Endocrine (2019) 66:563–572
https://doi.org/10.1007/s12020-019-02016-6

ORIGINAL ARTICLE

Check for updates

Common genetic variations of deiodinase genes and prognosis of brain tumor patients

Adomas Bunevicius[1,2,3] · Edward R. Laws[2,3] · Ausra Saudargiene[1] · Arimantas Tamasauskas[1] · Giorgio Iervasi[4] · Vytenis Deltuva[1] · Timothy R. Smith[2,3] · Robertas Bunevicius[5]

Received: 19 April 2019 / Accepted: 11 July 2019 / Published online: 26 August 2019

Abstract

Background Thyroid hormone (TH) metabolism can have prognostic significance in brain tumors. We studied the association of common variations in three deiodinase gene single-nucleotide polymorphisms (SNPs) with circulating TH concentrations and prognosis of brain tumor patients.

Methods Patients admitted for glioma and meningioma surgery between January, 2010 and September, 2011 were evaluated for functional status (Barthel Index or BI) and circulating free tri-iodothyronine (FT3), free thyroxine (FT4), and thyroid-stimulating hormone (TSH) concentrations. Ten common SNPs in the *DIO1* gene; five SNPs in the *DIO2* gene; and one SNP in the *DIO3* gene were genotyped. Follow-up continued until November, 2017.

Results In glioblastoma patients, the *DIO1* SNP rs2235544 CC genotype was associated with significantly lower risk of death at 2 years when compared to AA + CA genotypes after adjusting for patient gender, age, pre-operative functional status, adjuvant therapy, and extent of resection (HR = 0.34, 95% CI: 0.13–0.84, $p = 0.019$). The TT genotype vs. CC + TC genotypes of the *DI02* SNP rs12885300 was associated with increased mortality risk after adjusting for patient gender, age, pre-operative functional status, adjuvant therapy, extent of resection, and FT3/FT4 (HR = 3.13, 95% CI: 1.20–8.16, $p <$ 0.019). The C-allele of the *DI01* SNP rs2235544 was related to increased circulating free T3/ free T4 ratio in glioma and meningioma patients, indicating greater T4 to T3 conversion.

Conclusions SNPs of *DIO1* gene (rs2235544) and *DIO2* gene (rs12885300) have independent prognostic significance in glioblastoma patients. The C-allele of the *DIO1* (rs2235544) is associated with greater T4 to T3 conversion.

Keywords Glioma · Deiodinase · Glioblastoma · Survival

Introduction

Thyroid hormones (THs) are important for normal brain development and tissue metabolism in healthy individuals and in severely ill patients. Activity of the Hypothalamic-pituitary-thyroid (HPT) axis is closely controlled by the hypothalamic thyrotropin-releasing hormone (TRH) and via negative feedback of circulating THs on the hypothalamus and pituitary gland. TRH stimulates the anterior pituitary gland to synthetize and release thyroid-stimulating hormone (TSH) that in turn activates the thyroid gland to produce and release thyroxin (T4) and tri-iodothyronine (T3), the latter of which is the more potent of the two thyroid gland hormones. Circulating and tissue concentrations of T3 and T4 are closely regulated by balanced action of deiodinase enzymes [1, 2]. Deiodinase type 1 (D1) and type 2 (D2) enzymes convert T4 to T3 via 5′-deiodination resulting in increase of T3 production. Type 3 deiodinase (D3) deactivates THs by T4 to reversed T3 (rT3) and T3 to 3,3′-Diiodthyronine (3,3′-T2) conversion. The brain maintains tissue concentrations of THs by regulating TH transport across the blood–brain barrier and into neurons, and by T4 to T3 deiodination [3, 4]. Deiodinase enzyme functional activity, and consequentially circulating and tissue TH

✉ Adomas Bunevicius
a.bunevicius@yahoo.com

1 Neuroscience Institute, Lithuanian University of Health Sciences, Kaunas, Lithuania

2 Brigham and Women's Hospital, Boston, MA, USA

3 Harvard University, Boston, MA, USA

4 CNR - Institute of Clinical Physiology, Pisa, Italy

5 Lithuanian University of Health Sciences, Kaunas, Lithuania

concentration is defined by common genetic variations of the deiodinase enzyme encoding genes [5, 6].

Impairment of TH metabolism is common in critically ill patients, and has clinical-prognostic significance [7–9]. Reduced serum T3 concentration (as in the Low T3 syndrome) is the most commonly described pattern of impaired TH metabolism in critical illness and is attributed both to suppressed central drive of the HPT-axis, impaired T4 to T3 conversion and increased thyroid hormone inactivation by D3 [2, 10]. Down-regulation of D1 expression was documented in solid non-thyroidal cancers [11]. Also, deiodinase enzyme activity can be suppressed by inflammatory response that is common in cancer patients [2]. To date, the *DIO* genetic polymorphisms have received the most attention in patients with thyroid disorders, such as hypothyroidism and thyroid cancer [5, 11]. Studies exploring the importance of *DIO* enzyme genetic polymorphisms in patients with non-thyroidal disorders are lacking. The Low T3 syndrome is a common complication in brain tumor patients, and lower circulating T3 concentrations were linked to worse health-related quality of life [12], greater mood symptom issues, cognitive impairment, and shorter survival [13, 14]. Furthermore, it was shown that THs play a role in glioma cell signaling and proliferation [15, 16]. However, there are no studies investigating possible association of common genetic variations of deiodinase genes with circulating TH concentrations and prognosis of brain tumor patients.

We undertook this prospective study aiming to assess the possible role of common variations in the deiodinase genes in relation to circulating TH concentrations and prognosis of brain tumor patients. We selected SNPs of three deiodinase enzyme genes that covered a large proportion of common genetic variations across three genes.

Methods

Study design and patients

The Biomedical Research Ethics Committee at the Lithuanian University of Health Sciences, Kaunas, Lithuania approved the study and its consent procedures. Each patient gave written informed consent before enrollment in the study.

Patients for this prospective observational cohort study were recruited from the Department of Neurosurgery of Lithuanian University of Health Sciences Kaunas Clinics, Kaunas, Lithuania, in a period from January, 2010 until September, 2011. Consecutive adult patients admitted at our department were studied. The study exclusion criteria covered the established diagnosis of acute infections or documented chronic inflammatory disorders. Patients diagnosed with metastatic brain tumors and known thyroid disease were also excluded.

Before tumor surgery patients were prospectively evaluated for demographic characteristics (age and gender), functional status (Barthel Index or BI [17]) and previously received treatments(s) for brain tumor. Blood samples were drawn in the morning for genotyping and for evaluation of serum TSH, free T3 and free T4 concentrations. Extent of tumor resection was verified from operative reports and postoperative CT scans. All brain tumor patients had histological verification that was recorded from pathology reports. Information about adjuvant therapies was obtained from medical records.

Mortality data were obtained from the National Death Registry on November, 2017 by using the Lithuanian equivalent of social security number. Patients who survived longer than November 2017 were censored.

DNA extraction and genotyping

Blood samples were stored at −40 °C until analysis. Genomic DNA was extracted from peripheral blood leukocytes. Ten SNPs in the *DIO1* gene (rs10888818, rs12084242, rs11206237, rs11206244, rs2294511, rs4926616, rs731828, rs926456, rs2235544, and rs2294512); five SNPs in the *DIO2* gene (rs225011, rs2267873, rs225015, rs225014, and rs12885300); and one SNP in the gene *DIO3* (rs945006) were genotyped using predesigned TaqMan assays with a 7500TM real-time cycler, in accordance with the manufacturer's instructions (Life Technologies, CA, USA). Genotype assignments were confirmed with the SDS 2.0.5 software. After completion of genotyping 5% of samples in each genotype group were selected for repetitive analysis with a 100% concordance rate. Samples that failed to genotype were recorded as undetermined.

Assessment of HPT-axis hormone concentration

Blood samples were rapidly centrifuged and serum was frozen at −40 °C until analyses. Serum concentrations of free T3, free T4, and TSH (Tosoh AIA 600/21/1800) were assessed by radioimmunoassay. The reference values were as follows: free T3, 2–4 pg/ml; free T4, 7–17 pg/ml; and TSH, 0.25–4.5 μIU/ml.

Statistical analysis

Genotype frequencies were calculated and Hardy–Weinberg equilibrium was assessed for each SNP using a χ^2 goodness-of-fit test [18]. Linkage disequilibrium between SNPs was obtained using the EM algorithm [19]. The associations of all studies SNPs and overall survival of glioblastoma and

meningioma patients were estimated using the Kaplan–Meier methods and log-rank tests. Cox proportional hazards regression analyses were performed to evaluate the SNPs effect on the risk of death calculated as hazard ratios (HRs) with their corresponding 95% confidence intervals (CIs). Significant HRs were adjusted for patient gender, age, pre-operative functional status (BI score), adjuvant therapy (radiotherapy and chemotherapy), extent of resection, and free T3 to free T4 ratio.

Linear regression was used to assess the relationship between studied SNPs and the ratio of free T3 to free T4 in the total sample of brain tumor patients. Genotype was used as an independent variable and free T4 to free T3 ratio as the dependent variable. Significant associations were adjusted for gender and age. The level of statistical significance was set at $p < 0.05$. Data analyses were performed using IBM SPSS Statistics v.20 (IBM Corp., Armonk, NY, USA).

Results

We analyzed 60 glioblastoma (41.7% male), 8 anaplastic astrocytoma, 27 low-grade, and 140 meningioma patients (Table 1). The median age of glioblastoma patients was 58.5 years, and ranged between 30 and 82 years. Median age of total patient sample was 59 years.

The distribution of the analyzed genotypes matched the Hardy–Weinberg equilibrium in the glioblastoma patients and in the total sample (all $p > 0.05$) with the exception of *DI01* SNP rs11206237 and *DI02* SNP rs2267873 that were therefore excluded from further analysis.

Kaplan–Meier analyses and log-rank tests demonstrated that there was a certain trend toward significant association between *DI01* SNP rs2235544 with 2-year survival, and a significant association between *DI02* SNP rs12885300 with 2-year survival ($p = 0.042$) and overall survival time ($p = 0.041$) of glioblastoma patients (Figs. 1 and 2).

In addition, Kaplan–Meier analysis showed a near-significant trend for association between *DI01* SNP rs2294512 and 2-year survival (log-rank test $p = 0.052$): glioblastoma patients with AA genotype had higher overall survival than patients with GA and GG genotypes. However, due to a small size of AA genotype group (four cases) further analysis was not carried out.

For rs2235544 SNP, glioblastoma patients with AA and CA genotypes had lower overall survival than patients with CC genotype (264 days [95% CI: 209.2–318.8], 191 days

Table 1 Baseline characteristics

Characteristics	Glioblastoma	All patients
n	60	227
Male, *n* (%)	25 (41.7)	63 (30.1)
Age, median (range), years	58.5 (30–82)	59 (20–83)
Death, *n* (%)	59 (98.3)	91 (40.1)
Survival, median (range), days	249 (12–1755)	1613 (8–2056)
Gross total resection, *n* (%)	50 (83.3)	193 (85.0)
Subtotal resection *n* (%)	8 (13.3)	21 (9.3)
Biopsy, *n* (%)	0 (0)	1 (0.4)
Recurrent diseases, *n* (%)	15 (25.0)	66 (29.1)
Adjuvant treatment, *n* (%)	46 (76.6)	62 (27.3)
Radiotherapy, *n* (%)	44 (73.3)	62 (27.3)
Chemotherapy, *n* (%)	22 (36.7)	25 (11.0)
fT3/fT4, mean (SD)	0.28 ± 0.09	0.31 ± 0.10
TSH, median (range), iIUml	0.61 (0.006–13.02)	0.71 (0.006–13.02)

fT3 free tri-iodothyronine, *fT4* free thyroxine, *TSH* thyroid-stimulating hormone, *SD* standard deviation

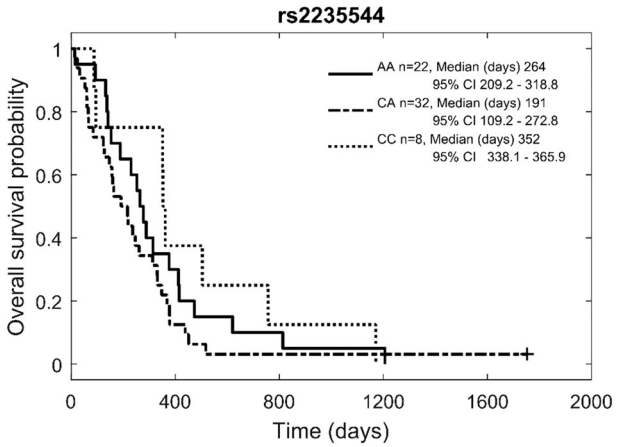

Fig. 1 Kaplan–Meier plot of overall survival for glioblastoma patients by genotype of *DI01* SNP rs2235544

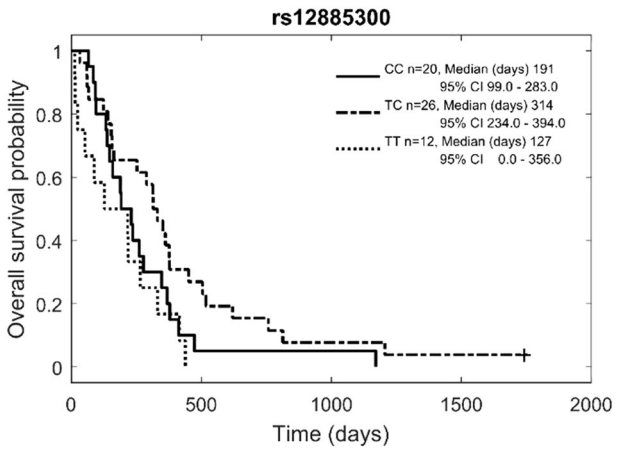

Fig. 2 Kaplan–Meier plot of overall survival for glioblastoma patients by genotype of *DI02 SNP* rs12885300

Table 2 Frequencies of *DI01* SNP rs2235544, *DI02* SNP rs12885300 genotypes and median survival day of glioblastoma patients

Model	Genotype	*n* (%)	Survival, median (range), days
Genotypes of *DI01* SNP rs2235544			
Codominant	A/A	20 (33.3)	271 (14–1206)
	C/A	32 (53.4)	204 (12–1755)
	C/C	8 (13.3)	357 (87–1171)
Dominant	A/A	20 (33.3)	271 (14–1206)
	C/A + C/C	40 (66.7)	227 (12–1755)
Recessive	A/A + C/A	52 (86.7)	233 (12–1755)
	C/C	8 (13.3)	357 (87–1171)
Overdominant	A/A + C/C	28 (46.7)	301 (14–1206)
	C/A	32 (53.3)	204 (12–1755)
Genotypes of *DI02* SNP rs12885300			
Codominant	C/C	20 (34.5)	215 (65–1171)
	T/C	26 (44.8)	322 (32–1755)
	T/T	12 (20.7)	171 (12–438)
Dominant	C/C	20 (34.5)	215 (65–1171)
	T/C + T/T	38 (65.5)	276 (12–1755)
Recessive	C/C + T/C	46 (79.3)	269 (32–1755)
	T/T	12 (20.7)	171 (12–438)
Overdominant	C/C + T/T	32 (55.2)	204 (12–1171)
	T/C	26 (44.8)	322 (32–1755)

[95% CI: 109.2–272.8], and 352 days [95% CI: 338.1–365.9], respectively; Fig. 1). The log-rank test for 1-year and overall survival was not significant ($p = 0.239$ and $p = 0.199$, respectively), however, it shows a tendency towards statistical significance for 2-year survival ($p = 0.077$, Fig. 1). For rs12885300 SNP, glioblastoma patients with CC and TC genotypes had higher overall survival than patients with the TT genotype (191 days [95% CI: 99.0–283.0], 314 days [95% CI: 234.0–394.0], and 127 days [95% CI: 0.0–356], respectively; log-rank test for 2-year and overall survival $p = 0.042$ and $p = 0.041$, Fig. 2).

Frequencies of *DI01* SNP rs2235544, *DI02* SNP rs12885300 genotypes and median survival days in glioblastoma patients are presented in Table 2. Cox proportional hazards regression analysis for 1-year, 2-year, and overall survival was carried out in glioblastoma patients for the rs2235544 and rs12885300 SNPs (Table 3). A recessive model showed that *DI01* SNP rs2235544 CC genotype patients had significantly lower risk of death at 2 years when compared to patients with AA + CA genotypes in the adjusted regression model (HR = 0.34, 95% CI: 0.13–0.84, $p = 0.019$) and a clear tendency to significance if free T3/free T4 was included in the model (HR = 0.39, 95% CI: 0.15–1.01, $p = 0.054$). In the overdominant model, the CA genotype when compared to AA + CC genotype was significantly associated with the increased hazards of death at 2 years in unadjusted and fully adjusted regression model (HR = 2.12, 95% CI: 1.13–4.00, $p = 0.020$). In the codominant and dominant models there were no significant associations between allele combination and survival.

The TC genotype of the *DI02* SNP rs12885300 was associated with a decreased overall risk of death when compared to CC genotype in the codominant model adjusted for gender, age, pre-operative functional status, adjuvant therapy, extent of resection, and free T3/free T4 (HR 0.26, 95% CI: 0.12–0.59, $p = 0.001$).

In the recessive model, the TT genotype vs. CC + TC genotypes significantly increased the overall risk of death in adjusted regression analysis (HR = 3.13, 95% CI: 1.20–8.16, $p = 0.019$). The overdominant model showed that the TC genotype vs CC + TT genotype was significantly associated with the decreased overall hazards of death (adjusted HR = 0.24, 95% CI: 0.11–0.50, $p < 0.001$).

After Bonferroni correction, glioblastoma patients carrying the DI02 SNP rs12885300 TC genotype showed a lower risk of death compared to those carrying the TT or CC genotypes (at the level of $p < 0.00625$ (0.05/8), 2 SNPs and 4 genetic models).

Analysis of linkage disequilibrium did not show evidence of a linkage disequilibrium between the polymorphisms *DI01* SNP rs2235544 and *DI02* SNP rs12885300 ($D' = 0.082$, $r^2 = 0.0057$) in glioblastoma patients.

In meningioma patients, only *DI01* SNP rs12084242 was associated with overall survival (log-rank test $p < 0.001$). Patients with AA genotype had lower overall survival than patients with TA and TT genotypes. Further analysis was not carried out due to a small size of AA genotype group (2 cases).

The DI01 SNP rs2235544 was associated with free T3/free T4 ratio of glioblastoma, anaplastic astrocytoma, low-grade patients, and meningioma patients (Table 4). The C-allele was related to the increased free T3 to free T4 ratio indicating greater T4 to T3 conversion. The association, however, did not remain significant after Bonferroni correction at the level of $p < 0.0125$ (0.05/4; two SNPs and two dependent variables, T3 to free T4 ratio and TSH levels; Table 4). The remaining SNPs were not associated with free T3 to free T4 ratio, nor with TSH concentration (Tables 4 and 5).

Discussion

This was the first study investigating possible clinical significance of deiodinase genetic polymorphisms in brain tumor patients. To date the majority of studies have analyzed *DIO* gene polymorphism general populations and in patients with thyroid gland disorders (hypothyroidism and

Table 3 Cox proportional hazards regression models for mortality in glioblastoma patients

Model	Genotype	Unadjusted		Adjusted[a]		Adjusted[b]	
		HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Genotypes of *DI01* SNP rs2235544, $n = 60$, H-W $p = 0.390$							
1 year							
Codominant	A/A	1		1		1	
	C/A	1.53 (0.78–2.99)	0.216	1.81 (0.84–3.88)	0.128	1.79 (0.82–3.92)	0.146
	C/C	0.77 (0.27–2.17)	0.623	0.53 (0.17–1.65)	0.270	0.57 (0.17–1.89)	0.360
Dominant	A/A	1		1		1	
	C/A + C/C	1.31 (0.69–2.52)	0.411	1.33 (0.64–2.77)	0.448	1.40 (0.65–2.99)	0.390
Recessive	A/A + C/A	1		1		1	
	C/C	0.60 (0.23–1.53)	0.281	0.36 (0.13–1.01)	0.052	0.39 (0.13–1.11)	0.078
Overdominant	A/A + C/C	1		1		1	
	C/A	1.65 (0.90–3.04)	0.106	2.27 (1.14–4.49)	0.019	2.17 (1.09–4.40)	0.028
2 years							
Codominant	A/A	1		1		1	
	C/A	1.54 (0.86–2.77)	0.149	1.75 (0.91–3.38)	0.094	0.73 (0.85–3.53)	0.129
	C/C	0.62 (0.25–1.56)	0.319	0.46 (0.17–1.26)	0.132	0.56 (0.19–1.58)	0.271
Dominant	A/A	1		1		1	
	C/A + C/C	1.24 (0.70–2.18)	0.458	1.23 (0.66–2.30)	0.520	1.29 (0.66–2.51)	0.462
Recessive	A/A + C/A	1		1		1	
	C/C	0.49 (0.21–1.15)	0.101	0.34 (0.13–0.84)	0.019	0.39 (0.15–1.01)	0.054
Overdominant	A/A + C/C	1		1		1	
	C/A	1.76 (1.03–3.03)	0.040	2.26 (1.24–4.12)	0.008	2.12 (1.13–4.00)	0.020
Overall 4.8 years							
Codominant	A/A	1		1		1	
	C/A	1.42 (0.81–2.51)	0.225	1.58 (0.84–2.96)	0.157	1.63 (0.81–3.26)	0.170
	C/C	0.74 (0.33–1.70)	0.484	0.60 (0.25–1.46)	0.259	0.66 (0.25–1.74)	0.401
Dominant	A/A	1		1		1	
	C/A + C/C	1.20 (0.70–2.05)	0.519	1.19 (0.65–2.17)	0.571	1.26 (0.66–2.41)	0.490
Recessive	A/A + C/A	1		1		1	
	C/C	0.62 (0.29–1.31)	0.206	0.46 (0.21–1.03)	0.060	0.49 (0.21–1.19)	0.114
Overdominant	A/A + C/C	1		1		1	
	C/A	1.56 (0.93–2.62)	0.096	1.89 (1.07–3.33)	0.029	1.88 (1.01–3.49)	0.046
Genotypes of *DI02* SNP rs12885300, $n = 58$, H-W $p = 0.5120$							
1 year							
Codominant	C/C	1		1		1	

Table 3 (continued)

Model	Genotype	Unadjusted		Adjusted[a]		Adjusted[b]	
		HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value
	T/C	0.67 (0.33–1.36)	0.265	0.37 (0.16–0.86)	0.020	0.32 (0.13–0.76)	0.010
	T/T	1.46 (0.66–3.25)	0.354	1.49 (0.48–4.62)	0.494	2.36 (0.69–8.01)	0.174
Dominant	C/C	1		1		1	
	T/C + T/T	0.85 (0.45–1.61)	0.612	0.46 (0.21–1.03)	0.057	0.43 (0.18–0.99)	0.048
Recessive	C/C + T/C	1		1		1	
	T/T	1.83 (0.90–3.74)	0.098	3.06 (1.15–8.14)	0.025	4.80 (1.57–14.65)	0.006
Overdominant	C/C + T/T	1		1		1	
	T/C	0.58 (0.31–1.01)	0.095	0.32 (0.16–0.67)	0.002	0.26 (0.11–0.57)	0.001
2 years							
Codominant	C/C	1		1		1	
	T/C	0.60 (0.32–1.12)	0.110	0.37 (0.18–0.76)	0.006	0.25 (0.11–0.56)	0.001
	T/T	1.45 (0.70–2.99)	0.320	1.35 (0.52–3.50)	0.537	1.59 (0.54–4.68)	0.398
Dominant	C/C	1		1		1	
	T/C + T/T	0.77 (0.44–1.36)	0.368	0.47 (0.24–0.93)	0.029	0.35 (0.16–0.75)	0.007
Recessive	C/C + T/C	1		1		1	
	T/T	1.93 (1.00–3.72)	0.049	2.62 (1.13–6.06)	0.024	3.51 (1.32–9.32)	0.012
Overdominant	C/C + T/T	1		1		1	
	T/C	0.53 (0.30–0.93)	0.027	0.34 (0.18–0.64)	0.001	0.22 (0.11–0.47)	<0.001
Overall 4.8 years							
Codominant	C/C	1		1		1	
	T/C	0.61 (0.33–1.11)	0.105	0.39 (0.20–0.77)	0.006	0.26 (0.12–0.59)	0.001
	T/T	1.45 (0.71–3.00)	0.310	1.26 (0.50–3.31)	0.624	1.45 (0.50–4.20)	0.499
Dominant	C/C	1		1		1	
	T/C + T/T	0.77 (0.44–1.33)	0.346	0.48 (0.25–0.91)	0.026	0.36 (0.17–0.78)	0.009
Recessive	C/C + T/C	1		1		1	
	T/T	1.93 (1.00–3.72)	0.049	2.40 (1.06–5.47)	0.037	3.13 (1.2–8.16)	0.019
Overdominant	C/C + T/T	1		1		1	
	T/C	0.54 (0.31–0.93)	0.026	0.36 (0.20–0.66)	0.001	0.24 (0.11–0.50)	<0.001

H-W *p p* value for Hardy–Weinberg equilibrium

[a]Adjusted for sex, age, XRT, CHT, BI1, and GTR

[b]Adjusted for sex, age, XRT, CHT, BI1, GTR, and fT_3/fT_4

thyroid cancer) and mental disorders. We found a prognostic significance of the *DIO1* gene (rs2235544) and *DIO2* gene (rs12885300) in glioblastoma patients. Our results showed that common variations in the *DIO1* gene (rs2235544) and *DIO2* gene (rs12885300) were associated with overall survival of glioblastoma patients independently

Table 4 fT_3/fT_4 ratio by genotype in glioma and meningioma patients

	Common homozygous		Heterozygous		Minor homozygous		*p* for trend	
SNP	Mean (95% CI)	*n*	Mean (95% CI)	*n*	Mean (95% CI)	*n*	Unadjusted	Adjusted[a]
DOI1								
rs2235544	0.29 (0.27–0.32)	68	0.30 (0.28–0.32)	93	0.34 (0.30–0.37)	41	**0.049**	**0.023**
rs10888818	0.31 (0.29–0.33)	135	0.31 (0.28–0.33)	64	0.26 (0.19–0.32)	6	0.523	0.538
rs12084242	0.31 (0.29–0.32)	179	0.28 (0.25–0.31)	18	0.29 (0.00–1.44)	2	0.302	0.412
rs11206244	0.31 (0.29–0.34)	76	0.31 (0.29–0.33)	97	0.28 (0.25–0.31)	29	0.248	0.159
rs2294511	0.31 (0.28–0.33)	84	0.31 (0.29–0.33)	94	0.29 (0.25–0.33)	28	0.449	0.498
rs4926616	0.31 (0.28–0.33)	74	0.31 (0.29–0.32)	105	0.31 (0.27–0.36)	24	0.869	0.951
rs731828	0.31 (0.29–0.33)	84	0.30 (0.28–0.33)	85	0.31 (0.27–0.35)	28	0.936	0.898
rs926456	0.31 (0.28–0.34)	53	0.31 (0.29–0.33)	92	0.31 (0.29–0.34)	53	0.800	0.850
rs2294512	0.30 (0.28–0.32)	100	0.31 (0.29–0.33)	87	0.31 (0.26–0.37)	18	0.465	0.414
DOI2								
rs225011	0.31 (0.29–0.33)	84	0.31 (0.28–0.33)	83	0.31 (0.28–0.35)	26	0.934	0.910
rs225015	0.31 (0.29–0.33)	109	0.30 (0.28–0.32)	82	0.32 (0.28–0.37)	15	0.077	0.107
rs225014	0.31 (0.29–0.33)	99	0.31 (0.29–0.33)	88	0.32 (0.28–0.36)	16	0.933	0.921
rs12885300	0.31 (0.28–0.33)	65	0.32 (0.30–0.34)	100	0.28 (0.25–0.31)	32	0.343	0.600
DOI3								
rs9450006	0.31 (0.29–0.32)	180	0.33 (0.28–0.38)	21	–	0	0.465	0.414

fT3 free tri-iodothyronine, *fT4* free thyroxine, *TSH* thyroid-stimulating hormone

[a]Adjusted for sex and age

Statistically significant *p* values (<0.05) are presented in bold

Table 5 TSH by genotype in glioma and meningioma patients

	Common homozygous		Heterozygous		Minor homozygous		*p* for trend	
SNP	Mean (95% CI)	*n*	Mean (95% CI)	*n*	Mean (95% CI)	*n*	Unadjusted	Adjusted[a]
DOI1								
rs2235544	0.97 (0.56–1.38)	68	1.21 (0.98–1.43)	94	1.05 (0.64–1.45)	41	0.647	0.566
rs10888818	1.13 (0.88–1.38)	136	1.08 (0.81–1.35)	64	0.45 (0.21–0.87)	6	0.785	0.817
rs12084242	1.07 (0.88–1.27)	180	0.89 (0.59–1.19)	18	3.09 (0.00–37.87)	2	0.689	0.562
rs11206244	1.06 (0.78–1.33)	76	1.24 (0.91–1.55)	98	0.78 (0.55–1.01)	29	0.670	0.699
rs2294511	1.11 (0.85–1.36)	84	1.03 (0.72–1.34)	94	1.22 (0.74–1.70)	29	0.775	0.666
rs4926616	1.29 (0.86–1.72)	74	0.92 (0.75–1.09)	105	1.18 (0.80–1.55)	25	0.577	0.589
rs731828	1.09 (0.85–1.34)	84	1.03 (0.70–1.35)	85	1.26 (0.74–1.78)	28	0.964	0.965
rs926456	1.15 (0.83–1.48)	54	1.07 (0.75–1.39)	92	1.05 (0.78–1.40)	53	0.922	0.865
rs2294512	1.18 (0.94–1.41)	100	1.03 (0.69–1.36)	87	0.85 (0.53–1.17)	18	0.243	0.240
DOI2								
rs225011	1.25 (0.87–1.63)	84	0.99 (0.77–1.21)	84	0.87 (0.65–1.09)	26	0.900	0.910
rs225015	1.21 (0.90–1.51)	109	1.00 (0.78–1.22)	83	0.83 (0.57–1.09)	15	0.945	0.937
rs225014	1.22 (0.88–1.55)	99	1.01 (0.80–1.23)	89	0.84 (0.59–1.08)	16	0.981	0.987
rs12885300	0.90 (0.72–1.10)	65	1.23 (0.91–1.56)	99	0.98 (0.56–1.40)	32	0.973	0.771
DOI3								
rs945006	1.09 (0.90–1.29)	181	1.15 (0.42–1.87)	21	–	0	0.904	0.887

[a]Adjusted for sex and age

from patient gender, age, functional status, adjuvant therapy, and circulating T3/T4 ratio. The selected genetic polymorphism of the *DIO3* was not associated with prognosis of glioma patients. The C-allele of the *DIO1*

(rs2235544) was associated with greater T4 to T3 conversion.

Our findings indicate that rs2235544 SNP of the *DIO1* is important for T4 to T3 conversion in patients with established brain tumor diagnosis. The C-allele of the *DIO1* gene (rs2235544) was associated with a higher ratio of circulating free T3 to free T4 hormones indicating greater enzymatic activity. This association was independent from patient gender and age. Our findings agree with previous studies showing that the C-allele of the rs2235544 is associated with greater enzymatic activity of D1 [20, 21]. For example, a study in 2513 subjects with normal thyroid function and 552 patients receiving T4 replacement therapy for hypothyroidism explored the association of circulating TSH, T3, and T4 concertation and T3/T4 ratio with SNPs of *DIO1* (7 SNPs), *DIO2* (7 SNPs), and *DIO3* (7 SNPs) genes [20]. They found that in both cohorts the C-allele of the rs2235544 was associated with greater T3/T4 ratio and free T3 concentration, and with lower free T4 and reversed T3 concentrations indicating greater enzymatic activity. The authors estimated that the rs2235544 SNP was responsible for ~2% of genetic variance of free T4 and 1.5% of free T3 concentrations. Meta-analysis of genome-wide association studies of serum TSH and free T4 concentrations in 17,520 euthyroid subjects also found that rs2235544 had the strongest association with T4 (substrate of D1 enzyme) concentrations among the six SNPs that were identified as significant and independent predictors of T4 concentrations. Our findings indicate that the rs2235544 SNP of the *DIO1* is important for T4 to T3 conversion in patients with established brain tumor diagnoses. Other investigated *DIO* SNPs were not associated with circulating T3/T4 ratio nor TSH concentration in our relatively small study, hence calling for future studies in larger brain tumor cohorts aiming to clarify the possible role of deiodinase enzyme genetic polymorphisms for circulating thyroid hormones levels.

Glioblastoma patients harboring CC genotype of the rs2235544 SNP had lower odds of death at 2 years relative to patients with AA and CA genotypes, and this association was independent from patient age, gender, functional status, extent of tumor resection, and adjuvant therapies. Longer survival of glioblastoma patients harboring the CC genotype of the rs2235544 SNP can be attributed to greater C-allele related enzymatic activity of D1. Indeed, it was previously documented that lower serum T3 concertation (possible due to reduced T4 to T3 conversion) is an independent predictor of shorter survival in glioblastoma patients [12–14]. The association of the rs2235544 SNP with patient survival remained at the limit of statistical significance after adjusting for circulating T3/T4 ratio. Brain has a capacity to maintain tissue T3 homeostasis within wide range of circulating TH levels [22] suggesting that circulating TH levels might not accurately correlate with brain tissue TH concentrations. Findings from preclinical studies suggest that THs play a role in glioma cell differentiation, proliferation, metabolism, and signaling; however, it remains to be clarified whether THs stimulate or inhibit glioma progression, since study findings were conflicting [15, 16]. Future studies should also clarify the relationship of circulating and brain tissue TH concentrations and the possible role of genetic polymorphism of proteins involved in TH transport and metabolism in this relationship. This information could help to identify novel therapeutic targets that could be employed to challenge glioma metabolism and progression.

CC and TC genotypes of the rs12885300 *DIO2* SNP were associated with lower mortality risk of glioblastoma patients when compared to the TT genotype in adjusted analyses. The T4/T3 ratio was not different as a function of rs12885300 genotypes. The rs12885300 was previously shown to influence pituitary sensitivity for negative feedback by free T4 in patients receiving treatment for differentiated thyroid carcinoma [23] and TSH-related thyroidal-free T4 secretion [24]. These findings suggest that regulation of the HPT-axis functioning can have important clinical-prognostic value in glioblastoma, and this observation awaits further validation.

This was the first study investigating possible clinical significance of deiodinase genetic polymorphisms in brain tumor patients. To date the majority of studies have analyzed *DIO* gene polymorphisms in general populations and in patients with thyroid gland disorders (hypothyroidism and thyroid cancer) and mental disorders. Studies investigating the prognostic role of *DIO* genetic polymorphism in patients without non-thyroidal somatic disorders are scant. For example, a study in 327 patients found that the G (Ala) allele of the Thr92Ala coding *DIO2* SNP (rs225014) was associated with reduced susceptibility for severe sepsis and severe sepsis–associated lung injury after adjustment for patient age and gender [25]. Studies exploring possible role of TH deiodination in cancer risk and prognosis are needed, especially because thyroid disorders can be easily diagnosed and managed using widely available and safe interventions.

We did not find an association between TH levels and the analyzed *DIO*2 and *DIO*3 SNPs. This can be explained by limited statistical power of our small sample size to observe the significant association or the effect of these deiodinases for circulating TH levels is small. It was previously reported that circulating TH concentrations were associated with rs11206244 and rs12095080 SNPs of the *DIO1* [26] and rs12885300 SNP of the *DIO2* [27]. However, these association were inconsistent across studies. Towards this end, meta-analyses clarifying possible phenotypic effects of *DIO2* and *DIO3* genetic polymorphisms are welcome. Deiodinase expression and functional activity in brain tumor cells remains unclear and was not addressed in our

study. Hence, the possible contribution of brain tumor deiodinase enzyme activity on thyroid hormone concentration in brain tissue, brain tumor tissue, and systemic circulation remain to be evaluated in the future. Given the increasing knowledge of brain tumor biology and development of clinically relevant molecular biomarkers (for example, isocitrate dehydrogenase mutation status) that are used for prognostication and treatment guidance of glioma patients, the possible role of *DIO* activity for brain tumor microenviroment warrants future research.

The study has limitations. Our sample was heterogeneous in terms of brain tumor histological diagnoses; therefore, the number of patients across different brain tumor histological diagnoses was small. This limitation prevented us from drawing definite conclusions, but call for larger studies and meta-analyses exploring the clinical significance of TH metabolism in brain tumors are warranted. Other important prognostic molecular biomarkers of glioma patients, such as Isocitrate dehydrogenase, were not considered, because they were not routinely performed at our department at the time of this study. Lack of a control group prevented the analysis of the possible association of thyroid deiodinase polymorphisms with brain tumor risk. Future studies should also attempt to evaluate *DIO* polymorphisms in tumor tissue samples in order to better understand the possible role of brain tumors to modulate tissue and circulating thyroid hormone concentrations.

Conclusions

Common variations in the *DIO1* gene (rs2235544) and *DIO2* gene (rs12885300) have prognostic significance in glioblastoma patients, independently from patient gender, age, functional status, adjuvant therapy, and circulating T3/T4 ratio. The selected genetic polymorphism of the *DIO3* was not associated with the prognosis of glioma patients. The C-allele of the *DIO1* (rs2235544) was associated with greater T4 to T3 conversion. Larger scale studies analyzing the clinical importance of TH metabolism in brain tumors should be designed.

Funding The study was supported by the Research Council of Lithuania (grant number: MIP-044/2015).

Author contributions RB and AB designed the study; AB, AT and VD acquired the data; AB, AS, ERL, GI, TRS analyzed the data; AB and AS wrote the manuscript; All authors critically reviewed the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. A.C. Bianco, D. Salvatore, B. Gereben, M.J. Berry, P.R. Larsen, Endocr. Rev. **23**, 38 (2002)
2. M.H. Warner, G.J. Beckett, J. Endocrinol. **205**, 1 (2010)
3. J.E. Silva, P.R. Larsen, J. Clin. Invest. **70**, 1110 (1982)
4. W.E. Visser, E.C.H. Friesema, J. Jansen, T.J. Visser, Trends Endocrinol. Metab. **19**, 50 (2008)
5. C.M. Dayan, V. Panicker, Nat. Rev. Endocrinol. **5**, 211 (2009)
6. H. Verloop, O.M. Dekkers, R.P. Peeters, J.W. Schoones, J.W.A. Smit, Eur. J. Endocrinol. **171**, R123 (2014)
7. G. Van den Berghe, Thyroid **24**, 1456 (2014)
8. X. Jiang, H. Xing, J. Wu, R. Du, H. Liu, J. Chen, J. Wang, C. Wang, Y. Wu, Sci. Rep. **7**, 16256 (2017)
9. R. Gao, J.-H. Liang, L. Wang, H.-Y. Zhu, W. Wu, J.-Z. Wu, Y. Xia, L. Cao, L. Fan, T. Yang, J.-Y. Li, W. Xu, Br. J. Haematol. **177**, 95 (2017)
10. L.J. DeGroot, *The Non-Thyroidal Illness Syndrome.* In: Feingold, K.R. Anawalt, B. Boyce, A. Chrousos, G. Dungan, K. Grossman, A. Hershman, J.M. Kaltsas, G. Koch, C. Kopp, P. Korbonits, M. McLachlan, R. Morley, J.E. New, M. Perreault, L. Purnell, J. Rebar, R. Singer, F. Trence, D.L. Vinik, A. Wilson, D.P. (Eds), Endotext. MDText.com, Inc., South Dartmouth, MA (2000).
11. A.L. Maia, I.M. Goemann, E.L.S. Meyer, S.M. Wajner, J. Endocrinol. **209**, 283 (2011)
12. A. Bunevicius, E.R. Laws, V. Deltuva, A. Tamasauskas, J. Neurooncol. **131**, 385 (2017)
13. A. Bunevicius, V. Deltuva, S. Tamasauskas, A. Tamasauskas, E. R. Laws, R. Bunevicius, J. Neurosurg. **118**, 1279 (2013)
14. A. Bunevicius, V.P. Deltuva, S. Tamasauskas, T. Smith, E.R. Laws, R. Bunevicius, G. Iervasi, A. Tamasauskas, Oncotarget **8**, 8648 (2017)
15. C. Perrotta, C. De Palma, E. Clementi, D. Cervia, Front. Cell. Neurosci. **9**, 236 (2015)
16. A. Liappas, L. Alexandros, I. Mourouzis, M. Iordanis, A. Zisakis, Z. Athanasios, K. Economou, E. Konstantinos, R.-W. Lea, L. Robert-William, C. Pantos, P. Constantinos, J. Thyroid Res. **2011**, 856050 (2011)
17. F.I. Mahoney, D.W. Barthel, Md State Med. J. **14**, 61 (1965)
18. S. Rodriguez, T.R. Gaunt, I.N.M. Day, Am. J. Epidemiol. **169**, 505 (2009)
19. T.R. Gaunt, S. Rodríguez, I.N. Day, BMC Bioinform. **8**, 428 (2007)
20. V. Panicker, C. Cluett, B. Shields, A. Murray, K.S. Parnell, J.R.B. Perry, M.N. Weedon, A. Singleton, D. Hernandez, J. Evans, C. Durant, L. Ferrucci, D. Melzer, P. Saravanan, T.J. Visser, G. Ceresini, A.T. Hattersley, B. Vaidya, C.M. Dayan, T.M. Frayling, J. Clin. Endocrinol. Metab. **93**, 3075 (2008)
21. E. Porcu, M. Medici, G. Pistis, C.B. Volpato, S.G. Wilson, A.R. Cappola, S.D. Bos, J. Deelen, M. den Heijer, R.M. Freathy, J. Lahti, C. Liu, L.M. Lopez, I.M. Nolte, J.R. O'Connell, T. Tanaka, S. Trompet, A. Arnold, S. Bandinelli, M. Beekman, S. Böhringer, S.J. Brown, B.M. Buckley, C. Camaschella, A.J.M. de Craen, G. Davies, M.C.H. de Visser, I. Ford, T. Forsen, T.M. Frayling, L. Fugazzola, M. Gögele, A.T. Hattersley, A.R. Hermus, A. Hofman, J.J. Houwing-Duistermaat, R.A. Jensen, E. Kajantie, M. Kloppenburg, E.M. Lim, C. Masciullo, S. Mariotti, C. Minelli, B.D. Mitchell, R. Nagaraja, R.T. Netea-Maier, A. Palotie, L. Persani, M.G. Piras, B.M. Psaty, K. Räikkönen, J.B. Richards, F. Rivadeneira, C. Sala, M.M. Sabra, N. Sattar, B.M. Shields, N. Soranzo, J.M. Starr, D.J. Stott, F.C.G.J. Sweep, G. Usala, M.M. van der

Klauw, D. van Heemst, A. van Mullem, S.H. Vermeulen, W.E. Visser, J.P. Walsh, R.G.J. Westendorp, E. Widen, G. Zhai, F. Cucca, I.J. Deary, J.G. Eriksson, L. Ferrucci, C.S. Fox, J.W. Jukema, L.A. Kiemeney, P.P. Pramstaller, D. Schlessinger, A.R. Shuldiner, E.P. Slagboom, A.G. Uitterlinden, B. Vaidya, T.J. Visser, B.H.R. Wolffenbuttel, I. Meulenbelt, J.I. Rotter, T.D. Spector, A.A. Hicks, D. Toniolo, S. Sanna, R.P. Peeters, S. Naitza. A meta-analysis of thyroid-related traits reveals novel loci and gender-specific differences in the regulation of thyroid function. PLoS Genet **9**, e1003266 (2013)

22. H.F. Escobar-Morreale, M.J. Obregón, F. Escobar del Rey, G. Morreale de Escobar, J. Clin. Invest. **96**, 2828 (1995)
23. H.C. Hoftijzer, K.A. Heemstra, T.J. Visser, S. le Cessie, R.P. Peeters, E.P.M. Corssmit, J.W.A. Smit, J. Clin. Endocrinol. Metab. **96**, E1527 (2011)
24. M.Y. Peltsverger, P.W. Butler, A.T. Alberobello, S. Smith, Y. Guevara, O.M. Dubaz, J.A. Luzon, J. Linderman, F.S. Celi, Eur. J. Endocrinol. **166**, 839 (2012)
25. S.-F. Ma, L. Xie, M. Pino-Yanes, S. Sammani, M.S. Wade, E. Letsiou, J. Siegler, T. Wang, G. Infusino, R.A. Kittles, C. Flores, T. Zhou, B.S. Prabhakar, L. Moreno-Vinasco, J. Villar, J.R. Jacobson, S.M. Dudek, J.G.N. Garcia, Am. J. Respir. Cell Mol. Biol. **45**, 1203 (2011)
26. R.P. Peeters, H. van Toor, W. Klootwijk, Y.B. de Rijke, G.G.J.M. Kuiper, A.G. Uitterlinden, T.J. Visser, J. Clin. Endocrinol. Metab. **88**, 2880 (2003)
27. R.P. Peeters, A.W. van den Beld, H. Attalki, H. van Toor, Y.B. de Rijke, G.G.J.M. Kuiper, S.W.J. Lamberts, J.A.M.J.L. Janssen, A. G. Uitterlinden, T.J. Visser, Am. J. Physiol. Endocrinol. Metab. **289**, E75 (2005)