Polymorphisms in genes encoding antioxidant enzymes (SOD2, CAT, GPx, TXNRD, SEPP1, SEP15 and SELS) and risk of chronic kidney disease in Japanese - cross-sectional data from the J-MICC study

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Chronic kidney disease (CKD) is well known as a strong risk factor for both of end-stage renal disease and cardiovascular disease. To clarify the association of polymorphisms in the genes encoding antioxidant enzymes (SOD2, CAT, GPx, TXNRD, SEPP1, SEP15 and SELS) with the risk of CKD in Japanese, we examined this association using the cross-sectional data of Japan Multi-Institutional Collaborative Cohort (J-MICC) Study. The subjects were 3,285 men and women, aged 35–69 years, selected from J-MICC Study participants for whom genotyping were conducted by multiplex polymerase chain reaction-based Invader assay. The prevalence of CKD was determined for CKD stages 3–5 (eGFR <60 ml/min/1.73 m2) when those with CKD risk estimation of this life-limiting disease in the near future.

Key Words: antioxidant enzymes, genetic predisposition to disease, single nucleotide polymorphisms, chronic kidney disease

C hronic kidney disease (CKD) is recently gathering attention as a potential risk factor for both of end-stage renal disease (ESRD) and cardiovascular disease (CVD). The prevalence of CKD is increasing in Japan, affecting about 19.1 million adult patients with CKD of stage ≥3.1 The community-based study in Hisayama town showed that the age-adjusted prevalence of stage 3 and 4 CKD increased from 4.1% in 1974 through 4.8% in 1988 to 8.7% in 2002 in men, from 7.3% in 1974 through 11.2% in 1988, to 10.7% in 2002 in women.2,3 Although the prevalence of CKD is known to be remarkably higher in our country of Japan compared to those in other countries,4 it is also reported that about 8 million adults are affected by this disease in the U.S.,5 suggesting that CKD is a major universal health problem, making its prevention an important issue worldwide.

Meanwhile, inflammation is shown to play an important role in the genesis of CKD mainly through the promotion of atherosclerosis; according to recent reports, inflammation is recognized in up to 50% of CKD predialysis and dialysis patients.6 Increased oxidative stress has been hypothesized to be an important link between inflammation and cellular damage, which may result in the development of cardiovascular disease as well as CKD.7 Oxidative stress results from increased concentrations of reactive oxygen species and/or a reduction in antioxidants.8 The antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) constitute the primary defense system against reactive oxygen species (ROS) and oxidative stress. In light of these biological backgrounds, relationships between genetic variations of the genes encoding antioxidant enzymes and various diseases associated with oxidative stress are attracting attention because of the hope that they might be of benefit in screening and possible individualized prevention of these diseases.6,8 Among them, SOD2 T201C (Val16Pro), CAT C-262T C/T demonstrated the OR for CKD of 0.67 (95% CI 0.43–1.06) with the marginally significant trend for decreased odds ratio with increasing numbers of T allele (p = 0.070). There were no significant associations between the other polymorphisms with CKD risk. The present study found a marginally significant trend of the decreased risk of CKD with increasing numbers of T allele of CAT, which may suggest the possibility of personalized risk estimation of this life-limiting disease in the near future.

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representative polymorphisms that are shown to modulate antioxidant enzyme capacities and studied well in association with the risks of various chronic diseases.\(^{3,9}\)

We conducted the Japan Multi-Institutional Collaborative Cohort (J-MICC) Study, a large genome cohort study to confirm and detect gene-environment interactions in lifestyle-related diseases, mainly cancer, launched in 2005 supported by a research grant for Scientific Research on Special Priority Areas of Cancer from the Japanese Ministry of Education, Culture, Sports, Science and Technology.\(^{10,11}\)

Considering the crucial role of the antioxidant enzymes in the atherosclerosis and the involvement of atherosclerosis in the etiology of CKD, it would be plausible to hypothesize that genetic polymorphisms modulating the defense system against oxidative stress will also affect CKD risk in humans. Accordingly, to clarify the association of polymorphisms in genes encoding antioxidant enzymes (\textit{SOD2}, \textit{CAT}, \textit{GPx}, \textit{TXNRD}, \textit{SEPP1}, \textit{SEPT3} and \textit{SELS}) with the risk of CKD in Japanese, we examined this association among the Japanese subjects using the cross-sectional data of this J-MICC study.

**Materials and Methods**

**Study subjects.** Subjects were the participants of the J-MICC Study, initially conducted in 10 areas in Japan, in which about 75,000 voluntarily enrolled participants aged 35–69 years provided their blood, health checkup data, and their lifestyle data based on the questionnaire after informed consent.\(^{10}\)

In this analysis, 4,519 randomly selected J-MICC Study participants (about 500 subjects from each of the 10 areas) were analyzed, for whom 108 selected polymorphisms were genotyped.\(^{11}\)

Firstly, six subjects were excluded due to withdrawal from the study. Serum creatinine (SCR) was measured in 3,327 respondents from 8 areas out of 10. Forty-two subjects were excluded because of genotyping error, and the remaining 3,285 subjects were included in the analyses.

**Evaluation of lifestyle exposure.** Lifestyle exposures were evaluated with a self-administered questionnaire checked by trained staffs. The questionnaire included items on smoking status, alcohol consumption and food consumption. Smoking status was classified as current, former or never, and level of exposure was evaluated in pack-years. Former smokers were defined as people who had quit smoking for at least 1 year. Alcohol consumption of each type of beverage was determined by average number of drinks per day, and then converted into the Japanese sake unit; ‘gou’ (180 ml), which is equivalent to 23 g of ethanol.

**Estimated glomerular filtration rate (eGFR) and definitions of CKD.** SCr was measured in all participants using the enzymatic method. The eGFR of each participant was calculated based on SCr, age, and sex using the following Japanese eGFR equation proposed by the Japanese Society of Nephrology: eGFR (ml/min/1.73 m\(^2\)) = 194 × SCr (mg/dl)\(^{-1.094} \times \text{age}^{-0.287}\) (for females\(^{12}\)) The prevalence of CKD was determined for CKD stages 3–5 (defined as eGFR \(<60\) ml/min/1.73 m\(^2\)).

**Genotyping of polymorphisms.** DNA was extracted from buffy coat with a BioRobot M48 Workstation (QIAGEN Group, Tokyo, Japan). The genotyping of \textit{SOD2} T201C (Val16Pro), \textit{CAT} C-262T, \textit{GPx1} Pro198Leu, \textit{GPx4} Ex7 + 77 C>T (Leu220Leu), \textit{GPx4} 273 bp 3’ of \textit{STP} [\textit{C>T}], \textit{TXNRD1} IVS1-181 [\textit{C>G}], \textit{SEPP1} 1345 G>A (3’UTR), \textit{SEPP1} 31,174 bp 3’ of \textit{STP}, \textit{SEPP1} 43,881 bp 3’ of \textit{STP}, \textit{SEPP1} 44,321 bp of 3’ of \textit{STP} [\textit{C>T}], \textit{SELS} G-105A and \textit{SEPT3} 5’UTR polymorphisms was conducted by the RIKEN institute using multiplex polymerase chain reaction-based Invader assay (Third Wave Technologies, Madison, WI) as described previously.\(^{13}\) The genotype distributions of all the 108 polymorphisms examined in this cross-sectional study are shown in the recently published data.\(^{13}\)

**Statistical analysis.** Logistic regression analysis was performed for estimating age- and sex-adjusted odds ratios (aORs) and 95% CI for CKD by genotype. All the other potential confounders of body mass index (BMI), systolic blood pressure, diastolic blood pressure, use of anti-hypertensive drugs, fasting plasma glucose, hemoglobin A1c (HbA1c), use of glucose-lowering drugs, total cholesterol, high density lipoprotein (HDL) cholesterol, triglyceride, use of lipid-lowering drugs, uric acid, past history of cardiovascular diseases, past history of cerebrovascular diseases, smoking status and drinking status were tested for change in estimate (CIE) to see if any of these covariates produces significant change in estimates.\(^{13}\) We decided not to include any of these variables because none of them fulfilled the criteria of CIE $\geq 0.1$. Gene-environment interactions were assessed by the logistic model, which included a multiplicative interaction term as well as variables for each genotype, age, sex, and smoking and drinking habits. Age adjustments in the analyses were done with ages regarded as continuous variables. Trend analyses by genotypes were done with genotypes for each polymorphism coded as ordinal-categorical variables. Differences in the distribution of the values of each characteristic variable between two groups (i.e., CKD (+) and CKD (−)) were examined by Student’s $t$ test or by the $\chi^2$ test. According to the Hardy-Weinberg’s equilibrium, which indicates an absence of discrepancy between genotype and allele frequencies, was checked using the $\chi^2$ test. Haplotype analysis using genotypes in two loci was calculated by the ‘haplologit’ command of STATA adjusted for age and sex based on the EM algorithm.\(^{13}\)

The linkage disequilibrium (LD) between the polymorphisms in two loci ($D'$ and $r^2$) was estimated by the ‘pwld’ command of STATA. All the calculations were done using the STATA version 10 (Stata Corp, College Station, TX).

**Results**

**Characteristics of the subjects and allele frequency of the \textit{SOD2}, \textit{CAT}, \textit{GPx}, \textit{TXNRD}, \textit{SEPP}, \textit{SEP} and \textit{SELS} polymorphisms.** The characteristics of the subjects are summarized in Table 1. The mean age ± SD was 56.7 ± 8.6 years, and the males were 48.7% in the whole subjects. Subjects with CKD accounted for 17.3% (568/3,285) of the entire study population. Age, systolic blood pressure, total cholesterol and uric acid were significantly higher in subjects with CKD, and use of anti-hypertensive/lipid-lowering drugs, past history of cardiovascular/ cerebrovascular diseases, and current smokers were more frequently observed in subjects with CKD, relative to those without CKD.

The genotype frequencies among the genotyped subjects were in accordance with Hardy-Weinberg’s equilibrium for all of the \textit{SOD2} T201C (Val16Pro) (C allele = 0.133 [minor allele frequency], $\chi^2$ = 1.036, $p$ = 0.3089), \textit{CAT} C-262T (T allele = 0.030, $\chi^2$ = 0.001, $p$ = 0.9777), \textit{GPx1} Pro198Leu (T allele = 0.075, $\chi^2$ = 4.767, $p$ = 0.0290), \textit{GPx4} Ex7 + 77 C>T (Leu220Leu) (T allele = 0.354, $\chi^2$ = 1.539, $p$ = 0.2148), \textit{GPx4} 273 bp 3’ of \textit{STP} [C>T] (T allele = 0.185, $\chi^2$ = 0.009, $p$ = 0.9226), \textit{TXNRD1} IVS1-181 [C>G] (G allele = 0.025, $\chi^2$ = 0.382, $p$ = 0.5365), \textit{SEPP1} 1345 G>A (3’UTR) (A allele = 0.383, $\chi^2$ = 0.033, $p$ = 0.8548), \textit{SEPP1} 31,174 bp 3’ of \textit{STP} (T allele = 0.352, $\chi^2$ = 0.000, $p$ = 0.9878), \textit{SEPP1} 43,881 bp 3’ of \textit{STP} (A allele = 0.383, $\chi^2$ = 0.052, $p$ = 0.8193), \textit{SEPP1} 44,321 bp of 3’ of \textit{STP} [C>T] (C allele = 0.456, $\chi^2$ = 1.432, $p$ = 0.2314), \textit{SELS} G-105A (A allele = 0.281, $\chi^2$ = 0.252, $p$ = 0.6157), and \textit{SEPT15} 3’UTR (T allele = 0.043, $\chi^2$ = 0.756, $p$ = 0.3845). Genotype call rate was more than 99.6% for each genotype among those with SCr data ($n$ = 3,327).

\textit{SOD2}, \textit{CAT}, \textit{GPx}, \textit{TXNRD}, \textit{SEPP1}, \textit{SEPT15} and \textit{SELS} polymorphisms and risk of CKD. When those with \textit{CAT} C-262T C/C were defined as reference, those with \textit{CAT} C-262T C/T demonstrated the OR for CKD of 0.67 (95% CI 0.43–1.06) with the marginally significant trend for decreased OR with the increasing number of \textit{T} allele ($p$ = 0.070). There were no significant associa-
tions between the polymorphisms in other polymorphisms in genes encoding antioxidant enzymes (SOD2 T201C (Val16Pro), CAT C-262T, GPx1 Pro198Leu, GPx4 Ex7 + 77 C>T (Leu220Leu), GPx4 273 bp 3' of STP [C>T], TXNRD1 IVS1-181 [C>G], SEPP1 1345 G>A (3'UTR), SEPP1 31,174 bp 3' of STP, SEPP1 43,881 bp 3' of STP, SEPP1 44,321 bp 3' of STP [C>T], SELS G-105A and SEPP1 1345 G>A (3'UTR)), revealed that A. Hishida et al. identified so far in humans, (23) GPx4 is the only GPx that can react with complex lipid hydroperoxides (GPx6). Of all the GPx family members, accumulated evidence suggests the particular importance of GPx1 and GPx4 in human chronic diseases. (25)

Table 1. Comparison of characteristics between subjects with and without CKD (n = 3,285)

|                          | CKD (+) (n = 568) | CKD (-) (n = 2,717) | p value |
|--------------------------|-------------------|---------------------|---------|
| eGFR (ml/min/1.73 m²)   | 53.6 ± 6.0        | 78.3 ± 12.5         | <0.001  |
| Age (y)                  | 60.4 ± 7.2        | 55.9 ± 8.7          | <0.001  |
| Male                     | 263 (46.3%)       | 1,337 (49.2%)       | 0.208   |
| Body mass index          | 23.5 ± 3.0        | 23.4 ± 3.3          | 0.464   |
| Systolic blood pressure  | 130.3 ± 19.8      | 128.2 ± 19.3        | 0.017   |
| Diastolic blood pressure | 79.0 ± 12.4       | 78.7 ± 11.9         | 0.608   |
| Use of anti-hypertensive | 154 (27.1%)       | 490 (18.0%)         | <0.001  |
| Fasting plasma glucose   | 99.0 ± 22.1       | 100.0 ± 20.9        | 0.351   |
| HbA1c (%)                | 5.22 ± 0.69       | 5.22 ± 0.66         | 0.971   |
| Use of glucose-lowering  | 32 (5.6%)         | 112 (4.1%)          | 0.110   |
| Total cholesterol        | 218.5 ± 33.9      | 211.0 ± 33.9        | <0.001  |
| HDL cholesterol          | 61.9 ± 16.0       | 63.3 ± 16.4         | 0.056   |
| Triglyceride (mg/dl)     | 107 (77–151)      | 104.5 (74–154)      | 0.951   |
| Use of lipid-lowering    | 73 (12.9%)        | 228 (8.4%)          | 0.001   |
| Uric acid (mg/dl)        | 5.55 ± 1.48       | 5.11 ± 1.33         | <0.001  |
| Cardiovascular diseases  | 34 (6.0%)         | 80 (2.9%)           | 0.001   |
| Cerebrovascular diseases | 31 (5.5%)         | 53 (2.0%)           | <0.001  |
| Current smokers          | 72 (12.7%)        | 489 (18.0%)         | 0.002   |
| Current drinkers         | 298 (52.5%)       | 1,522 (56.0%)       | 0.126   |

Results are expressed as means ± SD, n (%), or median (interquartile range). CKD = chronic kidney disease. CKD was defined as estimated glomerular filtration rate <60 ml/min/1.73 m². eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; HDL, high density lipoprotein.

Discussion

Oxidative stress along with inflammation is shown to promote kidney and vascular injury, and several factors are demonstrated to induce ROS in renal cells: e.g., inflammatory cytokines, Toll-like receptors, Angiotensin II, Bradykinin, arachidonic acid, thrombin, growth factors and mechanical pressure, in which NADPH oxidases, i.e., Nox enzymes are supposed to play key roles as the mediator of the ROS genesis. (16) Especially in ESRD patients, it is well-established that there is a high prevalence of acute-phase inflammation and oxidative stress, which are shown to lead to the high rate of cardiovascular morbidity and mortality, (47) suggesting the importance of oxidative stress in the CKD etiology. The present study suggested that CAT polymorphism, a key polymorphism in genes encoding antioxidant enzymes, is marginally significantly associated with the risk of CKD in Japanese. As far as we know, there is one report from Australia that investigated the association of genetic variations in antioxidant enzymes with CKD risk. (18) but this is the first one that investigated the roles of antioxidant enzyme polymorphisms in the risk of CKD in East Asian population. It is already reported that polymorphisms in both pro- and anti-inflammatory cytokines play important roles in the risk of CKD by way of atherosclerosis genesis through the modulation of the cytokine balance. (19) Our study results suggested the trend that the subjects with the T allele of CAT C-262T polymorphism was at a decreased risk of CKD, which was in line with our hypothesis and demonstrated the novel evidence that augmented antioxidant function due to this polymorphism may reduce the risk of CKD as well as other vascular diseases.

Superoxide anion, i.e., ROS, is dismutated by superoxide dismutases (SODs) to hydrogen peroxide that is catalyzed to water and oxygen by catalase or glutathione peroxidases (GPx). (20) There are three distinct isoforms of SOD identified and characterized in mammals: copper-zinc superoxide dismutase (Cu/Zn SOD) which is encoded by the SOD1 gene, manganese superoxide dismutase (MnSOD) encoded by the SOD2 gene, and extracellular superoxide dismutase (EC(S)D) encoded by the SOD3 gene. These forms of SOD elicit similar functions, although the characteristics of their structure, chromosome position, metal cofactor requirements, gene distribution, and cellular localization are distinct from one another. (21) Namely, SOD1 is present in red blood cells, SOD2 is primarily mitochondrial and SOD3 is extracellular. (22) Regarding GPx, 5 selenium-containing GPx’s have been identified so far in humans; (23) that is, GPx 1-4 and GPx6. All of them can react with H₂O₂ and soluble fatty acid hydroperoxides. GPx4 is the only GPx that can react with complex lipid hydroperoxides. (24) Of all the GPx family members, accumulated evidence suggests the particular importance of GPx1 and GPx4 in human chronic diseases. (23) GPx4 is a monomer that may facilitate modulation of the cytokine balance. (25)
Table 2. SOD2, CAT, GPx, TXN, SEPP1, SEP15 and SELS polymorphisms and risk of CKD

| Polymorphism       | Genotype | CKD (+) (n = 568) | CKD (-) (n = 2,717) | aOR (95% CI) | Trend p |
|--------------------|----------|-------------------|---------------------|--------------|---------|
| SOD2 Val16Pro (T201C) (rs4880) | T/T      | 442 (77.8%)       | 2,033 (74.8%)       | Reference    |         |
|                    | T/C      | 117 (20.6%)       | 628 (23.1%)         | 0.88 (0.70–1.10) | 0.198   |
|                    | C/C      | 9 (1.6%)          | 56 (2.1%)           | 0.77 (0.37–1.59) |         |
| CAT C–262T (rs1001179) | C/C      | 545 (96.0%)       | 2,546 (93.7%)       | Reference    |         |
|                    | C/T      | 23 (4.0%)         | 168 (6.2%)          | 0.67 (0.43–1.06) | 0.070   |
|                    | C/T      | 0 (0.0%)          | 3 (0.1%)            | 0 (-)        |         |
| GPx1 Pro198Leu (rs1050450) | C/C      | 489 (86.1%)       | 2,332 (85.8%)       | Reference    |         |
|                    | C/T      | 73 (12.9%)        | 364 (13.4%)         | 0.94 (0.72–1.24) | 0.951   |
|                    | C/T      | 6 (1.1%)          | 21 (0.8%)           | 1.37 (0.54–3.49) |         |
| GPx4 Ex7 + 77C>T (Leu220Leu) (rs713041) | C/C      | 247 (43.5%)       | 1,140 (42.0%)       | Reference    |         |
|                    | C/T      | 253 (44.5%)       | 1,217 (44.8%)       | 0.97 (0.80–1.19) | 0.340   |
|                    | T/T      | 68 (12.0%)        | 360 (13.2%)         | 0.85 (0.63–1.14) |         |
| GPx4 273 bp 3’ of STP [C>T] (rs2075710) | C/C      | 382 (67.3%)       | 1,802 (66.3%)       | Reference    |         |
|                    | C/T      | 165 (29.0%)       | 823 (30.3%)         | 0.96 (0.78–1.18) | 0.954   |
|                    | T/T      | 21 (3.7%)         | 92 (3.4%)           | 1.12 (0.68–1.84) |         |
| TXNRD1 IVS1–181 [C>G] (rs35009941) | C/C      | 539 (94.9%)       | 2,582 (95.0%)       | Reference    |         |
|                    | C/G      | 29 (5.1%)         | 132 (4.9%)          | 1.17 (0.97–1.41) | 0.879   |
|                    | G/G      | 0 (0.0%)          | 3 (0.1%)            | 1.02 (0.67–1.56) |         |
| SEPP1 1345G>A (3’ UTR) (rs7579) | G/G      | 224 (39.4%)       | 1,028 (37.8%)       | Reference    |         |
|                    | A/G      | 256 (45.1%)       | 1,292 (47.6%)       | 0.90 (0.73–1.10) | 0.720   |
|                    | A/A      | 88 (15.5%)        | 397 (14.6%)         | 1.00 (0.76–1.32) |         |
| SEPP1 31,174 bp 3’ of STP (rs12055266) | G/G      | 242 (42.6%)       | 1,138 (41.9%)       | Reference    |         |
|                    | G/A      | 245 (43.1%)       | 1,253 (46.1%)       | 0.90 (0.73–1.10) | 0.745   |
|                    | A/A      | 81 (14.3%)        | 326 (12.0%)         | 1.00 (0.76–1.32) |         |
| SEPP1 43,881 bp 3’ of STP (rs3797310) | G/G      | 224 (39.4%)       | 1,028 (37.8%)       | Reference    |         |
|                    | A/G      | 256 (45.1%)       | 1,291 (47.5%)       | 0.90 (0.73–1.10) | 0.716   |
|                    | A/A      | 88 (15.5%)        | 398 (14.6%)         | 1.00 (0.76–1.32) |         |
| SEPP1 44,321 bp of 3’ of STP [C>T] (rs2972994) | T/T       | 174 (30.6%)       | 780 (28.7%)         | Reference    |         |
|                    | C/T      | 273 (48.1%)       | 1,391 (51.2%)       | 0.87 (0.70–1.07) | 0.609   |
|                    | C/C      | 121 (21.3%)       | 546 (20.1%)         | 0.95 (0.73–1.24) |         |
| SELS G-105A (rs34713741) | G/G      | 284 (50.0%)       | 1,418 (52.2%)       | Reference    |         |
|                    | A/G      | 237 (41.7%)       | 1,080 (39.7%)       | 1.10 (0.90–1.33) | 0.339   |
|                    | A/A      | 47 (8.3%)         | 219 (8.1%)          | 1.11 (0.78–1.57) |         |
| SEP15 3’-UTR (rs5859) | G/G      | 520 (91.5%)       | 2,493 (91.8%)       | Reference    |         |
|                    | G/A      | 47 (8.3%)         | 217 (8.0%)          | 0.99 (0.70–1.38) | 0.900   |
|                    | A/A      | 1 (0.2%)          | 7 (0.3%)            | 0.85 (0.10–7.07) |         |

aOR: adjusted odds ratio (adjusted for age and sex); CKD: chronic kidney disease; SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; TXNRD, thioredoxin reductase; SEPP, selenoprotein P; SELS, selenoprotein S; SEP15, 15kDa selenoprotein.

Fig. 1. Linkage disequilibrium between the 4 SEPP1 polymorphisms.
its knockout mouse is lethal. Our study results revealed no significant association of CKD risk with the polymorphisms in these genes encoding antioxidant enzymes, SOD2 T201C (Val16Pro), GPx1 Pro198Leu, GPx4 Ex7 + 77 C>T (Leu220Leu) and GPx4 +273 bp 3’ of STP [C>T], suggesting that the roles of genetic variation of these antioxidants are limited in Japanese.

Meanwhile, CAT is an important endogenous antioxidant enzyme that detoxifies hydrogen peroxide to oxygen and water, which then helps prevent the formation of carbon dioxide bubbles in the blood, and thus limits the deleterious effects of ROS. CAT also utilizes hydrogen peroxide to breakdown toxins such as alcohol, phenol, formaldehyde, etc. In addition, CAT works in coordination with SOD to further prevent free-radical damage to human body. Based on all of these facts, CAT has been considered to be an important regulator of oxidative stress, which is involved in the genesis of various human chronic diseases. Of the several polymorphisms in CAT gene reported, one common polymorphism in the promoter region, CAT C-262T, is considered to be functional and relatively studied well in association with human diseases. Individuals with homozygous and heterozygous mutant (C allele) of CAT C-262T polymorphism are demonstrated to have increased levels of CAT mRNA and protein in erythrocytes in some studies, whereas one recent report from Egypt showed lower blood CAT activity in those with at least one T allele of CAT C-262T polymorphism compared with those with the CAT C-262T C/C genotype. Catalase is a common enzyme which catalyzes the decomposition of hydrogen peroxide to water and oxygen. Our study results revealed the marginally significant trend of the decreased risk of CKD with the increasing number of T allele of CAT C-262T polymorphism, suggesting that higher CAT activity due to this polymorphism lead to the less renal injury and CKD risk reduction in those with at least one T allele, if the reported association of the T allele of CAT C-262T polymorphism and higher CAT activity is true.

Of the remaining genes encoding antioxidant enzymes, TXNRD, SEPP1, SEP15 and SELS, mainly consists of selenoprotein genes through which selenium (Se) exerts its biological effects; Se is a micronutrient that is essential for human health, and there is an evidence that low Se status is associated with increased risk of colorectal cancer (CRC), whereas the higher Se intake will lower CRC morality, especially when Se status is low prior to supplementation. Among the selenoproteins presented, SEPP1 is a protein that transports Se to tissues, TXNRD1 is the one that functions in redox control, and SEP15 and SELS are the ones that are involved in inflammation. To the best of our knowledge, the present study is the first one that investigated the association of genetic variations in selenoproteins with the risk of human CKD, but it did not reach any statistical significance, suggesting that the roles of polymorphisms of these selenoproteins are limited in the etiology of CKD in Japanese.

There are some potential limitations in this study. At first, all of the CKD cases are diagnosed based on the SCr data, which might potentially be different from the actual GFR based on the renal measurement. Adjustments for multiple comparisons may be another issue; we decided not to adopt these adjustments in this study, because the present study was conducted under the exploratory context. In this study, the genotype frequencies of GPx1 Pro198Leu polymorphism among the entire subjects significantly deviated from the Hardy-Weinberg’s equilibrium, which may be explained by the type-I error randomly caused by the considerably small frequency of the minor allele (T allele = 0.075). Further investigations with better study designs in these aspects will also be required.

In summary, the present study found a marginally significant trend of the reduced risk of CKD with the increasing number of T allele of CAT C-262T polymorphism, which may suggest the future possibility of personalized risk estimation of this life-limiting disease in the near future.

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Abbreviations

| aOR       | adjusted odds ratio |
|-----------|---------------------|
| CAT       | catalase            |
| CKD       | chronic kidney disease |
| CVD       | cardiovascular disease |
| eGFR      | estimated glomerular filtration rate |
| ESRD      | end-stage renal disease |
| GPx       | glutathione peroxidase |
| J-MICC    | Japan Multi-Institutional Collaborative Cohort |
| LD        | linkage disequilibrium |
| ROS       | reactive oxygen species |
| SCr       | serum creatinine    |
| SELS      | selenoprotein S     |
| SEPP1     | selenoprotein P, plasma, 1 |
| SOD       | superoxide dismutase |
| TXNRD1    | thioredoxin reductase 1 |

Conflict of Interest

No potential conflicts of interest were disclosed.

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