

***NIS* mRNA Expression Level in Resected Thyroid Tissue as a Marker of Postoperative Hypothyroidism after Subtotal Thyroidectomy in Patients with Graves' Disease**

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Abstract. Subtotal thyroidectomy for Graves' disease sometimes leads to hypothyroidism or relapse during long-term follow-up in a significant proportion of patients. Factors predictive of postoperative hypothyroidism after subtotal thyroidectomy are not known. The objective of this study was to determine the relation between clinical features and expression of transcripts associated with thyroid hormone synthesis in resected thyroid tissues of patients with Graves' disease. Thyroid tissues were obtained from 65 patients with Graves' disease who underwent subtotal thyroidectomy. Expression of mRNAs from thyroglobulin (*Tg*), TSH receptor (*TSHR*), thyroid peroxidase (*TPO*), sodium/iodide symporter (*NIS*), and the Pendred's syndrome (*PDS*) genes were analyzed by quantitative reverse transcription-polymerase chain reaction. Uni- and multivariate analyses were performed to identify for postoperative hypothyroidism. We detected significant correlations between the *NIS* mRNA level and levels of free T_3 (fT_3) and free T_4 (fT_4) and between the *Tg* mRNA level and goiter weight before initial drug treatment. Mean levels of expression of all five mRNAs were significantly higher in patients who did not require L-thyroxine replacement therapy than in those who required replacement therapy at 6 months after surgery. In patients who did not require replacement therapy, a significant correlation was found between *NIS* mRNA expression and fT_4 levels. Univariate analysis revealed that decreased *NIS* mRNA expression ($NIS/PGK < 1.69$) and low TBII levels before initial treatment were significant of postoperative hypothyroidism. Multivariate analysis showed decreased expression of *NIS* mRNA ($NIS/PGK < 1.69$) to be an independent risk factor for L-thyroxine replacement after surgery (risk ratio, 3.26, confidence interval, 1.36–9.08, $p < 0.01$). *NIS* expression reflects the level of thyroid hormone synthesis in Graves' disease patients. Evaluation of *NIS* mRNA expression in thyroid tissues may help determine prognoses of Graves' disease patients, and therefore an appropriate treatment can be determined for each patient.

Key words: Graves' disease, *NIS* gene, Prognostic marker, Postoperative hypothyroidism, Subtotal thyroidectomy

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GRAVES' disease is one of the most common autoimmune diseases of the thyroid gland. There are three

established treatments for Graves' disease: antithyroid drugs therapy (ATD), radioiodine therapy, and surgery. Each treatment is valuable, yet each has its advantages and disadvantages. Therefore, choosing the best treatment for each Graves' disease patient can be difficult.

ATD treatment is the first choice for patients with Graves' disease in Japan but it often causes drug allergies: skin eruptions, liver dysfunction, arthralgia, or agranulocytosis. ATDs may be taken for at least 2 or 3 years before the disease is controlled, and relapse may occur after cessation of the drug or during drug therapy [1–3]. Radioiodine therapy is the second

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Abbreviations: *NIS*, sodium/iodide symporter; *Tg*, thyroglobulin; *TSHR*, thyroid stimulating hormone receptor; *TPO*, thyroid peroxidase; *PDS*, Pendred's syndrome; ATD, antithyroid drug; RT-PCR, reverse transcription-polymerase chain reaction; fT_3 , free triiodothyronine; fT_4 , free thyroxine; TSH, thyroid stimulating hormone; TBII, TSH binding inhibitory immunoglobulin.

choice treatment of Graves' disease in Japan. However, the effectiveness of radioiodine therapy varies widely between individuals, and approximately 50% of patients who undergo radioiodine treatment shift to a hypothyroid state after several years of radioiodine administration [4]. The third choice treatment for Graves' disease in Japan is surgery, usually subtotal thyroidectomy. Postoperative complications such as hypoparathyroidism and recurrent laryngeal nerve palsy may occur, but most of these complications are transient. Surgery can put hyperthyroidism into remission immediately, and the relapse rate after surgery is lower than that with ATD treatment. A number of patients shift to transient or perpetual hypothyroidism and require thyroid hormone replacement therapy. Some surgeons consider hypothyroidism to be the goal of surgical intervention in Graves' disease to minimize the chance of relapse of hyperthyroidism; however, our goal with surgical treatment is to achieve euthyroidism or slightly subclinical hypothyroidism that does not require L-thyroxine therapy. Subtotal thyroidectomy, rather than total or near-total thyroidectomy, is performed in our hospital. However, the factors predictive of postoperative hypothyroidism and relapse after surgery are not known.

During thyroid hormone synthesis, five major proteins, thyroglobulin (Tg), TSH receptor (TSHR), thyroid peroxidase (TPO), sodium/iodide symporter (NIS), and pendrin, encoded by the Pendred's syndrome (PDS) genes, are expressed specifically in thyroid follicular cells [5–9]. In thyroid carcinomas and non-functioning adenomas, levels of expression of the *NIS*, *TPO*, *Tg*, and *PDS* genes are reported to be decreased [10, 11]. In contrast, expression of *NIS* mRNA in thyroid epithelium is increased in patients with Graves' disease and in toxic adenomas [10, 11]. Some studies have addressed the relation between expression of mRNAs of the above mentioned genes and application of radioiodine treatment in patients with thyroid cancer [12, 13]. However, the relation between expression of mRNAs and the clinical features of Graves' disease has not been reported.

To address these matters, we analyzed the expression of *Tg*, *TSHR*, *TPO*, *NIS*, and *PDS* mRNAs in tissues of Graves' disease patients who underwent subtotal thyroidectomy and compared the gene expression profiles with clinical features and examinations. We also performed uni- and multivariate analyses to investigate whether the risk of postoperative hypo-

thyroidism can be estimated from expression of these genes. Furthermore, we performed fine-needle aspiration biopsy (FNAB) from 9 Graves' patients to get samples, analyzed expression of five mRNAs and compared the results to the mRNAs expression in the resected thyroid tissues.

Materials and Methods

Patients

We studied 65 consecutive patients with Graves' disease who underwent subtotal thyroidectomy at Noguchi Thyroid Clinic and Hospital Foundation during the period September 2002 through March 2003. The reason for thyroid surgery was ATD drug allergy in 21 patients and patient request in 44 patients. This study was approved by the Ethics Committee of Noguchi Thyroid Clinic and Hospital Foundation, and written informed consent was obtained from all patients. All patients were diagnosed clinically with Graves' disease before ATD treatment, and the diagnosis was confirmed histologically in specimens of resected thyroid glands. At 6 months after surgery, L-thyroxine replacement therapy was necessary in 35 patients because of postoperative hypothyroidism and unnecessary in 27 patients. We were unable to follow up with 3 patients.

Clinical examinations

Thyroid function tests and measurements of TSH binding inhibitory immunoglobulin (TBII) were performed before initial ATD treatment, 1 week before surgery, and 6 months after surgery. Serum free T₃ (fT₃), free T₄ (fT₄), and TSH concentrations were measured with ECL-IA kits (Roche Diagnostics, Berlin, Germany). TBII was measured by radioimmunoassay with RRA kits (Schering AG, Berlin, Germany). Ultrasonographic scanning was performed with a LOGIC500 (GE Yokogawa Medical, Tokyo, Japan) with a 7.5 MHz linear transducer before treatment. We measured the maximal length (a), breadth (b), and depth (c) of the goiter. Estimated thyroid weight (W, in grams) was calculated as the sum of both lobes and the isthmus, and the weight was calculated following the spherical ellipsoid formula, $W = (\pi/6)a \times b \times c$ [14, 15]. ¹³¹I uptake at 24 h in the thyroid gland was also determined.

Samples

Thyroid tissues from 65 patients with Graves' disease were collected. Tissues were frozen just after thyroidectomy and stored in liquid nitrogen until use. We also obtained six non-neoplastic thyroid tissues from patients with non-functioning follicular adenoma, and these samples were used as normal control samples. The resected thyroid was weighed during surgery, and the weight of the residual thyroid was estimated and recorded by comparing various sizes of phantoms. We also collected thyroid tissues from 9 patients with Graves' disease before subtotal thyroidectomy using fine-needle aspiration biopsy. 22G needle was used for aspiration.

RNA extraction and cDNA synthesis

Total RNA was isolated from thyroid tissues with an ISOGEN Kit (Wako Pure Chemical Industries, Osaka, Japan) according to the manufacturer's instructions. The RNA bands on agarose gels were visualized under UV illumination. cDNA synthesis was performed with murine leukemia virus reverse transcriptase (Roche Diagnostics) according to the manufacturer's protocol.

Reverse transcription-polymerase chain reaction (RT-PCR)

PCR amplification was performed in 50- μ l reactions containing 47.75 μ l PCR master mix, 2.5 units Taq DNA polymerase (Roche Diagnostics), 1.0 μ l template cDNA, and each 0.5 μ M sense and antisense primers. Primer sequences were *Tg*, sense, 5'-AGGGAAACG GCCTTTCTGAA-3' and antisense, 5'-GTGGAGAA GACGACGATTTC-3'; *TSHR*, sense, 5'-GCTTTTCA GGGACTATGCAATGAA-3' and antisense, 5'-AAG GGCAGTGACACTGGTTTGAGA-3'; *TPO*, sense, 5'-ACTGCACACGCTGTGGCTGC-3' and antisense, 5'-TGCAGTTTGGCTGGTCTTG-3'; *NIS*, sense, 5'-GCACCCAGGAACTCGTGATT-3' and antisense, 5'-CATTCCCAAGCTGAAGGCTCC-3'; *PDS*, sense, 5'-GAGCAATGCGGGTTCTTTGA-3' and antisense, 5'-TCTTCTTCCGTCAGCTCTGTTTCT-3'; *PGK*, sense, 5'-CAGTTTGGAGCTCCTGGAAG-3' and antisense, 5'-TGCAAATCCAGGGTGCAGTG-3'. Amplification conditions consisted of 20–42 cycles of 94°C for 1 min, 59°C for 1 min, and 72°C for 1 min. The exponential linear ranges of PCR reactions were

determined by analysis of a varying numbers of cycles for amplification of a fixed quantity of cDNA, and we chose the optimal cycle numbers for further studies. The numbers of cycles for amplification were 23 for *Tg* cDNA, 27 for *TSHR*, 25 for *TPO*, 31 for *NIS*, and 25 for *PDS*. *PGK* was used as a control for the cDNA integrity in the samples, and was amplified with 27 cycles of the above mentioned protocol. PCR products were resolved on 1% agarose gels and visualized by staining with ethidium bromide under UV illumination. ScanImage software (Scion Corp., Frederick, MD, USA) was used to quantify mRNA levels. We normalized expression of mRNA for each of the five genes to that of *PGK*. Identities of the PCR amplification products were confirmed by direct sequencing.

Statistical analysis

Expression of each gene mRNA was compared with each patient's clinicopathologic characteristics before initial treatment. These comparisons were all made with *t*-tests. The mRNA expression levels were also compared to the necessity or non-necessity for L-thyroxine replacement therapy. These comparisons were made with Mann-Whitney U-tests. We also analyzed the following clinical factors and expression of mRNAs using a Cox's proportional hazard model to assess association between clinical features and the need for the thyroid hormone replacement therapy after surgery: age, duration of ATD treatment, fT_3 , fT_4 , TBII, and goiter weight before initial ATD treatment, resected goiter weight at the time of surgery, estimated residual thyroid weight, and level of *NIS* mRNA expression (*NIS/PGK* above 1.69). A *p* value of less than 0.05 was considered statistically significant. JMP software (version 5.0; SAS Institute, Cary, NC, USA) was used for all data analyses.

Results

First, we analyzed the expression of *NIS* and *PGK* mRNA in tissues of 18 patients that were randomly chosen among 65 patients with Graves' disease using a SYBR Green-based quantitative realtime-PCR protocol. The relation between *NIS* mRNA expression in semi-quantitative RT-PCR performed in this study and those in realtime-PCR was evaluated, and a significant correlation was found ($p = 0.006$, $r = 0.62$).

Table 1. Clinical profiles of 65 patients with Graves' disease.

Age	
Median (year)	32
Range (year)	21–68
Gender	
Male/Female	22/43
Before initial treatment	
Free T ₃ (3.8–5.4 pmol/l)	32.4 ± 14.0
Free T ₄ (13–29 pmol/l)	75.9 ± 25.7
TSH (0.5–4.1 µU/ml)	<0.01
TBII (<15%)	47.1 ± 24.7
Ultrasonographically estimated goiter weight (g)	42.4 ± 27.7
Period of ATD treatment	
Median (month)	3.0
Range (month)	0.3–324.0
Drug allergy for ATD	
Present	21
Absent	44
Before thyroidectomy	
Free T ₃ (3.8–5.4 pmol/l)	5.6 ± 2.6
Free T ₄ (13–29 pmol/l)	16.7 ± 7.7
TSH (0.5–4.1 µU/ml)	1.0 ± 4.7
TBII (<15%)	36.6 ± 26.8
At thyroidectomy	
Resected thyroid weight (g)	47.9 ± 30.8
Estimated residual thyroid weight (g)	5.3 ± 1.0
6 months after thyroidectomy	
Free T ₃ (3.8–5.4 pmol/l)	4.0 ± 1.3
Free T ₄ (13–29 pmol/l)	14.1 ± 5.1
TSH (0.5–4.1 µU/ml)	15.6 ± 23.8
TBII (<15%)	21.4 ± 25.9
L-thyroxine replacement therapy	
Present	35
Absent	27
Unknown	3
L-thyroxine (µg/day)	52.8 ± 18.8

Clinical characteristics of the 65 Graves' patients who underwent subtotal thyroidectomy are summarized in Table 1. Results of quantitative RT-PCR for mRNA expression of *Tg*, *TSHR*, *TPO*, *NIS*, and *PDS* genes by RT-PCR in Graves' disease tissues and TSH levels before thyroidectomy are shown in Table 2. *NIS* expression was significantly higher in tissues obtained from Graves' disease patients than in the control tissues ($p < 0.01$). *Tg*, *TSHR*, and *TPO* mRNA levels in Graves' disease-affected tissues were slightly higher than those in control tissues, but the differences were not significant. *PDS* expression in the Graves' disease-

affected tissues was similar to that in control tissues. Average TSH levels were 1.06 ± 0.58 in Graves' disease group and 1.03 ± 0.58 in control group, with both being within normal range.

The relation between the expression of the five mRNAs and their clinical characteristics before initial ATD treatment was evaluated. For *fT₃* and *fT₄*, significant correlation with *NIS* mRNA expression were found (*fT₃*, $p < 0.02$; *fT₄*, $p < 0.04$) (Fig. 1a–b). A significant correlation between *Tg* mRNA expression and ultrasonographically estimated goiter weight before initial ATD treatment was found ($p < 0.01$) (Fig. 2). No significant correlation was detected between TBII and the expression of any of five genes. There was also no significant correlation between the TSH level at the time of surgery and expression of any of the five genes. There was no significant correlation between TSH level before surgery and *NIS* expression (Fig. 3).

Plots of mRNA expression status in relation to L-thyroxine replacement therapy 6 months after surgery are shown in Fig. 4. The mean level of expression of each of the five genes was significantly higher in patients who did not require L-thyroxine replacement therapy than in those who required replacement at 6 months after surgery. *NIS* expression correlated significantly with the *fT₄* level at 6 months after surgery in the non-replacement therapy group ($p < 0.01$, Fig. 5). A significant correlation was also observed between *NIS* expression and *fT₃* ($p < 0.01$), but there were no significant correlations between expression of the five mRNAs and other clinical factors, with respect to the requirement or non-requirement for L-thyroxine replacement therapy at 6 months after surgery. There were also no significant correlations between *fT₃*, *fT₄*, expression of the five genes and TSH levels at 6 months after surgery.

We performed univariate analysis of nine factors with respect to postoperative hypothyroidism. *NIS* expression (*NIS*/*PGK* < 1.69) and low TBII levels before initial treatment were shown to be significant factors predictive of postoperative hypothyroidism (Table 3). Multivariate analysis revealed that decreased *NIS* expression (*NIS*/*PGK* < 1.69) and low TBII before initial treatment were independent risk factors for postoperative hypothyroidism (hazard ratio for *NIS* mRNA, 3.26; 95% confidence interval [95% CI], 1.36–9.08; $p < 0.01$; hazard ratio for TBII before initial treatment, 0.97; [95% CI], 0.95–0.99; $p < 0.01$).

The relation between expression of the five mRNAs

Table 2. Quantitative evaluation of mRNA by RT-PCR and TSH level in thyroid tissues.

	n	Tg	TSHR	TPO	NIS	PDS
Graves' Disease	65	2.04 ± 0.75	1.10 ± 0.39	1.53 ± 0.61	1.69 ± 0.70	0.95 ± 0.38
Control	6	1.66 ± 0.18	0.97 ± 0.12	1.17 ± 0.22	0.47 ± 0.09	0.93 ± 0.18
<i>p</i>	NS	NS	NS	<i>p</i> <0.01	NS	

Each mRNA expression level of the five genes was corrected by *PGK* mRNA expression value. NS: not significant.

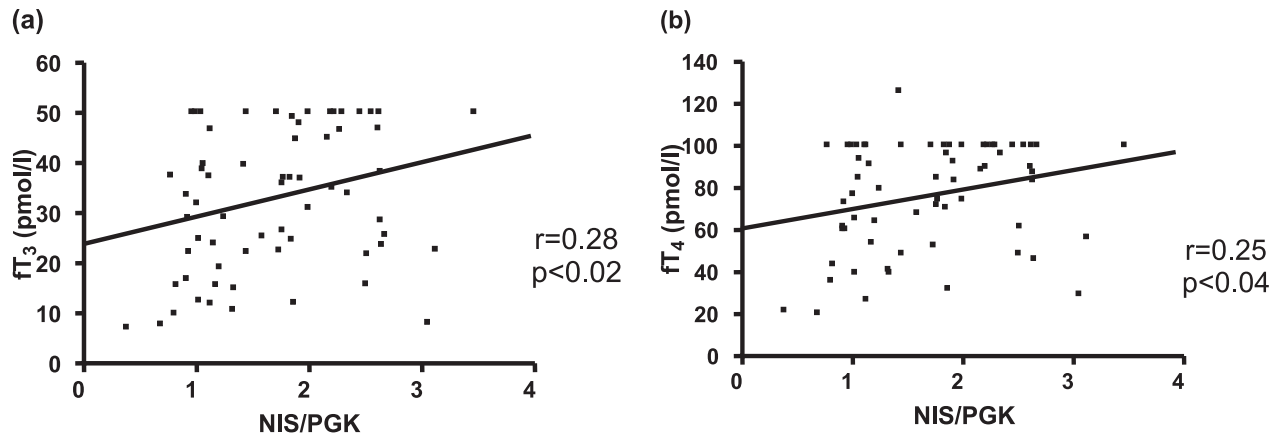


Fig. 1. (a) Significant relation between *NIS* mRNA expression and fT_3 levels before initial ATD treatment (*t*-test). (b) Significant relation between *NIS* mRNA expression and fT_4 levels before initial ATD treatment (*t*-test).

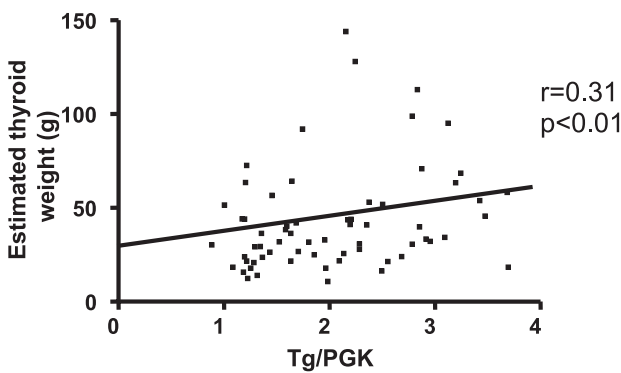


Fig. 2. Significant relation between *Tg* mRNA expression and ultrasonographically estimated goiter weight before initial ATD treatment (*t*-test).

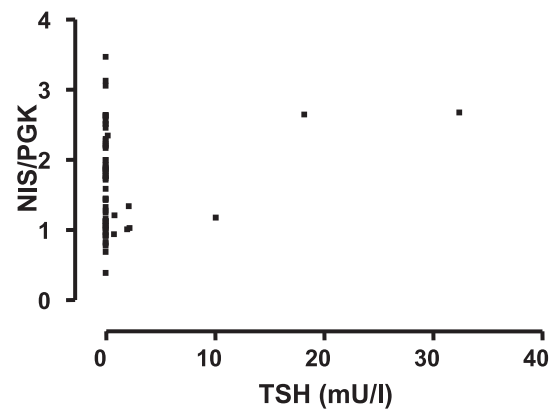


Fig. 3. There is no significant relation between the TSH level before surgery and *NIS* mRNA expression (*t*-test).

in resected thyroid tissues and those in FNAB samples was evaluated. A significant correlation with *NIS* mRNA expression were found ($p = 0.02$, Fig. 6). Significant correlations with *Tg*, *TSHR*, *TPO* and *PDS* mRNA expressions were also found (data not shown).

Discussion

We analyzed expression of five genes, *Tg*, *TSHR*, *TPO*, *NIS*, and *PDS*, that play important roles in thyroid hormone synthesis [5–9, 16, 17] and found that upregulation of these five genes is common in Graves' disease-affected tissues. Active transport of iodine mediated by *NIS* is the first step in thyroid hormone synthesis [8]. An increase of *NIS* expression affects

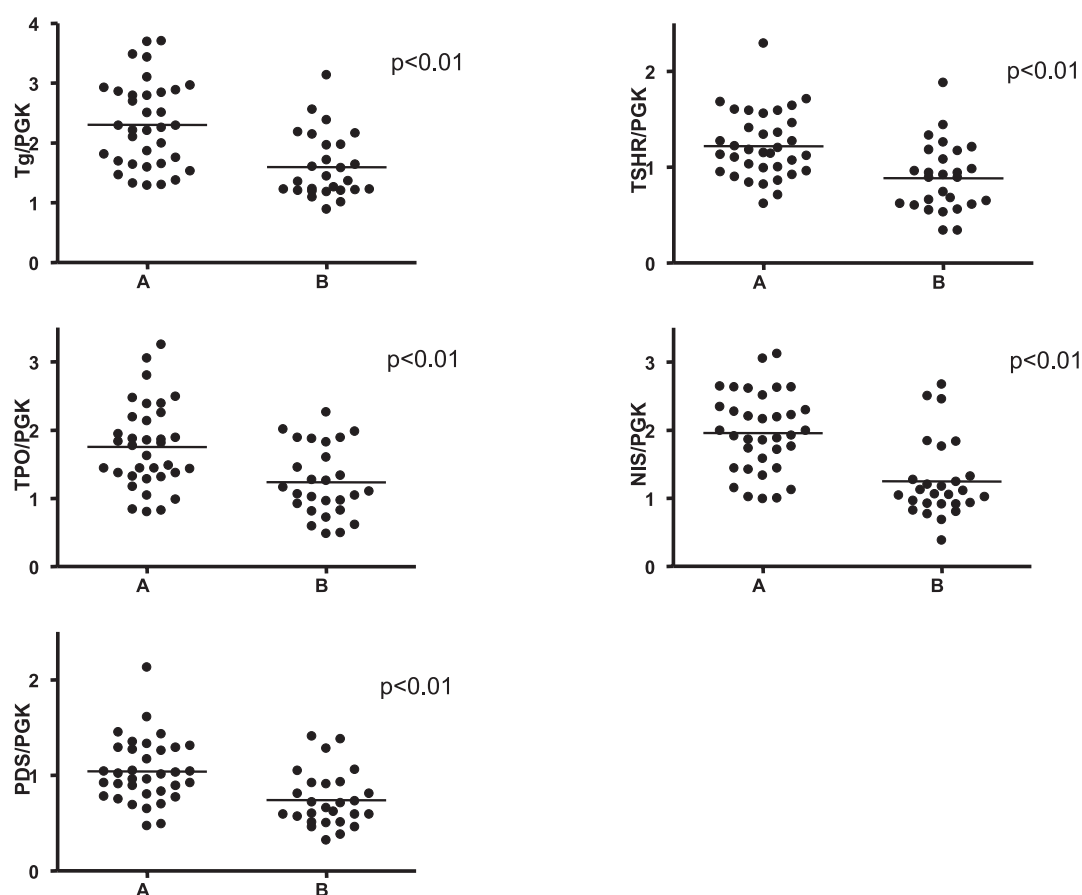


Fig. 4. Dot plots of mRNA expression of *Tg*, *TSHR*, *TPO*, *NIS* and *PDS* genes. **A**, Patients who did not require L-thyroxine replacement therapy after surgery, **B**, Patients who required L-thyroxine replacement therapy within 6 months after surgery. The line represents the median. Mean values of all five genes were significantly higher in patients who did not require replacement therapy than in those who required replacement therapy. P value is obtained by Mann-Whitney U-test.

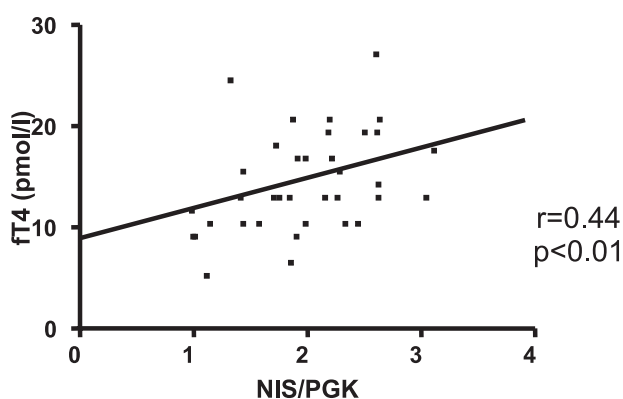


Fig. 5. Relationship between *NIS* mRNA expression and the fT₄ level at 6 months after surgery in the group of patients who did not require L-thyroxine replacement therapy. A significant relation between *NIS* mRNA and fT₄ levels is noted. P value is obtained by *t*-test.

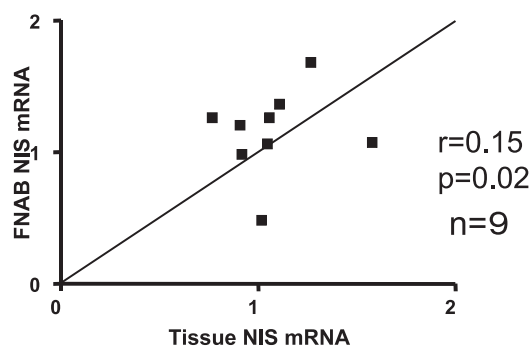


Fig. 6. Significant relation between *NIS* mRNA expression in resected thyroid tissues and *NIS* mRNA expression in fine-needle aspiration biopsy samples (*t*-test).

Table 3. Univariate and multivariate analysis for postoperative hypothyroidism in Graves' disease.

Variables	Hazard Ratio	95% Confidence Interval	<i>p</i> value
Univariate analysis			
Age	1.01	0.98–1.04	NS
Period of ATD treatment	1.00	0.98–1.00	NS
fT3 before initial treatment	0.96	0.92–1.00	NS
fT4 before initial treatment	0.88	0.74–1.06	NS
TBII before initial treatment	0.97	0.95–0.99	<0.01
Ultrasonographically estimated goiter weight	1.00	0.98–1.01	NS
Resected thyroid weight	1.00	0.98–1.01	NS
Estimated residual thyroid weight	0.73	0.51–1.02	NS
<i>NIS</i> mRNA expression ≤1.69	4.04	1.73–11.02	<0.01
Multivariate analysis			
Age	0.99	0.96–1.03	NS
TBII before treatment	0.97	0.95–0.99	<0.01
<i>NIS</i> mRNA expression ≤1.69	3.26	1.36–9.08	<0.01

NS: not significant

the mechanism for uptake of iodine in patients with Graves' disease. Increased *NIS* expression in Graves' disease-affected tissues has been reported by several researchers [10, 13, 16]. Sato *et al.* [16] reported that the amount of *NIS* protein was 3.1-fold higher in Graves' disease-affected tissue than in normal thyroid tissue, and therefore, the increased expression of *NIS* may contribute to development of Graves' disease. In the present study, only *NIS* mRNA was expressed at a significantly higher level in Graves' disease patients than in normal control. *NIS* mRNA expression is elevated to various degrees in Graves' disease patients and is correlated significantly with thyroid hormone levels before initial ATD treatment. These results suggest that overexpression of the *NIS* gene plays a major role in thyroid hormone synthesis.

TSH stimulation increases cAMP-mediated biosynthesis of *NIS* [18]. Expression of *NIS* mRNA and protein are reported to be upregulated by TSH stimulation in normal thyroid tissues [19, 20]. In Graves' disease patients, TSH receptor antibody stimulates TSHR. Our present results showed that *NIS* gene expression is not affected by the TSH level or anti-TSHR antibody level in Graves' disease patients, so the wide variability in the level of *NIS* gene expression may be due to the differences in the sensitivity of the *NIS* gene to stimulation by TSH and anti-TSHR antibody through TSHR. In the present study, we found that *NIS* gene expression is downregulated by ATDs. Genetic factors, such as the proximal promoters including thyroid transcription

factor 1 (*TTF1*), *NIS* TSH-responsive factor 1 (*NTF1*) or *NIS* upstream enhancers, iodine, cytokines and anti-*NIS* antibody, are known to regulate *NIS* gene expression [21–23]. These factors may also have influenced *NIS* expression in the present study.

It is reported that ATD treatment is not effective for patients with larger goiters [1–3]. Serum Tg levels are often high in patients with inflammatory thyroid diseases, benign or malignant thyroid tumors [5, 10, 24]. It has also been reported that *Tg* expression is increased in thyroid tumors [10, 24]. However, Lazar *et al.* [10] reported that *Tg* expression is normal in Graves' disease-affected tissue. Eszlinger *et al.* [25] reported that expression of *Tg* is decreased in peripheral blood of Graves' disease patients. In the present study, there was no significant correlation between *Tg* expression and thyroid hormone levels, but the *Tg* mRNA level correlated significantly with estimated goiter weight before initial ATD treatment. *Tg* expression may be an index of an increasing number of thyroid cells rather than an index of thyroid hormone synthesis.

The activity of Graves' disease is reported to be proportionate to the level of anti-TSHR antibody [3, 26]. Vitti *et al.* [2] reported that the relapse rate is dependent on the size of the goiter and the level of anti-TSHR antibody. We reported previously that the postoperative thyroid status of Graves' disease patients is determined by the responsiveness of thyrocytes to TSH *in vitro* [27]. Whether downregulation of *TSHR* is caused by continuous stimulation by anti-TSHR anti-

body during Graves' disease progress is unclear. In the present study, there was no significant correlation between *TSHR* expression and thyroid hormone levels before initial ATD treatment, but there was an inverse correlation between *TSHR* expression and thyroid hormone levels after ATD treatment. Eszlinger *et al.* [28] reported that regulators of G-protein signaling 2 (RGS2) inhibit signal transduction by *TSHR*. It may also have affected *TSHR* mRNA expression in the present study.

At 6 months after surgery, expression of each of the five genes was significantly lower in patients who required thyroid hormone replacement therapy than in patients who did not need replacement therapy. *NIS* expression correlated significantly with thyroid hormone levels in patients who did not need replacement therapy. Multivariate analysis revealed that decreased expression of *NIS* mRNA is an independent risk factor for postoperative thyroid hormone replacement. In patients with a large goiter and/or high TBII, the probability of cure by ATD treatment is thought to be low [1–3, 25]. We can estimate the prognosis of Graves' disease patients to some extent by comparing *NIS* expression with conventional factors, and with this information, appropriate treatment for each Graves' disease patient can be determined. At the Noguchi Thyroid Clinic and Hospital Foundation, 5.6% of patients shifted to postoperative hypothyroidism and 6.5% of

patients suffered a relapse of Graves' disease within 8.5 years following subtotal thyroidectomy (unpublished observation). Because only 1 of 65 patients in the present study experienced a relapse of Graves' disease during the observation period, we cannot speculate regarding the association of *NIS* expression with relapse of Graves' disease. It may be possible to predict the relapse of Graves' disease by measuring *NIS* expression. We found that the levels of *NIS* mRNA expression in thyroid tissues obtained by fine-needle aspiration biopsy are similar to levels in resected thyroid tissues. It may also be possible to predict the prognosis of Graves' disease patients prior to initial ATD treatment from measurements of *NIS* mRNA expression in fine-needle aspiration biopsy samples. In addition, our data in semi-quantitative RT-PCR are significantly correlated to those in SYBR Green-based quantitative realtime-PCR by comparing the results. Strissel *et al.* also reported that levels of mRNA expression analyzed in semi-quantitative RT-PCR is similar to those in quantitative realtime-PCR [29].

In conclusion, the level of *NIS* mRNA expression in thyroid tissues reflects the level of thyroid hormone synthesis in Graves' disease patients. We suggest that the prognosis of Graves' disease patients may be estimated by evaluating *NIS* expression, and thus appropriate treatment for each Graves' disease patient can be determined.

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