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Adriana Maria Pinto Gaspar da Rocha

TERT promoter mutations are a major indicator of poor outcome in differentiated thyroid carcinomas

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NOME

Adriana Maria Pinto Gaspar da Rocha

CARTÃO DE CIDADÃO OU PASSAPORTE (se estrangeiro)	E-MAIL	TELEFONE OU TELEMÓVEL
12064314	mimed08276@med.up.pt	918641845

NÚMERO DE ESTUDANTE	DATA DE CONCLUSÃO
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TÍTULO DISSERTAÇÃO

TERT promoter mutations are a major indicator of poor outcome in differentiated thyroid carcinomas

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À minha mãe e ao meu pai.

TERT Promoter Mutations Are a Major Indicator of Poor Outcome in Differentiated Thyroid Carcinomas

Miguel Melo,* Adriana Gaspar da Rocha,* João Vinagre,* Rui Batista, Joana Peixoto, Catarina Tavares, Ricardo Celestino, Ana Almeida, Catarina Salgado, Catarina Eloy, Patrícia Castro, Hugo Prazeres, Jorge Lima, Teresina Amaro, Cláudia Lobo, Maria João Martins, Margarida Moura, Branca Cavaco, Valeriano Leite, José Manuel Cameselle-Teijeiro, Francisco Carrilho, Manuela Carvalheiro, Valdemar Máximo, Manuel Sobrinho-Simões, and Paula Soares†

Context: Telomerase promoter mutations (*TERT*) were recently described in follicular cell-derived thyroid carcinomas (FCDTC) and seem to be more prevalent in aggressive cancers.

Objectives: We aimed to evaluate the frequency of *TERT* promoter mutations in thyroid lesions and to investigate the prognostic significance of such mutations in a large cohort of patients with differentiated thyroid carcinomas (DTCs).

Design: This was a retrospective observational study.

Setting and Patients: We studied 647 tumors and tumor-like lesions. A total of 469 patients with FCDTC treated and followed in five university hospitals were included. Mean follow-up (±SD) was 7.8 ± 5.8 years.

Main Outcome Measures: Predictive value of *TERT* promoter mutations for distant metastasization, disease persistence at the end of follow-up, and disease-specific mortality.

Results: *TERT* promoter mutations were found in 7.5% of papillary carcinomas (PTCs), 17.1% of follicular carcinomas, 29.0% of poorly differentiated carcinomas, and 33.3% of anaplastic thyroid carcinomas. Patients with *TERT*-mutated tumors were older ($P < .001$) and had larger tumors ($P = .002$). In DTCs, *TERT* promoter mutations were significantly associated with distant metastases ($P < .001$) and higher stage ($P < .001$). Patients with DTC harboring *TERT* promoter mutations were submitted to more radioiodine treatments ($P = .009$) with higher cumulative dose ($P = .004$) and to more treatment modalities ($P = .001$). At the end of follow-up, patients with *TERT*-mutated DTCs were more prone to have persistent disease ($P = .001$). *TERT* promoter mutations were significantly associated with disease-specific mortality [in the whole FCDTC ($P < .001$)] in DTCs ($P < .001$), PTCs ($P = .001$), and follicular carcinomas ($P < .001$). After adjusting for age at diagnosis and gender, the hazard ratio was 10.35 (95% confidence interval 2.01–53.24; $P = .005$) in DTC and 23.81 (95% confidence interval 1.36–415.76; $P = .03$) in PTCs.

Conclusions: *TERT* promoter mutations are an indicator of clinically aggressive tumors, being correlated with worse outcome and disease-specific mortality in DTC. *TERT* promoter mutations have an independent prognostic value in DTC and, notably, in PTC. **(*J Clin Endocrinol Metab* 99: E754–E765, 2014)**

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† Author affiliations are shown at the bottom of the next page.
* M.Me., A.G.d.R., and J.V. contributed equally to this work.

Abbreviations: AJCC, American Joint Committee on Cancer; ATC, anaplastic thyroid carcinoma; CI, confidence interval; DTC, differentiated thyroid carcinoma; FCDTC, follicular cell-derived thyroid carcinoma; FTC, follicular thyroid carcinoma; HR, hazard ratio; OR, odds ratio; PDTC, poorly differentiated thyroid carcinoma; PTC, papillary thyroid carcinoma.

Telomerase activation is known to be a hallmark of cancer (1), being detected in up to 80% of malignant tumors (2, 3). Some tumors may maintain their telomeres by an alternative mechanism, which is telomerase independent, designated as alternative lengthening of telomeres, which appears to maintain telomeres through recombination-based interchromosomal exchange of sequence information (4, 5). The maintenance of telomere length in cancer cells is thought to result more frequently from telomerase reexpression than from alternative lengthening of telomeres, although the mechanisms underlying such process remain largely unknown.

Thyroid tissue is a conditionally renewing tissue, which proliferates rarely in adult life (6). We have proposed that the embryonic remnants of the ultimobranchial body, the so-called solid cell nests of the thyroid, may represent the pool of thyroid stem cells because they express several stem cell markers, including telomerase (7). At variance with this, normal thyroid tissue is thought to be telomerase negative, thus raising the possibility that the reactivation of telomerase may be a useful marker of tumor development (8).

Several studies have examined telomerase activity in thyroid lesions and surrounding normal tissues using a PCR-based telomeric repeat amplification protocol assay for detection of telomerase activity and RT-PCR or quantitative real-time RT-PCR for the detection of *TERT* mRNA (for a review see reference 9). Thyroid carcinomas apparently display less frequent telomerase activation than other human carcinomas. Approximately 66% of all the thyroid carcinomas analyzed to date display telomerase activation that is more frequent in undifferentiated thyroid carcinomas than in differentiated carcinomas (9). Putting together the results obtained in the evaluation of telomerase activity by several authors, telomerase activity/expression is reported in 48% of papillary thyroid carcinomas (PTCs) and 71% of follicular thyroid carcinomas (FTCs). *TERT* copy number gain was described in familial PTCs (10). Capezzone et al (11) observed telomerase activity in most sporadic and familial malignant thyroid tumors as well as in some adenomas. No telomerase activity was observed in hyperplastic nodules or in normal thyroid tissue of patients with sporadic PTCs (11). Altogether, the aforementioned findings suggest that telomerase activity may contribute to a more aggressive behavior of thyroid tumors (12–14).

Recently highly frequent mutations in the promoter region of *TERT* were reported in melanomas, gliomas, and bladder and thyroid cancers (15–18). These mutations occur in two hot spot positions, located −124 and −146 bp upstream from the ATG start site (−124G>A and −146G>A, C>T on the opposite strand) and confer enhanced *TERT* promoter activity (1, 2) putatively by generating a consensus binding site (GGAA) for *ETS* transcription factors within the *TERT* promoter region. In thyroid tumors, these mutations were shown to be associated with aggressive features and with the presence of *BRAF* or *BRAF/RAS* mutations (15, 19, 20). The role played by *TERT* promoter mutations in the clinical course of thyroid tumors, response to the therapy and survival of cancer patients, remains to be addressed.

In the present study, we searched for the presence of telomerase promoter mutations in a large series of thyroid tumors and investigated the putative clinical significance of such somatic alterations.

Materials and Methods

All the procedures described in this study were in accordance with national and institutional ethical standards. Patients signed an informed consent form approved by the internal reviewing board.

Patient tissue samples

Six hundred forty-seven formalin-fixed, paraffin-embedded tissue samples from tumors and tumor-like lesions of the thyroid and from normal thyroid parenchyma localized at distance from the respective tumors were collected from the files of the Institute of Molecular Pathology and Immunology of the University of Porto (Porto, Portugal), corresponding to patients followed up in five university hospitals in Portugal and Spain. The histology of all tumor samples was revised by three pathologists (C.E., J.M.C.-T., M.S.-S.) according to the World Health Organization criteria (21). Data on the histological characteristics of the 647 samples and the frequency of *TERT* promoter mutations in each group are summarized in Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org. Twelve follicular adenomas

Institute of Molecular Pathology and Immunology of the University of Porto (M.Me., A.G.d.R., J.V., R.B., J.P., C.T., R.C., A.A., C.S., C.E., P.C., H.P., J.L., V.M., M.S.-S., P.S.), 4200-465 Porto, Portugal; Medical Faculty, University of Porto (A.G.d.R., C.T.), 4200-139 Porto, Portugal; Institute of Biomedical Sciences of Abel Salazar, University of Porto (J.V., A.A.), 4050-313 Porto, Portugal; Department of Pathology and Oncology, Medical Faculty, University of Porto (J.L., V.M., M.S.-S., P.S.), 4200-139 Porto, Portugal; Departments of Endocrinology, Diabetes, and Metabolism (M.Me., F.C., M.C.) and Pathology (M.J.M.), University and Hospital Center of Coimbra, 3000-075 Coimbra, Portugal; Unit of Endocrinology (M.Me., M.C.), Faculty of Medicine, University of Coimbra, 3000-548 Coimbra, Portugal; School of Allied Health Sciences, ESTSP - Escola Superior de Tecnologia da Saúde do Porto (R.C.), Polytechnic of Porto, 4400-330 Vila Nova de Gaia, Portugal; Portuguese Institute of Oncology (H.P.), Coimbra Center, 3000-075 Coimbra, Portugal; Department of Pathology (T.A.), Hospital Pedro Hispano, 4464-513 Matosinhos, Portugal; Department of Pathology (C.L.), Portuguese Institute of Oncology, Porto Center, 4200-072 Porto, Portugal; Center for Investigation of Molecular Pathobiology (M.Mo., B.C., V.L.) and Department of Endocrinology (V.L.), Portuguese Institute of Oncology, Lisbon Center, 1099-023 Lisbon, Portugal; Center for the Study of Chronic Diseases (M.Mo., B.C., V.L.), Faculty of Medical Sciences, University of Lisbon, 1099-085 Lisbon, Portugal; Department of Pathology (J.M.C.-T.), Clinical University Hospital, Servicio Gallego de Salud - SERGAS, Medical Faculty, University of Santiago de Compostela, 15705 Santiago de Compostela, Spain; and Department of Pathology (M.S.-S.), Hospital S. João, 4200-319 Porto, Portugal

and 27 PTCs from the Chernobyl-irradiated setting that we had previously studied (22) were also searched for the presence of *TERT* promoter mutations. Due to their etiopathogenic specificity (irradiation induced tumors) and lack of complete clinical information, they were not included in the subsequent clinicopathological analysis.

DNA extraction

DNA from formalin-fixed, paraffin-embedded tissues was retrieved from 10-μm sections after careful microdissection. DNA extraction was performed using the Ultraprep tissue DNA kit (AHN Biotechnologie) following the manufacturer's instructions.

PCR and Sanger sequencing

The genetic characterization of part of the tumors series regarding *BRAF*, *NRAS*, *RET/PTC*, and *PAX8/PPARG* had been previously reported; mutations were screened as previously described (23–25). To screen for *TERT* promoter mutations, we analyzed the hot spots previously identified by PCR followed by Sanger sequencing. *TERT* promoter mutation analysis was performed with the pair of primers forward *TERT*, CAGCGCTGCCTGAAACTC; and reverse *TERT*, GTCCTGCCCCTT CACCTT. Amplification of genomic DNA (25–100 ng) was performed by PCR using the QIAGEN multiplex PCR kit following the manufacturer's instructions (further details on the amplification will be given upon request). Sequencing reaction was performed with the ABI Prism BigDye terminator kit (PerkinElmer), and the fragments were run in an ABI prism 3100 genetic analyzer (PerkinElmer). The sequencing reaction was performed in a forward direction, and all the detected mutations were further validated by a new independent analysis in both strands (Supplemental Figure 1).

Patients' follow-up

Patients were treated and followed up in accordance with international protocols available at the time. Data regarding the number of radioiodine treatments and cumulative activity were retrieved from hospital records, together with other therapeutic procedures. For statistical analysis, we defined the category, additional treatments, in which we included other treatment modalities in addition to radioiodine, including surgery, external beam irradiation, and treatment with tyrosine kinase inhibitors (Supplemental Table 2). Patients were defined as being disease free at the end of follow-up if they had undetectable stimulated thyroglobulin (in the absence of thyroglobulin antibodies) and no imagiological evidence of disease. In the survival analysis, we have considered only deaths attributable to the thyroid carcinoma (disease-specific mortality).

Statistical analysis

Statistical analysis was conducted with SPSS version 20.0 (SPSS Inc). The results are expressed as a percentage or mean ± SD. Statistical analysis was performed both on the whole series of follicular cell-derived thyroid carcinomas (FCDTCs) and considering the different groups of tumors. A Fisher's exact test, *t* test (unpaired, two tailed), and ANOVA were used when appropriate. The predictive value of *TERT* promoter mutations and other factors [age, gender, histologic category, extrathyroidal extension, vascular invasion, lymph node metastases, staging I–IV (Union for International Cancer Control/American Joint Committee on Cancer [AJCC]), *BRAF* mutations, *RAS* mutations] for distant metastases and disease-free status at the end of follow-up were assessed using univariate and multivariate logistic regression models. Survival curves were plotted by the Kaplan-Meier method with the log-rank statistics. Multivariate survival analysis was performed using Cox regression. In the regression models, all the variables significantly associated with the specified outcome in the univariate model were included in the multivariate analysis. Mortality was expressed as a percentage and rate per person-year, the latter obtained dividing the number of thyroid cancer-specific deaths by the total follow-up time. Results were considered statistically significant at $P < .05$.

Results

TERT promoter mutations were not detected in normal parenchyma (n = 30) or in benign lesions (n = 81), such as a nodular goiter (hyperplastic lesions), lymphocytic thyroiditis, or follicular adenomas. *TERT* promoter mutations were also not detected in medullary thyroid carcinomas (n = 28) or in any of the 39 tumors from the Chernobyl series (Supplemental Table 1).

TERT promoter mutations were detected in 58 FCDTCs. The mutations were detected in 25 PTCs (7.5%), 12 FTCs (17.1%), nine poorly differentiated thyroid carcinomas (PDTCs; 29.0%), and 12 anaplastic thyroid carcinomas (ATCs; 33.3%) (Table 1). In differentiated thyroid carcinomas (DTCs), the overall prevalence of *TERT* promoter mutations was 9.2%. *TERT* promoter mutations were slightly more frequent in conventional PTCs (8.3%) than in cases of follicular variant of PTCs (6.8%). All PTCs and FTCs with oncocytic features as well as tall-cell PTCs and Warthin-like PTCs did not harbor *TERT* promoter mutations (Supplemental Table 1). Most of the mutated cases (48 of 58) presented the −124G>A mutation and the remaining 10 cases presented the −146 G>A.

Because *TERT* promoter mutations were only detected in FCDTCs, the subsequent clinicopathological analysis was restricted to these tumors (Table 1).

Relationship between *TERT* mutation and clinicopathological features

In the group of patients with DTCs, the presence of *TERT* promoter mutations was significantly associated with older age ($P < .001$) and larger tumor size ($P = .002$) (Table 2). Patients with tumors harboring *TERT* mutations had more distant metastases ($P < .001$) and higher stage ($P < .001$). No association was found with the presence of vascular invasion, extrathyroidal extension, or lymph node metastases.

A regression model was performed for factors associated with distant metastases in DTCs (Table 3). A total of

Table 1. Epidemiological, Histological, and Clinical Data of Patients With FCDTCs Included in the Study

	Total	PTC	FTC	PDTC	ATC
Total number	469	332	70	31	36
Age at diagnosis, y, n	469	332	70	31	36
Mean, y	48.2 ± 16.9	44.8 ± 15.6	51.8 ± 16.4	53.2 ± 18.4	68.7 ± 10.4
<45	211 (45.0)	178 (53.6)	23 (32.9)	8 (25.8)	2 (5.6)
≥45	258 (55.0)	154 (46.4)	47 (67.1)	23 (74.2)	34 (94.4)
Gender, n	459	327	68	29	35
Female	342 (74.5)	254 (77.7)	50 (73.5)	16 (55.2)	22 (62.9)
Male	117 (25.5)	73 (22.3)	18 (26.5)	13 (44.8)	13 (37.1)
Tumor size, cm, n	418	315	61	26	16
<2[a]	151 (36.1)	142 (45.0)	7 (11.5)	2 (7.7)	0
2–4	161 (38.5)	129 (41.0)	23 (37.7)	7 (26.9)	2 (12.5)
>4	106 (25.4)	44 (14.0)	31 (50.8)	17 (65.4)	14 (87.5)
Extrathyroidal extension, n	373	268	49	20	36
Present	231 (61.9)	176 (65.7)	8 (16.3)	12 (60.0)	35 (97.2)
Vascular invasion, n	325	257	53	8	7
Present	157 (48.3)	103 (40.1)	39 (73.6)	8 (100.0)	7 (100.0)
Lymph node metastasis, n	391	298	47	24	22
Present	234 (59.8)	202 (67.8)	8 (17.0)	14 (58.3)	10 (45.5)
Distant metastasis, n	337	263	31	20	23
Present	76 (22.6)	36 (13.7)	8 (25.8)	14 (70.0)	18 (78.3)
Stage (sixth UICC/AJCC), n	310	225	29	20	36
I	134 (43.2)	124 (55.1)	8 (27.6)	2 (10.0)	0
II	36 (11.6)	25 (11.1)	8 (27.6)	3 (15.0)	0
III	45 (14.5)	36 (16.0)	5 (17.2)	4 (20.0)	0
IV	95 (30.7)	40 (17.8)	8 (27.6)	11 (55.0)	36 (100.0)
TERT promoter, n	469	332	70	31	36
Wild-type	411 (87.6)	307 (92.5)	58 (82.9)	22 (71.0)	24 (66.7)
Mutation	58 (12.4)	25 (7.5)	12 (17.1)	9 (29.0)	12 (33.3)

Abbreviations: n, number of patients with available data for each feature; UICC, Union for International Cancer Control. Numbers in parentheses represent percentages within each category.

[a] Number of microcarcinomas (≤1.0 cm): total, 47 (11.2%); PTC, 43 (13.7%); FTC, 4 (6.6%); PDTC, 0, ATC, 0.

44 patients (15.0%) with DTCs had distant metastases detected during follow-up; the metastases were located in the lung (n = 28), bone (n = 8), lung and bone (n = 6), brain (n = 1), and lung and kidney (n = 1). *TERT* promoter mutations [odds ratio (OR) 5.36; $P < .001$], gender (OR 2.31; $P = .02$), tumor size (OR 1.28; $P = .009$), and vascular invasion (OR 3.94; $P < .001$) were associated with distant metastases. *TERT* promoter mutations were found to be a predictor of distant metastases irrespectively of gender and vascular invasion. When all the features associated with distant metastases in the univariate model were introduced in the multivariate regression, vascular invasion became the only independent predictive factor of distant metastases.

In patients with PTCs, the presence of *TERT* promoter mutations was associated with older age ($P < .001$), larger tumor size ($P = .005$), and higher stage ($P = .02$) (Table 2). Although patients with *TERT* mutation-positive PTCs had distant metastases more frequently than patients harboring *TERT* mutation-negative PTCs (27.8% vs 14.7%, respectively), the difference was not statistically significant ($P = .07$).

In patients with FTCs, the presence of *TERT* promoter mutations was associated with older age ($P = .004$), higher stage ($P = .007$), and distant metastases ($P < .001$).

In patients with PDTCs and ATCs, the presence of *TERT* promoter mutations was associated with older age ($P = .003$) and female gender ($P = .02$).

Taken *BRAF* and *RAS* together, 60.3% of *TERT*-mutated tumors were also mutated for *BRAF* or *RAS*. In PTCs, there was a significant association between the presence of *TERT* promoter mutations and the presence of *BRAF* mutation ($P = .008$) (Table 2). The *BRAF* mutation was not associated with increased lymph node or distant metastases or with patients' outcome (survival) (see Figure 2H). In patients with *TERT*-mutated tumors, the coexistence of *BRAF* mutations was not associated with more aggressive clinicopathological features or worse outcome (Supplemental Table 3 and Supplemental Figure 2). No associations were found concerning *RAS* mutated tumors.

None of the cases presenting *RET/PTC* (12 of 75) or *PAX8/PPARG* rearrangement (5 of 62) had *TERT* promoter mutations.

Relationship between *TERT* promoter mutations, clinicopathological features, and outcome

Mean follow-up time (±SD) of the patients was 7.8 ± 5.8 years (range 0.1–38.9 y). The total follow-up for the whole of FCDTC patients was 2967.5 person-years,

Table 2. Summary of Clinicopathological and Molecular Associations With *TERT* Promoter Mutations in Thyroid Cancers

Tumor Type	All Patients	*TERT* Wild Type	*TERT* Mutated	*P* Value
DTCs (n = 402)				
Clinicopathological				
Age at diagnosis, y (n = 402)	46.0 ± 16.0	44.5 ± 15.6	60.2 ± 12.7	**<.001**
Gender (n = 395)				
Female	304 (77.0)	278 (77.7)	26 (70.3)	NS (.31)
Male	91 (23.0)	80 (22.3)	11 (29.7)	
Mean tumor size, cm (n = 376)	2.7 ± 1.9	2.6 ± 1.8	3.6 ± 2.3	**.002**
Extrathyroidal extension (n = 317)[a]				
Present	184 (58.0)	167 (57.4)	17 (65.4)	NS (.43)
Vascular invasion (n = 310)[b]				
Present	142 (45.8)	129 (45.4)	13 (50.0)	NS (.65)
LN metastases (n = 345)				
Present	210 (60.9)	189 (61.0)	21 (60.0)	NS (.91)
Distant metastases (n = 294)				
Present	44 (15.0)	34 (12.5)	10 (43.5)	**<.001**
Stage (n = 254)				
I	132 (52.0)	129 (55.1)	3 (15.0)	**<.001**
II	33 (13.0)	31 (13.3)	2 (10.0)	
III	41 (16.1)	37 (15.8)	4 (20.0)	
IV	48 (18.9)	37 (15.8)	11 (55.0)	
Other genetic alterations				
BRAF mutation (n = 357)	148 (41.5)	130 (40.2)	18 (52.9)	NS (.15)
NRAS mutation (n = 365)	29 (7.9)	24 (7.3)	5 (14.3)	NS (.15)
PTCs (n = 332)				
Clinicopathological				
Age at diagnosis, y (n = 332)	44.8 ± 15.6	43.6 ± 15.3	58.4 ± 13.2	**<.001**
Gender (n = 327)				
Female	254 (77.7)	237 (78.5)	17 (68.0)	NS (.23)
Male	73 (22.3)	65 (21.5)	8 (32.0)	
Mean tumor size, cm (n = 315)	2.4 ± 1.5	2.3 ± 1.4	3.2 ± 2.2	**.005**
Extrathyroidal extension (n = 268)[a]				
Present	176 (65.7)	162 (64.8)	14 (77.8)	NS (.26)
Vascular invasion (n = 257)[b]				
Present	103 (40.1)	98 (40.7)	5 (31.3)	NS (.46)
LN metastases (n = 298)				
Present	202 (67.8)	184 (66.9)	18 (78.3)	NS (.26)
Distant metastases (n = 263)				
Present	36 (13.7)	31 (14.7)	5 (27.8)	NS (.07)
Stage (n = 225)				
I	124 (55.1)	121 (57.3)	3 (21.4)	**.02**
II	25 (11.1)	24 (11.4)	1 (7.1)	
III	36 (16.0)	32 (15.2)	4 (28.6)	
IV	40 (17.8)	34 (16.1)	6 (42.9)	
Other genetic alterations				
BRAF mutation (n = 301)	148 (49.2)	130 (46.9)	18 (75.0)	**.008**
NRAS mutation (n = 301)	15 (5.0)	14 (5.1)	1 (4.2)	NS (.85)
FTCs (n = 70)				
Clinicopathological				
Age at diagnosis, y (n = 70)	51.8 ± 16.4	49.3 ± 16.3	63.8 ± 11.0	**.004**
Gender (n = 68)				
Female	50 (73.5)	41 (73.2)	9 (75.0)	NS (.90)
Male	18 (26.5)	15 (26.8)	3 (25.0)	
Mean tumor size, cm (n = 61)	4.4 ± 2.6	4.4 ± 2.6	4.4 ± 2.5	NS (.96)
Extrathyroidal extension (n = 49)[a]				
Present	8 (16.3)	5 (12.2)	3 (37.5)	NS (.08)
Vascular invasion (n = 53)[b]				
Present	39 (73.6)	31 (72.1)	8 (80.0)	NS (.61)
LN metastases (n = 47)				
Present	8 (17.0)	5 (14.3)	3 (25.0)	NS (.39)

(*Continued*)

Table 2. Continued

Tumor Type	All Patients	*TERT* Wild Type	*TERT* Mutated	*P* Value
Distant metastases (n = 31)				
Present	8 (25.8)	3 (11.5)	5 (100.0)	**<.001**
Stage (n = 29)				
I	8 (27.6)	8 (34.8)	0	**.007**
II	8 (27.6)	7 (30.4)	1 (16.7)	
III	5 (17.2)	5 (21.7)	0	
IV	8 (27.6)	3 (13.1)	5 (83.3)	
Other genetic alterations				
BRAF mutation (n = 56)	0	0	0	[c]
NRAS mutation (n = 64)	14 (21.9)	10 (18.9)	4 (36.4)	NS (0.20)
Undifferentiated thyroid carcinomas (poorly differentiated + anaplastic) (n = 67)				
Clinicopathological				
Age at diagnosis, y (n = 67)	61.5 ± 16.2	58.3 ± 17.6	68.7 ± 9.6	**.003**
Gender (n = 64)				
Female	38 (59.4)	22 (50.0)	16 (80.0)	**.02**
Male	26 (40.6)	22 (50.0)	4 (20.0)	
Mean tumor size, cm (n = 42)	6.5 ± 3.2	6.3 ± 3.4	6.8 ± 2.9	NS (.62)
Extrathyroidal extension (n = 56)[a]				
Present	47 (83.9)	32 (82.1)	15 (88.2)	NS (.56)
Vascular invasion (n = 15)[b]				
Present	15 (100.0)	11 (100.0)	4 (100.0)	[c]
LN metastases (n = 46)				
Present	24 (52.2)	16 (57.1)	8 (44.4)	NS (.40)
Distant metastases (n = 43)				
Present	32 (74.4)	19 (70.4)	13 (81.3)	NS (.43)
Stage (n = 56)				
I	2 (3.6)	2 (5.3)	0	NS (.39)
II	3 (5.4)	3 (7.9)	0	
III	4 (7.1)	2 (5.3)	2 (11.1)	
IV	47 (83.9)	31 (81.5)	16 (88.9)	
Other genetic alterations				
BRAF mutation (n = 65)	10 (15.4)	6 (13.6)	4 (19.0)	NS (0.57)
NRAS mutation (n = 61)	17 (27.9)	9 (22.5)	8 (38.1)	NS (0.20)

Abbreviations: LN, lymph node; n, number of patients with available data for each feature; NS, not significant. Numbers in parentheses represent percentages within each category. Bold values indicate the result was statistically significant.

[a] Extrathyroidal extension was defined as having disease extending beyond the thyroid capsule, including either microscopic or macroscopic invasion.

[b] Vascular invasion was defined as the presence of intravascular tumor cells either covered by endothelium or associated with thrombus, affecting lymphatic or blood vessels within or beyond the tumor capsule.

[c] No statistics were computed due to constant numbers of one feature.

2760.8 person-years for DTC patients, 2342.9 person-years for PTC patients, and 417.9 person-years for FTC patients.

Patients with *TERT* promoter-mutated DTCs were submitted to more radioiodine treatments (2.7 ± 1.8 vs 1.8 ± 1.2; $P = .009$) with higher cumulative doses (15.8 ± 12.4 vs 9.0 ± 8.1 GBq; $P = .004$) as well as to more treatment modalities of other types (2.6 ± 2.5 vs 1.1 ± 1.5; $P = .001$) (Supplemental Table 2). A similar analysis was not performed for PDTCs and ATCs due to the heterogeneity of the therapeutic management of these two groups of patients.

At the end of follow-up, patients with DTCs harboring *TERT*-mutated tumors were more prone to have persistent disease (OR 5.10; $P = .001$) (Table 3). Male gender (OR 2.10; $P = .04$) and lymph node metastases (OR 1.95; $P = .04$) were other factors associated with disease persistence. In the multivariate model, *TERT* promoter mutations were independent predictors of persistent disease at the end of follow-up.

During follow-up, the overall disease-specific mortality was 11.7% in FCDTCs, 2.2% in DTCs, and 1.8% in the group of patients with PTCs (Table 4). The disease-specific mortality rate was 14.83 per 1000 person-years in FCDTCs, 2.53 per 1000 person-years in DTCs, and 2.13 per 1000 person-years in PTCs.

The Kaplan-Meier survival analysis showed that the presence of *TERT* promoter mutations was associated with increased disease-specific mortality in DTCs (log rank $P < .001$), PTCs (log rank $P = .001$), and FTCs (log rank $P < .001$) (Figure 1 and Table 4). Age at diagnosis and the pres-

Table 3. Predictive Factors for Distant Metastases and Disease Persistence at the End of Follow-Up in Differentiated Thyroid Carcinomas

	Distant Metastases (n = 294)					Persistent Disease (n = 211)				
		Univariate Analysis		Multivariate Analysis			Univariate Analysis		Multivariate Analysis	
	Presence (%)	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	Presence (%)	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Total	44 (15.0)					58 (27.5)				
Age, y										
<45	17 (11.5)	1 (Referent)	NS			25 (22.9)	1 (Referent)	NS		
≥45	27 (18.5)	1.75 (0.91–3.37)	(.09)			33 (32.4)	1.61 (0.87–2.96)	(.13)		
Gender										
Female	28 (12.4)	1	**.02**	1	**.03**	42 (24.9)	1	**.04**	1	**.03**
Male	16 (24.6)	2.31 (1.16–4.60)		2.32 (1.10–4.90)		16 (41.0)	2.10 (1.02–4.35)		2.46 (1.11–5.47)	
Tumor size[a]		1.28 (1.07–1.54)	**.009**	1.18 (0.97–1.44)	.10		1.18 (0.97–1.43)	NS (.10)		
Extrathyroidal extension										
No	14 (15.1)	1	NS			22 (24.4)	1	NS		
Yes	22 (14.3)	0.94 (0.46–1.94)	(.87)			28 (35.9)	1.73 (0.89–3.27)	(.11)		
Vascular invasion										
No	10 (7.2)	1	<**.001**	[b]		16 (20.0)	1	NS		
Yes	22 (23.4)	3.94 (1.77–8.78)				22 (32.8)	1.96 (0.93–4.13)	(.08)		
Lymph node metastases										
N0/Nx	17 (17.9)	1	NS			21 (23.3)	1	**.04**	1	**.03**
N1	27 (15.7)	0.85 (0.44–1.66)	(.64)			35 (37.2)	1.95 (1.03–3.71)		2.16 (1.10–4.25)	
BRAF[c]										
wt	21 (17.2)	1	NS			20 (22.7)	1	NS		
V600E	14 (12.1)	0.66 (0.32–1.37)	(0.26)			26 (37.1)	2.00 (1.00–4.03)	(.05)		
TERT										
wt	34 (12.5)	1	<**.001**	1	**.002**	48 (24.6)	1	**.001**	1	**.007**
mut	10 (43.5)	5.36 (2.18–13.18)		4.60 (1.73–12.21)		10 (62.5)	5.10 (1.76–14.78)		4.68 (1.54–14.27)	

Abbreviations: mut, mutated; n, number of patients with available data for each feature; NS, not significant; wt, wild type. Bold values indicate the result was statistically significant.

[a] Tumor size was included in the model as a continuous variable.

[b] Vascular invasion was not considered in the multivariate model displayed in the table. The OR of *TERT* promoter mutations for distant metastases adjusted for gender was 5.61 (2.24–16.06) ($P = .001$); the OR of *TERT* promoter mutations adjusted for tumor size was 4.30 (1.66–11.13) ($P = .003$); the OR of *TERT* promoter mutations adjusted for vascular invasion was 3.54 (1.06–11.82) ($P = .04$); the OR of *TERT* promoter mutations adjusted for vascular invasion and gender was 3.51 (1.05–11.72) ($P = .04$). When all the features associated with distant metastases in the univariate model (gender, tumor size, *TERT* mutational status, and vascular invasion) were included in the multivariate model, vascular invasion was the only independent predictor of distant metastases, with an OR adjusted for age, tumor size, gender, and *TERT* of 3.38 (1.42–8.04).

[c] *BRAF* analysis was performed only in the group of PTC.

ence of distant metastases were the other clinicopathological features significantly associated with patients' survival (Figure 2).

In the Cox regression analysis, the hazard ratio (HR) for mortality from DTC in patients harboring tumors with *TERT* promoter mutation was 19.42 [95% confidence interval (CI) 4.30–87.64]; after adjustment for age at diagnosis and gender, the HR was 10.35 (95% CI 2.01–53.24). In the subgroup of patients with PTCs with *TERT* promoter mutations, the HR for disease-specific mortality was 11.63 (95% CI 1.93–70.15); after adjustment for age at diagnosis and gender, the HR was 23.81 (95% CI 1.36–415.76). The low number of deaths in the group of patients with FTCs precluded a similar regression analysis.

Table 4. Thyroid Cancer-Specific Mortality and Hazard Ratios for Patients With *TERT* wt vs *TERT* Mutated Tumors

	Mortality, n , %				Mortality Rate (Deaths per 1000 Person-Years)		Hazard Ratio (95% CI)			
Type of Cancer	Overall	*TERT* wt	*TERT* Mutated	*P* Value[a]	*TERT* wt	*TERT* Mutated	Unadjusted	*P* Value	Adjusted[b]	*P* Value
DTC	7/323 (2.2)	3/298 (1.0)	4/25 (16.0)	<.001	1.17	21.17	19.42 (4.30–87.64)	<.001	10.35 (2.01–53.24)	.005
PTC	5/284 (1.8)	3/265 (1.1)	2/19 (10.5)	.001	1.36	13.64	11.63 (1.93–70.15)	.007	23.81 (1.36–415.76)	.03
FTC	2/70 (2.8)	0/58	2/12 (16.7)	<.001	0	47.23	[c]	[c]	[c]	[c]

Abbreviations: mut, mutated; wt, wild type.

[a] Log-rank *P* values.

[b] Adjusted for age and gender (Cox regression).

[c] Due to the low numbers, a regression analysis was not performed for FTC patients.

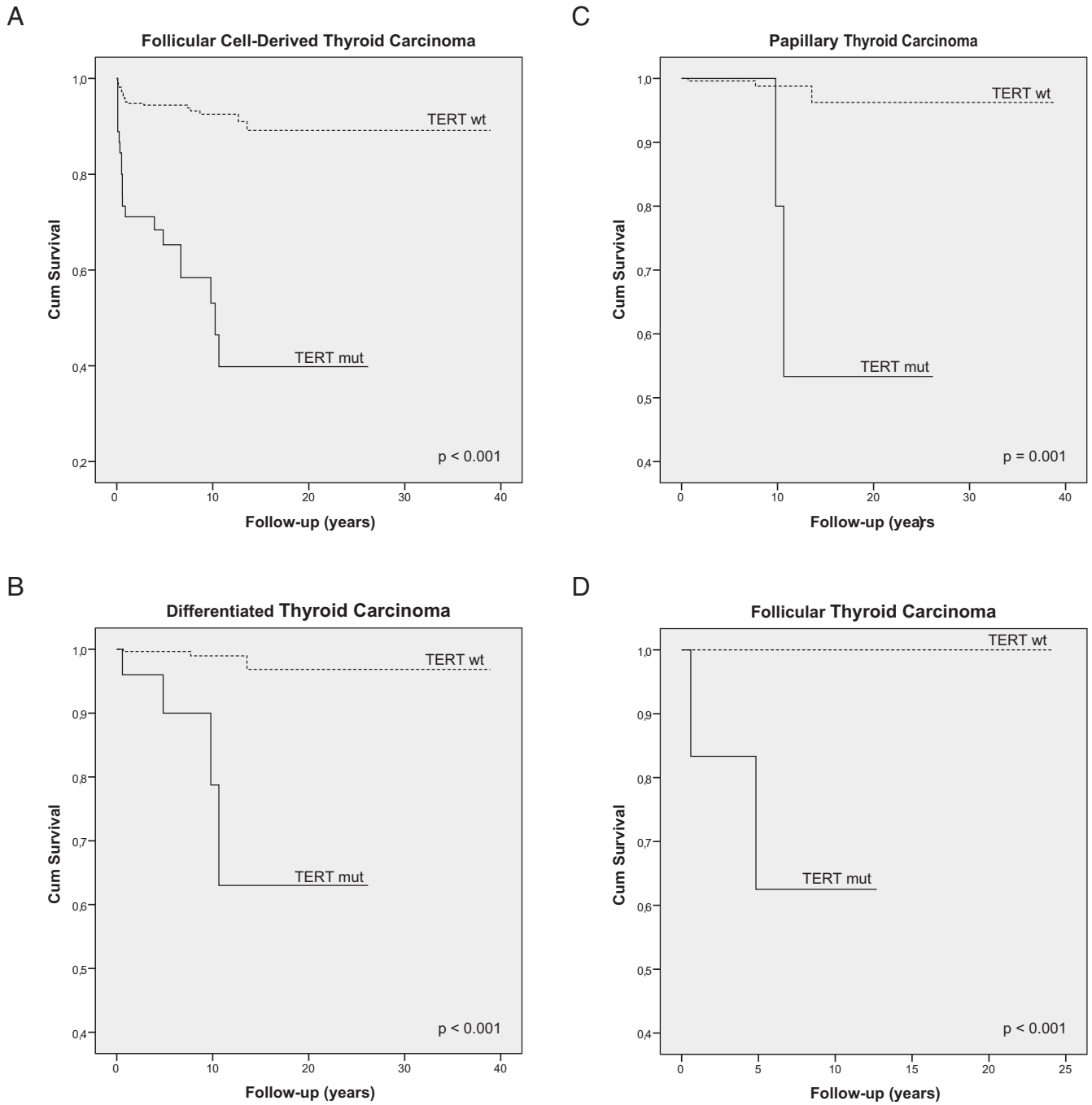

Figure 1. Kaplan-Meier survival curves of thyroid carcinoma-specific survival by *TERT* mutation status representing the whole follicular cell-derived thyroid carcinoma series (A), the group of patients with differentiated thyroid carcinoma (B), the subgroups of patients with PTCs (C), and the subgroup of patients with FTCs (D). TERT mut, *TERT* promoter mutation; TERT wt, *TERT* promoter wild type.

Discussion

In the present study, we show that *TERT* promoter mutations are an indicator of tumor aggressiveness in DTCs, being associated with distant metastases, worse response to treatment, and poor outcome.

TERT promoter mutations were present in 12.4% of the FCDTC tumors, being detected in PTCs and FTCs (7.5% and 17.1%, respectively), PDTCs and ATCs (29.0% and 33.3%, respectively). The frequencies of *TERT* promoter mutations in DTCs are similar to those recently reported by Liu et al (19) and lower than those reported by Landa et al (20) and Liu et al (26). *TERT* promoter mutations were not detected in normal thyroid or in benign lesions, such as nodular goiter, adenoma, or thyroiditis, or in medullary thyroid carcinomas. These negative findings fit with previous reports (15, 19, 20). No *TERT* promoter mutations were detected in benign or malignant tumors from Chernobyl-irradiated patients, suggesting that irradiation is not a strong etiological factor for the occurrence of such mutations.

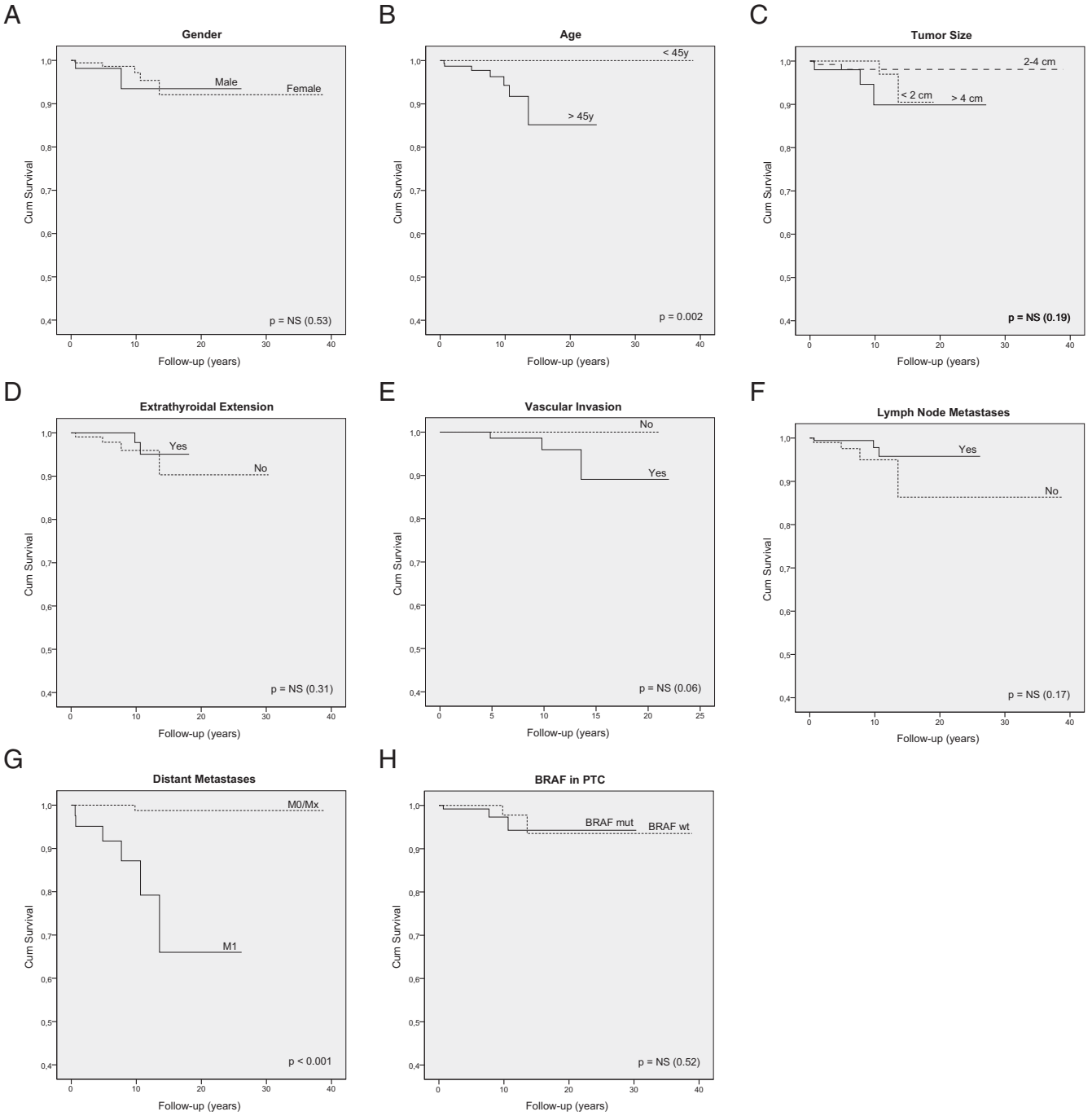

Figure 2. Kaplan-Meier survival curves of DTC-specific survival according to the different clinicopathological and molecular features: gender (A), age (B), tumor size (C), extrathyroidal extension (D), vascular invasion (E), lymph node metastases (F), distant metastases (G), and *BRAF* status in PTCs (H).

We did not detect *TERT* promoter mutations in any of the carcinomas displaying oncocytic features (Supplemental Table 1). The absence of mutations may reflect the small size of the series regarding oncocytic variant of PTCs, tall-cell PTCs, and Warthin-like PTCs. This does not hold true, however, regarding FTCs in which *TERT* promoter mutations were not detected in any of the 27 oncocytic cases (0%), in contrast to their presence in 12 of the 43 conventional FTCs (27.9%). We do not know how to explain this finding, which has to be confirmed in larger series, namely of oncocytic PTCs, but we believe it contributes to reinforce the assumption that oncocytic tumors of the thyroid differ from their nononcocytic counterparts with regard to several genetic and metabolic pathways (25, 27).

The relatively low number of microcarcinomas (11.2%; Table 1), all negative for *TERT* promoter mutations, and the higher percentage of cases with distant metastases (15% of DTCs) in comparison with data reported in some recent series (28, 29) support the assumption that one may be dealing in the present study with higher-stage

cases referred to academic centers. Further studies including lower-risk patients are necessary to establish a more accurate evaluation of the frequency of *TERT* promoter mutations in the different histotypes of FCDTCs.

In DTCs, the presence of *TERT* promoter mutations was associated with older age at diagnosis, larger tumors, higher frequency of distant metastases, and higher tumor stage. These results are in line with previous reports indicating that *TERT* promoter mutations were more prevalent in histological categories of thyroid carcinomas classically associated with more aggressive clinical behavior (15, 19, 20). The most important added value of the present study is that we found evidence showing that patients with *TERT* mutated tumors had decreased survival when compared with patients with tumors harboring wild-type *TERT* and, moreover, that this holds true for the whole DTC series as well as for PTCs and FTCs independently. The usefulness of *TERT* mutations as a prognostic marker is particularly relevant in DTCs due to the combination of two main reasons: only a small percentage of such carcinomas behave aggressively and may turn ultimately lethal, and there is a lack of good prognostic indicators in this setting.

TERT promoter mutations were more prevalent in PDTCs (29.0%) and ATCs (33.3%) than in DTCs. Although we did not find an association between *TERT* promoter mutations and mortality in this subset of less differentiated tumors (data not shown), the dismal outcome of most patients with poorly differentiated/undifferentiated carcinomas and their aggressive clinicopathological characteristics raises doubts about the prognostic utility of molecular markers, such as *TERT* promoter mutations, from a clinical standpoint. Furthermore, the small number of patients in each of the two categories does not allow a definitive conclusion on this issue. We also believe that the predictive value of *TERT* promoter mutations for disease-specific mortality in patients with FTCs should be clarified in larger series because we acknowledge the small number of events that occurred in our series due to the relatively low number of FTCs patients with appropriate follow-up.

Although the overall prognosis of patients with DTCs is usually good, with a 10-year survival rate higher than 85% in most series (30), the outcome of older patients with distant metastases is guarded, with a 5-year survival of approximately 30%–40% (31). Due to the clinical relevance of distant metastases, we performed a multivariate logistic regression evaluating the clinicopathological and molecular factors associated with distant metastasization. The presence of *TERT* promoter mutations was a significant predictor of distant metastases after adjusting for gender, tumor size, or vascular invasion, independently. When all the features associated with distant metastases in the univariated model (gender, tumor size, vascular invasion, and *TERT* mutational status) were included in the regression, vascular invasion became the only independent predictor. From the clinical standpoint, vascular invasion is information available only after surgery and all the others [age, gender, tumor size (estimated by ultrasound), and *TERT* mutational status (if feasible in fine needle aspiration cytology)] may in the near future be available for physicians before surgery, allowing a better risk stratification before the first treatment approach. For this reason, we decided to present the results of the multivariate model with and without the input of vascular invasion (Table 3).

The association of *TERT* promoter mutations with decreased survival in DTCs was kept irrespectively of age at diagnosis and gender. In addition to *TERT* promoter mutations and distant metastases, age at diagnosis was the only factor significantly associated with decreased survival in the present study. Age at diagnosis is a well-established predictor of mortality in DTCs, and this has been recognized by the Union for International Cancer Control/AJCC, setting age at diagnosis as a major determinant in the staging system (32). Even though male gender was not associated with decreased survival in our study, a large population-based cancer registry (Surveillance, Epidemiology and End-Results) showed that male gender may be associated with increased mortality (33). This finding fits with our results showing that male gender was significantly associated with distant metastases and disease persistence at the end of follow-up. These were the reasons that we decided to adjust the HR for both age at diagnosis and gender. The independent association of *TERT* promoter mutations with decreased survival holds true if we adjust the HR either for age at diagnosis (data not shown) or for both age at diagnosis and gender (Table 4).

Liu et al (26) recently published a report studying the frequency of *TERT* promoter mutations in a series of 107 FCDTCs (51 PTCs, 36 FTCs, and 20 ATCs) and its relationship with older age and shorter telomeres. The authors also found an association between *TERT* promoter mutations and distant metastases as well as shorter disease-specific survival in the whole FCDTC series and in PTCs. The results of this study fit in general with our findings. However, it should be emphasized that the report of Liu et al (26) was conducted in a small group of patients with extremely aggressive DTCs (51 PTCs and 36 FTCs, with a disease-specific mortality of 24.1%). At variance with the study by Liu et al, the disease-specific mortality in the present study is similar to those reported in most of the large recent series (34, 35). Furthermore, our study allowed a regression analysis in which the effect of *TERT* mutational status on survival could be determined and adjusted for age at diagnosis and gender. Taken together, we may say that, although performed in different populations, both studies indicate that *TERT* pro-

moter mutations may serve as a marker of aggressive disease and poor outcome in DTCs.

Our data show that DTC patients with *TERT* promoter-mutated tumors were less prone to be free of disease at the end of follow-up and were submitted to more radioiodine treatments with higher cumulative activities as well as to a greater number of other treatment modalities. Combining this information with the worse outcome of the patients, it is likely that, in the future, *TERT* promoter mutational status may be used to individualize treatment decisions, namely the type of surgery (if the molecular study proves to be feasible in fine needle aspiration cytology), the decision to perform radioiodine ablation, and the amount of ^{131}I to administer to patients. The latter issue is particularly relevant due to the current trend to reduce the number of patients submitted to radioiodine ablation and to use lower doses (36–38). International consensus and guidelines may also take into account *TERT* promoter mutational status to establish the best follow-up strategy for the individual patient.

Considering that DNA or RNA of *TERT* has been used as vaccines to induce T-helper cells and specific cytotoxic T lymphocyte responses against *TERT*-positive tumors (39, 40), it seems possible that, in the future, *TERT*-based immunotherapies may be tailored for patients with *TERT*-mutated carcinomas because these tumors carry a poor outcome, possibly because of a worse response to current therapies. Further studies are necessary to address this issue.

All the institutions involved in the present study followed international guidelines, but there were discrepancies regarding prophylactic central lymph node dissection. Taking into consideration that the role of such dissection is currently under debate and the existing differences from institution to institution of the present study, we did not investigate the implications of prophylactic lymph node dissection on the outcome.

TERT mutations were associated with *BRAF* mutations, highlighting the coexistence of activation of *BRAF* and of *TERT* genes, previously reported in melanoma (16) and thyroid (15). Horn et al (16) advanced that the mutation creates newly consensus binding sites for *TCF* subfamily transcription factors (Elk1 and Elk4) that can be activated by *BRAF*. Both in thyroid carcinoma and in melanoma, it seems that a background status of activated *BRAF* enhances the effects of *TERT* promoter mutation. Our previous results in *TERT* mRNA expression corroborated this assumption showing an increased *TERT* expression in tumors harboring *BRAF* and *TERT* mutation (15). However, the concurrence or coexistence in the current study of *TERT* and *BRAF* mutations was not associated with increased aggressiveness and worse outcome in comparison with the presence of *TERT* mutations alone (Supplemental Table 3 and Supplemental Figure 2). Nonetheless, these results should be viewed with caution due to the low number of patients in each of the two groups.

It remains to be fully clarified the biological meaning of the association between *TERT* promoter mutations and the metastatic nature of thyroid tumors. Being one of the hallmarks of cancer and enabling replicative immortality of cancer cells, *TERT* activation appears to be independent of the tumor microenvironment because there is no substantive evidence for stromal contributions to telomere stabilization in cancer cells (41). Our findings suggest that a link between telomerase activity and metastatic capacity may exist. We can speculate that this link may be inherent to the extended survival of *TERT*-mutated cells during the circulation phase and/or the homing in distant places, rather than to the increased invasiveness of such cells, but the discussion of this issue is beyond the scope of the present study.

We conclude that *TERT* promoter mutations are an indicator of clinical aggressiveness of follicular cell-derived thyroid carcinomas, being associated with distant metastases, worse response to treatment, and poor outcome. The detection of such mutations appears to be, per se, a promising prognostic indicator in DTCs and PTCs.

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Address all correspondence and requests for reprints to: Paula Soares, Institute of Molecular Pathology and Immunology of the University of Porto, Rua Dr Roberto Frias s/n, 4200-465 Porto, Portugal. E-mail: psoares@ipatimup.pt.

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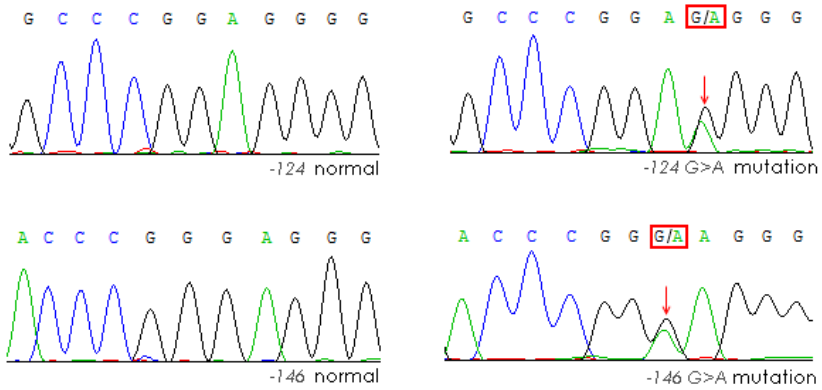
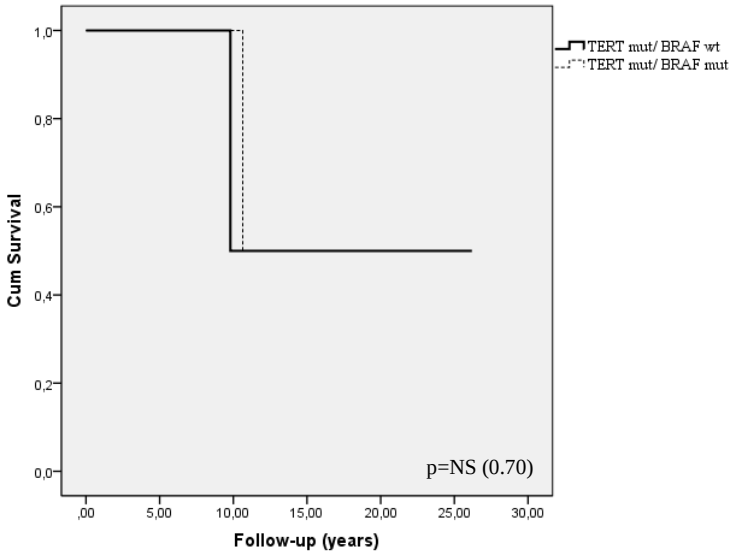
39. **Liao ZL, Tang XD, Lu MH, et al.** Antitumor effect of new multiple antigen peptide based on HLA-A0201-restricted CTL epitopes of human telomerase reverse transcriptase (hTERT). *Cancer Sci*. 2012; 103:1920–1928.
40. **Dosset M, Vauchy C, Beziaud L, Adotevi O, Godet Y.** Universal tumor-reactive helper peptides from telomerase as new tools for anticancer vaccination. *Oncoimmunology*. 2013;2:e23430.
41. **Hanahan D, Coussens LM.** Accessories to the crime: functions of cells recruited to the tumor microenvironment. *Cancer Cell*. 2012; 21:309–322.

Supplemental Figure 1 - Sequencing chromatographs of the *TERT* promoter locus in genomic tumor DNA obtained by Sanger sequencing (-124 normal, -124 G>A mutation, -146 normal, -146 G>A mutation)



Supplemental Figure 2 – Kaplan-Meier disease-specific survival curve for both groups (*TERT* mutated / *BRAF* wild-type and *TERT* mutated / *BRAF* mutated)



Supplemental Table 1 – Histological diagnoses of the 647 thyroid tissue samples searched for the presence of *TERT* promoter mutations

Histotypes	No. of cases	*TERT* n (%)
Normal thyroid	30	0 (0.0)
Lymphocytic thyroiditis	9	0 (0.0)
Nodular goiter	12	0 (0.0)
Follicular adenoma		
Conventional	53	0 (0.0)
Oncocytic variant	7	0 (0.0)
Follicular carcinoma		
Conventional	43	12 (27.9)
Oncocytic variant	27	0 (0.0)
Papillary carcinoma		
Conventional	230	19 (8.3)
Follicular variant	74	5 (6.8)
Oncocytic variant		
Papillary architecture	6	0 (0.0)
Follicular architecture	3	0 (0.0)
Solid variant	7	0 (0.0)
Warthin-like variant	6	0 (0.0)
Tall-cell variant	2	0 (0.0)
Mucoepidermoid carcinoma	4	1 (25.0)
Poorly differentiated carcinoma	31	9 (29.0)
Anaplastic carcinoma	36	12 (33.3)
Medullary carcinoma	28	0 (0.0)
Post-Chernobyl series		
Follicular adenomas	12	0 (0.0)
Papillary carcinoma	27	0 (0.0)

Supplemental Table 2 - Summary of treatment procedures in differentiated thyroid carcinomas

	All patients	***TERT* wild type**	***TERT* mutated**	**p value**
Number of radioiodine therapies	1.96±1.29	1.81±1.16	2.69±1.78	**0.009**
Cumulative activity (GBq/mCi)	9.8±8.9/ 264.1±240.4	9.0±8.1/ 242.8±218.1	15.8±12.4/ 425.8±334.8	**0.004**
Number of additional treatments*	1.36±1.73	1.13±1.52	2.56±2.45	**0.001**

*Number of additional treatments includes surgical procedures, external beam irradiation and treatment with tyrosine kinase inhibitors. Each surgical procedure was counted as one «additional treatment». Treatment with tyrosine kinase inhibitors was counted as one «additional treatment» if the patient took the drug for more than six months, and regardless of the utilization of more than one drug of the same class.

Supplemental Table 3 - Clinico-pathological data of patients with PTC harboring *TERT* promoter mutations and wild-type or mutated *BRAF*

	All patients	***TERT* mut / BRAF wt**	***TERT* mut / *BRAF* mut**	***p* value**
Clinico-pathological				
Age at diagnosis, y (n=24)	58.4±13.2	52.2±8.0	60.0±14.4	NS (0.22)*
Gender (n=24)				
Female	16 (66.7)	5 (83.3)	11 (61.1)	NS (0.32)
Male	8 (33.3)	1 (16.7%)	7 (38.9)	
Mean tumor size, cm (n=23)	3.2±2.2	2.9±2.1	3.3±2.3	NS (0.66)*
Extrathyroidal extension (n=18)				
Present	14 (77.8)	3 (60.0)	11 (84.6)	NS (0.26)
Vascular invasion (n=16)				
Present	5 (31.3)	2 (66.7)	3 (23.1)	NS (0.14)
LN metastases (n=23)				
Present	18 (78.3)	5 (83.3)	13 (76.5)	NS (0.73)
Distant metastases (n=18)				
Present	5 (27.8)	3 (50.0)	2 (16.7)	NS (0.14)
Stage (n=14)				
I	3 (21.4)	0	3 (37.5)	NS (0.29)
II	1 (7.1)	1 (16.7)	0	
III	4 (28.6)	2 (33.3)	2 (25.0)	
IV	6 (42.9)	3 (50.0)	3 (37.5)	
Clinical status at the end of follow-up (n=12)				
Disease persistence	6 (50.0)	4 (66.7)	2 (33.3)	NS (0.25)
Disease-specific mortality (n=19)	2 (10.5)	1 (16.7)	1 (7.7)	NS (0.70)**

* Due to low numbers in each group, Mann-Whitney U test was used

** Kaplan-Meier log-rank p values

Agradecimentos

À Professora Paula Soares, pelo convite para integrar esta equipa de investigação, pelos desafios e pelo apoio constante. Foi a Professora que me possibilitou trabalhar neste "mundo" da Ciência, uma experiência profissional incomparável… a curiosidade viciante!

Ao Professor Sobrinho Simões, pelo exemplo de educação, humanismo e excelência. É possível ser-se grande e humilde; é possível ser-se superior sem presunção ou arrogância. A simpatia…

A todo o grupo Cancer Biology, aos que cá estão e aos que cá estiveram, pelos ensinamentos, pela colaboração, pela ajuda… e pelas brincadeiras!

Ao Ipatimup e seus membros, por me terem ensinado o que é uma grande casa, o que é "vestir a camisola".

Ao Miguel, por ter aparecido quando menos esperava, mas quando mais precisava… por me ter mostrado que se pode fazer boa medicina e boa ciência ao mesmo tempo… por me ter mostrado que o nosso verdadeiro valor está no nosso carácter e não nas nossas mãos…

Por fim, à minha mãe e ao meu pai, aos meus irmãos, à minha restante e enorme família, que sempre me apoiaram em todo o meu percurso académico e profissional… a frase "já sabes, nós estamos aqui para o que for preciso" é um tesouro que nos protege e que se guarda no coração.

Anexos

Home

Instructions to Authors for *The Journal of Clinical Endocrinology & Metabolism*

Purpose and Scope

The Journal of Clinical Endocrinology & Metabolism (JCEM) publishes original research articles, reviews, and other special features related to endocrinology and metabolism in humans and human tissue.

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Expectation of Ethical Conduct

The Endocrine Society's mission is to advance excellence in endocrinology and be an integrative force in scientific research and medical practice. Such progress depends on integrity in the conduct of scientific research and truthful representation of findings. Specific guidelines regarding the Society's expectations for ethical conduct can be found in the Code of Ethics of The Endocrine Society and the Ethical Guidelines for Publications of Research.

The journal editors and publication oversight committees of The Endocrine Society are dedicated to upholding high ethical standards in its publications and expect authors and reviewers to do the same.

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General Information

Manuscripts must be written in idiomatic English and conform to the specifications described below. Papers that do not meet these requirements will be returned to the author for necessary revision before formal review. Manuscripts submitted to *JCEM* are usually evaluated by peer reviewers who remain anonymous; but the disposition of some manuscripts is determined by the editors alone. Authors of manuscripts requiring modifications have two (2) months to resubmit a revision of their paper. Manuscripts returned after more than two (2) months will be treated as new submissions. An unsolicited revision of a rejected manuscript will either be returned or treated as a new submission, at the editor's discretion.

In response to a growing demand for online content, the JCEM is posting three types of articles online only: Brief Reports, Hot Topics in Translational Endocrinology, and Advances in Genetics. The last two categories are chosen by the editors upon acceptance (see Dr. Wartofsky's Editorial) .

All papers accepted during each publishing year are eligible for The Endocrine Society and Pfizer, Inc. International Award for Excellence in Published Clinical Research in The Journal of Clinical Endocrinology & Metabolism (information at http://www.endo-society.org/awards/JournalAwards/index.cfm).

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Manuscript Categories

Reports of original research may be submitted to *JCEM* as an Original Article or Brief Report. Other special categories of manuscripts are described below. All manuscripts must adhere to the word count limitations, as specified below, for text only; the word count does not include the abstract, references, or figure/table legends. The word count must be noted on the title page, along with the number of figures and tables.

- **Original Articles** should be no longer than 3600 words and include no more than six figures and tables and 40 references. The Journal has a special interest in publishing results of major prospective randomized clinical trials, which may be eligible for submission through *Endocrine Trials Express*, a pathway for expedited manuscript review that aims to provide an initial editorial decision within two weeks. Authors who wish to request consideration by *Endocrine Trials Express* should contact the Managing Editor by e-mail (sherman@endo-society.org) before submitting their paper.
- **Brief Reports** are succinct descriptions of focused studies with important, but very straightforward, negative or confirmatory results. These manuscripts should be no longer than 1800 words and include no more than two figures and tables and 20 references.
- **Clinical Reviews** and other **Reviews** should address topics of importance to clinical endocrinologists and endocrine clinical investigators, including scholarly updates regarding the molecular and biochemical basis for normal physiology and disease states; the state-of-the-art in diagnosis and management of endocrine and metabolic disorders; and other topics relevant to the practice of clinical endocrinology. Authors considering the submission of uninvited reviews should contact the editors in advance to determine whether the topic that they propose is of current potential interest to the Journal. These manuscripts should be no longer than 4000 words and include no more than four figures and tables and 120 references. Authors should include a brief section describing the search strategies used to obtain information for the review.
- **Clinical Case Seminars** are descriptions of a case or small number of cases revealing novel and important insights into a condition's pathogenesis, presentation, and/or management. The case report is to be accompanied by a concise scholarly review of the literature regarding relevant aspects of the disorder. These manuscripts should be 2400 words or less, with no more than four figures and tables and 30 references.
- **Extensive Clinical Experiences** are learned descriptions of substantial clinical experience with a specific endocrine or metabolic disorder, or class of disorders, by a single clinical endocrinologist or facility. This experience should expose novel aspects of the condition's presentation, diagnosis, natural history, and/or treatment. These manuscripts should be no longer than 3600 words and include no more than four figures and tables and 40 references.
- **Position** and **Consensus Statements** related to the endocrine and metabolic health standards and healthcare practices may be submitted by professional societies, task forces, and other consortia. All such submissions will be subjected to peer review, must be modifiable in response to criticisms, and will be published only if they meet the Journal's usual editorial standards. These manuscripts should typically be no longer than 3600 words and include no more than six figures and tables and 120 references.
- **Controversies in Clinical Endocrinology** describe and justify different approaches to diagnosis and/or management of patients with an endocrine or metabolic condition. This feature typically consists of a pair of manuscripts authored by two individuals who thoughtfully describe their respective clinical perspectives on a problem, their related practices, and the rationale and evidence supporting them. The entire manuscript should be no longer than 2400 words and include no more than two figures and tables and 30 references.
- **Images in Endocrinology** are to be comprised of a single figure or two closely related figures that illustrate the value of visual information in clinical diagnosis of endocrine and metabolic disorders, with a caption that is 50 words or less, an accompanying commentary that is 250 words or less, and five or fewer references.
- **Commentaries** are essentially uninvited editorials, which should concisely address and take a well-reasoned position on a timely issue of importance to clinical endocrinologists and/or endocrine clinical investigators. These manuscripts should be no longer than 1200 words with no more than 10 references; no figures or tables are permitted.
- **Letters to the Editor** may be submitted in response to work that has been published in the Journal. **Letters** should be short commentaries related to specific points of agreement or disagreement with the published work. **Letters** are not intended for presentation of original data unrelated to a published article. **Letters** can only be submitted electronically via the Journal website, by clicking on the link entitled "Submit a Letter to the Editor" on the abstract page or the article itself. Letters should be no longer than 500 words with no more than five complete references, and may not include any figures or tables.

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Manuscript Submission Procedures

JCEM only uses electronic manuscript submission at Editorial Manager (http://jcem.edmgr.com).

If this is your first submission to an Endocrine Society Journal, click on "Register Now" to create an author account. If you already have an account from a previous submission to any of The Endocrine Society's Journals, enter your username and password to submit a new or revised manuscript. If you have forgotten your username and/or password, e-mail the editorial office (sherman@endo-society.org) for assistance.

Note that your author account is the same for *JCEM*, *Endocrinology*, *Molecular Endocrinology*, and *Endocrine Reviews*. Authors should be aware that in submitting a manuscript for consideration by *JCEM*, they are submitting their paper to The Endocrine Society Central Journals Office database, which is accessible by the Editors-in-Chief of all the Society's journals.

All submissions must include:

- A cover letter requesting that the manuscript be evaluated for publication in *JCEM* and any information relevant to your manuscript. Elsewhere on the submission form authors may suggest up to five specific reviewers and/or request the exclusion of up to three others.
- Assignment of Copyright and Disclosure of Potential Conflict of Interest is part of the online submission process. At the time of submission all co-authors will receive authorship verification emails to which they must respond. It is imperative that all co-authors are listed on the submission forms and their email address be correct.
- At least three key terms.
- Completed Disclosure Summary on the title page. For instructions on preparing the summary, see the following page (http://jcem.endojournals.org/site/author/RequiredForms.pdf).
- Authors are encouraged to submit a PDF for the initial submission. See the instructions on the JCEM homepage. If you do submit original files, Editorial Manager will create a PDF of your files, but it may take some time depending on the size of the files.

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Manuscript Preparation

General Format

The Journal requires that all manuscripts be submitted in a single-column format that follows these guidelines:

- All text should be double-spaced with 1-inch margins on both sides using 11-point type in Times Roman font.
- All lines should be numbered throughout the entire manuscript and the entire document should be paginated.
- All tables and figures must be placed after the text and must be labeled. Submitted papers must be complete, including the title page, abstract, figures, and tables. Papers submitted without all of these components will be placed on hold until the manuscript is complete.
- Authors are encouraged to cite primary literature rather than review articles in order to give credit to those who have done the original work.
- Any supplemental data intended for publication must meet the same criteria for originality as the data presented in the manuscript.

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Title Page

The title page should include the following:

- Full title (a concise statement of the article's major contents)
- Authors' names and institutions. At least one person must be listed as an author; no group authorship without a responsible party is allowed. A group can be listed in the authorship line, but only on behalf of a person or persons. All group members not listed in the authorship line must be listed in the Acknowledgments.
- Abbreviated title of not more than 40 characters for page headings
- At least three key terms for indexing and information retrieval
- Word count (excluding abstract, figure captions, and references)
- Corresponding author's e-mail and ground mail addresses, telephone and fax numbers
- Name and address of person to whom reprint requests should be addressed
- Any grants or fellowships supporting the writing of the paper
- Disclosure summary (see Disclosure of Potential Conflict of Interest form for instructions)
- Clinical Trial Registration Number, if applicable

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Structured Abstracts

All Original Articles, Brief Reports, Clinical Reviews, Clinical Case Seminars, Consensus and Position Statements, Controversies in Endocrinology, and Extensive Clinical Experiences should be submitted with structured abstracts of no more than 250 words. All information reported in the abstract must appear in the manuscript. The abstract should not include references. Write the abstract with a general medical audience in mind. Please use complete sentences for all sections of the abstract. Detailed instructions on writing Structured Abstracts are at http://jcem.endojournals.org/site/misc/Structured_Abstracts.xhtml.

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Introduction

The article should begin with a brief introductory statement that places the work to follow in historical perspective and explains its intent and significance.

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Materials and Methods

These should be described and referenced in sufficient detail for other investigators to repeat the work. The source of hormones, unusual chemicals and reagents, and special pieces of apparatus should be stated. For modified methods, only the modifications need be described.

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Results and Discussion

The Results section should briefly present the experimental data in text, tables, and/or figures. For details on preparation of tables and figures, see below. The Discussion should focus on the interpretation and significance of the findings with concise objective comments that describe their relation to other work in that area. The Discussion should not reiterate the Results.

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Acknowledgments

The Acknowledgments section should include the names of those people who contributed to a study but did not meet the requirements for authorship. The corresponding author is responsible for informing each person listed in the acknowledgment section that they have been included and providing them with a description of their contribution so they know the activity for which they are considered responsible. Each person listed in the acknowledgments must give permission - in writing, if possible - for the use of his or her name. It is the responsibility of the corresponding author to collect this information.

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References

References to the literature should be cited in numerical order (in parentheses) in the text and listed in the same numerical order at the end of the manuscript on a separate page or pages. The author is responsible for the accuracy of references. The number of references cited should be limited, as indicated above for each category of submission. Appropriate recent reviews should be cited whenever possible.

Examples of the reference style that should be used are given below. Further examples will be found in the articles describing the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (Ann Intern Med.1988; 108:258-265, Br Med J. 1988; 296:401-405). The titles of journals should be abbreviated according to the style used in the *Index Medicus*.

Journal articles and abstracts: List all authors. The citation of unpublished observations, of personal communications, and of manuscripts in preparation or submitted for publication is not permitted in the bibliography. Such citations should be inserted at appropriate places in the text, in parentheses and without serial number, or be presented in the footnotes. The citation of manuscripts accepted for publication but not yet in print is permitted in the bibliography provided the DOI (Digital Object Identifier) and the name of the journal in which they appear are supplied. Listing a manuscript as "in press" without a DOI and journal title is not permitted. If references to personal communications are made, authors are encouraged to keep written proof of the exchange. If it is necessary to cite an abstract because it contains substantive data not published elsewhere, it must be designated at the end of the reference [e.g., 68:313 (Abstract)].

Books: List all authors or editors.

Sample References

1. **Binoux M, Hossenlopp P** 1986 Insulin-like growth factor (IGF) and IGF-binding proteins: comparison of human serum and lymph. J Clin Endocrinol Metab 67:509-514
2. **MacLaughlin DT, Cigarros F, Donahoe PK** 1988 Mechanism of action of Mullerian inhibiting substance. Program of the 70th Annual Meeting of The Endocrine Society, New Orleans, LA, 1988, p 19
3. **Bonneville F, Cattin F, Dietemann J-L** 1986 Computed tomography of the pituitary gland. Heidelberg: Springer-Verlag; 15-16
4. **Burrow GN** 1987 The thyroid: nodules and neoplasia. In: Felig P, Baxter JD, Broadus AE, Frohman LA, eds. Endo crinology and metabolism. 2nd ed. New York: McGraw-Hill; 473-507

For general aid in the preparation of manuscripts, authors should consult: CBE Style Manual: A Guide for Authors, Editors and Publishers. 5th ed. Bethesda, MD: Council of Biology Editors; 1983.

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Tables

Tables must be constructed as simply as possible and be intelligible without reference to the text. Each table must have a concise heading. A description of experimental conditions may appear together with footnotes at the foot of the table. Tables must not simply duplicate the text or figures. The width of the table must be designed to occupy one or two journal columns, with no more than four table columns or 8-10 table columns, respectively.

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Figures and Legends

Please review the detailed instructions for preparing digital art at http://art.cadmus.com/da/index.jsp. E-mail queries can be sent to digitalart@cadmus.com. All figures must display the figure number.

Sizing the figure: The author is responsible for providing digital art that has been properly sized, cropped, and has adequate space between images. Plan the size of the figure to fill 1, 1.5, or 2 columns in the printed journal (see chart below for dimensions). In most cases, figures should be prepared for 1-column width. Produce original art at the size it should appear in the printed journal. (Note for PowerPoint users: The sizing instructions do not apply if you are submitting PowerPoint files for print production in Editorial Manager. On the submission page, check boxes to indicate that the figures are the correct size and resolution.)
1 column = 18 picas, 7.5 cm, 3.0 in
1.5. columns = 30 picas, 12.5 cm, 5.0 in
2 columns = 38 picas, 16.0 cm, 6.5 in

Lettering: At 100% size, no lettering should be smaller than 8 point (0.3 cm high) or larger than 12 point (0.4 cm high). Use bold and solid lettering. Lines should be thick, solid, and no less than 1-point rule. Avoid the use of reverse type (white lettering on a darker background). Avoid lettering on top of shaded or textured areas. Titles should be clear and informative. Keep wording on figures to a minimum, and confine any explanation of figures to their separate-page legends. Label only one vertical and one horizontal side of a figure. *Freehand lettering or drawing is unacceptable*.

Color Figures: Figures should now be submitted as RGB (red, green, blue) format. Saving color figures to this format will be more convenient for authors as RGB is the standard default on most programs. Color images will be preserved as RGB up until the time of printing and will be posted online in their original RGB form. Using RGB color mode for online images will be a significant improvement for figures that contain fluorescent blues, reds, and greens. Therefore the online journal will accurately reflect the true color of the images the way the author intended. For print, the images will be converted to CMYK through an automated color conversion process.

Shading: Avoid the use of shading, but if unavoidable, use a coarse rather than a fine screen setting (80-100 line screen is preferred). Avoid 1-20% and 70-99% shading; make differing shades vary by at least 20%, *i.e.*, 25%, 45%, 65%. Instead of shading, denote variations in graphs or drawings by cross-hatching; solid black; or vertical, horizontal, or diagonal striping. Avoid the use of dots.

Grouped figures: For grouped figures, indicate the layout in a diagram. Place grouped figures so that they can be printed in 1 column width with uniform margins. Indicate magnification in the legends and by internal reference markers in the photographs. Their length should represent the fraction or multiple of a micrometer, appropriate to the magnification.

Graphs: Graphs with axis measures containing very large or small numbers should convert to easily readable notations. *Example*: For an ordinate range of "counts per minute" values from 1,000 to 20,000, the true value may be multiplied by 10^{-3} (scale would read from 1 to 20) and the ordinate axis display "cpm ($x10^{-3}$)." Similarly, for a Scatchard plot with values ranging from 0.1 to 2 femtomolar (10^{-15} m), the scale may run from 0.1 to 2 with the abscissa labeled "m ($x10^{-15}$)." *Three-dimensional bar graphs will not be*

published if the information they refer to is only two-dimensional.

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Supplemental Data

Supplemental Data allows authors to enhance papers in *JCEM* by making additional substantive material available to readers. Supplemental Data may take the form of figures, tables, datasets, derivations, or videos, and is published only in *JCEM* online; it does not appear in the printed version of the journal. Authors who wish to include Supplemental Data should state so in the cover letter when the manuscript is submitted.

Supplemental Data files should be submitted through Editorial Manager at the time of manuscript submission, and will be reviewed along with the manuscript. The files should be uploaded in the field marked "Upload Supplemental Data Files", and should NOT be attached with the manuscript and figure files. Authors should refer to the Supplemental Data in the manuscript at an appropriate point in the text or figure/table legend.

The file formats listed below may be used for Supplemental Data. Provide a brief description of each item in a separate HTML or Word file (*i.e.*, figure or table legends, captions for movie or sound clips, etc.). Do not save figure numbers, legends, or author names as part of an image. File sizes should not exceed 5 MB. Images should not exceed 500 pixels in width or height. Do not use tabs or spaces for Word or WordPefect tables; please use the table functions available within these word processing programs to prepare tables. For web pages, provide a complete list of files and instructions for creating directories.

.htm, HTML*
.jpg, JPEG image*
.gif, Graphical image
.pdf, Adobe Portable Document Format
.xls, MS Excel Spreadsheet
.mov, Quick Time
.wav, Sound
.doc, MS Word 6 documents**
.txt, Plain ASCII*

*These files can be viewed directly on standard web browsers.
**MS Word may be used for text only.

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Units of Measure

Results should be expressed in metric units. Systeme Internationale (SI units) must be added in parentheses. Temperature should be expressed in degrees Celsius (*e.g.*, 28 C) and time of day using the 24-hour clock (*e.g.*, 0800 h, 1500 h).

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Standard Abbreviations

All nonstandard abbreviations in the text must be defined immediately after the first use of the abbreviation. The list of **Standard Abbreviations** is given in the link.

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Editorial Policies and Guidelines

Prior Publication

Failure to notify the editor that some results in the manuscript are being or have been previously published will result in placement of a notice in the journal that the authors have violated the Ethical Guidelines for Publication of Research in The Endocrine Society Journals. The journal publishes original research and review material. Material previously published in whole or in part shall not be considered for publication. This includes materials published in any form of mass communication. At the time of submission, authors must divulge in their cover letter all prior publications or postings of the material in any form of media. Abstracts or posters displayed for colleagues at scientific meetings need not be reported. Other postings of any part of the submitted material on web pages, as well as those essential for participation in required registries will be evaluated by the Editor-In-Chief, who shall determine if those postings are material enough to constitute prior publication.

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Authorship Criteria

An author should have participated in either the conception, planning, or execution of the work, the interpretation of the results and the writing of the paper. An acknowledgment accompanying the paper is appropriate recognition for others who have contributed to a lesser extent, e.g., provision of clones, antisera or cell lines, or reading and reviewing manuscripts in draft. The signature of each author on the Affirmation of Originality and Copyright Release form that must be submitted with the manuscript indicates that all authors have had a part in the writing and final editing of the report, all have been given a copy of the manuscript, all have approved the final version of the manuscript, and all are prepared to take public responsibility for the work, sharing responsibility and accountability for the results. Medical writers can be legitimate contributors, and their roles, affiliations, and potential conflicts of interest should be described when submitting manuscripts. These writers should be acknowledged on the byline or in the Acknowledgments section in accord with the degree to which they contributed to the work reported in the manuscript. Failure to acknowledge these contributors would mean that the manuscript could have been "ghost-written," which is not allowed.

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Guidelines for considering authors of non-research articles who have a potential COI

The editors of The Endocrine Society's journals appreciate the importance of assuring unbiased authorship of editorials, reviews, and other non-research features involving selection of evidence to be discussed and perspectives to be presented. Consequently, special care is taken in choosing authors for such articles to assure their views are balanced and unencumbered, and that the Society's policies on disclosure of conflicts of interest are implemented.

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Obligations of Reviewers

The critical and confidential review of manuscripts is an essential element of research publications. Every scientist has an obligation to contribute to the peer review process by serving as a reviewer. Among the obligations of reviewers is the commitment to providing an expert, critical, and constructive scientific and literary appraisal of research reports in their fields of knowledge, skills, and experience in a fair and unbiased manner. In order to facilitate the prompt sharing of scientific results, it is also the obligation of each reviewer to complete their assignments promptly, within the editor's deadline. Should a delay in their review occur, the reviewer has the obligation to notify the editor at once. Reviewers should not review a manuscript if: 1) they do not think that they are competent to assess the research described, 2) they believe there is a conflict of interest or personal or professional relationship with the author(s) that might bias their assessment of the manuscript, or (3) there is any other situation that could bias their review. Employment at the same institution as one of the authors does not automatically represent a conflict. Having previously reviewed the article for another journal does not disqualify a reviewer, although the editor should be informed so the reviewer's perspective can be considered. In circumstances when reviewers need to recuse themselves, they should notify the editor promptly, preferably with an explanation. If reviewers are uncertain whether they should recuse themselves, they should consult with the editor.

The reviewer should strive to provide accurate, detailed, and constructive criticisms, and the review should be supported by appropriate references, especially if unfavorable. The reviewer should also note whether the work of others is properly cited. If the reviewer notes any substantial resemblance of the manuscript being reviewed to a published paper or to a manuscript submitted at the same time to another journal, they should promptly report this to the editor.

No part of the manuscript under review should ordinarily be revealed to another individual without the permission of the editor. If a reviewer consults a colleague on a particular point, this fact, and the name of the collaborator or consultant, should be reported to the editor, preferably in advance. With these exceptions, a reviewer must obtain through the editor written permission from the authors to use or disclose any of the unpublished content of a manuscript under review.

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Experimental Subjects

To be considered, all clinical investigations described in submitted manuscripts must have been conducted in accordance with the guidelines in The **Declaration of Helsinki** and must have been formally approved by the appropriate institutional review committees or its equivalent. All manuscripts must indicate that IRB approval was acquired; and that when informed consent was required by the IRB, that this was obtained from subjects in experiments involving humans. Investigators must disclose potential conflict of interest to study participants and should indicate in the manuscript that they have done so. The study populations should be described in detail. In many studies details of age, race, and sex are important. However, subjects must be identified only by number or letter, not by initials or names. Photographs of patients' faces should be included only if scientifically relevant. Authors must obtain written consent from the patient for use of such photographs. For further details, see the Ethical Guidelines.

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Experimental Animals

A statement confirming that all animal experimentation described in the submitted manuscript was conducted in accord with accepted standards of humane animal care, as outlined in the Ethical Guidelines, should be included in the manuscript.

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Clinical Trials Registration

For clinical trial reports to be considered for publication in the Journal, the Endocrine Society requires their prospective registration, as endorsed by the International Conference of Medical Journal Editors. We recommend use of www.clinicaltrials.gov. The Society's full Position Statement on Clinical Trials Registration is at the following web site: http://jcem.endojournals.org/site/misc/ClinicalTrials.pdf. All trials beginning after January 1, 2007 must have been prospectively registered before enrollment of the first subject. All trials begun before that date must be retroactively registered before submission. Please note that the Clinical Trial Registration number should be provided clearly on the title page of the manuscript.

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Genetic and Genome-Wide Association Studies

To ensure rigor in genetic and genome-wide association studies and permit readers to assess their biological and clinical significance, submitted manuscripts describing such work should generally conform to the following study design criteria, which will be applied by the Journal's reviewers and editors in their evaluations.

Sample Size and Multiple Testing: Studies should include sufficient samples to have the power to detect an effect. In addition, since multiple hypotheses are often tested (*e.g.*, multiple SNPs, substratification, and multiple phenotypes), analyses and interpretations should account for the influence of such multiple testing on the findings' biological and clinical significance.

Validation Samples: The most rigorous association studies should include both a testing (or training) sample set and an independent validation series.

Functional Data: Functional data strengthen association data if the functional assay(s) have demonstrable relevance to the associated phenotype. In some instances, association studies with a single testing sample set and highly relevant functional data may be acceptable without an independent validation series.

Single Genetic Marker (e.g., SNP) versus Whole Gene/Genome Studies: Single SNP studies are acceptable when the particular

SNP has strong prior claims for involvement in the phenotype of interest. However, it is desirable to examine genetic variation at least across and flanking the gene of interest when this is feasible.

Negative Association Studies: Well-designed and executed association studies that demonstrate significant negative findings will be considered if the gene in question has clear relevance to disease pathogenesis or has been implicated in prior published association studies.

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Microarray Expression Studies

Genome-wide expression studies require both technical validation and an independent validation series. Technical validation entails application of a different technique (*e.g.*, RT-PCR of single genes or immunohistochemistry) to confirm the differential expression detected by genome-wide expression. An independent validation series of samples should be utilized to confirm the differential expression noted by genome-wide analysis of the initial testing sample set.

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Nomenclature and Technical Requirements

The value of study data is enhanced if, where relevant, manuscripts:

- Use standard terminology for variants, providing rs numbers for all variants reported. These can be easily derived for novel variants uncovered by the study. Where rs numbers are provided, the details of the assay (primer sequences, PCR conditions, etc.) should be described very concisely.
- Describe measures taken to ensure genotyping accuracy, *e.g.*, percentage of genotype calls, number of duplicate samples that were genotyped, and percentage concordance.
- Provide approved GDB/HUGO approved gene names, in the appropriate cases and italics.
- Provide linkage disequilibrium (LD) relationships between typed variants.
- Provide information and a discussion of departures from Hardy-Weinberg equilibrium (HWE). The calculation of HWE may help uncover genotyping errors and impact on downstream analytical methods that assume HWE.
- Provide raw genotype frequencies in addition to allele frequencies. It is also desirable to provide haplotype frequencies.
- Provide the criteria they have used to select tagSNPs.
- Denote the boundaries considered when studying SNPs within a gene of interest. For example, "gene X and 100 kb upstream of the first translational start site and 150 kb downstream of the stop codon."

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Manuscripts Reporting New Amino Acid or Nucleotide Sequence

Manuscripts reporting amino acid or nucleotide sequences of proteins with sequences already known from other tissues or species will be considered only if they provide new biological insight. Manuscripts dealing with partial sequence data are not likely to be considered. The Endocrine Society has established policy that deals with submission of new protein or nucleic acid sequences. When a manuscript is accepted that contains novel sequences, such sequences must be deposited in the appropriate database (such as GenBank) and an accession number obtained before the manuscript is sent to the printer. It is recommended that the following statement containing the assigned accession number be inserted as a footnote: "These sequence data have been submitted to the DDBJ/EMBL/GenBank databases under accession number UI2345."

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Standards for Steroid Nomenclature

The 3 major classes of mammalian sex hormones - androgens, estrogens, and progestins (or progestagens or gestagens) - are defined by their biological activities, which are mediated via the well-defined androgen, estrogen and progesterone (or progestin) receptors. The principal bioactive sex steroid and natural ligand for each class is testosterone (or 5α-dihydrotestosterone), estradiol and progesterone, respectively. Androgen(s), estrogen(s) and progestin(s) are classes of compounds with hormonal activity, and not the names of individual steroids. Synthetic steroids or extracts can be considered as members of a generic steroid class (androgens, estrogens, progestins), but are distinct from the natural cognate ligand itself. Synthetic hormones or extracts of biological origin of each class may also have agonist, antagonist or mixed bioactivity in one or more classes. Therefore, the terms androgens, estrogens and progestins (or progestagens or gestagens) should be used when referring to the class of hormones, whereas when a specific natural or synthetic steroid is being used or assayed, the particular compound must be specified.

Apart from accepted trivial names, steroids should be named according to the systematic nomenclature of the IUPAC convention on Nomenclature of Steroids (Moss et al Pure & Applied Chemistry 61:1783-1822, 1989) at first mention in a single footnote defining all letter abbreviations. Subsequently, generic or trivial names or letter abbreviations, but not trade-names, should be used.

Examples of accepted trivial names include: cholesterol, estrone, 17α and 17β estradiol (estradiol is also acceptably used as the trivial name for 17β estradiol), estriol, aldosterone, androsterone, etiocholanolone, dehydroepiandrosterone, testosterone, 5α dihydrotestosterone, 5β dihydrotestosterone, androstenedione, pregnenolone, progesterone, corticosterone, deoxycorticosterone, cortisone, and cortisol.

Trivial names may be modified by prefixes or suffixes indicating substituents (as in 17-hydroxyprogesterone for 17-hydroxy-4-pregnene-3,20-dione), double bonds (as in 7-dehydrocholesterol for 5,7-cholestadien-3-ol) and epimeric configurations of functional groups provided the locus of epimerization is indicated (as in 11-epicortisol for 11α21-trihydroxypregn-4-en-3-one).

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Manuscripts Reporting Novel Compounds

Manuscripts describing experiments with new compounds must provide their chemical structures. For known compounds, the source and/or literature reference to the chemical structure and characterization must be provided.

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Validation of Data and Statistical Analysis

Assay validation: Bioassay and radioimmunoassay potency estimates should be accompanied by an appropriate measure of the precision of these estimates. For bioassays, these usually will be the standard deviation, standard error of the mean, coefficient of variation, or 95% confidence limits. For both bioassays and radioimmunoassays, it is necessary to include data relating to within-assay and between-assay variability. If all relevant comparisons are made within the same assay, the latter may be omitted. Authors should be aware that the precision of a measurement depends upon its position on the dose-response curve.

In presenting results for new assays, it is necessary to include data on the following: 1) within-assay variability; 2) between-assay variability; 3) slope of the dose-response curve; 4) mid-range of the assay; 5) least-detectable concentration (concentration resulting in a response two standard deviations away from the zero dose response); 6) data on specificity; 7) data on parallelism of standard and unknown and on recovery; and 8) comparison with an independent method for assay of the compound. When radioimmunoassay kits are utilized or hormone measurements are conducted in other than the authors' laboratories and the assay is central to the study, data regarding performance characteristics should be included.

Pulse analysis: Data from studies of pulsatile hormone secretion should be analyzed using a validated, objective pulse detection algorithm. The algorithm used should require that false-positive rates of pulse detection be defined in relation to the measurement error of the data set being analyzed, and the methods used to determine the measurement error should be described. The author(s) also should describe the methods used: 1) to deal with missing or undetectable values; 2) to determine peak frequency, interpeak interval, and pulse amplitude; and 3) for statistical comparisons of peak parameters.

Data analysis: It is the author's responsibility to document that the results are reproducible and that the differences found are not due to random variation. No absolute rules can be applied, but in general quantitative data should be from no fewer than three replicate experiments. Appropriate statistical methods should be used to test the significance of differences in results. The term "significant" should not be used unless statistical analysis was performed, and the probability value used to identify significance (*e.g.*, $P > 0.05$) should be specified.

When several *t* tests are employed, authors should be aware that nominal probability levels no longer apply. Accordingly, the multiple *t* test, multiple range test, or similar techniques to permit simultaneous comparisons should be employed. Also, in lieu of using several *t* tests, it is often more appropriate to utilize an analysis of variance (ANOVA) to permit pooling of data, increase the number of degrees of freedom, and improve reliability of results. Authors should use appropriate nonparametric tests when the data depart substantially from a normal distribution. Analysis of variance tables should not be inserted in manuscripts. F values with the degrees of freedom as subscripts together with the *P* values are sufficient.

In presenting results of linear regression analyses, it is desirable to show 95% confidence limits. When data points are fitted with lines (as in Scatchard or Lineweaver-Burk plots), the method used for fitting (graphical, least squares, computer program) should be specified. If differences in slopes and/or axis intercepts are claimed for plotted lines, these should be supported by statistical analysis.

Authors should include in the manuscript a list of the software used for statistical analyses.

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Digital Image Integrity

When preparing digital images, authors must adhere to the following guidelines as stated in the CSE's White Paper on Promoting Integrity in Scientific Journal Publications:

- No specific feature within an image may be enhanced, obscured, moved, removed, or introduced.
- Adjustments of brightness, contrast, or color balance are acceptable if they are applied to the entire image and as long as they do not obscure, eliminate, or misrepresent any information present in the original.
- The grouping of images from different parts of the same gel, or from different gels, fields, or exposures must be made explicit by the arrangement of the figure (e.g., dividing lines) and in the figure legend.

Deviations from these guidelines will be considered as potential ethical violations.

Note that this is an evolving issue, but these basic principles apply regardless of changes in the technical environment. Authors should be aware that they must provide original images when requested to do so by the Editor-in-Chief who may wish to clarify an uncertainty or concern.

[Please see paper of Rossner and Yamada (Journal of Cell Biology, 2004, 166:11-15), which was consulted in developing these policy issues, for additional discussion, and the CSE's White Paper on Promoting Integrity in Scientific Journal Publications, published by the Council of Science Editors, 2006.]

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Publication and Production Guidelines

Proofs and Reprints

Proofs and a reprint order form are sent to the corresponding author unless the Editorial Office is advised otherwise. The author should designate by footnote on the title page of the manuscript the name and address of the person to whom reprint requests should be directed. Questions about reprints should be directed to June Billman, Account Manager at Cenveo Publisher Services, (June.Billman@cenveo.com) (preferred), 410-943-3086 (direct) or 1-866-487-5625 (toll-free).

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Publication and Color Costs

There is no submission fee for The Endocrine Society journals.

There will be a charge of $95 per page for members of The Endocrine Society and $115 per printed page for non-members. There will be a charge of $235 per color figure for members of The Endocrine Society and $735 per color figure for non-members. For more information on the benefits of membership in The Endocrine Society, please visit the Member Benefits page of The Endocrine Society's website. Authors must submit usable digital art that passes Cadmus's Rapid Inspector. Queries on page charges may be

directed to June Billman at Cenveo Publisher Services, (June.Billman@cenveo.com) (preferred), 410-943-3086 (direct) or 877-705-1373 (fax).

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NIH Deposits

For articles that were funded by NIH, accepted manuscripts will be submitted to PubMed Central. These manuscripts will be made freely available online twelve months after print publication. NIH will contact the author to confirm submission.

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Open Choice Option

The Endocrine Society's Open Choice program was developed to allow researcher authors the ability to provide immediate, open and free access to their work. For a growing number of our authors, providing open access is a condition of funding. For others, they simply want to have their latest findings available to the scientific domain without delay. Still others believe that paying to make their article free in the first 12 months of publication is not a worthwhile use of their grant monies.

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