Functional polymorphisms in antioxidant genes in Hurthle cell thyroid neoplasm - an association of GPX1 polymorphism and recurrent Hurthle cell thyroid carcinoma

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Background. Hurthle cells of the thyroid gland are very rich in mitochondria and oxidative enzymes. As a high level oxidative metabolism may lead to higher level of oxidative stress and can be associated with an increased risk for cancer, we investigated whether common functional polymorphisms in antioxidant genes (SOD2, CAT, GPX, GSTP1, GSTM1 and GSTT1) are associated with the development or clinical course of Hurthle cell thyroid carcinoma (HCTC).

Methods. A retrospective study was performed in 139 patients treated by thyroid surgery for a Hurthle cell neoplasm. HCTC, Hurthle cell thyroid adenoma (HCTA) or Hurthle cell thyroid nodule (HCTN) were diagnosed by pathomorphology. DNA was extracted from cores of histologically confirmed normal tissue obtained from formalin-fixed paraffin-embedded specimens and genotyped for investigated polymorphisms. Logistic regression was used to compare genotype distributions between patient groups.

Results. HCTC, HCTA and HCTN were diagnosed in 53, 47 and 21 patients, respectively. Metastatic disease and recurrence of HCTC were diagnosed in 20 and 16 HCTC patients, respectively. Genotypes and allele frequencies of investigated polymorphisms did not deviate from Hardy-Weinberg equilibrium in patients with HCTC, HCTA and HCTN. Under the dominant genetic model we observed no differences in the genotype frequency distribution of the investigated polymorphisms when the HCTA and HCTN group was compared to the HCTC group for diagnosis of HCTC or for the presence of metastatic disease. However, GPX1 polymorphism was associated with the occurrence of recurrent disease (p = 0.040).

Conclusions. GPX1 polymorphism may influence the risk for recurrent disease in HCTC.

Key words: Hurthle cell thyroid carcinoma; Hurthle cell neoplasia; thyroid; oxidative stress; antioxidant genes

Introduction

Hurthle cell thyroid carcinoma (HCTC) is a rare type of differentiated thyroid cancer (DTC). Traditionally, HCTC was regarded as a subtype of follicular thyroid cancer, while new evidence indicates that HCTCs may have a distinct molecular profile compared to other DTCs.¹

Clinically, and compared to other DTCs, HCTCs are considered more aggressive, with worse prognosis, requiring more stringent follow-up. HCTCs are also more likely to metastasize to neck soft tissue and distant sites, are more iodine resistant and have higher tumour-related mortality.¹⁻⁴ A definitive way to differentiate a HCTC from a benign Hurthle cell thyroid adenoma (HCTA) is based on vascular and/or transcapsular invasion.⁵⁻⁶ For HCTA, a lobectomy is a sufficient surgical procedure. However, if a HCTC is diagnosed on histologic sections after a lobectomy, then a complete...
thyroidectomy is performed as a second surgical procedure. Therefore, when a follicular neoplasm is detected with a cytological analysis of material obtained by fine-needle aspiration biopsy, the use of predictive clinical or genetic markers has been proposed, before deciding on the extent of the thyroid surgical procedure.

A Hurthle (oncocytic) cell has abundant granular eosinophilic cytoplasm, which has such an appearance because of the accumulation of a large number of mitochondria. A full-blown Hurthle cell has 4000 to 5000 mitochondria, while a human cell rich in mitochondria (oocyte) has about 1500 mitochondria only. Enzyme histochemistry studies have shown that Hurthle cells contain high concentrations of oxidant enzymes.

The respiratory redox chain in the mitochondria is considered the major source of reactive oxygen species (ROS) and other free radicals in the cell. ROS and other free radicals can oxidize target cellular proteins, membrane lipids, nucleic acids and damage their cellular structure and function. Effective protective mechanisms, comprising antioxidative molecules and compartmentalization of potentially toxic molecules, have been developed to maintain a balance between generation and detoxification of reactive oxygen species (ROS) under physiological conditions. In case of excessive ROS oxidative stress occurs. To prevent this, complex defence mechanisms including many enzymes, proteins and antioxidants are involved. Antioxidant enzymes such as manganese superoxide dismutase (Mn-SOD), glutathione peroxidase (GPX) and catalase (CAT) directly eliminate ROS, while glutathione-S-transferases (GSTs) detoxify cytotoxic secondary metabolites. Numerous functional polymorphisms in the genes coding for antioxidant enzymes have been described that may also modify their ROS detoxification capacity.

Oxidative stress and ROS have been associated with several cancers and also many complex diseases like cardiovascular disease, diabetes mellitus and neurodegenerative disorders. Several studies also found a connection between oxidative stress and thyroid diseases including neoplasia and thyroid cancer. However all these studies have been done on papillary thyroid carcinoma and/or follicular thyroid carcinoma. As Hurthle cells are very rich in mitochondria and oxidative enzymes, it is possible that antioxidant enzymes may have an important role in defence against oxidative stress. To our knowledge, there are no data in the literature about oxidative stress and HCTC or HCTA. Furthermore, there are no data about the association between HCTC/HCTA and polymorphisms of genes coding for antioxidant enzymes.

**Patients and methods**

**Patients**

A retrospective study included Slovenian patients treated by thyroid surgery for a Hurthle cell neoplasm at the Institute of Oncology Ljubljana. The medical records of all the patients were reviewed and a total of 167 patients with cytological features for a Hurthle cell neoplasm were selected for molecular analysis. As 28 patients had no sufficient formalin-fixed and paraffin-embedded (FFPE) material for DNA extraction, they were excluded from the study. Eventually, 139 patients were included.

All the patients had a Hurthle cell neoplasm diagnosed by fine-needle aspiration cytology and the majority of fine-needle aspiration biopsies were ultrasound guided. The cytological criteria for Hurthle cell neoplasms were hypercellularity, with a predominance of Hurthle cells (at least 75%), few or no lymphocytes, and scant or no colloid. Cytological slides were examined by cytopathologist, experienced in thyroid pathomorphology.

Final diagnosis of HCTC/HCTA/other was obtained by definitive histology of thyroid tissue obtained by surgical procedure. The histological features for HCTC were based on vascular invasion and/or transcapsular invasion. Histology slides were examined by a pathologist, experienced in thyroid pathomorphology.

All patients with HCTC diagnosis were regularly monitored for possible recurrent or metastatic disease. The median follow-up time was 105 (1–337) months.

The study was reviewed and approved by the Slovenian Ethics Committee for Research and Medicine (No: KME 32/12/11) and was carried out according to the Declaration of Helsinki. The study was also approved by the Institute of Oncology Ljubljana Protocol Review Board.

**Methods**

Hematoxylin and eosin (H&E) stained slides from FFPE samples were examined by a pathologist, experienced in thyroid pathomorphology, to confirm the diagnosis and to select areas representative of normal tissue. Two to three cores (1 mm in diameter) of histologically confirmed normal tissue were obtained from each specimen for DNA extraction using a QiaAmp Mini kit (Qiagen, Hilden,
Germany) according to the manufacturer’s instructions.

Genotyping of SNPs in SOD2 rs4880 (c.47C>T; p.Val16Ala), CAT rs1001179 (c.-262C>T; c.-262G>A), GPX1 rs1050450 (c.599C>T; p.Pro200Leu), GSTP1 rs1695 (c.341C>T; p.Ile105Val) and GSTP1 rs1138272 (c.313A>G; p.Ala114Val) was carried out using a fluorescence-based competitive allele-specific (KASPar) assay (Kbiosciences, Herts, UK) according to the manufacturer’s instructions. Amplifications were performed in a PCR system 9700 AB (Applied Biosystems, California, USA) as recommended by the manufacturer (Kbiosciences). Fluorescence was measured on a 7500 Real Time PCR System AB and allele discrimination data analyzed with 7500 System SDS Software (both Applied Biosystems).

GSTM1 and GSTT1 gene deletions were detected using a multiplex PCR simultaneously amplifying GSTM1, GSTT1 and BGLO genes as described previously.27 With this approach, we could identify homozygous GSTM1 or GSTT1 gene deletion, but we were not able to distinguish between carriers of one or two copies of each gene. Genotyping was repeated in 20% of samples to check for genotyping accuracy.

Statistical analysis

Median and interquartile ranges were used to describe central tendency and variability of continuous variables, while frequencies were used to describe the distribution of categorical variables. A standard chi-square test was used to assess the deviation from Hardy-Weinberg equilibrium (HWE). Logistic regression was used to compare genotype distributions between patient groups and to calculate odds ratios (ORs) and 95% confidence intervals (CIs). All statistical analyses were carried out using IBM SPPS Statistics version 19.0 (IBM Corporation, Armonk, NY, USA). A dominant genetic model was used in all statistical analyses and the level of statistical significance was set at 0.05. Haplotype analysis was performed using Thesias software28 as previously described.29

Results

In total 139 patients with cytological features for Hurthle cell neoplasm were included in the study. The female to male sex ratio was 3.8:1. Median (range) age was 54 (42–66) years. Median diameter of the tumour was 28 (20–45) mm. The final diagnosis was established by definitive histology of the thyroid tissue obtained by a surgical procedure. Patients were diagnosed as follows: 53 (38.1%) had HCTC, 47 (33.8%) HCTA, 21 (15.1%) Hurthle cell thyroid nodule (HCTN), 11 (7.9%) multinodular goiter, 4 (2.9%) follicular thyroid adenoma, while 2 (1.4%) patients had lymphocytic thyroiditis. In 46 (33%) patients, concomitant disease was recorded: 31 (22.3%) had Hashimoto thyroiditis, 12 (8.6%) diabetes mellitus, 7 (5.0%) Graves’ disease, and 4 (2.9%) patients had other malignant disease not present in the thyroid tissue.

Only patients with a final diagnosis of HCTC, HCTA or HCTN were selected for molecular analysis. The group of patients with HCTA or HCTN was compared to the group of patients with HCTC. Altogether 20 of 53 (37.7%) patients with HCTC had metastatic disease. Recurrent disease was observed in 16 (30.2%) patients with HCTC. The clinical and demographic characteristics of those patients are summarized in Table 1.

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TABLE 1. Clinical and demographic characteristics of patients with Hurthle cell neoplasms

|                  | HCTA + HCTN | HCTC |
|------------------|-------------|------|
| Number [N] (%)   | 68 (56.2)   | 53 (43.8) |
| Median age [years] (range) | 49.5 (38.5–57.8) | 62 (45.5–70.5) |
| Gender F/M [N] (%) | 58/10 (85.3/14.7) | 37/16 (69.8/30.2) |
| Median tumor diameter [mm] (range) | 26.0 (16.0–34.8) | 40.0 (25.5–65.0) |
| Metastasis [%]   | /           | 20 (37.7) |
| Recurrence [%]   | /           | 16 (30.2) |
| Concomitant disease N [%] | 16 (23.5) | 20 (37.7) |
| Hashimoto thyroiditis | 11 (16.2) | 12 (22.6) |
| Diabetes mellitus | 1 (1.5)    | 7 (13.2) |
| Graves’ disease  | 2 (2.9)    | 3 (5.7) |
| Non-thyroid Malignancy | 2 (2.9) | 2 (3.8) |

F= female; HCTA = Hurthle cell thyroid adenoma; HCTC = Hurthle cell thyroid carcinoma; HCTN = Hurthle cell thyroid nodule; M = male
HCTN are shown in Table 3. The observed genotype frequencies did not deviate from Hardy-Weinberg equilibrium in the whole cohort of patients (p > 0.050, Table 3).

The association of SOD2, CAT, GPX1 and GST polymorphisms with diagnosis of Hurthle cell neoplasm and with the presence of metastatic or recurrent disease are presented in Table 4. These associations were also adjusted for clinical parameters. Since gender, age and tumour diameter were correlated in a multivariable model, only tumour diameter was used for adjustment.

Under the dominant genetic model, no significant differences in the genotype frequency distribution of the investigated polymorphisms were observed when the HCTA and HCTN group was compared to the HCTC group (all p > 0.050). These polymorphisms were also not associated with metastatic disease (all p > 0.050). However, GPX1 polymorphism was associated with the presence of recurrent disease (p = 0.040). The association of GPX1 polymorphism and recurrent disease was even greater when adjusted for tumour diameter (p = 0.036).

Table 2. Association of clinical and demographic characteristics with Hurthle cell thyroid neoplasms, metastatic disease and recurrent disease

|                      | HCTA+HCTN versus HCTC | Metastatic disease | Recurrent disease |
|----------------------|------------------------|--------------------|-------------------|
|                      | OR (95% CI) p<sup>a</sup> | OR (95% CI) p<sup>a</sup> | OR (95% CI) p<sup>a</sup> |
| Gender               | 2.51 (1.03–6.12) 0.043  | 2.08 (0.63–6.90) 0.230 | 2.42 (0.70–8.37) 0.163 |
| Age                  | 1.04 (1.01–1.06) 0.004  | 1.07 (1.02–1.12) 0.005 | 1.05 (1.01–1.10) 0.026 |
| Tumor diameter       | 1.05 (1.02–1.07) < 0.001 | 1.09 (1.04–1.14) < 0.001 | 1.04 (1.01–1.07) 0.005 |
| Concomitant disease  | 1.97 (0.90–4.34) 0.092  | 0.83 (0.26–2.63) 0.749 | 0.83 (0.26–2.63) 0.523 |

CI = confidence interval; HCTA = Hurthle cell thyroid adenoma; HCTC = Hurthle cell thyroid carcinoma; HCTN = Hurthle cell thyroid nodule; OR = odds ratio; <sup>a</sup> = p < 0.05 was considered statistically significant

Table 3. Genotype frequencies in patients with Hurthle cell neoplasms

| Gene   | Polymorphism                          | Genotype | All patients (%) | P<sub>HWE</sub> HCTA+HCTN (%) | HCTC (%) |
|--------|---------------------------------------|----------|------------------|-------------------------------|----------|
| SOD2   | rs4880; c.47C>T; p.Val16Ala           | CC       | 26 (21.7)        | 0.903                         | 12 (17.9) |
|        |                                       | CT       | 59 (49.2)        | 34 (50.7)                     | 25 (47.2) |
|        |                                       | TT       | 35 (29.2)        | 21 (31.3)                     | 14 (26.4) |
| CAT    | rs1001179; c.-262C>T; c.-262G>A       | CC       | 70 (58.3)        | 0.907                         | 35 (52.2) |
|        |                                       | CT       | 43 (35.8)        | 30 (44.8)                     | 13 (24.5) |
|        |                                       | TT       | 7 (5.8)          | 2 (3)                         | 5 (9.4)  |
| GPX1   | rs1050450; c.599C>T; p.Pro200Leu      | CC       | 63 (52.1)        | 0.424                         | 35 (51.5) |
|        |                                       | CT       | 51 (42.1)        | 32 (47.1)                     | 19 (35.8) |
|        |                                       | TT       | 7 (5.8)          | 1 (1.5)                       | 6 (11.3)  |
| GSTP1  | rs1695; c.341C>T; p.Ile105Val         | CC       | 54 (44.6)        | 0.653                         | 28 (41.2) |
|        |                                       | CT       | 52 (43.0)        | 32 (47.1)                     | 20 (37.7) |
|        |                                       | TT       | 15 (12.4)        | 6 (11.8)                      | 7 (13.2)  |
| GSTP1  | rs1138272; c.313A>G; p.Ala114Val      | AA       | 103 (85.1)       | 0.159                         | 58 (85.3) |
|        |                                       | AG       | 16 (13.2)        | 8 (11.8)                      | 8 (15.1)  |
|        |                                       | GG       | 2 (1.7)          | 2 (2.9)                       | 0 (0)    |
| GSTM1  | Gene deletion                         | Wild type| 55 (50.9)        | /<sup>a</sup>                 | 33 (50.8) |
|        |                                       | Gene deletion | 53 (49.1) | 22 (51.2) |
| GSTT1  | Gene deletion                         | Wild type| 93 (86.1)        | /<sup>a</sup>                 | 54 (83.1) |
|        |                                       | Gene deletion | 15 (13.9) | 39 (10.7) |

HCTA = Hurthle cell thyroid adenoma; HCTC = Hurthle cell thyroid carcinoma; HCTN = Hurthle cell thyroid nodule; HWE = Hardy-Weinberg equilibrium

<sup>a</sup> HWE could not be evaluated for GSTM1 and GSTT1 as we were not able to distinguish between carriers of one or two copies of each gene.
Haplotype analysis was performed to assess the combined effect of SNPs within the GSTP1 gene. As shown in Table 5, no associations were observed between GSTP1 haplotypes and diagnosis of HCTA/HCTN versus HCTC, the presence of metastatic disease or the occurrence of recurrent disease.

**Discussion**

In the present study, we investigated whether common functional polymorphisms in antioxidant genes could be used as molecular markers for the development of HCTC or its clinical course in patients with Hurthle cell neoplasms.

In patients with cytological features for Hurthle cell neoplasm, different final diagnoses are made by definitive histology of thyroid tissue obtained by a surgical procedure. In our study group, 87% of patients with cytological features for Hurthle cell thyroid neoplasm had HCTC, HCTA or HCTN and were eligible for our study. Patient groups with benign HCTA and HCTN were combined and compared to a group with HCTC. The malignancy rate in our HCTC group was 44% and within the incidence rate of malignancy reported in the literature, where it ranged from 13% to 70%.

**TABLE 4.** Association of SOD2, CAT, GPX1 and GST polymorphisms with diagnosis of Hurthle cell neoplasm, presence of metastatic disease and occurrence of recurrent disease

| Gene   | Genotype | Diagnosis (HCTA+HCTN vs. HCTC) | Metastatic disease | Recurrent disease |
|--------|----------|---------------------------------|--------------------|-------------------|
|        |          | OR (95% CI) | p<sup>a</sup> OR-adj (95% CI) p-adj<sup>b</sup> | OR (95% CI) | p<sup>a</sup> OR-adj (95% CI) p-adj<sup>b</sup> | OR (95% CI) | p<sup>a</sup> OR-adj (95% CI) p-adj<sup>b</sup> |
| SOD2   | rs4880   | CC 0.61 (0.25–1.61) 0.254 | 0.65 (0.25–1.67) 0.373 | 1.14 (0.30–4.60) 0.772 | 0.856 (0.12–6.09) 0.706 | 1.14 (0.29–4.26) 0.782 | 0.82 (0.18–3.62) 0.778 |
|        |          | CT+TT 0.55 (0.27–1.11) 0.292 | 0.81 (0.36–1.81) 0.600 | 0.34 (0.09–1.24) 0.102 | 0.57 (0.11–2.89) 0.499 | 1.25 (0.37–4.25) 0.721 | 2.95 (0.66–13.1) 0.155 |
| CAT    | rs1011179| CC 0.65 (0.27–1.49) 0.095 | 1.02 (0.46–2.24) 0.962 | 0.63 (0.29–1.93) 0.417 | 0.72 (0.15–3.52) 0.682 | 0.25 (0.07–0.94) 0.040 | 0.19 (0.04–0.89) 0.036 |
| GPX1   | rs1050450| CC 0.73 (0.35–1.50) 0.388 | 0.92 (0.37–1.62) 0.628 | 1.30 (0.43–3.96) 0.646 | 2.40 (0.47–12.13) 0.291 | 0.46 (0.14–1.52) 0.202 | 0.49 (0.13–1.89) 0.300 |
| GSTP1  | rs1695   | CC 0.73 (0.35–1.50) 0.388 | 0.92 (0.37–1.62) 0.628 | 1.30 (0.43–3.96) 0.646 | 2.40 (0.47–12.13) 0.291 | 0.46 (0.14–1.52) 0.202 | 0.49 (0.13–1.89) 0.300 |
| GSTP1  | rs1138272| AA 1.03 (0.38–2.63) 0.952 | 1.15 (0.39–3.45) 0.800 | 0.99 (0.21–4.67) 0.988 | 1.24 (0.17–9.19) 0.836 | 0.29 (0.03–2.54) 0.261 | 0.24 (0.02–2.64) 0.244 |
| GSTM1  | Wild type| 0.98 (0.46–2.13) 0.968 | 0.91 (0.39–2.12) 0.819 | 1.59 (0.47–5.39) 0.456 | 1.40 (0.23–8.57) 0.716 | 1.32 (0.38–4.64) 0.666 | 1.24 (0.28–5.41) 0.774 |
| GSTT1  | Wild type| 0.50 (0.15–1.70) 0.269 | 0.44 (0.11–1.62) 0.257 | 1.44 (0.18–11.29) 0.730 | 0.83 (0.02–39.34) 0.923 | 2.00 (0.25–15.85) 0.512 | 1.42 (0.12–20.98) 0.798 |

**TABLE 5.** Association of GSTP1 haplotypes and diagnosis of Hurthle cell neoplasm, presence of metastatic disease and occurrence of recurrent disease

| Haplotype | Estimated frequency | Diagnosis (HCTA+HCTN vs. HCTC) | Metastatic disease | Recurrent disease |
|-----------|---------------------|---------------------------------|--------------------|-------------------|
|           |                     | OR (95% CI) | p<sup>a</sup> | OR (95% CI) | p<sup>a</sup> | OR (95% CI) | p<sup>a</sup> |
| AC        | 0.68                | Reference | Reference | Reference |
| GC        | 0.25                | 0.88 (0.49–1.60) 0.686 | 1.04 (0.38–2.86) 0.935 | 0.45 (0.13–1.64) 0.230 |
| GT        | 0.07                | 0.83 (0.33–2.13) 0.704 | 0.99 (0.21–4.72) 0.988 | 0.28 (0.03–2.89) 0.288 |

CI = confidence interval; HCTA = Hurthle cell thyroid adenoma; HCTC = Hurthle cell thyroid carcinoma; HCTN = Hurthle cell thyroid nodule; OR = odds ratio; <sup>a</sup> = p less than 0.05 was considered statistically significant; <sup>b</sup> = adjusted for tumor diameter
tumour diameter (69 versus 30 mm and 62 versus 30 mm, respectively) or were significantly older (67 versus 53 years and 65 versus 54 years, respectively) at initial diagnosis than the HCTC patients that did not have metastatic or recurrent disease. However, it has to be noted that our HCTC group with metastatic or recurrent disease was relatively small compared to non-metastatic or non-recurrent HCTC group.

To establish whether common functional polymorphisms in genes coding for antioxidant genes could be used as molecular markers for the development of HCTC or its clinical course, we investigated associations between SOD2, CAT, GSTP1, GSTM1, GSTT1 and GPX1 genotypes and the clinical characteristics of patients with definite diagnosis of HCTC, HCTA or HCTN.

CAT -262C>T genotype frequencies observed in our patient group were in accordance with those previously published for a healthy population.34-36 In our study CAT -262C>T polymorphism was not associated with HCTC, or metastatic or recurrent disease. To our knowledge CAT -262C>T has not been studied in HCTC, but higher CAT activity has been associated with papillary thyroid carcinoma and follicular carcinoma.34-37 It has been demonstrated that CAT -262C>T polymorphism influences the binding of transcriptional factors and is associated with a decrease in enzyme expression35,38,39, but also with higher CAT activity.34,40

Also SOD2 Val16Ala genotype frequencies in our patients with Hurthle cell neoplasms were similar to frequencies previously reported in Caucasian patients.41-44 In our study SOD2 Val16Ala polymorphism was not associated with the occurrence of HCTC, or with metastatic or recurrent disease. To our knowledge this polymorphism has not been studied in HCTC yet. SOD2 Val16Ala polymorphism leads to less efficient transport of SOD2 into mitochondrial matrix in vitro45, but association studies of SOD2 in thyroid cancers gave inconclusive results. Two groups showed an increased SOD2/SOD level or activity in follicular and papillary thyroid cancer, while one group found no change of SOD activity in papillary thyroid cancer.37,46,47 On the other hand a reduced level of SOD2 was found in poorly differentiated thyroid cancers.48

Frequencies of GSTP1, GSTM1 and GSTT1 polymorphisms in our patients were similar to previously reported studies.49 However, in the HCTC group we noticed a lower percentage of GSTT1 gene deletion, compared to the HCTN/HCTA group. GSTP1 genotypes and haplotypes as well as GSTT1 and GSTM1 deletions were not associated with the occurrence of HCTC and neither with metastatic nor recurrent disease. Our findings are in agreement with a previous study that also found no association between GSTM1 and GSTT1 polymorphisms and HCTC.50 Both GSTP1 Ile105Val and GSTP1 Ala114Val decrease enzymatic activity51,52, while GSTM1 and GSTT1 deletion polymorphisms result in the complete loss of enzymatic activity in homozygous carriers.53 Some previous studies have shown possible associations of GSTP1, GSTM1 or GSTT1 polymorphisms, or a combination of GST1 and GSTM1 null allele with papillary and/or follicular thyroid cancer54-58, while others found no association between these polymorphisms and primary or secondary thyroid cancers.59,62

Frequencies of GPX1 Pro198Leu genotypes in our study group were also similar to the ones previously reported.63 We did not find any association of GPX1 Pro198Leu polymorphism with the occurrence of HCTC or with metastatic disease, even though several groups have found decreased activity or decreased expression of GPX1 in thyroid carcinomas54,47,64,65, while one group reported increased levels of GPX1 in papillary thyroid carcinoma.57 Several groups also reported that Leu variant could lead to lower GPX1 activity in patients with lung cancer, breast cancer, prostate cancer, bladder cancer and some other cancers.66-68 We observed an interesting association between GPX1 Pro198Leu polymorphism and lower probability for recurrent disease. Our findings are consistent with a previous report on the association of GPX1 198Leu variant with lower risk of recurrence in cancer patients.69 A possible explanation may be that some HCTC therapies (radioiodine ablation and radiotherapy) are large ROS generators with antineoplastic effects and may also influence the patient’s prognosis after these treatments. As GPX1 198Leu variant is associated with reduced removal of ROS and their secondary products produced by some HCTC therapies, patients with variant allele may have a better prognosis and longer recurrence-free survival time.

To sum up, in our study we did not find any association between common functional polymorphism antioxidant genes (SOD2, CAT, GPX1, GSTP1, GSTM1, and GSTT1) and the development of HCTC. A possible explanation could be that these polymorphisms may influence an initial and shared phase of HCTC and HCTA/HCTN development. Common functional polymorphisms in SOD2, CAT, GSTP1, GSTM1 or GSTT1 were also
not associated with metastatic or recurrent disease development, while GPX1 Pro198Leu polymorphism may modulate the risk of HCTC recurrence. However, the group of patients with recurrent disease was relatively small, so it is possible that the results may result from sampling error. Ideally, our findings relating both to statistically significant associations and not significant associations, should be confirmed in an independent sample cohort. Because of the rarity of these tumours, it was impossible to perform a validation study in a single institution. Further research in a larger group is needed before we can conclude that GPX1 Pro198Leu polymorphism could be used as an additional molecular marker in clinical practice to support decisions about follow-up procedures in patients with HCTC.

Conclusions

In conclusion, GPX1 Pro198Leu polymorphism may influence the risk for recurrent disease in HCTC, however, these results must be validated in an independent sample cohort.

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