

# 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 neoplasm; 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.<sup>1</sup>

Clinically, and compared to other DTCs, HCTCs are considered more aggressive, with worse prog-

nosis, 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.<sup>1-4</sup> A definitive way to differentiate a HCTC from a benign Hurthle cell thyroid adenoma (HCTA) is based on vascular and/or transcapsular invasion.<sup>5-9</sup> 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<sup>4,10</sup> or genetic markers<sup>11</sup> has been proposed, before deciding on the extent of the thyroid surgical procedure.

A Hurthle (oncocyctic) 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.<sup>7,12</sup> Enzyme histochemistry studies have shown that Hurthle cells contain high concentrations of oxidant enzymes.<sup>13</sup>

The respiratory redox chain in the mitochondria is considered the major source of reactive oxygen species (ROS) and other free radicals in the cell.<sup>14</sup> 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.<sup>15,16</sup> 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.<sup>17</sup>

Oxidative stress and ROS have been associated with several cancers and also many complex diseases like cardiovascular disease, diabetes mellitus and neurodegenerative disorders.<sup>15,18</sup> Several studies also found a connection between oxidative stress and thyroid diseases including neoplasia and thyroid cancer.<sup>16,19-25</sup> 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.<sup>4,10</sup> 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.<sup>26</sup> 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.<sup>26</sup> 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.<sup>4</sup>

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.<sup>27</sup> 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 SPSS 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 software<sup>28</sup> as previously described.<sup>29</sup>

## 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

**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

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%) multi nodular 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 (30.0%) patients with HCTC. The clinical and demographic characteristics of those patients are summarized in Table 1.

The patients from the HCTC group had a different gender (F/M) ratio ( $p = 0.043$ ), were older ( $p = 0.004$ ) and had a larger tumour diameter ( $p < 0.001$ ) in comparison to the patients from the HCTA or HCTN group (Table 2). In the HCTC group, independent risk factors for both metastatic disease and recurrent disease were the patient’s age and tumour diameter as shown by logistic regression analysis (Table 2).

Genotype frequencies of the investigated polymorphisms in patients with HCTC, HCTA and

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 less than 0.05 was considered statistically significant

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 3. Genotype frequencies in patients with Hurthle cell neoplasms

Gene	Polymorphism	Genotype	All patients (%)	P <sub>HWE</sub>	HCTA+HCTN (%)	HCTC (%)
<i>SOD2</i>	rs4880; c.47C>T; p.Val16Ala	CC	26 (21.7)	0.903	12 (17.9)	14 (26.4)
		CT	59 (49.2)		34 (50.7)	25 (47.2)
		TT	35 (29.2)		21 (31.3)	14 (26.4)
<i>CAT</i>	rs1001179; c.-262C>T; c.-262G>A	CC	70 (58.3)	0.907	35 (52.2)	35 (66.0)
		CT	43 (35.8)		30 (44.8)	13 (24.5)
		TT	7 (5.8)		2 (3)	5 (9.4)
<i>GPX1</i>	rs1050450; c.599C>T; p.Pro200Leu	CC	63 (52.1)	0.424	35 (51.5)	28 (52.8)
		CT	51 (42.1)		32 (47.1)	19 (35.8)
		TT	7 (5.8)		1 (1.5)	6 (11.3)
<i>GSTP1</i>	rs1695; c.341C>T; p.Ile105Val	CC	54 (44.6)	0.653	28 (41.2)	26 (49.1)
		CT	52 (43.0)		32 (47.1)	20 (37.7)
		TT	15 (12.4)		8 (11.8)	7 (13.2)
<i>GSTP1</i>	rs1138272; c.313A>G; p.Ala114Val	AA	103 (85.1)	0.159	58 (85.3)	45 (84.9)
		AG	16 (13.2)		8 (11.8)	8 (15.1)
		GG	2 (1.7)		2 (2.9)	0 (0)
<i>GSTM1</i>	Gene deletion	Wild type	55 (50.9)	/°	33 (50.8)	22 (51.2)
		Gene deletion	53 (49.1)		32 (49.2)	21 (48.8)
<i>GSTT1</i>	Gene deletion	Wild type	93 (86.1)	/°	54 (83.1)	39 (90.7)
		Gene deletion	15 (13.9)		11 (16.9)	4 (9.3)

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

° 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.

**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 <sup>b</sup> (95% CI)	p-adj <sup>b</sup>	OR (95% CI)	p <sup>a</sup>	OR-adj <sup>b</sup> (95% CI)	p-adj <sup>b</sup>	OR (95% CI)	p <sup>a</sup>	OR-adj <sup>b</sup> (95% CI)	p-adj <sup>b</sup>
SOD2 rs4880	CC	0.61	0.264	0.65	0.373	1.12	0.856	0.72	0.706	1.11	0.878	0.82	0.788
	CT+TT	(0.25-1.46)		(0.25-1.67)		(0.32-4.00)		(0.12-4.09)		(0.29-4.26)		(0.18-3.62)	
CAT rs1001179	CC	0.56	0.129	0.81	0.600	0.34	0.102	0.57	0.499	1.25	0.721	2.95	0.155
	CT+TT	(0.27-1.18)		(0.36-1.81)		(0.09-1.24)		(0.11-2.91)		(0.37-4.25)		(0.66-13.1)	
GPX1 rs1050450	CC	0.95	0.882	1.02	0.962	0.63	0.417	0.72	0.682	0.25	0.040	0.19	0.036
	CT+TT	(0.46-1.94)		(0.46-2.24)		(0.20-1.93)		(0.15-3.52)		(0.07-0.94)		(0.04-0.89)	
GSTP1 rs1695	CC	0.73	0.388	0.82	0.628	1.30	0.646	2.40	0.291	0.46	0.202	0.49	0.300
	CT+TT	(0.35-1.50)		(0.37-1.82)		(0.43-3.96)		(0.47-12.13)		(0.14-1.52)		(0.13-1.89)	
GSTP1 rs1138272	AA	1.03	0.952	1.15	0.800	0.99	0.988	1.24	0.836	0.29	0.261	0.24	0.244
	AG+GG	(0.38-2.83)		(0.39-3.45)		(0.21-4.67)		(0.17-9.19)		(0.03-2.54)		(0.02-2.64)	
GSTM1	Wild type	0.98	0.968	0.91	0.819	1.59	0.456	1.40	0.716	1.32	0.666	1.24	0.774
	Gene deletion	(0.46-2.13)		(0.39-2.12)		(0.47-5.39)		(0.23-8.57)		(0.38-4.64)		(0.28-5.41)	
GSTT1	Wild type	0.50	0.269	0.44	0.257	1.44	0.730	0.83	0.923	2.00	0.512	1.42	0.798
	Gene deletion	(0.15-1.70)		(0.11-1.82)		(0.18-11.29)		(0.02-39.34)		(0.25-15.85)		(0.1-20.98)	

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

**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.; HCTC = Hurthle cell thyroid carcinoma; HCTA = Hurthle cell thyroid adenoma; HCTN = Hurthle cell thyroid nodule; OR = odds ratio <sup>a</sup> - p less than 0.05 was considered statistically significant

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%<sup>30</sup> up to 70%.<sup>31</sup> A significant difference in age was observed between the HCTA+HCTN group and the HCTC group, with patients in the HCTC group being nearly 12 years older and having a significantly larger median size of initial tumour (26 versus 40 mm). These findings are consistent with previous reports.<sup>10,32,33</sup> We also found a small gender difference, with a significantly larger F/M ratio in the HCTA+HCTN group as compared to the HCTC group. The two groups did not differ regarding the presence of concomitant disease. Metastases were diagnosed in 38% of patients with HCTC. Furthermore, 30% of patients developed a recurrent disease. These two groups of patients had a significantly larger initial

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.<sup>34-36</sup> 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.<sup>24,37</sup> It has been demonstrated that *CAT* -262C>T polymorphism influences the binding of transcriptional factors and is associated with a decrease in enzyme expression<sup>35,38,39</sup>, but also with higher *CAT* activity.<sup>34,40</sup>

Also *SOD2* Val16Ala genotype frequencies in our patients with Hurthle cell neoplasms were similar to frequencies previously reported in Caucasian patients.<sup>41-44</sup> 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 vitro<sup>45</sup>, 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.<sup>37,46,47</sup> On the other hand a reduced level of *SOD2* was found in poorly differentiated thyroid cancers.<sup>48</sup>

Frequencies of *GSTP1*, *GSTM1* and *GSTT1* polymorphisms in our patients were similar to previously reported studies.<sup>49</sup> 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.<sup>50</sup> Both *GSTP1* Ile105Val and *GSTP1* Ala114Val decrease enzymatic activity<sup>51,52</sup>, while *GSTM1* and *GSTT1* deletion polymorphisms result in the complete loss of enzymatic activity in homozygous carriers.<sup>53</sup> Some previous studies have shown possible associations of *GSTP1*, *GSTM1* or *GSTT1* polymorphisms, or a combination of *GSTT1* and *GSTM1* null allele with papillary and/or follicular thyroid cancer<sup>54-58</sup>, while others found no association between these polymorphisms and primary or secondary thyroid cancers.<sup>59-62</sup>

Frequencies of *GPX1* Pro198Leu genotypes in our study group were also similar to the ones previously reported.<sup>63</sup> 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 carcinomas<sup>24,47,64,65</sup>, while one group reported increased levels of *GPX1* in papillary thyroid carcinoma.<sup>37</sup> 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.<sup>66-68</sup> 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.<sup>69</sup> 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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## References

- Chindris AM, Casler JD, Bernet VJ, Rivera M, Thomas C, Kachergus JM, et al. Clinical and molecular features of Hurthle cell carcinoma of the thyroid. *J Clin Endocrinol Metab* 2015; **100**: 55-62.
- Cannon J. The significance of hurthle cells in thyroid disease. *Oncologist* 2011; **16**: 1380-7.
- Barnabei A, Ferretti E, Baldelli R, Procaccini A, Spriano G, Appetecchia M. Hurthle cell tumours of the thyroid. Personal experience and review of the literature. *Acta Otorhinolaryngol Ital* 2009; **29**: 305-11.
- Petric R, Gazic B, Besic N. Prognostic factors for disease-specific survival in 108 patients with Hurthle cell thyroid carcinoma: a single-institution experience. *BMC Cancer* 2014; **14**: 777.
- Baloch ZW, LiVolsi VA. Our approach to follicular-patterned lesions of the thyroid. *J Clin Pathol* 2007; **60**: 244-50.
- Hedinger C, Williams ED, Sobin LH. The WHO histological classification of thyroid tumors: a commentary on the second edition. *Cancer* 1989; **63**: 908-11.
- Rosai J. Tumors of the thyroid gland. In: Rosai J, Carcangiu ML, editors. Atlas of Tumor Pathology. Washington: Armed Forces Institute of Pathology; 1992. p. 31-50.
- Baloch ZW, Fleisher S, LiVolsi VA, Gupta PK. Diagnosis of "follicular neoplasm": a gray zone in thyroid fine-needle aspiration cytology. *Diagn Cytopathol* 2002; **26**: 41-4.
- Baloch ZW, LiVolsi VA. Fine-needle aspiration of the thyroid: today and tomorrow. *Best Pract Res Clin Endocrinol Metab* 2008; **22**: 929-39.
- Strazisar B, Petric R, Sesek M, Zgajnar J, Hocevar M, Besic N. Predictive factors of carcinoma in 279 patients with Hurthle cell neoplasm of the thyroid gland. *J Surg Oncol* 2010; **101**: 582-6.
- Nikiforov YE, Carty SE, Chiosea SI, Coyne C, Duvvuri U, Ferris RL, et al. Highly accurate diagnosis of cancer in thyroid nodules with follicular neoplasm/suspicious for a follicular neoplasm cytology by ThyroSeq v2 next-generation sequencing assay. *Cancer* 2014; **120**: 3627-34.
- Sobrinho-Simões M, Máximo V, Castro IV, Fonseca E, Soares P, Garcia-Rostan G, et al. Hurthle (oncocytic) cell tumors of thyroid: etiopathogenesis, diagnosis and clinical significance. *Int J Surg Pathol* 2005; **13**: 29-35.
- Asa SL. My approach to oncocytic tumours of the thyroid. *J Clin Pathol* 2004; **57**: 225-32.
- Villanueva I, Alva-Sánchez C, Pacheco-Rosado J. The role of thyroid hormones as inducers of oxidative stress and neurodegeneration. *Oxid Med Cell Longev* 2013; **2013**: 218145.
- Xing M. Oxidative stress: a new risk factor for thyroid cancer. *Endocr Relat Cancer* 2012; **19**: C7-11.
- Karbownik-Lewińska M, Kokoszko-Bilska A. Oxidative damage to macromolecules in the thyroid - experimental evidence. *Thyroid Res* 2012; **5**: 25.
- Klaunig JE, Kamendulis LM, Hocevar BA. Oxidative stress and oxidative damage in carcinogenesis. *Toxicol Pathol* 2010; **38**: 96-109.
- Thanan R, Oikawa S, Hiraku Y, Ohnishi S, Ma N, Pinlaor S, et al. Oxidative stress and its significant roles in neurodegenerative diseases and cancer. *Int J Mol Sci* 2014; **16**: 193-217.
- Young O, Crotty T, O'Connell R, O'Sullivan J, Curran AJ. Levels of oxidative damage and lipid peroxidation in thyroid neoplasia. *Head Neck* 2010; **32**: 750-6.
- Wang D, Feng JF, Zeng P, Yang YH, Luo J, Yang YW. Total oxidant/antioxidant status in sera of patients with thyroid cancers. *Endocr Relat Cancer* 2011; **18**: 773-82.
- Marcocci C, Leo M, Altea MA. Oxidative stress in Graves disease. *Eur Thyroid J* 2012; **1**: 80-7.
- Klubo-Gwiedzinska J, Jensen K, Bauer A, Patel A, Costello J Jr, Burman KD, et al. The expression of translocator protein in human thyroid cancer and its role in the response of thyroid cancer cells to oxidative stress. *J Endocrinol* 2012; **214**: 207-16.
- Tabur S, Aksoy ŞN, Korkmaz H, Ozkaya M, Aksoy N, Akarsu E. Investigation of the role of 8-OHdG and oxidative stress in papillary thyroid carcinoma. *Tumour Biol* 2015; **36**: 2667-74.
- Lassoued S, Mseddi M, Mnif F, Abid M, Guermazi F, Masmoudi H, et al. A comparative study of the oxidative profile in Graves' disease, Hashimoto's thyroiditis, and papillary thyroid cancer. *Biol Trace Elem Res* 2010; **138**: 107-15.
- Karger S, Krause K, Engelhardt C, Weidinger C, Gimm O, Dralle H, et al. Distinct pattern of oxidative DNA damage and DNA repair in follicular thyroid tumours. *J Mol Endocrinol* 2012; **48**: 193-202.
- Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, et al. American Thyroid Association (ATA) Guidelines Taskforce on Thyroid Nodules and Differentiated Thyroid Cancer; Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. *Thyroid* 2009; **19**: 1167-214.
- Jazbec J, Aplenc R, Dolzan V, Debeljak M, Jereb B. GST polymorphisms and occurrence of second neoplasms after treatment of childhood leukemia. *Leukemia* 2003; **17**: 2540-2.
- Tregouet DA, Garelle V. A new JAVA interface implementation of THESIAS: testing haplotype effects in association studies. *Bioinformatics* 2007; **23**: 1038-9.
- Erčulj N, Kovač V, Hmeljak J, Franko A, Dodič-Fikfak M, Dolžan V. The influence of gemcitabine pathway polymorphisms on treatment outcome in patients with malignant mesothelioma. *Pharmacogenet Genomics* 2012; **22**: 58-68.
- Carcangiu ML, Bianchi S, Savino D, Voinick IM, Rosai J. Follicular Hurthle cell tumors of the thyroid gland. *Cancer* 1991; **68**: 1944-53.

31. Elliott DD, Pitman MB, Bloom L, Faquin WC. Fine-needle aspiration biopsy of Hurthle cell lesions of the thyroid gland: A cytomorphologic study of 139 cases with statistical analysis. *Cancer* 2006; **108**: 102-9.
32. Lopez-Penabad L, Chiu AC, Hoff AO, Schultz P, Gaztambide S, Ordoñez NG, et al. Prognostic factors in patients with Hurthle cell neoplasms of the thyroid. *Cancer* 2003; **97**: 1186-94.
33. Zhang YW, Greenblatt DY, Repplinger D, Bargren A, Adler JT, Sippel RS, et al. Older age and larger tumor size predict malignancy in hurthle cell neoplasms of the thyroid. *Ann Surg Oncol* 2008; **15**: 2842-6.
34. Forsberg L, Lyrenäs L, de Faire U, Morgenstern R. A common functional C-T substitution polymorphism in the promoter region of the human catalase gene influences transcription factor binding, reporter gene transcription and is correlated to blood catalase levels. *Free Radic Biol Med* 2001; **30**: 500-5.
35. Bastaki M, Huen K, Manzanillo P, Chande N, Chen C, Balmes JR, et al. Genotype-activity relationship for Mn-superoxide dismutase, glutathione peroxidase 1 and catalase in humans. *Pharmacogenet Genomics* 2006; **16**: 279-86.
36. Gavalas NG, Akhtar S, Gawkrödger DJ, Watson PF, Weetman AP, Kemp EH. Analysis of allelic variants in the catalase gene in patients with the skin depigmenting disorder vitiligo. *Biochem Biophys Res Commun* 2006; **345**: 1586-91.
37. Sadani GR, Nadkarni GD. Role of tissue antioxidant defence in thyroid cancers. *Cancer Lett* 1996; **109**: 231-5.
38. Ahn J, Gammon MD, Santella RM, Gaudet MM, Britton JA, Teitelbaum SL, et al. Associations between breast cancer risk and the catalase genotype, fruit and vegetable consumption, and supplement use. *Am J Epidemiol* 2005; **162**: 943-52.
39. Ahn J, Nowell S, McCann SE, Yu J, Carter L, Lang NP, et al. Associations between catalase phenotype and genotype: modification by epidemiologic factors. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 1217-22.
40. Chistiakov DA, Savost'yanov KV, Turakulov RI, Titovich EV, Zilberman LI, Kuraeva TL, et al. A new type 1 diabetes susceptibility locus containing the catalase gene (chromosome 11p13) in a Russian population. *Diabetes Metab Res Rev* 2004; **20**: 219-24.
41. Akyol O, Yanik M, Elyas H, Namli M, Canatan H, Akin H, et al. Association between Ala-9Val polymorphism of Mn-SOD gene and schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry* 2005; **29**: 123-31.
42. Galecki P, Pietras T, Szymraj J. Manganese superoxide dismutase gene (MnSOD) polymorphism in schizophrenics with tardive dyskinesia from central Poland. *Psychiatr Pol* 2006; **40**: 937-48.
43. Zai CC, Tiwari AK, Basile V, de Luca V, Müller DJ, Voineskos AN, et al. Oxidative stress in tardive dyskinesia: genetic association study and meta-analysis of NADPH quinone oxidoreductase 1 (NQO1) and Superoxide dismutase 2 (SOD2, MnSOD) genes. *Prog Neuropsychopharmacol Biol Psychiatry* 2010; **34**: 50-6.
44. Bošković M, Vovk T, Saje M, Goričar K, Dolžan V, Kores Plesničar B, et al. Association of SOD2, GPX1, CAT, and TNF genetic polymorphisms with oxidative stress, neurochemistry, psychopathology, and extrapyramidal symptoms in schizophrenia. *Neurochem Res* 2013; **38**: 433-42.
45. Sutton A, Khoury H, Prip-Buus C, Cepanec C, Pessayre D, Degoul F. The Ala16Val genetic dimorphism modulates the import of human manganese superoxide dismutase into rat liver mitochondria. *Pharmacogenetics* 2003; **13**: 145-57.
46. Nishida S, Akai F, Iwasaki H, Hosokawa K, Kusunoki T, Suzuki K, et al. Manganese superoxide dismutase content and localization in human thyroid tumours. *J Pathol* 1993; **169**: 341-5.
47. Akinci M, Kosova F, Cetin B, Sepici A, Altan N, Aslan S, et al. Oxidant/antioxidant balance in patients with thyroid cancer. *Acta Cir Bras* 2008; **23**: 551-4.
48. Russo D, Bisca A, Celano M, Talamo F, Arturi F, Scipioni A, et al. Proteomic analysis of human thyroid cell lines reveals reduced nuclear localization of Mn-SOD in poorly differentiated thyroid cancer cells. *J Endocrinol Invest* 2005; **28**: 137-44.
49. Li J, Long J, Hu Y, Tan A, Guo X, Zhang S. Glutathione S-transferase M1, T1, and P1 polymorphisms and thyroid cancer risk: a meta-analysis. *Cancer Epidemiol* 2012; **36**: e333-40.
50. Stankov K, Landi S, Gioia-Patricola L, Bonora E, Volante M, Papotti M, et al. GSTT1 and M1 polymorphisms in Hurthle thyroid cancer patients. *Cancer Lett* 2006; **240**: 76-82.
51. Ali-Osman F, Akande O, Antoun G, Mao JX, Buolamwini J. Molecular cloning, characterization, and expression in Escherichia coli of full-length cDNAs of three human glutathione S-transferase Pi gene variants. Evidence for differential catalytic activity of the encoded proteins. *J Biol Chem* 1997; **272**: 10004-12.
52. Watson MA, Stewart RK, Smith GB, Massey TE, Bell DA. Human glutathione S-transferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution. *Carcinogenesis* 1998; **19**: 275-80.
53. Hayes JD, Strange RC. Glutathione S-transferase polymorphisms and their biological consequences. *Pharmacology* 2000; **61**: 154-66.
54. Siraj AK, Ibrahim M, Al-Rasheed M, Abubaker J, Bu R, Siddiqui SU, et al. Polymorphisms of selected xenobiotic genes contribute to the development of papillary thyroid cancer susceptibility in the Middle Eastern population. *BMC Med Genet* 2008; **9**: 61.
55. Ho T, Zhao C, Zheng R, Liu Z, Wei Q, Sturgis EM. Glutathione S-transferase polymorphisms and risk of differentiated thyroid carcinomas: a case-control analysis. *Arch Otolaryngol Head Neck Surg* 2006; **132**: 756-61.
56. Granja F, Morari J, Morari EC, Correa LA, Assumpção LV, Ward LS. GST profiling may be useful in the screening for thyroid nodule malignancy. *Cancer Lett* 2004; **209**: 129-37.
57. Lemos MC, Coutinho E, Gomes L, Carrilho F, Rodrigues F, Regateiro FJ, et al. Combined GSTM1 and GSTT1 null genotypes are associated with a lower risk of papillary thyroid cancer. *J Endocrinol Invest* 2008; **31**: 542-5.
58. Gaspar J, Rodrigues S, Gil OM, Manita I, Ferreira TC, Limbert E, et al. Combined effects of glutathione S-transferase polymorphisms and thyroid cancer risk. *Cancer Genet Cytogenet* 2004; **151**: 60-7.
59. Gonçalves AJ, Carvalho LH, Serdeira K, Nakai MY, Malavasi TR. Comparative analysis of the prevalence of the glutathione S-transferase (GST) system in malignant and benign thyroid tumor cells. *Sao Paulo Med J* 2007; **125**: 289-91.
60. Kweon SS, Shin MH, Kim HN, Kim SH, Kang HC. Polymorphisms of methyl-ene-tetrahydrofolate reductase and glutathione S-transferase are not associated with the risk of papillary thyroid cancer in Korean population. *Mol Biol Rep* 2014; **41**: 3793-9.
61. Hernández A, Céspedes W, Xamena N, Surrallés J, Creus A, Galofré P, et al. Glutathione S-transferase polymorphisms in thyroid cancer patients. *Cancer Lett* 2003; **190**: 37-44.
62. Vodusek AL, Goricar K, Gazic B, Dolzan V, Jazbec J. Antioxidant defence-related genetic variants are not associated with higher risk of secondary thyroid cancer after treatment of malignancy in childhood or adolescence. *Radial Oncol* 2016; **50**: 80-6.
63. Méplan C, Dragsted LO, Ravn-Haren G, Tjønneland A, Vogel U, Hesketh J. Association between polymorphisms in glutathione peroxidase and seleno-protein P genes, glutathione peroxidase activity, HRT use and breast cancer risk. *PLoS One* 2013; **8**: e73316.
64. Takano T, Hasegawa Y, Matsuzuka F, Miyauchi A, Yoshida H, Higashiyama T, et al. Gene expression profiles in thyroid carcinomas. *Br J Cancer* 2000; **83**: 1495-502.
65. Hasegawa Y, Takano T, Miyauchi A, Matsuzuka F, Yoshida H, Kuma K, et al. Decreased expression of glutathione peroxidase mRNA in thyroid anaplastic carcinoma. *Cancer Lett* 2002; **182**: 69-74.
66. Chen J, Cao Q, Qin C, Shao P, Wu Y, Wang M, et al. GPX-1 polymorphism (rs1050450) contributes to tumor susceptibility: evidence from meta-analysis. *J Cancer Res Clin Oncol* 2011; **137**: 1553-61.
67. Brigelius-Flohé R, Kipp A. Glutathione peroxidases in different stages of carcinogenesis. *Biochim Biophys Acta* 2009; **1790**: 1555-68.
68. Yuzhalin AE, Kutikhin AG. Inherited variations in the SOD and GPX gene families and cancer risk. *Free Radic Res* 2012; **46**: 581-99.
69. Zhao H, Liang D, Grossman HB, Wu X. Glutathione peroxidase 1 gene polymorphism and risk of recurrence in patients with superficial bladder cancer. *Urology* 2005; **66**: 769-???