GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Analysis of glutathione peroxidase 1 gene polymorphism and Keshan disease in Heilongjiang Province, China

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Genet. Mol. Res. 10 (4): 2996-3001 (2011)
Received February 7, 2011
Accepted July 12, 2011
Published December 2, 2011
DOI http://dx.doi.org/10.4238/2011.December.2.1

ABSTRACT. Keshan disease (KD) is an endemic cardiomyopathy associated with selenium deficiency. Recent studies indicate that glutathione peroxidase 1 (GPx1) mutation decreases GPx activity in myocardial cells and increases the risk of KD. To further clarify the correlation between GPx1 polymorphism and KD, we analyzed GPx1 polymorphism, blood selenium levels and GPx activity in KD patients and healthy controls in Heilongjiang Province. Four and 24 new mutation loci in the promoter and the exon region, respectively, of the GPx1 gene were found in the subjects, in contrast with the previously reported loci. There were no significant differences in the mutation frequency of these loci between the KD group and controls (chi-square test; $P > 0.05$). However, the mutation frequency of exon 474 was higher in the KD group (7/36) than in controls (2/41), and GPx activity was lower in the mutation group (90.475 ± 23.757 U/L) than in the non-mutation group (93.947 ± 17.463 U/L). Further investigation is necessary to clarify a possible causality between GPx1 exon 474 mutation and KD.

Key words: Keshan disease; Selenium; Glutathione peroxidase 1; Gene polymorphism

INTRODUCTION

Keshan disease (KD) is an endemic cardiomyopathy associated with selenium deficiency, named after Keshan County in Heilongjiang Province, where the first epidemic was identified in 1935. It is categorized clinically into four groups: acute, sub-acute, chronic, and latent. Epidemiological studies have shown that KD is distributed in a narrow low-selenium belt from Northeast to Southwest China (Yu, 1999). KD is mainly found in agricultural residents in the endemic belt, with higher incidence in women of childbearing age or pre-school children. Recently, the incidence of acute and sub-acute cases has been obviously reduced, and most cases at present are the chronic or latent type (Yang et al., 2010).

Accumulating evidence has shown a significant KD causality for selenium deficiency. Low selenium content has been found not only in the soil or food in the endemic region, but also in the blood or hair in KD patients in contrast to the healthy population. Furthermore, the incidence of acute and sub-acute cases may decrease dramatically after selenium supplementation (Chen, 1986). As one of the vital antioxidant elements, selenium is incorporated into selenium-containing proteins to fulfill its biological functions. For example, glutathione peroxidase (GPx) is an essential antioxidant enzyme with the active center containing selenocysteine. GPx activity decreases greatly in the case of selenium scarcity, leading to a decrease in systemic antioxidant capacity and myocardial apoptosis due to oxidative damage (Hamanishi et al., 2004).

GPx was the first selenium-containing enzyme found in mammals and the most abundant selenium-containing protein in most cells (Rotruck et al., 1973). GPx catalyzes the reduction of hydrogen peroxide to eliminate the harmful reactive oxygen species from the tissue and protects biological membranes and large molecular structures from oxidative damage. GPx activity is regulated by hepatic selenium level, and the biological function of selenium is largely based on the antioxidant activity of GPx (Lei et al., 2007). Recent studies indicate that SNP Pro198Leu may decrease GPx activity in rat myocardial cells and increase the risk of KD (Lei et al., 2009). In order to further clarify the correlation between GPx1 polymorphism and KD, we analyzed GPx1 polymorphism and blood selenium and GPx activity in KD patients in Heilongjiang Province.

MATERIAL AND METHODS

Subjects

KD patients and healthy controls were enrolled from Fuyu County in Heilongjiang Province, a severe KD endemic region, and subjected to physical examination after they have provided informed consent. KD was diagnosed based on KD Diagnosis Criteria (National Standard No. GB17021-1997), and paired controls up to 5 years old with the same gender and comparable economic and living standards were selected from the healthy population.

Determination of blood selenium

Anticoagulated blood samples (1 mL) were obtained from the subjects, and blood selenium was analyzed with hydride generation atomic fluorescence spectrometry according to National Standard methods (GB/T 5009.93-2003).

Determination of blood GPx activity

Anticoagulated blood (20 µL) was diluted with distilled water to 1 mL, and blood GSH-PX activity was detected with a dithiobis nitrobenzoic acid (DNTB) kit (Nanjing Jiancheng Bioengineering Institute).

PCR and sequencing

Genomic DNA was purified from blood samples with a Whole Blood Genomic DNA extraction kit (Hanzhou Bori). Primers were designed for the promoter (GenBank sequence No. AY327818) and the exon (Chinese GPx1 genetic sequence) (He and Yuan, 2001) and synthesized by Invitrogen. The primer sequences were: for promoter, 5'-ACTTCCTGGCCTAGCTCACCCTGG-3' (forward) and 5'-AAGGAGCAG CAGGCATGTCTGGTC-3' (forward), 574 bp; for exon, 5'-ATGTGTGCTGCTCGGCTAGC-3' (up) and 5'-GGCACAGCTGGGCCCTTGAG-3' (reverse), 606 bp. PCR was performed with a PCR amplification kit (TaKaRa) and the products were subjected to bidirectional dideoxy-mediated sequencing (Invitrogen).

Sequence alignment

Sequence alignment was performed with Lasergene 6 to find the potential mutation locus.

Statistical analysis

All quantitative data are reported as means ± SD. The *t*-test and the chi-square test were carried out with the SPSS 13.0 statistics package, with $P < 0.05$ considered to be significant.

RESULTS

Blood selenium level

Seventy-two subjects were analyzed for blood selenium level, including 38 patients and 34 controls. No statistical difference was observed between the KD group and controls (Table 1).

Table 1. Blood selenium level (mg/L) in the KD group and controls.

Group	N	Mean ± SD	*t*-test	P
KD	38	0.048 ± 0.013	1.475	0.145
Control	34	0.052 ± 0.015		

KD = Keshan disease.

Blood GPx activity

Blood GPx activity was determined in 88 subjects, including 47 KD patients and 41 controls. The *t*-test revealed a significant difference in GPx activity between the two groups, with lower GPx activity in the KD group (Table 2).

Table 2. GPx activity (U/L) in the KD group and controls.

Group	N	Mean ± SD	*t*-test	P
KD	47	89.313 ± 22.254	3.131	0.002
Control	41	102.070 ± 13.625		

KD = Keshan disease.

GPx1 genetic polymorphism

Seventy-seven subjects were analyzed for GPx1 gene polymorphism including 41 controls and 36 KD patients. Four and 24 mutated loci were detected in the promoter and the exon regions of the GPx1 gene, respectively (Table 3). The chi-square test indicated no statistical significance in locus mutation frequency between the KD group and controls ($P > 0.05$). A higher mutation frequency in exon 474 was detected in the KD group, with a lower GPx activity in the mutation group than in the non-mutation group, despite no statistical significance (Table 4).

Table 3. GPx1 gene polymorphism in KD group and controls.

	Mutated locus	KD group	Control	χ^2	P
Promoter	A42 blank	3 (36)	1 (41)	1.352	0.245
	C59T	1 (36)	2 (41)	0.226	0.635
	C60A	5 (36)	3 (41)	0.889	0.346
	A158G	2 (36)	2 (41)	0.018	0.894
Exon	C26G	2 (36)	2 (41)	0	1
	G28C	0 (36)	4 (41)	1.989	0.158
	G31C	2 (36)	2 (41)	0	1
	A35G	2 (36)	3 (41)	0	1
	G46T	4 (36)	4 (41)	0	1
	C48T	2 (36)	1 (41)	0.013	0.908
	C51T	1 (36)	1 (41)	0	1
	G63A	6 (36)	9 (41)	0.341	0.559
	G101C	4 (36)	1 (41)	1.161	0.281
	A111 blank	2 (36)	1 (41)	0.013	0.908
	G129C/blank	3 (36)	2 (41)	0.023	0.88
	C224G	6 (36)	8 (41)	0.104	0.747
	A245G	2 (36)	0 (41)	0.658	0.417
	G264Y/A/C	3 (36)	0 (41)	1.678	0.195
	T474A	7 (36)	2 (41)	2.655	0.103
	T552blank/A	7 (36)	10 (41)	0.273	0.602
	C567A/G	3 (36)	0 (41)	1.678	0.195
	T569C/G	1 (36)	3 (41)	0.145	0.703
	C570G	2 (36)	2 (41)	0	1
	A572G/C	1 (36)	2 (41)	0	1
	G574 blank/C/T	1 (36)	1 (41)	0	1
	T578C/G	1 (36)	2 (41)	0	1
	T581 blank/C	0 (36)	3 (41)	1.135	0.287
	T583G/C	0 (36)	3 (41)	1.135	0.287

KD = Keshan disease.

Table 4. GPx1 activity (U/L) in the locus 474 mutation group and the non-mutation group.

Group	N	Mean ± SD	*t*-test	P
Mutation group	8	90.475 ± 23.757	0.473	0.638
Non-mutation group	55	93.947 ± 17.463		

DISCUSSION

Previous studies have shown decreased blood selenium level and GPx activity in the KD populations in endemic regions compared to those in a normal area, although the differences were not significant (Group KDPaT, 1976; Zhu et al., 1982). The mean blood selenium level of 0.05 ppm in the subjects enrolled in this study was higher than the level of 0.02 ppm reported in the endemic population in the 1970s and 1980s, but was much lower than the level of 0.136 ppm in the healthy population in the non-endemic region or the level of 0.054-0.079 ppm in Egypt, a region with the lowest selenium level reported by the World Health Organization (WHO, 1973; Group KDPaT, 1976). Mean blood selenium level was lower in the KD group than in controls (0.048 *vs* 0.052 mg/L), although there was no statistical significance. These results indicate a low selenium level in the endemic population and the possible link between low selenium level and KD.

Notably, we found that GPx activity was significantly lower in the KD group than in controls. Since the difference in blood selenium level between these two groups was not significant, these data indicate that other factors may influence GPx activity. Given the significant correlation between GPx1 activity and its gene polymorphism (Matsuzawa et al., 2005), we hypothesized that GPx1 gene polymorphism could contribute to the difference in GPx activity between these two groups.

Four loci involved in the genetic polymorphism of GPx1 have been identified, including 602A/G, 2C/T, Ala5/Ala6, and Pro198Leu, which are implicated in cancer, diabetes or coronary heart disease (Kote-Jarai et al., 2002; Hu and Diamond, 2003; Hamanishi et al., 2004; Venardos et al., 2007). In particular, the Pro198Leu SNP has been shown to result in a lower GPx activity. Nevertheless, a previous study found no difference in Pro198Leu mutation frequency of GPx1 between the disease group and the control group in the Shaanxi Province, but found significant differences in mutation frequency of three other loci between the endemic population and the non-endemic population (Lei et al., 2009).

In the present study, we found 4 and 24 new mutation loci in the promoter and exon regions of the GPx1 gene, respectively, in relation to the previously reported loci. The chi-square test revealed no significant differences in the frequency of any of these foci between the KD group and the control group ($P > 0.05$), suggesting no variation in distribution frequency of GPx1 polymorphism between KD patients and healthy controls in the endemic region, consistent with a previous report (Lei et al., 2009). No polymorphism of the Pro198Leu SNP in GPx1 was detected in our study. However, the mutation frequency of exon 474 was higher in the KD group (7/36) than in controls (2/41), and GPx activity was lower in the mutation group (90.475 ± 23.757 U/L) than in the non-mutation group (93.947 ± 17.463 U/L), although these differences were not significant, perhaps due to the limited sample size in this study.

In summary, our study demonstrates that blood selenium level and GPx activity are lower in KD patients than healthy controls in a KD endemic region. We found that the mutation frequency of exon 474 is higher in the KD group than in controls. Further investigation is necessary to clarify the causality between GPx1 exon 474 mutation and Keshan disease.

Conflicts of interest

No competing financial interests exist.

ACKNOWLEDGMENTS

Research supported by the Harbin Innovative Talent's Specific Research Fund (#2007RFQXS073).

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