Association between GPX5 gene polymorphism and selenium concentration in liver and kidney of wild boars from West Pomerania province, Poland

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Abstract

The aim of the experiment was to detect polymorphism in the GPX5 gene and to determine associations between individual genotypes and selenium concentration in liver and kidney of wild boars from West Pomerania province in Poland. The polymorphism in GPX5 gene was detected using the PCR-RFLP method with specific primers and the restriction enzyme HinfI. Two different alleles of the GPX5 gene were identified - allele 1B (0.37) and 2B (0.63). The relationships between the GPX5 genotypes and concentrations of selenium in liver and kidney were analyzed, revealing statistically significant (P<0.05) differences between wild boars carrying different GPX5 genotypes in both genders. Obtained results indicated that wild boars with the 2B2B genotype had the highest selenium concentration in livers and kidneys compared to 1B1B and 1B2B animals and these differences were statistically significant (P<0.05).

Introduction

The physiological functions of selenium in mammals are thought to be mainly due to its presence as selenocysteine in ~25 selenoproteins. Several of these proteins are enzymes belong to the group of the selenoperoxidases, whose function is to protect enzymes and other cellular components from oxidative stress. The glutathione peroxidase (GPX) catalyses the reduction of oxidized lipids to metabolites which are nontoxic. There are few isoenzymes of GPX encoded by different genes that are found in different cell fractions and tissues of the body (Arthur, 2000). One of them is GPX5 which is the selenium-independent glutathione peroxidase with a relative molecular mass of 24-25 kDa. Although lack of selenocysteine residue GPX5 retaining its antioxidiant properties. Moreover it could function as a back-up system for other selenium-dependent GPXs (Vernet et al., 1999). GPX5 is expressed mainly in the epididymis and spermatozoa under androgenic control, however this protein was also found in kidney and liver (Drevet et al., 2006). GPX5 connects with the sperm surface during epididymal pass through and protects the spermatozoa from peroxide-mediated attack during their maturation (Aitken, 2009). Gene encoding glutathionylase 5 (GPX5) in pigs is localized within the major histocompatibility complex (SLA), which is assigned to chromosome 7 (Bertani et al., 1999). The porcine GPX5 gene consists of 5 exons; the transcript length is 1443 base pairs (bp) whereas protein is composed of 219 aminoacids (Ensembl). Two polymorphic sites have been identified and analyzed so far in this gene: substitution A>G, recognized by the HinfI restriction enzyme and deletion/insertion of 522bp (Bertani et al., 1999).

The purpose of this study was to estimate relative genotype and allele frequencies of single nucleotide polymorphism (SNP) in intron 1 (c.81+1896A>G, Stscafla10.2:7:242235514) of the GPX5 gene and to determine the association between individual variants of gene and selenium level in liver and kidney of wild boars came from West Pomerania. The level of selenium in soil in West Pomeranian province is known and it is very low in comparison to other provinces. For example, in the area of West Pomerania its concentration amounts 0.050-0.193 mg/kg, whereas in soils of the southern part of Poland it amounts to 0.060-0.818 µg/g. Different variants of GPX5 gene may be associated with modulation of selenium level in wild boars.

Materials and methods

The study was carried out in a pedigree herd of 172 wild boars, which were shot during the hunting seasons of 2005-2008 compliance with the hunting limits set in the West Pomeranian region. After the shooting prepared liver and kidney were transported to a wild game collection center. Selenium concentration were measured by Piłarczyk et al. (2010), who investigated its level depending on season of the year, age, sex, and body weight. Genomic DNA was extracted from the organs using EZ-10 Spin Column Genomic DNA Mini Preps Kit (Bio-Basic). Genotypes of the GPX5 were determined by PCR-RFLP with primers and thermal conditions proposed by Buske et al. (2006). PCR reactions were performed in total volume 25 µL using 100 ng DNA, 0.2 µM of each primer, 100 µM of dNTP mix, 1.5 mM MgCl2 and 0.6 U Taq DNA polymerase (MBI Fermentas) in standard PCR buffer. Digestion of PCR product was performed with 5 U of HinfI restriction endonuclease (MBI Fermentas) at 37°C overnight. The restriction fragment of DNA were separated by electrophoresis in 2% agarose gel stained with ethidium bromide. After electrophoresis, the gels were illuminated by UV rays and the genotypes were recorded. The lengths of restriction fragments detected during the experiment were: 298, 94 for allele 1B and 234, 94bp for allele 2B. Fragments with lengths 64, 53, 33 and 23bp were not clearly visible on gels. Nomenclature of analyzed polymorphism according to Bertani et al. (1999) was given as follows: 1B1B genotype (GG), 1B2B (AG) and 2B2B (AA).

The analysis of relationship between the GPX5 genotypes and selenium concentrations were examined using the SAS/STAT User’s Guide procedure PROC GLM (SAS, 2000). The following model was as follows:

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Results and discussion

An analysis of the frequencies of the GPX5 genotypes in a studied population of wild boars revealed that the 1B2B genotype was the most frequent genotype in a herd, with a frequency of 0.50. The 1B1B genotype was the least frequent (0.12), whereas the frequency of the 2B2B genotype was 0.38. Alleles 1B and 2B, however, occurred with a frequency of 0.37 and 0.63, respectively (Table 1). In a studied population deviation from Hardy-Weinberg equilibrium (HWE) was observed (P ≤ 0.05).

Makowski et al. (2004) reported higher frequency for the 2B2B genotype (0.51) and similar for the 1B2B genotype (0.46) in boars analyzed in relation to semen characteristics. From among nine different breeds and their crosses 1B1B genotype was only found in the Hampshire (0.50) and in Duroc x Pietrain crossbred (0.08). Since number of Hampshire boars was very low (n=4) the results obtained are not reliable and could not be discussed. In the examined wild boars, the 1B1B genotype frequency was somewhat higher in relation to above mentioned Duroc x Pietrain crossbred. In the case of investigations carried out by Buske et al. (2006) on crossbred (Large White) sows x Leicome boars, the 1B1B genotype had also the lowest frequency (0.06). Heterozygous genotype however occurred with lower frequency, while 2B2B genotype with higher frequency in comparison to wild boars.

In the studied herd, allele 2B was characterized by a higher frequency (0.63) than allele 1B (0.37). A similar allele frequency in a herd of Hampshire x Pietrain pigs was obtained by Kmie et al. (2007) and it amounted to 0.32 and 0.68 for allele 1B and 2B, respectively. Associations between the GPX5/Hinf polymorphism and selenium concentrations in the livers and kidneys of the examined animals were analyzed (Table 2).

A mean of selenium concentration in the livers of wild boars with the 2B2B genotype was statistically (P≤0.05) higher than in animals carrying 1B1B genotypes in male as well as in female. The same result was observed regarding selenium concentration in kidney. The male and female wild boars with the 2B2B genotype have statistically (P≤0.05) higher selenium concentration than the ones with the 1B1B genotypes.

The mean of selenium concentration in the kidneys of the examined wild boars was also analyzed. On the basis of the presented results, it can be concluded that the selenium concentration in the kidneys of females was higher than the one of males. Moreover, individuals with the 2B2B genotype were characterized by the highest selenium concentration in both sexes.

GPX5 gene polymorphism was studied in wild boars for the first time. Only Zhang et al. (2010) analyzed GPX5, FUT1, FSH and PRLR genes in F1 hybrid pigs derived from crosses between wild boars and large white pigs. Results showed that GPX5 variants were not associated with individual weight at birth and 30 days. Interestingly, authors indicated that introduction of wild boar blood did not significantly change the hereditary basis of swine. GPX5 polymorphism in pigs was analyzed mainly in relation to reproductive traits - semen characteristics and litter size. Conducted analysis showed that animals with 2B2B genotype characterized higher ejaculate volume, sperm alive percentage, live sperm content and number of insemination doses in respect to 1B1B genotype (Kmie et al., 2007). Other results showed that 2B2B genotype is associated with better quality of semen but it depends on breed as well as it is not associated with litter size (Mackowski et al., 2004; Buske et al., 2006). Latest investigations showed that GPX5 gene variants are associated with number of functional tests in Italian Large White pigs (Dall’Olio et al., 2012).

On the basis of the data obtained from the analysis of the association between the GPX5 polymorphism and the selenium content in the liver and kidney of wild boars from West Pomerania it could be initially assume that the 2B2B genotype is a favorable one but it should be verified in further investigations.

Conclusions

Two alleles and three genotypes of the GPX5 gene were identified in wild boar herd from West Pomerania province in Poland. Statistical analysis showed that individual genotypes were associated with selenium concentrations in the liver and kidney (P≤0.05).

The present study shows that different variants of GPX5 gene may influence selenium concentrations in wild boars.
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