Three Novel Genes Associated with Longevity Found in European Bison

Evžen Korec (director@zootabor.eu)
Zoologická zahrada Tábor a.s
Lenka Ungrová
Institute of Molecular Genetics
Jiří Hejnar
Institute of Molecular Genetics
Adéla Grieblová
Zoologická zahrada Tábor a.s
Kateřina Zelená
Zoologická zahrada Tábor a.s

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Abstract

Background: Longevity-related genes have been found in mice, humans and in several other animal species. The goal of this study was to perform genetic analysis of long-lived European bisons with the aim to find genes that are associated with longevity.

Results: SNPs with particular nucleotides were significantly overrepresented in long-lived European bisons in three genes: BCKDHB, FER1L6 and SERPINI2.

In SERPINI2, the longevity-associated SNP localizes to an exon. In the protein coded by the SERPINI2 gene, amino acid leucine present in the reference European bisons is replaced by tryptophan in the long-lived European bisons.

Conclusions: Three genes, BCKDHB, FER1L6 and SERPINI2, were proved to be associated with longevity in European bisons.

Background

European bison (*Bison bonaus*) was in the past one of the key large herbivore species in european lowlands with an areal range across the whole Europe and east Asia. It became extinct in the wild at the beginning of the 20th century due to intensive unlimited hunting, poaching and habitat fragmentation [1]. Only several dozen animals survived in zoos and private farms. In 1920, an attempt was made to restore the species from which two genetic lineages of European bison have been diversified. Although the lowland line (L) of European bison originated from only seven founders of *B. b. bonasus* subspecies, approximately 80% of the genes in the contemporary population came from as few as two founders. Thus, the average inbreeding coefficient in the L line is almost 50% [2]. The *B. b. caucasicus* subspecies survives in hybrid form as the lowland-Caucasian line (LC), which originated from 12 animals and the inbreeding coefficient of the LC line is now 28% [2]. The genus *Bison* has been studied from the evolutionary [3–5], genetic [6–9], behavioral [10, 11] and conservational [12, 13] points of view. The European bison is an exceptional genetic model of a large mammal with a high degree of inbreeding.

In our previous study we were the first to determine the median lifespan of the European bison as only 3.54 years. The median lifespan of females (6.01 years) exceeded more than twice the median lifespan of males (2.71 years). Along with American bison (*Bison bison*), the genus Bison has the biggest sex-related difference in longevity among mammals [14]. Despite the low median lifespan, some European bisons live up to 28 years [15, 16]. Both these facts together with a high degree of inbreeding make the European bison an interesting model for studying the longevity.

Longevity-associated genes have been described in humans, mice and in other animals [17–19]. However, no genes associated with longevity have been described in European bison so far. The aim of this study was to identify longevity-associated genes in the European bison using GWAS (genome-wide association study).

Methods

Hair samples with roots from European bison individuals were obtained from various breeders during years the 2016–2020. Samples were divided into two groups defined by the age of the examined bisons. The group of long-lived bisons contained samples from individuals older than 14 years, which were considered as long-lived according to the previous study on the European bison longevity [14]. For the reference group, we sampled bisons aged between 3–5 years. Overall, 22 samples of long-lived bisons and 20 samples of reference bisons were used for this study. Since the reference group could contain the long–living individuals, monitoring of this group will continue to uprate our results afterwards.

DNA was isolated from hair samples using a Qiagen DNeasy Blood & Tissue Kit and the standard phenol–chloroform DNA isolation protocol. DNA was eluted in 20 µl to 100 µl elution solution. The concentration and purity of isolated DNA was checked using a spectrophotometer. The required length of 5,000 base pairs for SNP genotyping was checked in 2% agarose gel. Suitable samples were diluted or concentrated to the required concentration of DNA for 20–30 ng/µl. Samples were genotyped using Illumina BovineHD BeadChip at Neogen laboratory, 4131 N. 48th St. Lincoln, NE 68504, USA. This chip allows analysis of more than 770,000 SNPs.

Statistical analysis and the necessary steps preceding association analysis were performed using PLINK v1.90b6.16 [20]. The received data were checked according to commonly used quality parameters. Firstly, SNPs that were missing in more than 10% (geno 0.1) of the samples were excluded from further analysis. From the rest of the markers, those that were missing in more than 1% (geno 0.01) of all samples were also excluded. All samples kept for further analysis had more than 95% of SNP markers genotyped (mind 0.05). After this

first step of data cleaning 546,352 variants and 27 bisons passed the data clean up. From the association analysis were also excluded SNPs with minor allele frequencies lower than 5% (maf 0.05). 13,080 SNP variants and 27 bisons passed for further genome-wide association analysis (GWAS). Manhattan plot for visualization of association analysis was constructed in R Studio [21].

According to the result of GWAS, genomic position of all candidate SNPs was checked in the Bos taurus UMD 3.1.1 reference genome. Four candidate SNPs and their close surrounding, located in four genes, were PCR amplified and sequenced in 40 samples including those used for GWAS. Samples were sequenced in SEQme s.r.o., 26301 Dobris, Czech Republic.

Statistical significance of the distribution of candidate SNPs within the long-lived and reference group was tested in R Studio [21] using Fisher's exact test [22].

Results

GWAS

Of the 23 SNPs that passed the set significance threshold (1.0e-04), seven SNPs were located within the described genes in the Bos taurus UMD 3.1.1 reference genome. According to the results of GWAS, we selected three SNPs located in the intron region with the highest p value for further sequencing (Fig. 1). Next, one SNP located in the exon region, which passed through the significance threshold of 1.0e-03 was also sequenced in the whole sample panel (Fig. 1). Results from the association analysis for the four selected SNPs and their position in particular genes are shown in Table 1.

Table 1
Table of the GWAS results for four candidate SNPs sorted by the highest P-value.

CHR	SNP	BP	A1	F_A	F_U	A2	CHISQ	P	OR	GENE	POSITION
9	BovineHD0900005530	20313162	T	0.7727	0.125	G	23.02	1.61E-06	23.8	BCKDHB	intron
14	BovineHD1400007066	24361242	G	0.5	0	A	20.09	7.38E-06	NA	XKR4	intron
14	ARS-BFGL-NGS-82859	17517923	T	0.4545	0	C	17.85	2.39E-05	NA	FER1L6	intron
1	BovineHD0100047129	100794258	G	0.4091	0.0313	T	12.33	4.45E-04	21.5	SERPINI2	exon

Sequencing

For the most significantly longevity-associated SNP (BovineHD0900005530) according to the GWAS results, located in an intron of the BCKDHB gene, association of nucleotide T with longevity was significant according to Fisher's exact test (P-value = 0.0424) after successful sequencing of 17 long-lived bisons and 12 reference bisons. Genotype TT was found in 53% of the long-lived bisons and only in 17% of reference samples. Thirty-five % of the long-lived bisons were heterozygous with genotype TG in comparison with 25% heterozygous reference samples. Only 12% of the long-lived bisons had genotype GG compared to 58% of the reference samples (Fig. 2).

SNP ARS-BFGL-NGS-82859 located in an intron of the FER1L6 gene was successfully sequenced in 20 long-lived bisons and 15 reference bisons. Association of allele T with longevity was significant according to Fisher's exact test (P-value = 0.009786). Twenty-five % of the long-lived bisons has homozygous genotype TT compared to zero individuals from the reference group. Heterozygous genotype TC was found in 45% of the long-lived samples and in 20% of the reference samples. Genotype CC was present in 30% of the long-lived bisons and in 80% of the reference bisons (Fig. 2).

In the second exon of the SERPINI2 gene in SNP BovineHD0100047129, allele G is significantly associated with longevity according to Fisher's exact test (P-value = 0.0391). This was determined by DNA sequencing of 16 long-lived bisons and 12 reference bisons. Nineteen % of the long-lived group had homozygous genotype GG compared to zero individuals from the reference group. Heterozygous genotype GT was found in 25% of the long-lived bisons compared to zero individuals from the reference group. Homozygous genotype TT was found in 56% of the long-lived group and in 100% of the reference group (Fig. 2). With this nucleotide substitution T ◊ G, amino acid leucine present in the reference group was replaced by tryptophan in the long-lived group.

Even though SNP BovineHD1400007066 located in an intron of the XKR4 gene had a low P-value in GWAS, after sequencing 17 long-lived bisons and 11 reference bisons, association of allele G with longevity was not significant according to Fisher's exact test. Genotype GG and heterozygous genotype GA was found in 17% of the long-lived group compared to zero individuals from the reference group. Homozygous genotype AA was found in 66% of the long-lived group and in 100% of the reference group (Fig. 2).

Discussion

European bison is the only species of large mammal that has a very high inbreeding coefficient; which reaches 50% in the L line and 28% in the LC line [2]. The high inbreeding coefficient is due to the fact that the rescue of the species was carried out by crossing a very small number of founder animals. Uniformity should prevail in the population of the European bison and variability should be minimal. However, this assumption is not valid at all if we examine the lifespan of individual animals. The lifespan of individual animals is highly variable. Although the median lifespan is only 3.54 years [14], some animals live to 28 years [15, 16]. Very interesting is the fact, that the median lifespan of females (6.01 years) exceeded more than twice the median lifespan of males (2.71 years). The genus Bison has the biggest sex-related difference in longevity among mammals [14].

Statistical evaluation of the lifespan of individual animals does not correspond with the normal distribution that is common for most animal species [14].

The European bison thus represents a very interesting model for the study of longevity-associated genes. Identifying genes associated with longevity in European bison could be useful in long-term conservation of this species and could improve current and future reintroduction programs thanks to selective breeding and deeper knowledge about its genetic background.

We used GWAS followed by confirmation of the results by sequencing to study these genes. The association of the BCKDHB gene with longevity has not been described so far. The BCKDHB gene encodes the E1 beta subunit of the branched-chain keto acid dehydrogenase, which is a multienzyme complex associated with the inner membrane of mitochondria. This enzyme complex is active in the catabolism of branched-chain amino acids. Mutations of this gene have been associated with the maple syrup disease type 1B, a disease characterized by a maple syrup odor of the urine, mental and physical retardation, feeding problems and dihydrolipoamide dehydrogenase deficiency [23].

The association of the FER1L6 gene with longevity has not been described so far. The FER1L6 gene (FER-1 like family member 6) is associated with diseases including cerebellar ataxia type 43 [24] and Miyoshi muscular dystrophy [25].

Determining the causal relationship between a particular nucleotide substitution and longevity can be crucial in identifying the predisposition for longevity at the molecular genetic level. SNPs located in gene exons are of greatest importance. We detected that the SNP associated with longevity in the SERPINI2 gene is located in an exon. This nucleotide substitution leads to an amino acid change resulting in the tryptophan presence in the long-lived European bisons, while leucine is present in the reference bisons.

Substitution of one amino acid can lead to a change in the structure of the protein produced, which can then cause a change in its function. Analysis of the structure of such proteins will be the subject of our further research.

The SERPINI 2 gene (SERPIN family I member 2) encodes a member of a family of proteins that acts as an inhibitor of serine protease. These proteins act in the regulation of a variety of physiological processes including coagulation, fibrinolysis, development, malignancy and inflammation [26]. Expression of the encoded protein is downregulated in pancreatic and breast cancer and it is associated with acinar cell apoptosis and pancreatic insufficiency when absent in mice [27]. SERPINI2 deficient mice are growth retarded, have abnormal immunity and reduced lifespan [28]. Association of the SERPINI2 gene with lifespan that was also found in the mice may suggest that the association of this gene with longevity is more general.

This study has its limits due to the low number of sampled European bisons. It will be useful to confirm these results in a study with a larger number of samples. However, the limited sample size still allowed statistical analyses to be performed and to determine the statistical significance of our findings.

Conclusions

Three genes, BCKDHB, FER1L6 and SERPINI2, proved to be associated with longevity in European bisons were identified using GWAS and DNA sequencing.

In BCKDHB and FER1L6 genes, the longevity-associated SNP is localized in an intron.

In the SERPINI2 gene, the longevity-associated SNP is localized in an exon.

Abbreviations

L line
lowland line
LC line
lowland-Caucasian line
GWAS
Genome-wide association study
SNP
single nucleotide polymorphism

Declarations

Acknowledgments

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Author Contributions

Conceptualization, Evžen Korec; Methodology, Lenka Ungrová, Jiří Hejnar and Adéla Grieblová; Project administration, Evžen Korec; Supervision, Evžen Korec and Jiří Hejnar; Validation, Evžen Korec and Jiří Hejnar; Visualization, Lenka Ungrová; Writing – original draft, Evžen Korec and Lenka Ungrová; Writing – review & editing, Jiří Hejnar and Kateřina Zelená. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials

The datasets used in this study are available from https://www.ebi.ac.uk/eva/?Study-Browser&browserType=sgv accession number: PRJEB51724.

Ethics approval and consent to participate

All samples were obtained non-invasively. Owners of the European bisons collected and provided all samples. Bison hair samples were collected not directly for this study. The hair samples were collected for the needs of the owners of the animals, only then the owners sent the samples to be used for this study. All owners approved the experimental protocols beforehand and all methods were performed in accordance with the relevant guidelines and regulations. Informed consent and permission to use the provided samples in this study was obtained from all owners.

Consent for publication

Not applicable.

Competing Interest

The authors declare no competing interests.

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Figures

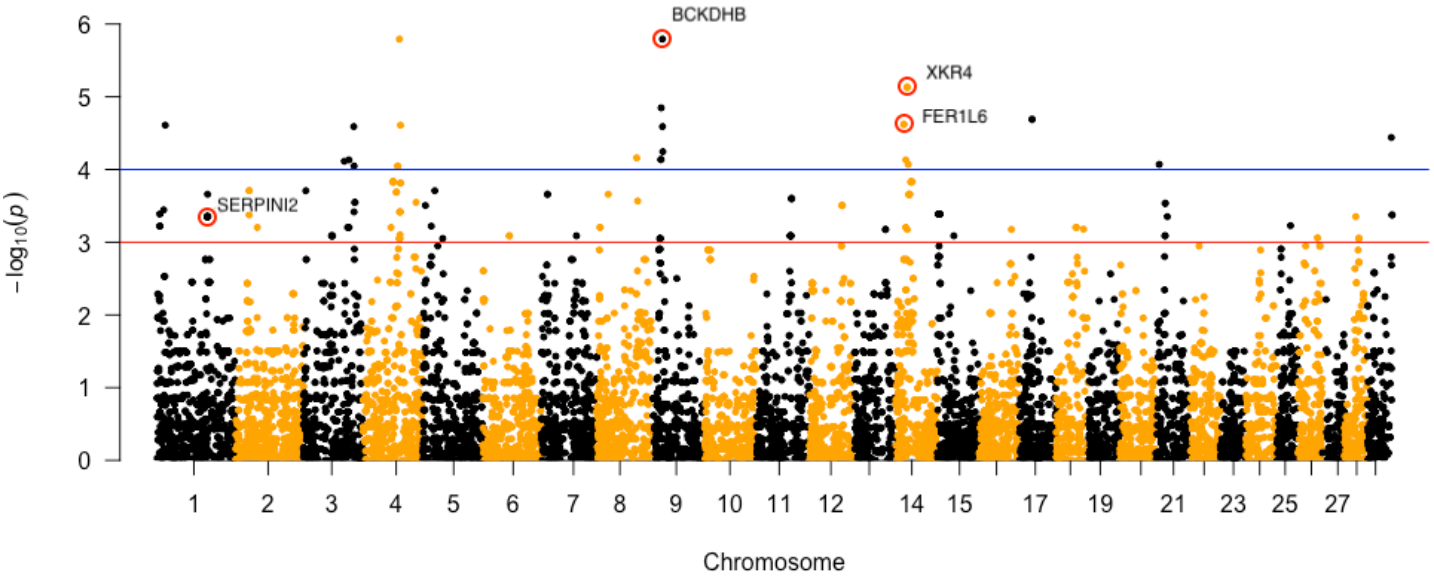

Figure 1

Manhattan plot of the GWAS results. The blue line represents a significance threshold of 1.0e-04. The red line represents a significance threshold of 1.0e-03. SNPs chosen for further analyses are circled.

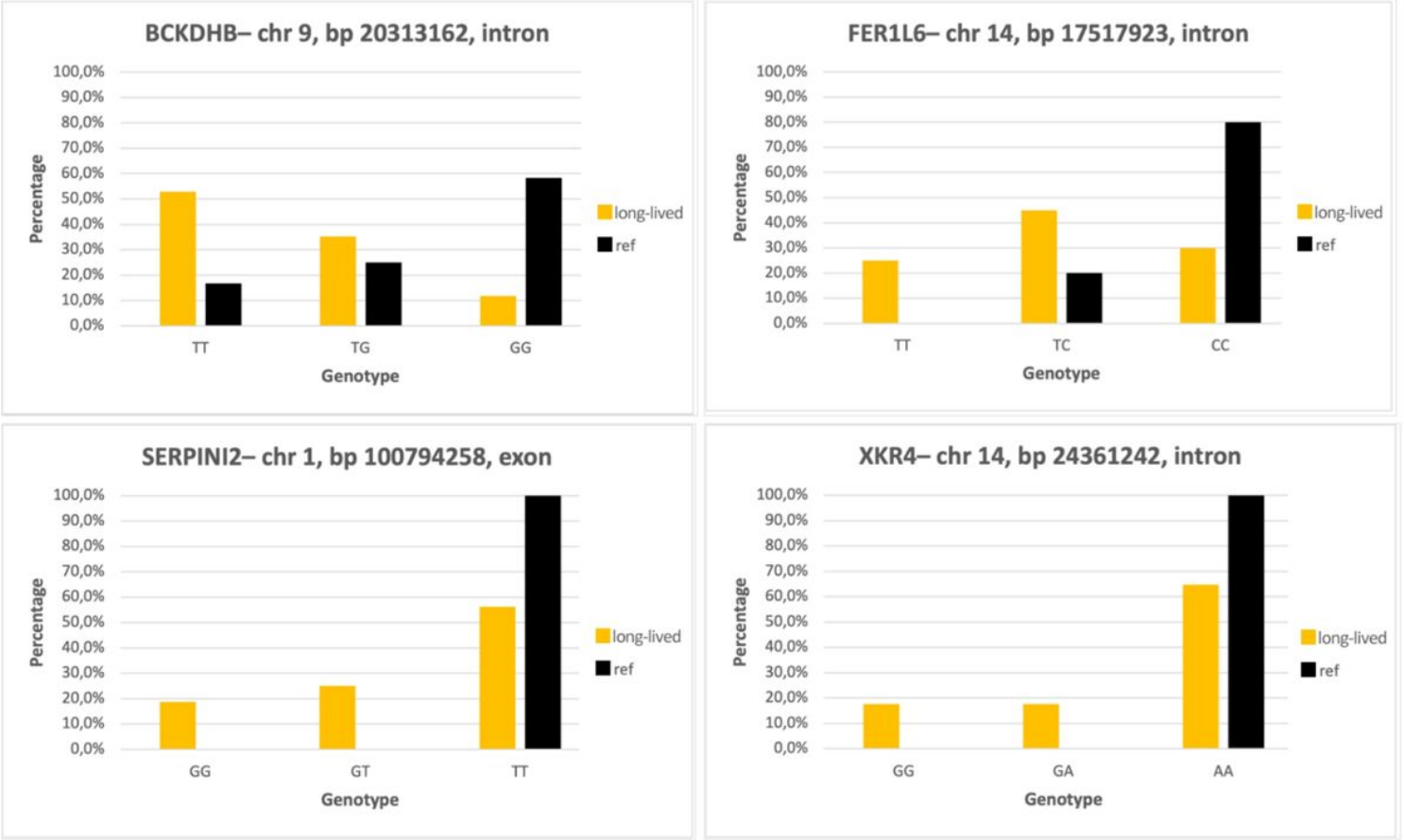

Figure 2

Distribution of genotypes in SNPs of investigated genes. Orange columns = long-lived group, black columns = reference group.

Supplementary Files

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