | 0.361 | 94 | 23.50 | 0.072 | 0.296 |
| | *ID* | 200 | 45.25 | | 318 | 45.95 | | 198 | 49.50 | | |
| | *DD* | 138 | 31.22 | | 198 | 28.61 | | 108 | 27.00 | | |
| *HECW1* Ya5NBC182 | *II* | 189 | 42.19 | 0.47 | 250 | 45.29 | 0.041 | 125 | 45.29 | < 0.001 | < 0.001 |
| | *ID* | 203 | 45.31 | | 219 | 39.67 | | 128 | 46.38 | | |
| | *DD* | 56 | 12.50 | | 83 | 15.04 | | 23 | 8.33 | | |
| *SEMA6A* Yb8NBC597 | *II* | 18 | 3.76 | 0.47 | 33 | 5.84 | 0.120 | 23 | 7.57 | 0.005 | 0.037 |
| | *ID* | 156 | 32.57 | | 167 | 29.56 | | 105 | 34.54 | | |
| | *DD* | 305 | 63.67 | | 365 | 64.60 | | 176 | 57.89 | | |
| *CDH4* Yb8NBC516 | *II* | 145 | 39.94 | 0.19 | 191 | 39.63 | 0.074 | 100 | 35.09 | 0.483 | 0.216 |
| | *ID* | 144 | 39.67 | | 216 | 44.81 | | 124 | 43.51 | | |
| | *DD* | 74 | 20.39 | | 75 | 15.56 | | 61 | 21.40 | | |
| *STK38L* Ya5ac2145 | *II* | 10 | 2.06 | 0.06 | 12 | 2.11 | 0.004 | 4 | 1.28 | 0.008 | 0.522 |
| | *ID* | 78 | 16.05 | | 102 | 17.96 | | 63 | 20.19 | | |
| | *DD* | 398 | 81.89 | | 454 | 79.93 | | 245 | 78.53 | | |
| *PKHD1L1* Yb8AC702 | *II* | 119 | 23.20 | 0.54 | 133 | 20.75 | 0.421 | 72 | 20.06 | 0.025 | 0.160 |
| | *ID* | 284 | 55.36 | | 346 | 53.98 | | 186 | 51.81 | | |
| | *DD* | 110 | 21.44 | | 162 | 25.27 | | 101 | 28.13 | | |
| *TEAD1* Ya5ac2013 | *II* | 137 | 27.45 | 0.05 | 157 | 27.79 | 0.966 | 97 | 29.39 | 0.024 | 0.024 |
| | *ID* | 224 | 44.89 | | 250 | 44.25 | | 161 | 48.79 | | |
| | *DD* | 138 | 27.66 | | 158 | 27.96 | | 72 | 21.82 | | |
| *PLAT* TPA25 | *II* | 134 | 23.76 | 0.57 | 157 | 24.30 | 0.591 | 87 | 23.71 | 0.561 | 0.126 |
| | *ID* | 253 | 44.86 | | 296 | 45.82 | | 158 | 43.05 | | |
| | *DD* | 177 | 31.38 | | 193 | 29.88 | | 122 | 33.24 | | |
| *COL13A1* Ya5ac1986 | *II* | 309 | 55.28 | 0.47 | 363 | 54.18 | 0.038 | 229 | 57.11 | 0.937 | 0.081 |
| | *ID* | 205 | 36.67 | | 263 | 39.25 | | 137 | 34.16 | | |
| | *DD* | 45 | 8.05 | | 44 | 6.57 | | 35 | 8.73 | | |
| *LAMA2* Ya5-MLS19 | *II* | 128 | 21.84 | 0.13 | 129 | 19.03 | 0.095 | 60 | 14.81 | < 0.001 | < 0.001 |
| | *ID* | 262 | 44.71 | | 310 | 45.72 | | 224 | 55.31 | | |
| | *DD* | 196 | 33.45 | | 239 | 35.25 | | 121 | 29.88 | | |

Note. *n* – group volume; *p* – genotype frequency; $P_{HW}$ – *P*-value of Hardy-Weinberg test; $P\chi^2$ – *P*-value of $\chi 2$ Pearson test; * – results relative to the middle-aged group; ** – results relative to the senile age group

**Table 4.** Association between Alu polymorphism of aging and longevity candidate genes and all-cause mortality

| Gene Alu polymorphism | Genotype | Total group | | Men | | Women | |
|---|---|---|---|---|---|---|---|
| | | *HR* (95% $CI_{HR}$) | *P* | *HR* (95% $CI_{HR}$) | *P* | *HR* (95% $CI_{HR}$) | *P* |
| *ACE* Ya5ACE | *ID* | 1.11 (0.88–1.4) | 0.396 | 1.08 (0.72–1.63) | 0.701 | 1.09 (0.8–1.48) | 0.577 |
| | *II* | 0.98 (0.74–1.3) | 0.911 | 0.99 (0.59–1.66) | 0.974 | 0.88 (0.61–1.26) | 0.475 |
| *HECW1* Ya5NBC182 | *ID* | 0.67 (0.49–0.93) | 0.015 | 0.61 (0.33–1.1) | 0.102 | 0.65 (0.44–0.98) | 0.037 |
| | *II* | 0.71 (0.52–0.98) | 0.038 | 0.63 (0.35–1.14) | 0.126 | 0.7 (0.47–1.04) | 0.076 |
| *SEMA6A* Yb8NBC597 | *ID* | 0.98 (0.79–1.22) | 0.854 | 1.06 (0.72–1.56) | 0.784 | 0.97 (0.73–1.28) | 0.825 |
| | *II* | 0.63 (0.39–1.02) | 0.058 | 0.62 (0.18–2.13) | 0.447 | 0.7 (0.41–1.19) | 0.184 |
| *CDH4* Yb8NBC516 | *ID* | 1.11 (0.88–1.59) | 0.276 | 0.78 (0.44–1.37) | 0.380 | 1.28 (0.88–1.85) | 0.198 |
| | *II* | 1.16 (0.86–1.57) | 0.327 | 0.95 (0.54–1.66) | 0.857 | 1.16 (0.8–1.69) | 0.438 |
| *STK38L* Ya5ac2145 | *ID* | 0.98 (0.76–1.28) | 0.907 | 0.88 (0.57–1.36) | 0.559 | 0.97 (0.68–1.39) | 0.879 |
| | *II* | 2.07 (1.11–3.86) | 0.022 | 2.38 (1.04–5.46) | 0.041 | 1.55 (0.54–4.46) | 0.415 |
| *PKHD1L1* Yb8AC702 | *ID* | 1.07 (0.82–1.4) | 0.632 | 1.46 (0.89–2.41) | 0.138 | 1.02 (0.72–1.44) | 0.919 |
| | *II* | 1.11 (0.82–1.51) | 0.501 | 1.55 (0.88–2.73) | 0.132 | 0.91 (0.62–1.35) | 0.651 |
| *TEAD1* Ya5ac2013 | *ID* | 0.91 (0.71–1.16) | 0.443 | 0.95 (0.61–1.49) | 0.835 | 0.83 (0.6–1.15) | 0.261 |
| | *II* | 0.74 (0.57–0.98) | 0.035 | 0.79 (0.47–1.31) | 0.353 | 0.72 (0.51–1.02) | 0.066 |
| *PLAT* TPA25 | *ID* | 1.12 (0.89–1.42) | 0.323 | 0.76 (0.5–1.15) | 0.199 | 1.36 (0.99–1.85) | 0.056 |
| | *II* | 1.16 (0.89–1.51) | 0.283 | 0.93 (0.59–1.46) | 0.764 | 1.29 (0.91–1.83) | 0.151 |
| *COL13A1* Ya5ac1986 | *ID* | 1.17 (0.72–1.92) | 0.53 | 1.55 (0.59–4.09) | 0.379 | 1.01 (0.56–1.85) | 0.962 |
| | *II* | 0.99 (0.61–1.62) | 0.989 | 1.31 (0.49–3.52) | 0.586 | 0.81 (0.45–1.44) | 0.472 |
| *LAMA2* Ya5-MLS19 | *ID* | 0.92 (0.73–1.15) | 0.473 | 1.25 (0.84–1.86) | 0.261 | 0.84 (0.62–1.13) | 0.243 |
| | *II* | 1.08 (0.81–1.44) | 0.609 | 1.41 (0.8–2.49) | 0.238 | 0.92 (0.63–1.35) | 0.672 |

Note. *HR* – hazard rate, CI – confidence interval, *P* – significance level indicator

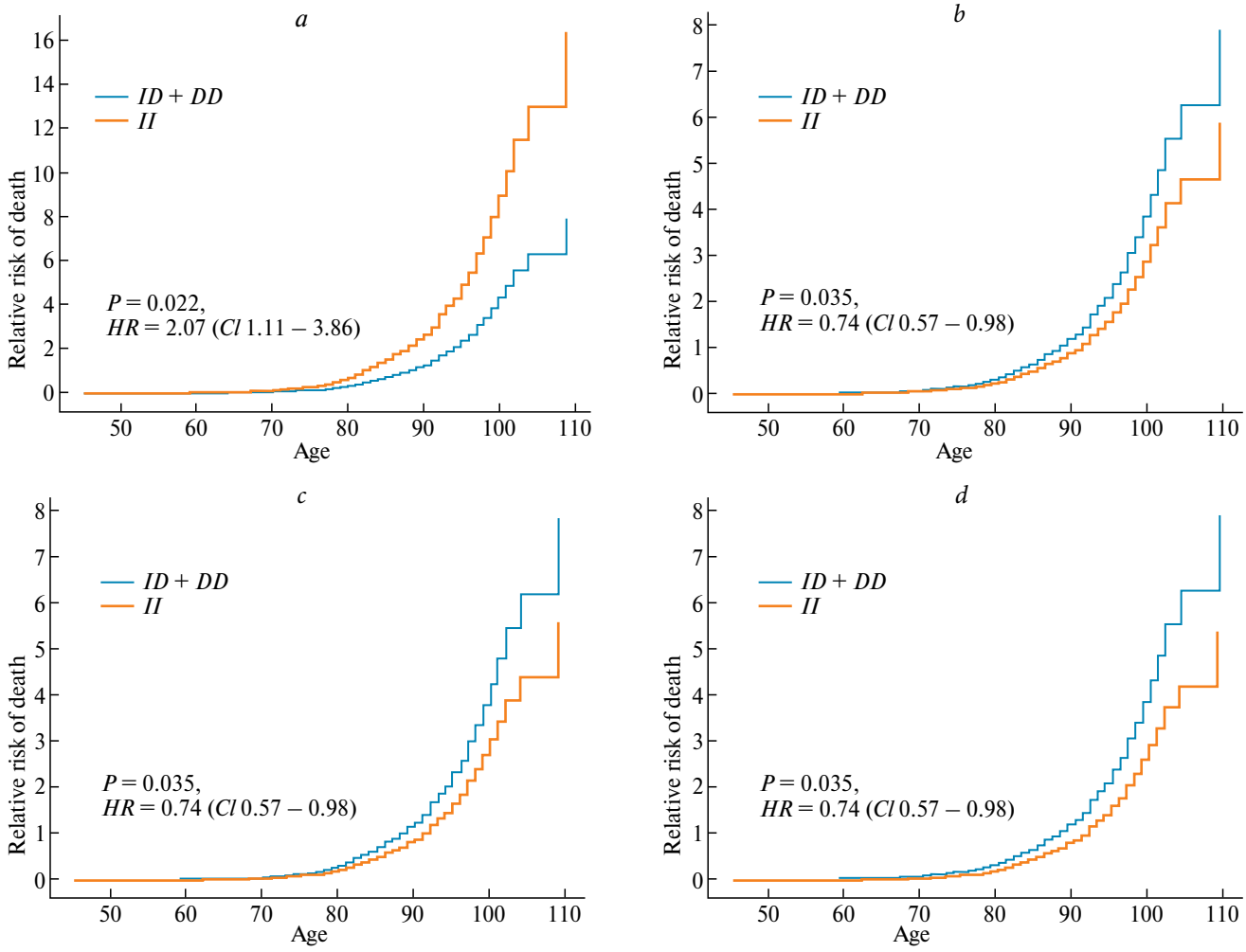


**Fig. 1.** Cumulative risk of all-cause mortality in the general group, associated with Alu insertion in the *STK38L* (*a*), *TEAD1* (*b*) and *HECW1* genes (*c* and *d*)

insertion genotype of the *STK38L* gene showed a more pronounced association with the risk of death from all causes (Ya5ac2145*II, *HR* = 2.38, *P* = 0.041, Fig. 2). The presence of Alu insertions in the *TEAD1* and *HECW1* genes, although reducing the risk of death, does not reach the level of statistical significance (CI 0.47–1.31 and CI 0.61–1.49 for genotypes Ya5ac2013*II and Ya5ac2013*ID of the *TEAD1* gene respectively; CI 0.35–1.14 and CI 0.33–1.11 for genotypes Ya5NBC182*II and Ya5NBC182*ID of the *HECW1* gene respectively, Table 4). In women, the presence of the heterozygous genotype of the *HECW1* gene is associated with a reduced risk of death from all causes (Ya5NBC182*ID, *HR* = 0.65, *P* = 0.037, Fig. 3).

Survival analysis in groups of individuals differentiated by causes of death showed an association of Alu insertions in the *SEMA6A*, *TEAD,1* and *LAMA2* genes with a reduced risk of death in various pathological phenotypes. The presence of Alu insertion in a heterozygous variant in the *SEMA6A* gene is associated with a reduced risk of death from cerebrovascular diseases (Yb8NBC597*ID, *HR* = 0.54, *P* = 0.016, Fig. *4a*). The risk of mortality from cardiovascular pathologies is reduced in carriers of the homozygous Alu insertion genotype in the *TEAD1* gene (Ya5ac2013*II, *HR* = 0.57, = 0.016, Fig. 4b). Also, a reduction in mortality risk is associated with Alu insertion in the *LAMA2* gene among individuals with polymorbidity (Ya5-MLS19*ID, *HR* = 0.36, *P* = 0.03, Fig. *4c*).

## DISCUSSION

Within the framework of human aging and longevity research, a survival analysis was conducted among individuals who reached an age exceeding the average

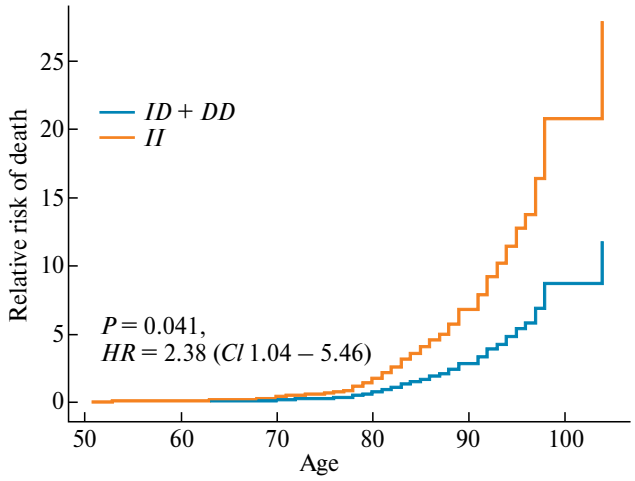


**Fig. 2.** Cumulative risk of all-cause mortality in the male group, associated with Alu insertion in the *STK38L*

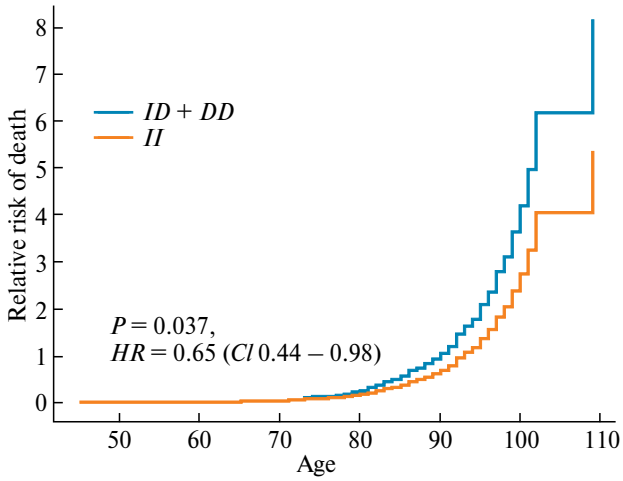


**Fig. 3.** Cumulative risk of all-cause mortality in the female group, associated with Alu insertion in the *HECW1*

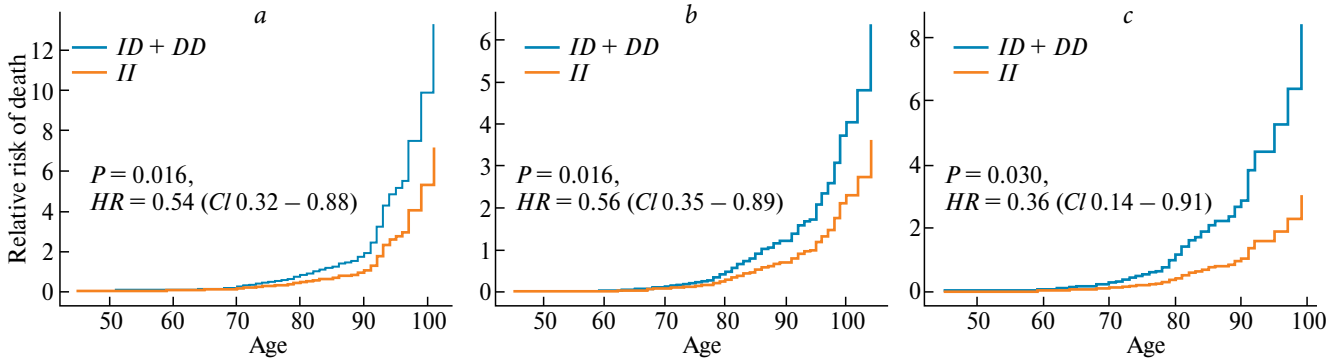


**Fig. 4.** Cumulative mortality risk in groups differentiated by causes of death: from cerebrovascular diseases, associated with Alu insertion in the *SEMA6A* gene *(a)*, from cardiovascular diseases, associated with Alu insertion in the *TEAD1* gene *(b)*, in polymorbidity, associated with Alu insertion in the *LAMA2* gene *(c)*

population indicator, using Alu polymorphic variants of genes for key structural and functional proteins as predictors. According to the obtained results, the Alu insertion genotype Ya5ac2145 of the *STK38L* gene is associated with mortality risk, while for Alu insertion genotypes Ya5NBC182 of the *HECW1* gene and Ya5ac2013 of the *TEAD1* gene, a protective effect on survival has been demonstrated. Additionally, Alu insertion loci Yb8NBC597 of the *SEMA6A* gene and Ya5-MLS19 of the *LAMA2* gene were associated with survival in various clinical phenotypes. Genes associated with survival in the senile phenotype are involved in regulatory pathways controlling apoptosis and autophagy.

HECW1 is a C2 and WW domain-containing protein of the E3 ubiquitin ligase family, and belongs to the NEDD4 family of transcription factors (TF), which regulate cytoplasmic translation, ribonucleoprotein complex and ribosome biogenesis, as well as KEGG pathways, including Akt, p53, autophagy and apoptosis [24]. Like other members of the NEDD4 family, HECW1 binds to LC3 — a key protein of the autophagy system, thereby participating in the regulation of this cellular process. An inhibitory effect of NEDD4 knockdown on autophagy has been demonstrated in cancer cells [25]. Moreover, HECW1 enhances the pro-apoptotic activity of p53 independently of its catalytic activity [26]. In general, E3 ubiquitin ligase-controlled protein degradation plays a fundamental role in self-renewal, maintenance, and differentiation of cancer stem cells [27]. Thus, HECW1 activity is essential for the development of pathological phenotype that forms with age. The HECW1 protein is abundantly present in neuronal tissues and, through its participation in protein homeostasis, is a key element in normal and pathological

development of the nervous system [28]. Interestingly, there is an inverse correlation between oncological and neurodegenerative diseases, the key cause of which may be the localization of p53 in the cell, which affects apoptosis and autophagy processes differently [29]. In this work, an association of survival and longevity with the Alu insertion Ya5NBC182 in the *HECW1* gene was established. It can be assumed that Alu transposons, by influencing gene activity, are the molecular basis for the adaptive plasticity of nervous system tissues. However, the tissue-specific nature of HECW1 involvement in the complex network of apoptosis and autophagy regulation, especially in the later stages of human life, requires further comprehensive study.

TF TEAD1 and protein kinase STK38L are members of the conservative Hippo pathway, which regulates organ size and tissue homeostasis [30]. Recent studies have demonstrated the role of the Hippo signaling cascade in stimulating apoptosis and autophagy [10]. Deletion of genes related to autophagy and interacting with Hippo kinase cascades is associated with an increasing propensity for spontaneous development of various diseases [31]. TEAD1 protein (TEA domain TF) is one of the main downstream nuclear effectors of Hippo signaling. It is able to bind to the DNA consensus sequence 5'-CATTCC-3', known as the MCAT element [32]. Through interaction with various cofactors, such as YAP (yes-associated protein) and TAZ (transcriptional coactivator with PDZ binding motif), TEAD binds to MCAT-containing genes that regulate cell growth. TEAD protein expression has been shown to be enhanced in various types of cancer and correlates with poor survival in cancer patients [11]. Additionally, TEAD regulates the expression of multiple genes involved in cardiovascular system development and, being the main molecular component of the YAP/TAZ signaling pathway, is involved in pathophysiological processes that contribute to cardiovascular diseases [33]. It can be assumed that the Alu insertion Ya5ac2013 reduced activity of the *TEAD1* gene, involved in the control of proliferation and apoptosis, under conditions of senescent phenotype contributes to survival and protection against heart and vascular pathologies.

Additional enzymes of the Hippo pathway include the NDR protein kinase family, particularly NDR1/STK38 and NDR2/STK38L [34, 35]. These kinases regulate a wide range of age-sensitive cellular processes, such as cell cycle control, intercellular communication, apoptosis, autophagy, nutrient homeostasis, etc. [36]. The involvement of the STK38 kinase in systemic metabolism was experimentally demonstrated: on a high-fat diet the expression of STK38 was significantly increased, which, in turn, led to the development of inflammation and insulin resistance [37]. It has been established that *STK38/STK38L* acts as a main factor in response to stress and plays an important role in autophagy [10]. The expression of the *STK38L* gene decreases under the influence of stress factors, and the degree of decrease correlates with chronological age [36]. The association we identified with the risk of death from all causes in the study group of the Alu insertion Ya5ac2145 in the *STK38L* gene, associated with decreased gene activity, is consistent with the presented literature data.

In our work, we established an association with survival against the background of polymorbid status of the genotype heterozygous for Alu insertion in the *LAMA2* gene, which encodes the main component of the basal membrane laminin. When studying the role of laminin gene expression in the formation of various aging processes, contradictory results were demonstrated, which can be explained by the tissue specificity of this protein [12]. Interestingly, in muscles with a deficiency of the α2-chain of laminin, the expression of autophagy genes increases [13]. From the perspective of adaptation and survival in old age, the results of this work can be considered as an interaction of various compensatory mechanisms.

The involvement of Alu insertion polymorphism of the *SEMA6A* gene in survival during cerebrovascular events directly confirms the role of semaphorin-6 in the structural and functional organization of the nervous system [14]. In addition, many studies have shown that semaphorins affect cell proliferation, migration, and apoptosis by acting on components of the vascular wall and thereby participating in many pathological processes of the circulatory system [15].

Thus, Alu polymorphisms, for which associations with survival and mortality have been established, are localized in genes involved in the processes of apoptosis and autophagy. Recent advances in understanding the temporal and spatial consequences of autophagy dysregulation for tissue homeostasis have revealed a complex and multifactorial relationship between autophagy and aging. Being a highly conserved pathway for the destruction of defective cellular components, autophagy acts as an important endogenous mechanism providing relief from cellular stress states, while chronic activation of autophagy can lead to cell death [38]. In general, our data are consistent with the concept of age-dependent decrease in the number of proteins associated with autophagy and providing transport to lysosomes, which indicates impaired autophagy as one of the important factors of organism aging [34]. Alu retrotransposons can influence gene function in many different ways, mainly leading to decreased

gene expression levels. This suggests that this type of genetic polymorphism may be associated with a number of pathological age-dependent phenotypes and, accordingly, with the duration and quality of human life.

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## ETHICS DECLARATION

The study was approved by the Ethics Committee of IBG UFRC RAS on June 06, 2024, protocol No. 8.

## STATEMENT OF COMPLIANCE WITH ETHICS REQUIREMENTS

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the Declaration of Helsinki 1964 and its later amendments or comparable ethical standards.

Informed voluntary consent was obtained from each participant included in the study. All examined individuals were adults.

## CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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