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ORIGINAL ARTICLE

Expression of iodine metabolism genes in human thyroid tissues: evidence for age and BRAF^{V600E} mutation dependency

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Summary

Context Children present a higher susceptibility to developing thyroid cancer after radioiodine exposure and also a higher frequency of functional metastases than adults.

Objective To assess the mRNA expression of the sodium/iodide (Na^+/I^-) symporter (NIS), the Pendred syndrome gene (PDS), thyroperoxidase (TPO), thyroglobulin (Tg) and TSH receptor (TSH-R) in normal thyroid tissues (NTTs) and papillary thyroid carcinomas (PTCs) among different age groups.

Methods Analysis included 59 samples: 21 NTTs and 38 PTCs, of which 21 were the classic type (CPTC) and 17 the follicular variant (FVPTC). Patients were divided into three age groups: I ($n = 16$) 5–21 years, II ($n = 13$) 22–59 years, and III ($n = 10$) 60–91 years. The relative mRNA expression of the five target genes was determinate by quantitative reverse transcription polymerase chain reaction (QRT-PCR).

Results Expression of all genes was significantly higher in NTTs than in PTCs, and it was not age dependent in the NTT group. Among PTCs, the mean expression of PDS, TPO and TSH-R was significantly lower in group II than in group I. PDS, TPO and Tg expression was significantly lower in classic PTCs than in FVPTCs. The difference was related to a higher frequency of the BRAF^{V600E} mutation in the former group.

Conclusions The finding of higher PDS, TPO and TSH-R mRNA expression in paediatric *vs.* adult primary tumour tissues supports the hypothesis that this might contribute to the increased functional activity of metastases in the paediatric group. The finding that mRNA expression of the target genes in NTT was not age dependent does not provide an explanation for the higher susceptibility in the paediatric group.

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Introduction

The major established environmental risk factor for the development of differentiated thyroid carcinoma (DTC), particularly papillary thyroid carcinoma (PTC), is ionizing radiation, whether from an external (cancer radiotherapy) or an internal (radioiodine exposure) source.[1–4] After the Chernobyl accident, internal ionizing radiation became a well-documented risk factor for developing DTC.[5,6] Younger individuals, particularly those < 5 years of age, are more sensitive to the effect of ionizing radiation than adults.[1,3,7]

The mechanisms responsible for the higher risk of thyroid cancer after radiation exposure in children than in adults remain unknown. A general decrease in proliferative activity of thyroid cells with increasing age has been suggested to explain, in part, the higher risk of radiation-related thyroid cancer in children compared with adults.[8] However, the low rate of cell cycling during late foetal life and within the first years after birth, as reported by the same authors, suggests that additional factors predisposing to a higher risk in children are likely to exist.

The above evidence and results from immunostaining studies[9] documenting an age-dependent variation in follicular size and in the expression of the sodium/iodide (Na^+/I^-) symporter (NIS) and pendrin in normal thyroid tissues (NTTs) led us to consider that differences in expression of NIS and other proteins involved in iodine trafficking and metabolism might be responsible for the higher susceptibility to developing thyroid cancer secondary to radioiodine in childhood, and likewise for the higher prevalence of functional metastases in paediatric DTC.

The present study was therefore designed to compare the mRNA expression levels of NIS, Pendred syndrome gene (PDS), thyroperoxidase (TPO), thyroglobulin (Tg) and TSH receptor (TSH-R) in PTCs and NTTs of patients at various ages (ranging from 5 to 91 years). The expression levels were assessed by quantitative reverse transcription polymerase chain reaction (QRT-PCR).

Materials and methods

Tissue samples

We analysed 59 samples from 39 patients (28 females, 11 males) who underwent total thyroidectomy at the Portuguese Cancer Centre

of Lisbon between 2000 and 2007. Thirty-eight samples were representative of PTCs and 21 of NTTs. Normal samples were obtained from the adjacent nontumour tissues or from the contralateral lobe (multifocal or bilateral tumours were excluded). Samples were paired (tumour/normal) in 20 cases. From one case, only normal tissue was available.

At surgery, 16 patients were aged < 22 years (group I), 13 were aged 22–59 years (group II) and 10 were > 59 years old (group III); all had a normal serum TSH level and none were being treated with thyroid hormone.

Samples were immediately frozen in liquid nitrogen at the time of surgery. They were obtained in accordance with protocols approved by the institutional review board and informed consent was obtained for the study together with the consent for surgery.

Radioiodine therapy was performed 4–6 weeks after surgery. Prior to ^{131}I therapy, urinary iodine was evaluated in all patients. Only patients presenting values < 100 μg/l were considered illegible for treatment.

Tumours were classified according to the World Health Organization (WHO) classification:[10] 21 were classified as classic PTC (CPTC) and 17 corresponded to the follicular variant (FVPTC).

RNA extraction and cDNA synthesis

Total RNA was isolated from frozen tissues by TRIzol reagent (Invitrogen, Paisley, UK), according to the manufacturer's protocol. RNA was quantified by UV spectrophotometry (optical density measured at 260 nm). cDNA was synthesized from 2 μg of total RNA at 37 °C for 90 min using oligo(dT) primers (Invitrogen) and reverse transcriptase (Superscript II, Invitrogen).

QRT-PCR

The mRNA expression levels of NIS, PDS, TPO, Tg and TSH-R were quantified by QRT-PCR using TaqMan probes on an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). For each gene, specific primers and probes were selected from the Assay-On-Demand products (P/N 4331182; Hs00166567_m1 (NIS), Hs00166504_m1 (PDS), Hs00174927_m1 (TPO), Hs00794359_m1 (Tg), Hs00174910_m1 (TSH-R); Applied Biosystems). To normalize the differences in the amount of total cDNA used in each reaction, we performed the amplification of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA as endogenous control (Pre-Developed TaqMan Assay Reagents; P/N 4326317E; Applied Biosystems). Gene expression assays have a 6-carboxy-fluorescein (FAM) (NIS, PDS, TPO, Tg and TSH-R) or a VIC (GAPDH) reporter dye at the 5′ end of the TaqMan MGB probe, and a nonfluorescent quencher at the 3′ end of the probe. QRT-PCR was performed in a total reaction volume of 20 μl containing 1 × TaqMan Universal PCR Master Mix (P/N 4304437; Applied Biosystems), 1 × Assays-On-Demand Gene Expression Assay Mix and 2 μl of cDNA diluted in RNase-free water. All reactions were performed in a 96-well reaction plate (MicroAmp Optical 96-Well Reaction Plate, Applied Biosystems). Samples were heated for 2 min at 50 °C, followed of 10 min at 95 °C and amplified for 50 cycles of 15 s at 95 °C and 1 min at 60 °C. cDNA from Human Thyroid Total RNA (total RNA from normal tissue of human thyroids pooled from 64 male/female Caucasians, aged 15–61 years; cause of death: sudden death; Clontech, Mountain View, CA) was serially diluted in dH_2O and amplified in parallel to establish a standard curve for relative quantification. As calibrator we also used cDNA from Human Thyroid Total RNA. Expression data were analysed by the standard curve method accordingly to User Bulletin no. 2 (Applied Biosystems). Target relative expression values, obtained from a standard curve, were normalized to the relative amounts of GAPDH mRNA, which were obtained from a similar standard curve. Then these normalized values were compared with the normalized expression in a reference sample (calibrator) to calculate a fold difference value. The calibrator becomes the 1× sample, and all other quantities were expressed as an *x*-fold difference relative to the calibrator. All QRT-PCR experiments, including a control with no template, were performed in triplicate.

Mutational analysis for BRAF

To amplify exon 15 of BRAF, we used primers as described previously.[11] The most common $BRAF^{V600E}$ mutation was sought by automatic sequencing of PCR products. Sequencing was performed with the ABI PRISM® Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) and data were analysed using the ABI PRISM 3130 Genetic Analyser.

Statistical analysis

Statistical analysis was carried out using GraphPad Prism statistical software (San Diego, CA). Values are expressed as mean ± SEM. Variables were compared using the unpaired *t*-test (two-tailed), the Mann–Whitney test, one-way analysis of variance (ANOVA) test or the Kruskal–Wallis test plus Dunn's Multiple Comparison Test, as appropriate. Relationships among variables were sought using Pearson's or Spearman's correlation coefficients. Nonparametric tests were used when the data were not normally distributed. Categorical variables were analysed with either Fisher's exact test or the χ^2-test. Statistical significance was accepted at $P < 0{\cdot}05$.

Results

NTTs and PTCs

Expression of the five target genes in NTTs (NIS: 0·5192 ± 0·0815; PDS: 1·939 ± 0·2966; TPO: 1·811 ± 0·2193; Tg: 1·988 ± 0·2152; TSH-R: 1·459 ± 0·1933) was significantly higher than that observed in PTCs (NIS: 0·0227 ± 0·0073; PDS: 0·5603 ± 0·1183; TPO: 0·3246 ± 0·1140; Tg: 0·5628 ± 0·1171; TSH-R: 0·8633 ± 0·1329) (Fig. 1). The differences remained significant when the analysis was performed between paired samples only (data not shown).

mRNA expression by age group

Samples were compared by age group (I: 5–21 years; II: 22–59 years; III: 60–91 years) according to the age of patients at surgery.

Table 1. mRNA expression levels in normal thyroid tissues according to the age group (mean (SEM), [min–max])

Group	*n*	NIS	PDS	TPO	Tg	TSH-R
I	5	0·6353 (0·1088)	1·936 (0·3722)	2·485 (0·4857)	2·214 (0·4084)	1·667 (0·1165)
(5–21 years)		[0·3343–0·8891]	[1·044–3·295]	[1·424–4·243]	[1·175–3·252]	[1·432–2·099]
II	10	0·5410 (0·1468)	1·488 (0·3530)	1·535 (0·3086)	1·815 (0·3351)	1·283 (0·2161)
(22–59 years)		[$1{\cdot}54 \times 10^{-3}$–1·170]	[0·01806–3·525]	[$4{\cdot}996 \times 10^{-3}$–2·908]	[0·05681–3·211]	[0·3062–2·406]
III	6	0·3863 (0·1210)	2·695 (0·7635)	1·708 (0·3566)	2·087 (0·4244)	1·579 (0·5945)
(60–91 years)		[$2{\cdot}99 \times 10^{-3}$–0·7975]	[0·3964–5·981]	[0·7993–3·286]	[1·051–3·621]	[0·8485–4·530]
P-value		NS	NS	NS	NS	NS

n, number of samples; NS, not significant. The results are expressed as an *x*-fold difference relative to the calibrator. *P*-values determined by one-way ANOVA test with the Tukey Multiple Comparison Test.

Table 2. mRNA expression levels in papillary thyroid carcinomas according to the age group (mean (SEM), [min–max])

Group	*n*	NIS	PDS	TPO	Tg	TSH-R
I	15	0·0290 (0·0149)	0·6658 (0·1413)	0·2651 (0·0662)	0·4512 (0·0673)	0·9657 (0·1128)
(5–21 years)		[$9{\cdot}44 \times 10^{-6}$–0·2131]	[0·1246–2·184]	[0·0287–0·8886]	[0·1214–1·037]	[0·4377–1·935]
II	13	0·0124 (0·0101)	0·1555 (0·0346)	0·0684 (0·0303)	0·3653 (0·0780)	0·5193 (0·0632)
(22–59 years)		[$3{\cdot}40 \times 10^{-4}$–0·1334]	[0·0051–0·3618]	[0·0040–0·3538]	[0·0734–0·9489]	[0·2229–0·9805]
III	10	0·0266 (0·0110)	0·9285 (0·3596)	0·7468 (0·4019)	0·9870 (0·4069)	1·157 (0·4592)
(60–91 years)		[$6{\cdot}92 \times 10^{-5}$–0·1066]	[$3{\cdot}02 \times 10^{-4}$–3·377]	[$3{\cdot}58 \times 10^{-6}$–4·081]	[0·0714–3·875]	[0·0291–4·966]
P-value		NS	0·0022	0·0121	NS	0·0349

n, number of samples; NS, not significant. The results are expressed as an *x*-fold difference relative to the calibrator. *P*-values determined by the Kruskal–Wallis test [with Dunn's Multiple Comparison Test: PDS (I *vs.* II $P < 0{\cdot}01$; II *vs.* III $P < 0{\cdot}05$); TPO (I *vs.* II $P < 0{\cdot}05$); TSH-R (I *vs.* II $P < 0{\cdot}05$)].

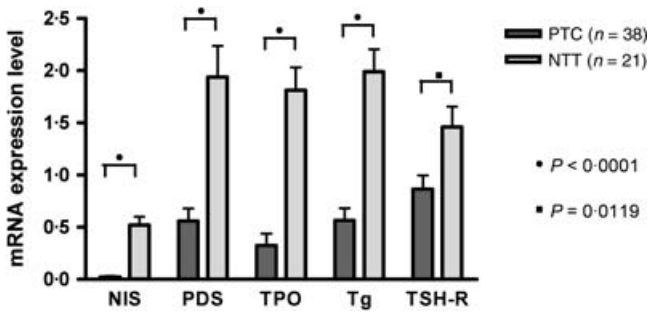

Fig. 1 mRNA expression levels of NIS, PDS, TPO, Tg and TSH-R genes in papillary thyroid carcinomas (PTCs) and normal thyroid tissues (NTTs). The results are expressed as an *x*-fold difference relative to the calibrator. Analysis was performed using the Mann–Whitney test (NIS, PDS, TPO, Tg) and the unpaired *t*-test (TSH-R). n, number of samples.

Table 3. Correlations between PDS, TPO, Tg and TSH-R expression levels in carcinomas and normal thyroid tissues

	Papillary thyroid carcinomas		Normal thyroid tissues	
Genes	*r*	*P*	*r*	*P*
PDS–TPO	0·6759	< 0·0001	0·5784	0·0060
PDS–Tg	0·5074	0·0011	0·5314	0·0132
PDS–TSH-R	0·5326	0·0006	0·7474	< 0·0001
TPO–Tg	0·6026	< 0·0001	0·6364	0·0019
TPO–TSH-R	0·4897	0·0018	0·6061	0·0036
Tg–TSH-R	0·6766	< 0·0001	0·6367	< 0·0001

Papillary thyroid carcinomas (*r*, Spearman); normal thyroid tissues (*r*, Pearson).

Among the normal thyroid samples, no differences in the levels of expression of the various genes were found between the three age groups (Table 1). Among the carcinomas, analysis by age group disclosed a remarkable decrease in expression of all five genes in group II. The mean expression of PDS, TPO and TSH-R was significantly lower in group II than in group I. The mean expression of PDS was also significantly lower in group II than in group III. By contrast, NIS and Tg expression was not significantly different between age groups (Table 2, Fig. 2).

Relationships between expression levels of NIS, PDS, TPO, Tg and TSH-R

Significant correlations were found between the expression of PDS, TPO, Tg and TSH-R in NTTs and PTCs (Table 3). By contrast, the NIS expression was not correlated with the expression of the other genes in either the NTTs or the PTCs.

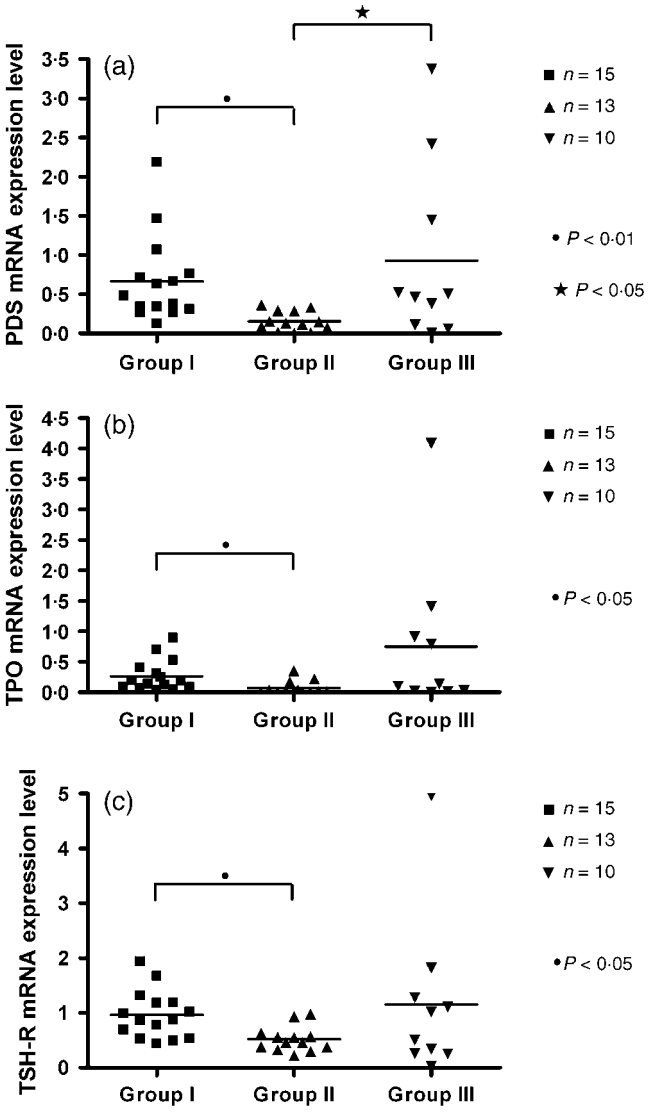

Fig. 2 Age-group analysis of (a) PDS, (b) TPO and (c) TSH-R mRNA expression levels in papillary thyroid carcinomas. Results are expressed as an *x*-fold difference relative to the calibrator. Analysis was performed using the Kruskal–Wallis test plus Dunn's Multiple Comparison Test. Solid lines, mean of mRNA expression levels; n, number of samples.

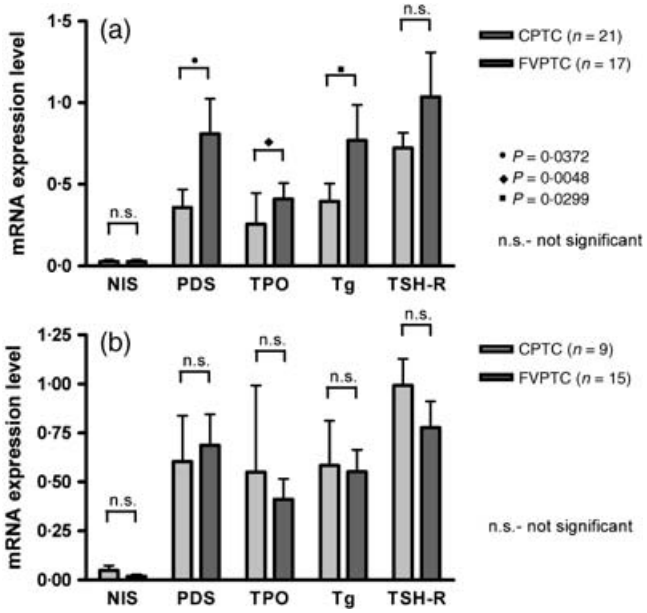

Fig. 3 mRNA expression levels of NIS, PDS, TPO, Tg and TSH-R genes in classic PTC (CPTC) *vs.* the follicular variant of PTC (FVPTC). (a) Comparison between histological subtypes. (b) Reanalysis after exclusion of the subset of $BRAF^{V600E}$ mutant cases. The results are expressed as an *x*-fold difference relative to the calibrator. Analysis was performed using the Mann–Whitney test or the unpaired *t*-test, as appropriate. n, number of samples.

$BRAF^{V600E}$ mutation

The $BRAF^{V600E}$ mutation was present in 14/38 PTCs (36·8%). The frequency of the $BRAF^{V600E}$ mutation (Table 4) was different among the age groups [6·6% in group I, 61·5% in group II and 50% in group III ($P = 0·0067$)] and also between the histological subtypes [57·1% in CPTC *vs.* 11·8% in FVPTC ($P = 0·0063$)].

Expression patterns in the classic and follicular variants of PTC

Expression of PDS, TPO and Tg was significantly decreased in carcinomas classified as CPTCs (Fig. 3a). However, a lower expression of NIS was observed in the follicular variant of the PTC group without reaching a level of significance. Re-evaluation, after exclusion of the BRAF-positive cases, disclosed no differences between the two histological subtypes (Fig. 3b).

First ^{131}I therapy

Thirty-four patients underwent ^{131}I treatment after thyroidectomy (14 in group I, 11 in group II, and 9 in group III). Whole-body scan after the first ^{131}I treatment revealed lung metastases in seven patients, five from group I and two from group III (Table 4). Evaluation of serum Tg, before and after ^{131}I treatment, is depicted in Fig. 4.

Discussion

Only 10% of all thyroid cancers occur in individuals younger than 21 years.[13] DTC (i.e. papillary and follicular thyroid carcinoma) accounts for the vast majority of cases and medullary thyroid carcinoma (MTC) accounts for most of the rest.[14–16] Undifferentiated carcinomas are extremely rare.[17]

In childhood, the thyroid presents a higher susceptibility to the carcinogenetic effect of ionizing radiation than in adulthood. Childhood DTC is associated with more locally aggressive and more frequent distant disease than its adult counterpart.[18] The lungs are almost the sole distant metastatic site in children and pulmonary metastases are nearly always functional.[19,20]

Table 4. Clinical, histological, TNM classification and $BRAF^{V600E}$ mutation data

	Patient no.	Gender	Age at diagnosis (years)	TNM classification	Histopathology	$BRAF^{V600E}$ mutation	Radioiodine uptake (after first course of ^{131}I)
Group I	1	M	5	T4aN1bMx	FVPTC	–	Neck/mediastinum
	2	F	9	T1N1aMx	FVPTC	–	Neck/mediastinum
	3	F	9	T4aN1bMx	CPTC	–	Neck/mediastinum
	4	F	10	T1N1bMx	CPTC	–	Neck/mediastinum
	5	F	11	T3N1bMx	FVPTC	–	Neck/mediastinum Lungs (bilateral, diffuse)*
	6	M	12	T4aN1bMx	CPTC	–	Neck/mediastinum Lungs (bilateral, diffuse)*
	7	M	13	T3N1bMx	CPTC	–	Neck/mediastinum Lungs (bilateral, diffuse)*
	8	F	15	T3N1bMx	CPTC	–	Neck/mediastinum Lungs (bilateral, diffuse)*
	9	F	15	T2N0Mx	FVPTC	–	Neck/mediastinum
	10	M	17	T4aN1bMx	FVPTC	–	Neck/mediastinum Lungs (bilateral, diffuse)*
	11	F	17	T2N0Mx	FVPTC	–	NIT
	12	F	19	T3N0Mx	FVPTC	–	Neck
	13	F	19	T2N0Mx	FVPTC	–	Neck
	14	F	21	T3N0Mx	CPTC	+	Neck
	15	F	21	T3N1bMx	CPTC	–	Neck
Group II	16	F	24	T2N0Mx	CPTC	+	Neck
	17	F	27	T2NxMx	CPTC	–	Neck
	18	M	29	T2NxMx	FVPTC	–	Neck
	19	F	29	T3NxMx	FVPTC	+	Neck
	20	F	30	T2NxMx	FVPTC	–	Neck/mediastinum
	21	M	30	T1N0Mx	CPTC	+	NIT
	22	F	34	T1N0Mx	CPTC	+	NIT
	23	F	38	T3N0Mx	CPTC	–	Neck/mediastinum
	24	F	38	T3N0Mx	CPTC	+	Neck
	25	M	44	T2NxMx	CPTC	+	Neck/mediastinum
	26	F	50	T2N0Mx	CPTC	+	Neck
	27	F	53	T1NxMx	FVPTC	–	Neck
	28	F	58	T3N1aMx	CPTC	+	Negative
Group III	29	M	61	T1NxMx	FVPTC	+	Neck
	30	F	64	T3NxMx	CPTC	+	Neck
	31	F	67	T3NxMx	CPTC	+	Neck
	32	F	69	T2NxMx	FVPTC	–	Neck
	33	M	71	T2NxMx	FVPTC	–	Neck
	34	M	73	T3N1bMx	FVPTC	–	Neck/mediastinum Lungs (focal)*
	35	F	75	T1NxM1	FVPTC	–	Neck/mediastinum Lungs (focal)†
	36	F	77	TxN1aMx	CPTC	–	Neck
	37	F	81	T3NxMx	CPTC	+	Neck
	38	M	91	T3N1bMx	CPTC	+	NIT

M, male; F, female; CPTC, classic PTC; FVPTC, follicular variant of PTC; –, without mutation; +, with mutation; NIT, no iodine therapy.
*Computed tomography (CT) negative; †CT positive. TNM classification according to Sobin *et al.*[12]

We hypothesized that the higher susceptibility to developing thyroid cancer secondary to radioiodine and the higher frequency of functional metastases, observed in children, might be the consequence of a higher efficiency in the processes of transport, organification and storage of iodide. Expression of NIS and other iodine transport-related molecules has been found to be reduced in adult DTC[21–25] but it is unclear whether expression is greater in childhood DTC. We therefore investigated in a series of PTCs and NTTs, by QRT-PCR,

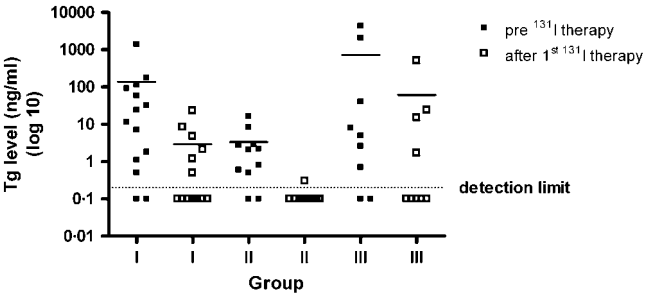

Fig. 4 Evaluation of serum Tg levels in patients who underwent ^{131}I treatment (14 in group I, 11 in group II, and 9 in group III). Dotted line, detection limit (0·2 ng/ml); solid lines, mean levels.

the expression of five thyroid-specific genes coding for key elements in iodine metabolism:[26] NIS, which is an integral plasma membrane glycoprotein present at the basolateral plasma membrane that mediates active I^- transport into the thyroid follicular cells; pendrin (PDS gene product), which is a Cl^-/I^- transporter responsible by the efflux of I^- across the cytoplasm towards the colloid; TPO, which catalyses the oxidation and incorporation of I^- into some tyrosil residues within the Tg molecule; and TSH-R, as all these steps are stimulated by TSH. The results were analysed according to the patients' age.

We identified a significant reduction in mRNA expression, for all five genes, in PTCs compared with NTTs. The correlations among PDS, TPO, Tg and TSH-R levels in PTCs were similar to those observed in NTTs. The NIS gene expression was not related to that of the other genes, in both NTTs and PTCs. These results suggest that PTCs maintain the normal regulatory systems for these genes, albeit at a lower level of expression.

An age-based analysis of the mRNA expression levels led us to the following observations. First, among normal thyroid tissues (21 samples), the mRNA expression levels of the target genes were not age dependent. Second, among the PTCs (38 samples), the patients presenting the lowest mRNA expression levels were those aged between 22 and 59 years. The PDS expression was significantly lower in these patients than in younger or older patients. The TPO and TSH-R expression was significantly lower in these patients than in younger patients. Furthermore, the underexpression of genes was independently associated with age, regardless of the histological subtype of papillary carcinoma. Unexpectedly, NIS expression was not significantly different between age groups. Of interest, the mean mRNA expression levels observed among the oldest patients were not significantly different from those observed in the youngest patients; probably because the former group was very heterogeneous and included patients either with very low or very high levels of expression.

Although limited by the small number of NTTs in groups I and III, the first observation does not support an age-related risk of developing thyroid cancer based on differences in NIS, PDS, TPO, Tg and TSH-R expression as assessed by RT-PCR. These results are in contrast to those presented by Faggiano *et al.*[9] for NIS and PDS, as assessed by immunohistochemistry, suggesting an age-dependent expression.

On the contrary, the second observation suggests that the impaired iodine metabolism documented in the PTCs is variable among age groups. A significant difference was observed between patients aged 22–59 years and younger patients, markedly in those < 19 years, whereas the expression profile among the oldest patients was highly variable. Although the behaviour of the metastases will be similar to that of the primary tumour, we therefore speculate that this may be the underlying explanation for the higher prevalence of lung metastases (Fisher's exact test, $P = 0{\cdot}046$) and, eventually, better response to ^{131}I therapy in paediatric PTCs compared to adults aged 22–59 years. Failure to demonstrate differences in NIS expression among groups does not exclude this possibility. Indeed, iodide transported into the cell that is not oxidized and organified diffuses back into the extracellular fluid. PDS and TPO genes coding for key proteins involved in iodide thyroidal metabolism presented higher expression levels in patients under 22 years than among adults aged 22–59 years. The finding of a significant difference between groups I and II concerning TSH-R expression and the fact that upregulation of thyroid NIS expression, by TSH, has been demonstrated in human thyroid primary cultures[27,28] are likely to produce different results under TSH stimulation.

After ^{131}I treatment, and on thyroxine suppressive therapy, 57% of patients in group I achieved an undetectable level of Tg and the mean value observed in those with detectable Tg was 6·7 ng/ml; in group II the corresponding values were 91% and 0·3 ng/ml. Analysis of these results requires consideration of the TNM classification (Table 4) of patients included in each group as well as the pre-^{131}I Tg values (160 ng/ml in group I *vs.* 4·1 ng/ml in group II). Overall, we would say that the results in group I probably reflect the effect of ^{131}I on tumour tissue whereas the response observed in group II probably corresponds to the ablation of remnant thyroid tissue.

In addition, we compared the expression levels of NIS, PDS, TPO, Tg and TSH-R observed in tumours classified as FVPTC relative to those observed in CPTC. The FVPTC designation includes tumours with the typical nuclear features of PTC and a predominant or exclusive follicular growth pattern. Whether this entity, with a variable clinical course,[29] is closer to PTCs or to the follicular thyroid adenoma/follicular thyroid carcinoma group is still a matter of debate, although some evidence favours the latter hypothesis.[30,31] We documented a higher frequency of BRAF mutations in CPTC than in FVPTC (57·1% *vs.* 11·8%), as reported by others.[32–34] Comparison of gene expression between the two subtypes led us to conclude that there was a lower expression of PDS, TPO and Tg in CPTCs. Re-evaluation, after exclusion of the BRAF-positive cases, abolished the differences. These findings are in close agreement with a previous study showing a reduced TPO expression in BRAF mutant PTCs[35] and reinforce the hypothesis that mutational status may play a role in gene expression variation.

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