TABLE 5. FACTORS ASSOCIATED WITH THE FREQUENCY OF IRREGULAR TYPE OF MARGINS, LOGISTIC REGRESSION ANALYSIS

Factor	*Comparison*	*Odds ratio*	*95% CI*	p-*Value*[a]
Age at accident	By 1-year increment	0.95	0.84–1.08	0.434
Latent period	By 1-month increment	0.98	0.96–0.99	0.009
Nodule size on ultrasound	Larger than 10 mm vs. smaller	1.17	1.02–1.37	0.042
N stage				
	N1a vs. N0	10.88	2.09–89.9	0.010
	N1b vs. N0			0.072

[a]Based on the likelihood ratio test.
CI, confidence interval.

Clinical, ultrasonic, and pathological characteristics with respect to nodule echogenicity

Tumors were hypoechoic in 54 patients, isoechoic in 22, and mixed in 17 (Table 6). The age at diagnosis was significantly higher ($p = 0.048$) and latent period was significantly longer ($p = 0.015$) in the patients with isoechoic tumors (usually no sign of malignancy) than in those with hypoechoic or mixed-type tumors. The frequency of isoechoic tumors was significantly higher in male patients ($p = 0.003$).

Heterogeneous US structure was significantly more frequent in mixed-type tumors ($p = 0.021$). Irregular margins were more frequent in hypoechoic or mixed-type tumors ($p = 0.051$). The presence of halo was significantly more frequent in isoechoic tumors ($p < 0.001$), while calcifications occurred more frequently in the mixed-type tumors ($p < 0.001$). Tumor size on pathology was significantly larger in the mixed-type tumors ($p = 0.023$).

Three factors—sex, halo, and calcification—were found by logistic regression analysis to be associated with the frequency of hypoechoic tumors (Table 7). It was significantly higher in female patients ($p = 0.012$), in the nodules without halo ($p = 0.003$) or calcifications ($p = 0.005$).

Discussion

Easy detection of thyroid nodules on US, besides of obvious benefits, brings about a variety of associated problems. These are the differential diagnosis, follow-up of a great number of small nodules occurring in 3–50% of population, and establishment of evidence-based criteria for FNAB in different age groups (1,2,7,22). Regarding the latter, the Consensus of Society of Radiologists in US and the American Thyroid Association recommend FNAB for the nodules measuring 1 cm or greater because of "uncertainty as to whether diagnosis of smaller cancers improves life expectancy as well as a concern that assessment of small nodules would lead to an excessive number of biopsies" (9,22). Nodules not exceeding 1 cm in diameter are recommended for follow-up or evaluation in case of suspicious US findings, history of head and neck irradiation, or documented thyroid cancer in consanguine relative(s). Recent guidelines of the American Association of Clinical Endocrinologists and Associazione Medici Endocrinologi also recommend evaluation of thyroid nodules smaller than 1 cm in radiation-exposed patients (23). With regard to the young individuals who were internally irradiated by iodine isotopes, substantiated recommendations for management of small thyroid nodules need further study, in our opinion.

In the present work we specifically focused on a group of young patients with radiation-induced papillary thyroid carcinoma. Nodules in all cases had been initially detected due to the implementation of post-Chernobyl US screening programs in Belarus; all nodules exceeding 5 mm were subjected to FNAB and cytological examination. Unlike in other studies, here we not addressed the differential cancer diagnosis but attempted to determine clinicopathological and ultrasonic criteria with regard to tumor size, ultrasonographic margin type, and nodule echogenicity, that is, with the most important US parameters taken into account for clinical decision making.

The results of analyses demonstrated an association of tumor size (under vs. over 10 mm) with tumor margin type and bilateral lymph node involvement. With increasing nodule size, irregular tumor margins (a usual suspicious sign) and bilateral lymph node involvement (an indicator of tumor aggressiveness) became more frequent. Similar to our findings, Pellegriti *et al.* reported a progressive accumulation of signs of tumor aggressiveness (multifocality, bilaterality, extrathyroidal invasion, and nodal disease) with increasing nodule size at presentation (18). Also, in patients with cancers larger than 1 cm, a close relationship between tumor size and aggressiveness has been demonstrated (24–26). Apparently, smaller tumors display more benign characteristics on US, but these should not be misleading as nodule size *per se* is not a predictor of malignancy (8,13,27) and may not necessarily correlate with an indolent clinical course or lower risk of recurrence (10,18).

Type of margins in our series correlated not only with tumor size, but also with other US features, in particular lymph node involvement and duration of latency. The frequency of irregular margins (a usual suspicious sign) was increasing in parallel with those of heterogeneous structure, hypoechoic or mixed echogenicity (all worrisome), and lymphadenopathy (tumor aggressiveness). Patients with irregular tumor margins were younger at diagnosis and had shorter latent period and more aggressive tumors. Of note, from the molecular studies of Chernobyl thyroid cancers, it is known that mentioned characteristics correlate with the type of genetic abnormality underlying malignancy. RET/PTC3 rearrangements were particularly prevalent in the aggressive tumors developing after the shorter latency; these tumors frequently had solid morphological structure (28,29). In our series, solid morphology was more frequently observed in the tumors with irregular margins (as well as in the hypoechoic ones), but significance of difference was not reached perhaps because of the small number of such cases. It would

TABLE 6. CLINICAL, ULTRASONOGRAPHIC, AND PATHOLOGICAL PARAMETERS BY TUMOR ECHOGENICITY

Parameters	*Group*			p-*Value*[a]
	Hypoechoic n = *54*	*Isoechoic* n = *22*	*Mixed* n = *17*	
Clinical characteristics				
Sex				
Male	22 (40.7)[b]	18 (81.8)	7 (41.2)	
Female	32 (59.3)	4 (18.2)	10 (58.8)	0.003
Age at the time of accident (years)	5.2 ± 4.1[c]	6.7 ± 6.2	5.1 ± 4.2	0.87
Age at the time of diagnosis (years)	14.9 ± 5.8	19.4 ± 8.1	16.8 ± 5.5	0.048
Latent period (months)	117.7 ± 47	151.9 ± 45	139.9 ± 47	0.015
Ultrasonographic characteristics				
Nodule size (mm)[d]	14.7 ± 7.1	14.7 ± 4.8	16.6 ± 6.7	0.13
Nodule volume, mL	1.2 ± 1.90	1.0 ± 0.87	1.9 ± 4.1	0.32
Number of nodules				
Solitary	48 (88.9)	17 (77.3)	13 (76.5)	
Multiple	6 (11.1)	5 (22.7)	4 (23.5)	0.38
Structure				
Homogeneous	25 (47.2)	11 (50.0)	2 (11.8)	
Heterogeneous	28 (52.8)	11 (50.0)	15 (88.2)	0.021
Unknown	1	0	0	
Margins				
Regular	9 (17.0)	9 (40.9)	2 (11.8)	
Irregular	44 (83.0)	13 (59.1)	15 (88.2)	0.051
Unknown	1	0	0	
Halo				
Absent	53 (98.2)	12 (54.5)	15 (88.2)	
Present	1 (1.8)	10 (45.5)	2 (11.8)	<0.001
Calcifications				
Absent	53 (98.2)	18 (81.8)	12 (70.6)	
Present	1 (1.8)	4 (18.2)	5 (29.4)	<0.001
Lymphadenopathy				
Absent	27 (50.0)	15 (68.2)	11 (64.7)	
Present	27 (50.0)	7 (31.8)	6 (35.3)	0.29
Hypoechoic	20 (37.0)	6 (27.3)	1 (4.5)	
Isoechoic	6 (11.1)	1 (4.5)	1 (5.9)	0.76
Unknown	1	0	4	
Pathological characteristics				
Tumor size (mm)[d]	11.0 ± 5.6	11.7 ± 4.1	14.4 ± 5.4	0.023
T stage				
T1	50 (92.6)	22 (100.0)	13 (76.5)	
T2	3 (5.6)	0 (0.0)	1 (5.9)	
T3	1 (1.8)	0 (0.0)	3 (17.6)	0.063
N stage				
N0	23 (43.4)	6 (27.3)	4 (23.5)	
N1a	17 (32.1)	5 (22.7)	4 (23.5)	
N1b	13 (24.5)	11(50.0)	9 (53.0)	0.14
Unknown	1	0	0	
Histopathological variant				
Solid	7 (13.0)	1 (4.5)	2 (11.8)	
Papillary and follicular	47 (87.0)	21 (95.5)	15 (88.2)	0.86
Invasiveness				
Intrathyroidal	46 (85.2)	19 (86.4)	14 (82.4)	
Extrathyroidal	8 (14.8)	3 (13.6)	2 (17.6)	0.92
Unknown	0	0	1	

[a]Based on Wilcoxon rank-sum or Kruskal-Wallis test for continuous variables and Fisher's exact test for categorical variables; patients without available information were excluded.
[b]Number of patients with percentage within parentheses.
[c]Mean ± standard deviation.
[d]Largest measurement.

TABLE 7. FACTORS ASSOCIATING WITH NODULE HYPOECHOGENICITY, LOGISTIC REGRESSION ANALYSIS

Factor	*Comparison*	*Odds ratio*	*95% CI*	p-*Value*[a]
Sex	Male vs. female	0.26	0.08–0.72	0.012
Halo	Absent vs. present	26.37	4.24–522.49	0.003
Calcifications	Absent vs. present	25.42	3.64–525.31	0.005

[a]Based on the likelihood ratio test.
CI, confidence interval.

be interesting to analyze a correlation between US, clinicopathological, and molecular features, first of all RET/PTC3 and RET/PTC1 rearrangements, in a specially designed study.

The irregular nodule margins on US are common in thyroid malignancy (1,3,9–11,13–16,30,31). An analysis done by Papini *et al.* demonstrated that independent risk factors of cancer at US of nonpalpable thyroid nodules were irregular or blurred nodule margins, intranodular vascular pattern, and microcalcifications (13). Another study has identified nodule margins and shape, internal echo level, but not "strong echoes" (calcifications) as important characteristics in differentiating papillary thyroid carcinoma from benign neoplasm (10). Our findings are in agreement with these studies only for the tumors exceeding 10 mm in diameter. The importance of irregular margins as criterion of malignancy was significantly weaker in smaller tumors. Technically, it is not surprising that irregular margins are found less frequently in very small thyroid nodules by US because of limited resolution of the latter.

The associations between cancer nodule size, type of margin, and latent period are also worth noting. The longer latent period was characteristic to the nodules with regular margins, which may reflect the difficulties of early diagnosis of slowly growing tumors. Radiation-induced thyroid carcinomas developing after the shorter period of latency are often in advanced stage, are aggressive at presentation, and display elevated risk of recurrence despite the small size (5,32–34). It is therefore necessary to take into account that the nodules detected after the longer latency may display less unusual suspicious signs on US, yet they may well be malignant.

Regarding tumor echogenicity, hypoechoic nodules are more likely to be malignant in contrast to those lacking such characteristics (10,11,15–17), especially if nodule size exceeds 15 mm (35). In our series hypoechogenicity was associated with female sex, and the absence of both halo and calcifications, while isoechogenicity with male sex (this observation is difficult to explain). Note also that nodule hypoechogenicity was more frequently observed in younger patients and after the shorter period of latency which together are supportive of the overall suspicious nature of this US feature.

From the practical point of view, the clinical decision to biopsy a small thyroid nodule in children or young patients is not simple and has individual and overall healthcare cost implications (36). If we used nodule diameter of 10 mm as cut-off for FNAB, we would have missed 25 (26.6%) cases of thyroid cancer in our young patients. Papini *et al.* obtained a very similar result in a prospective study of 402 nodules (13). If FNAB had been restricted to the nodules exceeding 10 mm in size, cytological evaluation would have been omitted in 38.7% of the actually diagnosed malignancies. They proposed a cut-off diameter of 8 mm for nonpalpable nodules to be subjected to cytological evaluation, while follow-up of the smaller lesions was recommended. The findings presented here support the results of prior studies that showed that nodule size greater or smaller than 10 mm was not an effective arbitrary criterion for FNAB (8,13,37,38) as well as that probability of malignancy in thyroid nodules measuring less than 1 cm is not lower than in larger lesions (39). From this point of view, our results also support the necessity of evaluation of small thyroid nodules in radiation-exposed patients recommended by the American Thyroid Association (9) and the American Association of Clinical Endocrinologists and Associazione Medici Endocrinologi (23).

In addition to the nodule size issues, if we biopsied only nodules with irregular margins, 20 (21.7%) cases would have been missed. If we biopsied only usual suspicious hypoechoic nodules, we would have missed 22 isoechoic tumors and 17 nodules with mixed echogenicity (41.9% cases combined). Further, as shown in our previous study, the relative proportion of cancer nodules among all thyroid nodules detected in young individuals affected by the Chernobyl fallouts decreases with time after exposure due to the increasing prevalence of nodular goiter, nonverified small solid nodules, and cysts (3). In some sense this may predispose to the intuitive expectation of a benign rather than cancerous nodule to be developing after the longer latency. In our opinion, physicians need to be aware of that the situation is more complex.

This study demonstrates that important US features considered usual for thyroid cancer are less frequent in smaller tumors and that thyroid cancers developing with a greater latency after radiation are less likely to have worrisome US features. We therefore propose that in young individuals with a history of internal radiation exposure FNAB should be recommended even for small thyroid nodules (i.e., whenever technically accessible) with any margin type and echogenicity.

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Disclosure Statement

The authors declare that no competing financial interests exist.

References

1. Hegedus L 2004 Clinical practice. The thyroid nodule. N Engl J Med **351**:1764–1771.
2. Reiners C, Wegscheider K, Schicha H, Theissen P, Vaupel R, Wrbitzky R, Schumm-Draeger PM 2004 Prevalence of thyroid disorders in the working population of Germany: ultrasonography screening in 96,278 unselected employees. Thyroid **14**:926–932.
3. Drozd V, Polyanskaya O, Ostapenko V, Demidchik Y, Biko I, Reiners C 2002 Systematic ultrasound screening as a

significant tool for early detection of thyroid carcinoma in Belarus. J Pediatr Endocrinol Metab **15**:979–984.
4. Imaizumi M, Usa T, Tominaga T, Akahoshi M, Ashizawa K, Ichimaru S, Nakashima E, Ishii R, Ejima E, Hida A, Soda M, Maeda R, Nagataki S, Eguchi K 2005 Long-term prognosis of thyroid nodule cases compared with nodule-free controls in atomic bomb survivors. J Clin Endocrinol Metab **90**:5009–5014.
5. Bucci A, Shore-Freedman E, Gierlowski T, Mihailescu D, Ron E, Schneider AB 2001 Behavior of small thyroid cancers found by screening radiation-exposed individuals. J Clin Endocrinol Metab **86**:3711–3716.
6. Shevchuk VE, Gurachevsky GV (eds) 2006 20 Years after the Chernobyl Catastrophe. Publishing House Belarus, Minsk, Belarus, p 250.
7. Baskin HJ 2004 Thyroid ultrasound—just do it. Thyroid **14**:91–92.
8. Frates MC, Benson CB, Doubilet PM, Kunreuther E, Contreras M, Cibas ES, Orcutt J, Moore FD Jr., Larsen PR, Marqusee E, Alexander EK 2006 Prevalence and distribution of carcinoma in patients with solitary and multiple thyroid nodules on sonography. J Clin Endocrinol Metab **91**:3411–3417.
9. Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, McIver B, Sherman SI, Tuttle RM 2006 Management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid **16**:109–142.
10. Shimura H, Haraguchi K, Hiejima Y, Fukunari N, Fujimoto Y, Katagiri M, Koyanagi N, Kurita T, Miyakawa M, Miyamoto Y, Suzuki N, Suzuki S, Kanbe M, Kato Y, Murakami T, Tohno E, Tsunoda-Shimizu H, Yamada K, Ueno E, Kobayashi K, Kobayashi T, Yokozawa T, Kitaoka M 2005 Distinct diagnostic criteria for ultrasonographic examination of papillary thyroid carcinoma: a multicenter study. Thyroid **15**:251–258.
11. Tae HJ, Lim DJ, Baek KH, Park WC, Lee YS, Choi JE, Lee JM, Kang MI, Cha BY, Son HY, Lee KW, Kang SK 2007 Diagnostic value of ultrasonography to distinguish between benign and malignant lesions in the management of thyroid nodules. Thyroid **17**:461–466.
12. Wienke JR, Chong WK, Fielding JR, Zou KH, Mittelstaedt CA 2003 Sonographic features of benign thyroid nodules: interobserver reliability and overlap with malignancy. J Ultrasound Med **22**:1027–1031.
13. Papini E, Guglielmi R, Bianchini A, Crescenzi A, Taccogna S, Nardi F, Panunzi C, Rinaldi R, Toscano V, Pacella CM 2002 Risk of malignancy in nonpalpable thyroid nodules: predictive value of ultrasound and color-Doppler features. J Clin Endocrinol Metab **87**:1941–1946.
14. Koike E, Noguchi S, Yamashita H, Murakami T, Ohshima A, Kawamoto H, Yamashita H 2001 Ultrasonographic characteristics of thyroid nodules: prediction of malignancy. Arch Surg **136**:334–337.
15. Rago T, Vitti P, Chiovato L, Mazzeo S, De Liperi A, Miccoli P, Viacava P, Bogazzi F, Martino E, Pinchera A 1998 Role of conventional ultrasonography and color flow-doppler sonography in predicting malignancy in 'cold' thyroid nodules. Eur J Endocrinol **138**:41–46.
16. Chan BK, Desser TS, McDougall IR, Weigel RJ, Jeffrey RB Jr. 2003 Common and uncommon sonographic features of papillary thyroid carcinoma. J Ultrasound Med **22**:1083–1090.
17. Peccin S, de Castsro JA, Furlanetto TW, Furtado AP, Brasil BA, Czepielewski MA 2002 Ultrasonography: is it useful in the diagnosis of cancer in thyroid nodules? J Endocrinol Invest **25**:39–43.
18. Pellegriti G, Scollo C, Lumera G, Regalbuto C, Vigneri R, Belfiore A 2004 Clinical behavior and outcome of papillary thyroid cancers smaller than 1.5 cm in diameter: study of 299 cases. J Clin Endocrinol Metab **89**:3713–3720.
19. Brunn J, Block U, Ruf G, Bos I, Kunze WP, Scriba PC 1981 Volumetrie der Schilddrüsenlappen mittels Real-Time-Sonographie. Dtsch Med Wochenschr **106**:1338–1340.
20. Sobin LH, Wittekind CH (eds) 2002 International Union Against Cancer. TNM Classification of Malignant Tumors, sixth edition. John Wiley & Sons, Inc., New York, p 239.
21. SAS Institute 1999 SAS/STAT User's Guide Version 8. SAS Institute, Cary, NC.
22. Frates MC, Benson CB, Charboneau JW, Cibas ES, Clark OH, Coleman BG, Cronan JJ, Doubilet PM, Evans DB, Goellner JR, Hay ID, Hertzberg BS, Intenzo CM, Jeffrey RB, Langer JE, Larsen PR, Mandel SJ, Middleton WD, Reading CC, Sherman SI, Tessler FN 2005 Management of thyroid nodules detected at US: Society of Radiologists in Ultrasound consensus conference statement. Radiology **237**:794–800.
23. AACE/AME Task Force on Thyroid Nodules 2006 American Association of Clinical Endocrinologists and Associazione Medici Endocrinologi medical guidelines for clinical practice for the diagnosis and management of thyroid nodules. Endocr Pract **12**:63–102.
24. Hay ID, Grant CS, van Heerden JA, Goellner JR, Ebersold JR, Bergstralh EJ 1992 Papillary thyroid microcarcinoma: a study of 535 cases observed in a 50-year period. Surgery **112**:1139–1146; discussion 1146–1147.
25. Mazzaferri EL, Massoll N 2002 Management of papillary and follicular (differentiated) thyroid cancer: new paradigms using recombinant human thyrotropin. Endocr Relat Cancer **9**:227–247.
26. Cappelli C, Castellano M, Braga M, Gandossi E, Pirola I, De Martino E, Agosti B, Rosei EA 2007 Aggressiveness and outcome of papillary thyroid carcinoma (PTC) versus microcarcinoma (PMC): a mono-institutional experience. J Surg Oncol **95**:555–560.
27. Alexander EK, Heering JP, Benson CB, Frates MC, Doubilet PM, Cibas ES, Marqusee E 2002 Assessment of nondiagnostic ultrasound-guided fine needle aspirations of thyroid nodules. J Clin Endocrinol Metab **87**:4924–4927.
28. Nikiforov YE, Rowland JM, Bove KE, Monforte-Munoz H, Fagin JA 1997 Distinct pattern of ret oncogene rearrangements in morphological variants of radiation-induced and sporadic thyroid papillary carcinomas in children. Cancer Res **57**:1690–1694.
29. Rabes HM, Demidchik EP, Sidorow JD, Lengfelder E, Beimfohr C, Hoelzel D, Klugbauer S 2000 Pattern of radiation-induced RET and NTRK1 rearrangements in 191 post-Chernobyl papillary thyroid carcinomas: biological, phenotypic, and clinical implications. Clin Cancer Res **6**: 1093–1103.
30. Takashima S, Fukuda H, Nomura N, Kishimoto H, Kim T, Kobayashi T 1995 Thyroid nodules: re-evaluation with ultrasound. J Clin Ultrasound **23**:179–184.
31. Kim EK, Park CS, Chung WY, Oh KK, Kim DI, Lee JT, Yoo HS 2002 New sonographic criteria for recommending fine-needle aspiration biopsy of nonpalpable solid nodules of the thyroid. AJR Am J Roentgenol **178**:687–691.
32. Williams ED, Becker D, Dimidchik EP, Nagataki S, Pinchera A, Tronko ND 1996 Effects on the thyroid in populations exposed to radiation as a result of the Chernobyl accident.

In: Demir M (ed) One Decade after Chernobyl: Summing up the Consequences of the Accident. International Atomic Energy Authority, Vienna, pp 207–230.
33. Farahati J, Demidchik EP, Biko J, Reiners C 2000 Inverse association between age at the time of radiation exposure and extent of disease in cases of radiation-induced childhood thyroid carcinoma in Belarus. Cancer **88**:1470–1476.
34. Demidchik YE, Demidchik EP, Reiners C, Biko J, Mine' M, Saenko VA, Yamashita S 2006 Comprehensive clinical assessment of 740 cases of surgically treated thyroid cancer in children of Belarus. Ann Surg **243**:525–532.
35. Lyshchik A, Drozd V, Demidchik Y, Reiners C 2005 Diagnosis of thyroid cancer in children: value of gray-scale and power doppler US. Radiology **235**:604–613.
36. Rosen JE, Stone MD 2006 Contemporary diagnostic approach to the thyroid nodule. J Surg Oncol **94**:649–661.
37. Davies TF 2006 Is consensus a good thing in the management of thyroid nodules? Thyroid **16**:205.
38. Burman KD 2006 Micropapillary thyroid cancer: should we aspirate all nodules regardless of size? J Clin Endocrinol Metab **91**:2043–2046.
39. Berker D, Aydin Y, Ustun I, Gul K, Tutuncu Y, Isik S, Delibasi T, Guler S 2008 The value of fine-needle aspiration biopsy in subcentimeter thyroid nodules. Thyroid **18**:603–608.

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Lack of GNAQ Hotspot Mutation in Papillary Thyroid Carcinomas

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Dear Editor:

Papillary thyroid carcinoma (PTC) is the most common malignant tumor in endocrine organs. PTCs have characteristic gene mutations leading to the activation of mitogen-activated protein kinase (MAPK) signaling pathway. Those include *RET/PTC* rearrangements and point mutations in *BRAF* and *RAS* family genes. They are found in approximately 60–70% of all PTCs and rarely overlap in the same tumor (1–3). The absence of coexistence of those mutations provides strong genetic evidence for the requirement of constitutively active MAPK signaling for transformation to PTC. However, the remaining 30–40% of PTC cases do not have mutations in those genes, suggesting that other factors may contribute to constitutive activation of the MAPK pathway.

Similar to PTCs, most melanomas harbor oncogenic mutations in the MAPK signaling components—in particular, *BRAF* and *RAS*; however, a subset of melanomas including uveal melanoma does not have mutations in those genes. Very recently, frequent somatic mutations in the *GNAQ* gene have been identified in uveal melanomas and blue naevi (4,5). *GNAQ* encodes members of the q class of G-protein α-subunits involved in mediating signals from G-protein-coupled receptors. Several patterns of mutations have been found (at nucleotides 625–627, wild-type CAA to CTA, TTA, CCA, CAT, CGA or TAT), but all the mutations exclusively occur in codon 209 in the RAS-like domain, which corresponds to codon 61 of RAS, and result in constitutive activation of the MAPK pathway in melanocytes (4). There is no equivalent of RAS codon 12 in GNAQ. Signaling from GNAQ to MAPK seems to be transmitted through diacylglycerol/protein kinase C (4,6). The *GNAQ* mutations have not been described in other human neoplasms so far. However, $GNAQ^{Q209L}$ mutation, which is indeed dominant type of mutation found in blue naevi and uveal melanomas, was already shown to have an ability to transform NIH3T3 cells (7).

Although it remains to be tested whether GNAQ can activate the MAPK pathway in thyroid cells, GNAQ seems to be a good candidate as a target of oncogenic mutation in PTCs. In this study, therefore, the possibility that *GNAQ* mutation plays a role in PTC pathogenesis was explored.

First of all, we investigated Russian PTC samples that were obtained at the time of surgery in the Medical Radiological Research Center of Russian Academy of Medical Sciences (Obninsk, Russia); none of the patients had a history of radiation exposure. An informed consent was obtained from each individual according to ethical guidelines for the use of human materials for scientific purposes effective in Medical Radiological Research Center of Russian Academy of Medical Sciences. Protocol for the present study was approved by the Committee for Ethical Issues of Human Genome Analysis in Nagasaki University. All samples were collected at the time of primary surgery and did not include recurrence. Forty-two PTC frozen samples from 36 patients (36 primary tumors and 6 metastatic lymph nodes, no multiple cancer) without *RET/PTC1*, *RET/PTC3*, $BRAF^{V600E}$, or hotspot mutations (codons 12, 13, and 61) in three *RAS* genes (*HRAS*, *KRAS*, and *NRAS*) were subjected to DNA extraction (conventional proteinase K/phenol protocol). The same set of samples was used for the analysis of *RAP1* mutation, and the clinicopathological characteristics of the patients were described previously (8). Amplification of the *GNAQ* gene was then performed by genomic PCR using AmpliTaq Gold (Applied Biosystems, Foster City, CA). The sequences of used primers are same as previously described (4). PCR products were then treated with ExoSAP-IT (USB, Cleveland, OH), and sequence analysis was performed with a Big Dye Terminator sequencing kit v3.1 (Applied Biosystems) and an ABI3100 automated sequencer (Applied Biosystems). As a result, no mutation was found around hotspot codon 209 in the *GNAQ* gene.

We next used 46 Japanese PTC samples collected at Kuma Hospital (Kobe, Japan). All samples were primary tumors from different individuals. An appropriate informed consent was obtained from each individual, and the protocol was

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approved by the ethics committees of Nagasaki University and Kuma Hospital. We performed the sequence analysis using all 46 samples regardless of existence of other oncogenes. Of note, among 46 samples, there were 36 *BRAF*^{V600E}, 3 *RET/PTC1*, and no *RET/PTC3* nor *RAS* mutation (without overlap). Again, no mutations were identified in the *GNAQ* gene.

Based on these findings, we conclude that the *GNAQ* hotspot mutation is unlikely to play an important role in the molecular pathogenesis of PTCs.

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References

1. Frattini M, Ferrario C, Bressan P, Balestra D, De Cecco L, Mondellini P, Bongarzone I, Collini P, Gariboldi M, Pilotti S, Pierotti MA, Greco A 2004 Alternative mutations of BRAF, RET and NTRK1 are associated with similar but distinct gene expression patterns in papillary thyroid cancer. Oncogene **23**:7436–7440.
2. Kimura ET, Nikiforova MN, Zhu Z, Knauf JA, Nikiforov YE, Fagin JA 2003 High prevalence of BRAF mutations in thyroid cancer: genetic evidence for constitutive activation of the RET/PTC-RAS-BRAF signaling pathway in papillary thyroid carcinoma. Cancer Res **63**:1454–1457.
3. Soares P, Trovisco V, Rocha AS, Lima J, Castro P, Preto A, Maximo V, Botelho T, Seruca R, Sobrinho-Simoes M 2003 BRAF mutations and RET/PTC rearrangements are alternative events in the etiopathogenesis of PTC. Oncogene **22**:4578–4580.
4. Van Raamsdonk CD, Bezrookove V, Green G, Bauer J, Gaugler L, O'Brien JM, Simpson EM, Barsh GS, Bastian BC 2009 Frequent somatic mutations of GNAQ in uveal melanoma and blue naevi. Nature **457**:599–602.
5. Onken MD, Worley LA, Long MD, Duan S, Council ML, Bowcock AM, Harbour JW 2008 Oncogenic mutations in GNAQ occur early in uveal melanoma. Invest Ophthalmol Vis Sci **49**:5230–5234.
6. Hubbard KB, Hepler JR 2006 Cell signalling diversity of the Gqalpha family of heterotrimeric G proteins. Cell Signal **18**:135–150.
7. Kalinec G, Nazarali AJ, Hermouet S, Xu N, Gutkind JS 1992 Mutated alpha subunit of the Gq protein induces malignant transformation in NIH 3T3 cells. Mol Cell Biol **12**:4687–4693.
8. Matsuse M, Mitsutake N, Rogounovitch T, Saenko V, Nakazawa Y, Rumyantsev P, Lushnikov E, Suzuki K, Yamashita S 2009 Mutation analysis of RAP1 gene in papillary thyroid carcinomas. Endocr J **56**:161–164.

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Question

3．免疫不全症で問題となる真菌症は？

Answer

免疫不全があると，さまざまな真菌感染症が発症しやすくなる。免疫不全には「好中球減少症」，抗体や補体などが関与する「液性免疫」不全，Tリンパ液などが関与する「細胞性免疫」不全がある。それぞれで起こりやすい真菌症の種類が異なるとされるが，実際の臨床でみられるのはこれらの複合免疫不全であり，診療にあたってはこれらの真菌症すべてを検討する必要がある。

好中球減少症は，末梢血好中球が500/μL以下となった場合にリスクがあり，初期にはカンジダによる菌血症や播種性カンジダ症（肝脾膿瘍）の頻度が高い。症状としては，発熱以外の特徴的なものはないが，カンジダ肝脾膿瘍では季肋部痛を訴えることがある。また，播種性病変では7～10日ほど経過してカンジダ眼内炎による視覚障害を訴えることがある。カンジダと同様の経過をとり，より重篤な真菌症として稀にトリコスポロン感染症がみられる。

好中球減少が高度で遷延する場合は，侵襲性アスペルギルス症がみられるようになる。主な病変部位は肺であり，発熱の検索には胸部単純X線写真や胸部CTが必須である。稀に副鼻腔や眼窩に膿瘍を形成することがある。また，同様の経過・所見を呈する稀なものとして接合菌症（いわゆるムーコル症）があり，より急速で難治性の経過をとる。

造血幹細胞移植では，移植後早期にはカンジダが，数週間以降の中・後期にはアスペルギルス症のリスクがある。

液性免疫不全では，化膿性細菌感染が主な病原体であり真菌感染は特徴的とはいえないが，カンジダ症を中心とした真菌の考慮が必要な場合もある。

細胞性免疫不全は，代表的な疾患としてHIV/AIDS（human immunodeficiency virus/acquired immunodeficiency syndrome）があげられる。日常的に

遭遇する疾病として，糖尿病や透析患者でも細胞性免疫の低下がみられる。また，副腎皮質ステロイドホルモン薬や関節リウマチに対するインフリキシマブなどの免疫抑制薬が普遍的に投与されるようになってきており，細胞性免疫不全は日常的に注意が必要になってきている。

糖尿病では皮膚真菌症，深在性皮膚真菌症のほかに，カンジダ症（口腔，食道，膣，カンジダ血症）がみられやすい。また，稀ではあるが特徴的とされる真菌症に接合菌症（肺，鼻脳型）が知られている。

HIV/AIDS やステロイド薬・免疫抑制薬投与例では，1）ニューモシスチス肺炎，2）カンジダ症（口腔，食道，膣），3）クリプトコックス症（肺感染以外；髄膜炎や全身播種など），4）アスペルギルス症（肺や副鼻腔，脳）などが注意すべき真菌症としてあげられる。このほか，HIV/AIDS では日本では稀であるが，5）ヒストプラズマ症，6）コクシジオイデス症，7）マルネッフェイ型ペニシリウム症も輸入感染症としてみられることがある。

ニューモシスチス肺炎は，以前原虫に分類されていた *Pneumocystis jirovecii*（以前は *carinii*）による重症肺炎で，HIV/AIDS では AIDS 発症診断の 4 ～ 6 割が本症の発症による。死亡率は 10 ～ 30%と重篤な肺炎である。ニューモシスチスは真菌に属するものの，一般的な抗真菌薬は無効で，ST 合剤（sulfamethoxazole/trimethoprim：スルファメトキサゾール／トリメトプリム）やペンタミジンによって治療を行う。

カンジダ症は，最も頻度が高い HIV 関連真菌症で，口腔内に粘膜の発赤を伴った白苔がみられることが多い（口腔カンジダ症）。この病変が食道まで波及し，胸部の中心付近の痛み（胸骨裏面痛）や嚥下時痛をきたした場合が食道カンジダ症で，HIV 患者の場合は AIDS 発症とみなされる。このほか，女性では膣カンジダ症もよく認められる。

クリプトコックス症は，免疫不全が高度になるほど，一般的な肺病変よりも髄膜炎の頻度が高くなる。HIV 感染者では頭痛や嘔気といった髄膜刺激症状が軽度で，局所所見を伴わないまま衰弱や意識障害が進むという経過をとることも少なくないため，注意が必要である。

アスペルギルス症は，高度の細胞性免疫不全状態で肺や副鼻腔の感染が起こりやすい。本症は，AIDS 指標疾患ではないものの HIV/AIDS でも同様であり，高度免疫不全で死亡した例の解剖所見では，肺などにアスペルギルスの病変が認められることが少なくない。

HIV にみられる真菌症で問題となる点は，免疫不全が持続しているため治療への反応が悪く，長期治療が必要な点である。再燃・再発が多いため，免疫不全が改善しない限り生涯の再発予防（2 次予防）が必要である場合が多い。

また，口腔・食道カンジダ症などのように，繰り返し治療が必要となる場合もあり，頻回に薬剤にさらされるため，薬剤に対する耐性を獲得することがある。アゾール系薬（フルコナゾールなど）に対する *Candida albicans* の耐性化がよく知られており，この耐性はアゾール系薬すべてに交差耐性を示す。治療は，軽度耐性の場合は投与量増量で対応するが，高度耐性の場合はキャンディンやポリエンなど異なる系統の薬剤による治療が必要となる。

（安岡　彰）

Question

44. 口腔・食道カンジダ症の治療法は？

Answer

カンジダは口腔・食道の常在菌であるため，喀痰や口腔・食道拭い液などの微生物検査で陽性となっても，感染症を発症しているとは言えない。口腔カンジダ症・食道カンジダ症は，肉眼的あるいは内視鏡によって，口腔や食道に菌の増殖した淡黄白色の“白苔”の付着が確認され，粘膜の発赤，びらん，増殖性変化を伴った場合のみが治療対象となる（図1，図2）。

口腔カンジダ症は，軽度の免疫不全（高齢，糖尿病，副腎皮質ステロイドホルモンの吸入など）に加えて，口腔の保湿が十分でない場合に発症する可能性

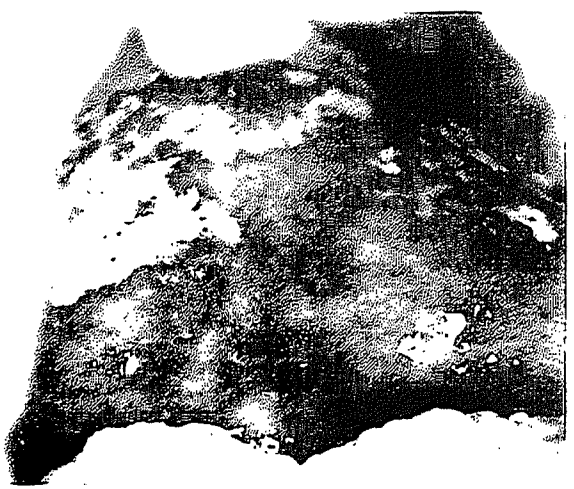

図1　口腔カンジダ症
軟口蓋から硬口蓋にかけて乳白色の白苔が付着し，周辺は発赤・びらんが認められる。舌にも厚い白苔がみられる。
（筆者提供）

図2　食道カンジダ症
食道は全周性に厚い白苔に覆われ，正常粘膜は認められない。（筆者提供）

がある。また，HIV 感染症では，免疫不全前駆症として重要であり，繰り返す口腔カンジダ症をみた場合は，HIV (human immunodeficiency virus) 抗体検査をすすめるべきである。

食道カンジダ症は，ほとんどは HIV 感染者でみられ，AIDS (acquired immunodeficiency syndrome) 発症疾患の 1 つとされている。多くの患者では口腔カンジダ症もみられ，これに加えて前胸部の中心の痛み(胸骨裏面痛)が認められれば，臨床的には食道カンジダ症と診断される。しかし，HIV 感染者に上部消化管内視鏡を行ってみると，口腔にはカンジダの病変がなくても高度の食道病変がみられる場合もあるため，胸骨裏面痛や嚥下痛，嚥下困難，原因不明の痩せがみられる場合には積極的な内視鏡検査がすすめられる。

口腔カンジダ症の治療には，アゾール系抗真菌薬の経口的全身投与と，トローチやシロップ剤による局所投与がある。前者はすみやかに効果が期待できるが，肝障害などの副作用を考慮する必要があり，後者は全身の副作用はほとんどみられないが，効果発現までに数日を要し，また 1 日 4 ～ 6 回の服用が必

要と，やや煩雑である。投与法および投与量は次の通りである。

- フルコナゾール（ジフルカン®）
  100 mg　1 日 1 回　7 ～ 14 日
- イトラコナゾール（イトリゾール® 液またはカプセル）
  200 mg　1 日 1 回　7 ～ 14 日
- クロトリマゾールトローチ（エンペシド® トローチ）
  1 回 1 錠 1 日 5 回，口腔内で溶解
- ミコナゾールゲル（フロリード® ゲル）
  1 回 10 ～ 20 mL を口腔内に含んだ後，嚥下 1 日 4 回

食道カンジダ症には口腔カンジダ症で用いられる局所治療薬は無効で，アゾール系抗真菌薬の経口投与が行われる。経口摂取困難な場合は，治療開始数日は点滴投与も行われる。フルコナゾール（ジフルカン® またはプロジフ®）100 ～ 200 mg/ 日を 1 日 1 回，またはイトラコナゾール注（イトリゾール®注）200 mg 1 日 1 回（最初の 2 日は 1 日 2 回）を 1 ～ 3 週間投与する。

いずれのカンジダ症も，口腔・食道にびらんや潰瘍を形成する疾患との鑑別が重要となる。鑑別を要するものとして，口腔では単純ヘルペスや特発性の口腔アフタ，梅毒・クラジミアの咽頭病変など，食道ではサイトメガロウイルス食道潰瘍や単純ヘルペス，HIV ウイルスによる食道潰瘍などがあげられる。

HIV にみられる口腔・食道カンジダ症は反復性であるが，比較的容易に治療が可能であり，通常，発症予防投薬は行わない。しかし，治療終了後すぐに再発する場合や難治例では，フルコナゾール 100 ～ 200 mg/ 日またはイトラコナゾール液またはカプセル 100 ～ 200 mg/ 日の持続的な予防投薬が考慮される。

また，HIV 感染者のカンジダ症では，アゾール系抗真菌薬を長期投与することになるため，アゾール系抗真菌薬に対する耐性を獲得することがある。一旦耐性化すると，同じ系統の薬剤に対しては交差耐性を獲得している可能性があるため，ミカファンギンや脂質製剤を含むアムホテリシン B など異なった系統の薬剤に変更し，治療することを考慮する。しばらく他剤で治療を行うと，再び感受性が回復することが多い。（Q 3・Q 45 参照）。（安岡　彰）