Clinical Cancer Research

Association between GWAS-Derived rs966423 Genetic Variant and Overall Mortality in Patients with Differentiated Thyroid Cancer

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Abstract

Purpose: Five germline genetic variants (rs116909374, rs965513, rs944289, rs966423, and rs2439302) have been associated in genome-wide association studies (GWAS) with increased risk of differentiated thyroid cancer (DTC), but their role in mortality of patients has not been established. Also, no preoperative marker of the clinical outcome of thyroid cancer had yet been identified. The aim of the study was to investigate the relationship between the variants and overall mortality in patients with DTC.

Experimental Design: Retrospective study of 1,836 patients (1,643 women, 193 men) with median age at diagnosis of 49 years and overall median follow-up time of 8.7 years after initial treatment at a single comprehensive cancer center between 1990 and 2013.

Results: Among 5 variants, rs966423 was associated with increased mortality, which was 6.4% (33 of 518) versus 3.7% (47 of 1,259) in TT carriers versus CC/CT carriers ($P = 0.017$). The HR of TT versus TC/CC carriers was 1.6 [95% confidence interval (CI), 1.02–2.49; $P = 0.038$] after adjustment for age at diagnosis and sex. Importantly, the association of rs966423 with mortality remained valid when clinicopathologic risk factors were included in the model (HR, 1.89; 95% CI, 1.14–3.13; $P = 0.014$). Higher rs966423–associated patient mortality of TT versus CC/CT carriers was also observed in interaction with angioinvasion (adjusted HR, 3.48; 95% CI, 1.67–7.22; $P < 0.001$), lymph node metastasis (adjusted HR, 3.47; 95% CI, 1.16–10.4; $P = 0.018$), extrathyroidal invasion (adjusted HR, 2.07; 95% CI, 1.15–3.73; $P = 0.013$).

Conclusions: The presence of the rs966423-TT genotype was associated with a significant increase in overall mortality of patients with DTC. Contrary to *BRAF* mutation and other somatic changes, the status of germline rs966423 is known before the treatment and might be used in the management of mortality risk by means of modification of therapy. *Clin Cancer Res; 22(5); 1111–9. ©2015 AACR.*

Introduction

Differentiated thyroid carcinomas (DTC) constitute the most prevalent group of thyroid cancer. Two major histological subtypes: papillary (PTC) and follicular (FTC) thyroid carcinomas account for approximately 80% and 15% of all cases, respectively. DTC is generally considered a disease of low mortality as 90% of patients survive 10 years or more. Still, a significant number of patients die within first few years regardless of treatment, which usually consists of total or near total thyroidectomy, central or centrolateral neck lymphadenectomy, followed by thyroid ablation using low (30 mCi) or high (100–250 mCi) radioiodine (^{131}I). All efforts are made to identify clinical or molecular markers characteristic for the patients with poor prognosis that would allow for offering them with a more aggressive therapy (1). Several factors have been described; unfortunately they are either of low effect like male sex and older age, or cannot be determined prior to the surgical treatment, like tumor angioinvasion, capsule invasion, or *BRAF* somatic mutation. Distant metastasis, the strongest indicator of poor prognosis, usually protrudes later. In a search of pretreatment clinical markers determining the poor prognosis of patients with thyroid cancer, we tested the impact of five inherited polymorphisms on overall survival. All five markers (rs116909374, rs965513, rs944289, rs966423, and rs2439302) have been previously shown by genome-wide association studies (GWAS) to increase the risk of differentiated thyroid cancer; however their impact on the clinical outcome has not been established (2–4).

Several large case–control studies, including hypothesis-free GWAS, have convincingly demonstrated genetic factors predisposing to DTCs (2–6). Still, there are no data showing the effect

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Translational Relevance

The study shows that a naturally occurring variant (rs966423) reported in genome-wide association studies (GWAS) as predisposing to thyroid cancer is associated with a significant increase in overall mortality of patients with thyroid cancer and importantly remains valid after adjustment for sex, age, lymph node metastasis, extrathyroidal invasion, angioinvasion, and distant metastasis [adjusted HR, 1.89; 95% confidence interval (CI), 1.14–3.13; $P = 0.014$]. The impact of the rs966423 variant is even more significant when combined with other clinicopathologic risk factors, for example, lymph node metastases (HR, 3.47; 95% CI, 1.16–10.4, $P = 0.018$). Contrary to *BRAF* mutation and other somatic changes putatively associated with patients' mortality, the status of germline rs966423 is known before the treatment and might be used in the management of mortality risk by means of modification of therapy; nevertheless, it requires further investigation in large prospective studies before it is ready for clinical application.

of SNPs on the survival rates of patients with thyroid cancer. Recent studies showed that somatic mutations are important predictors of the clinical outcome, as two nucleotide substitutions within the promoter of telomerase reverse transcriptase *TERT* gene were shown to occur with higher frequency in highly aggressive thyroid carcinomas (7, 8). Another recent study proposed the well-known *BRAF* V600E mutation as a determinant of the cancer-related mortality of patients with PTC (9), possibly due to its involvement in the radioiodine treatment response. However, the same study revealed that the significance of the association between *BRAF* V600E and PTC-related mortality is lost when the survival model includes other determinants like lymph node metastasis, extrathyroidal invasion, and distant metastases. Similarly, many other studies failed to prove such association (10).

In our previous study, we revealed a cumulative, additive effect of rs116909374, rs965513, rs944289, rs966423, and rs2439302 on the thyroid cancer risk (4). Because the influence of polymorphic variants on the overall survival has never been established, we analyzed the association of these SNPs with the mortality of patients with thyroid cancer. This is significant, as in contrary to somatic mutations, germline variants can be prognostic markers of clinical outcome prior to treatment.

Materials and Methods

Study patients

Patients eligible for the study included 1,836 patients from northern and central Poland, treated and followed up at the Maria Skłodowska-Curie Memorial Cancer Center-Institute of Oncology in Warsaw (Warsaw, Poland), during the years 1990–2013. Two pathologists, based on World Health Organization criteria, confirmed the diagnosis of DTC. Patient treatment consisted in a total or near-total thyroidectomy followed by radioactive iodine ablation and thyrotropin suppression. Follow-up or survival time indicates the time from the initial thyroidectomy to patient's death or, if the patient stayed alive, to the July 1, 2014, when the survival data were confirmed at the Polish Ministry of Internal Affairs. The demographic and clinical information, including pathologic characteristics and median follow-up time of specific subgroups of patients, are summarized in Table 1.

Study design

The study was conducted on the basis of genotyping data and retrospective clinical data of the patients treated for thyroid cancer in a single comprehensive cancer center, the Maria Skłodowska-Curie Memorial Cancer Center-Institute of Oncology in Warsaw. The Institutional Review Board has approved the study and all subjects gave written informed consent before participation. Genotyping of the five analyzed SNPs (rs116909374, rs965513, rs944289, rs966423, rs2439302) in blood-derived DNA of patient with DTC was performed using the Sequenom technology, as described previously (4), and clinicopathologic data were collected during medical examinations as well as from medical archives.

Statistical analyses

Patient mortality was determined by dividing the number of deaths by the number of patients in each analyzed group. The mortality rates within each genotype were compared using the Fisher exact test. The number of deaths was divided by the sum of total follow-up years to produce the rates per person-year, and the 95% confidence intervals (CI) were calculated using the Poisson regression. The general genotype-dependent survivals were estimated and compared using the Kaplan–Meier survival curves, log-rank tests, and Cox proportional hazard regression analyses that censored patients at the time of the database inquire (July 1, 2014) or the follow-up period of 20 years. Proportional hazards regressions were adjusted by stratification for age at diagnosis (<45 and ≥45 years old) and sex using the counting process formulation of Andersen and Gill (11). Subgroup analyses were not adjusted for multiple comparisons and should be considered exploratory. All P values are 2-sided and significance was set at $P < 0.05$. All statistical analyses were performed using the R software package version 3.0.2.

Results

Relationship between 5 GWAS-derived variants and overall mortality

The study included 1,836 patients (1,643 women and 193 men) with a median age at diagnosis of 48.6 years (interquartile range, 38–57 years) and an overall median follow-up time of 8.7 years (interquartile range, 5–12.5 years). The frequencies of each genotype in the 5 GWAS variants: rs116909374, rs965513, rs944289, rs966423, and rs2439302 are provided in Table 1. The overall mortality of all cases was 4.63% (85 of 1,836). There were 53 deaths among cases with conventional variant of PTC (3.71%), 15 deaths among follicular variant of PTC (6.17%), 10 deaths among patients with FTC (6.94%), and 7 with insular FTC (35%). Mortality percentages and deaths per 1,000 person-years for all 5 genetic variants are reported in Table 1.

Among the five variants, rs966423 was associated with increased mortality reaching 6.4% (33 of 518) in TT-carriers versus 3.7% (47 of 1,259) in CC- or CT-carriers ($P = 0.017$; Table 2). Deaths per 1,000 person-years were 6.81 (95% CI, 4.69–9.56) in TT-carriers versus 4.01 (95% CI, 2.95–5.34) in CC/CT-carriers; the HR was 1.6 (95% CI, 1.02–2.49; $P = 0.038$) after adjustment for age at diagnosis and sex. After dividing patients according to the histopathologic variants with lower numbers of cases and deaths in specific subgroups, no significant results were observed

Table 1. Demographic characteristics and follow-up time of patients

			Age at diagnosis, median (interquartile range), y			Follow-up, median (interquartile range), y		
Factor	**Number of patients**	**Number of deaths (%)**	**All patients**	**Survivors**	**Patients who died**	**All patients**	**Survivors**	**Patients who died**
All	1,836	85 (4.63)	48.58 (37.58–57.25)	47.92 (36.92–56.46)	65.92 (56.25–72.25)	8.67 (5–12.5)	8.75 (5.08–12.67)	6.75 (3.67–11.08)
Gender								
Female	1,643	70 (4.26)	48.58 (37.71–57)	48.08 (37.42–56.33)	65.92 (56.27–72.08)	8.75 (5.17–12.67)	8.83 (5.25–12.75)	7.12 (3.85–11.15)
Male	193	15 (7.77)	48.83 (35.58–58.83)	47 (35.04–57.96)	62.92 (56.33–74.62)	7.58 (4.25–11.58)	7.83 (4.33–11.9)	6.08 (3.04–9.88)
Subtype								
PTC cv	1,429	53 (3.71)	48.83 (37.67–57.08)	48.42 (37.31–56.5)	65.92 (56–73.33)	8.67 (5.08–12.5)	8.67 (5.17–12.5)	7.67 (4.08–11.83)
PTC fv	243	15 (6.17)	44.5 (35.79–55.29)	43.33 (34.58–53.58)	62.75 (55.21–71.21)	8.75 (4.75–11.92)	8.83 (4.81–11.9)	7.17 (2.83–11.29)
FTC	144	10 (6.94)	51 (38.9–60.52)	49.46 (38.1–58.65)	68.46 (62.79–72)	9.17 (4.25–14.67)	9.29 (4.33–15.04)	5.25 (3.54–8.71)
FTC insulare	20	7 (35)	58.33 (47.25–67.46)	62.58 (38.58–68)	56.67 (53.29–62.25)	5.71 (4.21–8.52)	6.67 (4.83–10.33)	2.75 (2.58–5.71)
Stage								
I	987	26 (2.63)	49.5 (39.71–56.75)	49.08 (39.33–56.5)	57.79 (53.56–67.52)	8.33 (4.83–11.67)	8.33 (4.83–11.67)	8.04 (4.6–11.83)
II	156	6 (3.85)	45.92 (37.21–57.02)	44.71 (36.21–55.83)	67.12 (66.48–73.52)	9.08 (6.4–13.33)	9.17 (6.75–13.46)	4.67 (3.29–7.42)
III	386	16 (4.15)	49.29 (34.77–59.1)	48.75 (34.5–58.31)	69.29 (56.6–72.02)	8.79 (4.69–12.5)	8.83 (4.69–12.65)	7.12 (4.73–10.77)
IV	232	33 (14.22)	45.67 (31.06–59.58)	40.75 (28.71–53.33)	67.83 (59.83–75.92)	8.38 (4.5–12)	9 (5.12–12.12)	5.75 (2.75–8.58)
pT								
pT1a	713	17 (2.38)	48.75 (39–56.17)	48.5 (38.92–56)	58.17 (55.17–66.08)	8.08 (4.75–11.42)	8.08 (4.75–11.42)	6.75 (3.92–10.58)
pT1b	411	13 (3.16)	47.67 (35.04–57.08)	47.38 (34.83–56.58)	65.92 (49.08–67.92)	8.58 (5.17–12.25)	8.67 (5.17–12.25)	7.67 (4.08–12)
pT2	194	8 (4.12)	45.25 (34.4–56.83)	44.29 (34–55.83)	67.12 (64.04–69.77)	8.96 (5.69–13.12)	9.08 (5.79–13.31)	5.71 (3.54–8.15)
pT3	372	31 (8.33)	49.96 (37.1–59.94)	48.83 (35.83–58.58)	69.08 (56.38–75.33)	8.58 (4.67–12.17)	8.92 (4.83–12.42)	5.17 (2.79–9.29)
pT4	47	9 (19.15)	57.08 (41.67–72.29)	50.92 (36.33–66.73)	72.33 (65.33–76.33)	8.67 (6.21–11)	9.17 (6.44–11.17)	7.08 (6.08–7.75)
pN								
N0	1,426	53 (3.72)	49.75 (39.92–57.48)	49.33 (39.58–57)	62.42 (55.17–69.83)	8.75 (5.33–12.67)	8.83 (5.33–12.75)	7.08 (4.42–11.08)
N1a	178	6 (3.37)	41.46 (29.71–54.06)	39.67 (29.27–53.35)	67.79 (65.96–74.88)	8.54 (4.33–11.5)	8.58 (4.4–11.52)	1.83 (1.33–8.9)
N1b	174	18 (10.34)	40.46 (28.04–54.6)	38.62 (27.21–49.98)	67.38 (57.54–76.23)	8.38 (4.75–12)	8.67 (5.08–12.23)	5.46 (3.35–8.9)
pM								
M0	1,728	65 (3.76)	48.42 (37.65–56.83)	47.92 (37.25–56.33)	65.33 (55.5–72.58)	8.79 (5.23–12.67)	8.83 (5.29–12.75)	7.67 (4.08–11.33)
M1	56	15 (26.79)	57.04 (36.92–67.15)	50.33 (31–59.92)	66.08 (62.04–69.54)	5.75 (3.65–9.15)	6.08 (4.17–10.25)	3.42 (2.75–6.58)
Multifocality								
s	1,279	59 (4.61)	48.42 (36.79–57.21)	47.67 (36–56.42)	64.17 (55.58–71.62)	8.75 (5.29–12.33)	8.83 (5.33–12.42)	6.67 (3.58–10.42)
m	473	17 (3.59)	49.58 (38.33–57.75)	48.71 (38.08–56.77)	68.42 (57.42–73.33)	8.08 (4.5–11.67)	8.08 (4.5–11.81)	4.83 (2.25–11.17)
Size, mm								
0–9	626	15 (2.4)	48.62 (38.77–56)	48.42 (38.71–55.62)	62.08 (55.58–68.96)	7.92 (4.83–11)	7.92 (4.83–11.04)	5.67 (3.38–9.75)
10–19	563	20 (3.55)	49.67 (37.62–58.5)	49.25 (37.21–57.58)	66.12 (56.94–72.33)	8.33 (4.79–11.58)	8.42 (4.83–11.58)	6.25 (3.23–8.31)
20–29	216	8 (3.7)	44.67 (32.42–57.08)	44 (31.69–56.15)	69.25 (48.38–75.67)	8.96 (5.81–12.69)	9 (5.9–12.79)	7.29 (4.21–10.25)
30–39	98	8 (8.16)	48.46 (38.15–60.65)	46.58 (36.42–56.33)	67.58 (64.48–75.1)	8.79 (5.15–12.65)	9.04 (5.15–13.19)	6.88 (4.94–8.4)
>40	159	21 (13.21)	50.33 (37.96–62.21)	48.38 (35.71–59.5)	64.17 (50.33–75.33)	8.58 (4.38–12.62)	8.92 (4.56–13.69)	5.17 (2.83–9.5)
Angioinvasion								
N	1,298	42 (3.24)	48.5 (38.08–56.58)	48.17 (37.67–56.25)	61.38 (55.25–69.75)	8.83 (5.5–12.65)	8.92 (5.5–12.69)	7.62 (4.33–11.04)
T	403	30 (7.44)	49.25 (34.12–59.88)	47.83 (33.42–58.58)	67.83 (58.21–75.19)	8.42 (4.38–11.5)	8.58 (4.5–11.75)	5.46 (2.5–9.27)
Capsule								
N	1,056	29 (2.75)	47.75 (37.4–56)	47.42 (36.92–55.83)	55.17 (47.5–66.08)	8.83 (5.31–12.75)	8.83 (5.33–12.75)	8.42 (4.58–12.17)
T0	361	20 (5.54)	49.58 (39–59.33)	48.67 (37.58–57.25)	66.62 (61.35–73.48)	8.08 (4.83–11.5)	8.25 (4.83–11.58)	7.17 (3.58–10.71)
T1	321	27 (8.41)	51.17 (37.33–61.17)	49.42 (36–59.17)	69.83 (61.92–76.5)	8.42 (4.83–11.5)	8.83 (5.08–11.71)	4.83 (2.58–7.46)
rs944289								
CC	259	12 (4.63)	48.42 (35.71–57.46)	47.17 (35.04–56.17)	67.38 (60.85–74.96)	8.92 (5.29–12.58)	9.08 (5.33–12.83)	8.17 (2.83–9.33)
CT	798	41 (5.14)	49 (38.58–57.06)	48.58 (38–56.42)	61.33 (54–69.83)	8.67 (5.08–12.5)	8.75 (5.17–12.58)	7.17 (3.92–12)
TT	705	27 (3.83)	47.33 (36.92–57.42)	46.88 (36.44–56.73)	67.33 (58.88–73.62)	8.33 (4.75–12.33)	8.42 (4.83–12.48)	6.08 (3.38–10.12)
rs966423								
TT	518	33 (6.37)	48.42 (37.35–57.06)	47.42 (36.58–55.75)	66.92 (56.33–72.58)	8.54 (5.1–12.94)	8.58 (5.25–13.08)	5.67 (2.83–8.92)
CT	883	32 (3.62)	48.67 (38.25–57.88)	48.42 (37.79–57.12)	64 (53.92–72.19)	8.75 (5.08–12.46)	8.83 (5.08–12.5)	7.58 (4.6–12.04)
CC	376	15 (3.99)	48.25 (36.27–56.54)	47.75 (36–56.08)	64.17 (55.79–70.88)	8.5 (4.73–12.27)	8.58 (4.75–12.33)	5.75 (3.58–7.79)
rs2439302								
CC	504	22 (4.37)	48.75 (36.79–58.17)	48.25 (35.1–57.06)	61.71 (56.48–70.52)	8.33 (4.67–11.79)	8.33 (4.69–11.73)	7.58 (4.92–11.77)
GC	878	38 (4.33)	48.5 (36.96–57.25)	47.92 (36.4–56.5)	66.92 (56.85–73.04)	8.67 (5–12.81)	8.75 (5–12.94)	6.88 (2.98–11.71)
GG	371	16 (4.31)	47.75 (38.79–56.33)	47.33 (38.5–55.83)	64.17 (51.98–72.92)	9 (5.12–12.42)	9 (5.46–12.5)	5.58 (3.19–9.79)
rs116909374								
CC	1,709	77 (4.51)	48.5 (37.5–57.33)	47.88 (36.73–56.5)	66.33 (56.67–72.33)	8.58 (4.92–12.42)	8.67 (5–12.5)	6.75 (3.42–11.08)
CT	108	7 (6.48)	49.04 (38.21–56.21)	48.42 (38.08–56.17)	54.17 (50.21–58.12)	9.38 (6.29–13.27)	9.67 (6.42–13.33)	6.33 (4.71–10.08)
TT	1	0 (0)	36.25 (36.25–36.25)	36.25 (36.25–36.25)	NA	24 (24–24)	24 (24–24)	NA
rs965513								
AA	372	14 (3.76)	47.42 (35.88–57.02)	47.12 (35.31–56.5)	64.04 (49.23–70.46)	9.08 (5.21–13.33)	9.21 (5.33–13.48)	5.75 (3.04–8.77)
AG	888	42 (4.73)	48.33 (37.81–56.5)	47.33 (37.27–55.75)	67.58 (59.85–74.67)	8.33 (4.83–12.33)	8.42 (4.83–12.33)	7.12 (3.46–11.96)
GG	518	23 (4.44)	50.08 (38.25–58)	49.58 (37.67–57.46)	59.25 (56.29–68.71)	8.58 (5.08–12.17)	8.67 (5.08–12.25)	7.58 (3.88–10.42)

Table 2. Overall mortality, person-years of follow-up, and HRs for risk genotypes

SNP	Mortality, *n*/total (%)				Person-years follow-up	Deaths per 1,000 person-years (95% CI)		Unadjusted		Adjusted	
	Overall	Risk homozygotes	Heterozygotes/ homozygotes	*P*		Risk homozygotes	Heterozygotes/ homozygotes	Log-rank *P*	HR (95%CI)	Log-rank *P*	HR (95%CI)
rs944289 (CC vs. CT/TT)	80/1,762 (4.5)	12/259 (4.6)	68/1,503 (4.5)	0.873	16,413 (13,931 ± 2,482)	4.83 (2.5–8.44)	4.88 (3.79–6.19)	0.986	0.99 (0.54–1.84)	0.751	1.11 (0.6–2.05)
rs966423 (TT vs. CT/CC)	80/1,777 (4.5)	33/518 (6.4)	47/1,259 (3.7)	0.017	16,557 (11,709 ± 4,848)	6.81 (4.69–9.56)	4.01 (2.95–5.34)	0.022	1.67 (1.07–2.61)	0.038	1.6 (1.02–2.49)
rs2439302 (CC vs. GC/GG)	76/1,753 (4.3)	22/504 (4.4)	54/1,249 (4.3)	1	16,268 (11,774 ± 4,494)	4.9 (3.07–7.41)	4.59 (3.45–5.98)	0.709	1.1 (0.67–1.81)	0.792	1.07 (0.65–1.76)
rs116909374 (CC vs. CT/TT)	84/1,818 (4.6)	77/1,709 (4.5)	7/109 (6.4)	0.343	17,019 (1,112 ± 15,907)	4.84 (3.82–6.05)	6.29 (2.53–12.97)	0.572	0.8 (0.37–1.74)	0.674	0.85 (0.39–1.84)
rs965513 (GG vs. GA/AA)	79/1,778 (4.4)	23/518 (4.4)	56/1,260 (4.4)	1	16,594 (11,874 ± 4,720)	4.87 (3.09–7.31)	4.72 (3.56–6.12)	0.781	1.07 (0.66–1.75)	0.813	1.06 (0.65–1.73)

(Supplementary Table S1). For the largest subgroup consisting of PTC cases, the mortality was 5.3% (25 of 468) in TT-carriers versus 3.3% (38 of 1,146) in CC/CT-carriers ($P = 0.065$).

The independent association of clinical factors and rs966423 with increased overall mortality

We observed a significantly higher risk of death associated with several factors that are known to increase the mortality of patients with thyroid cancer, including male gender, age older than 45 years, tumor size > 30 mm, lymph node metastases, distant metastases, angioinvasion, and extrathyroidal invasion (Fig. 1). In the multivariate model, comprising such factors as sex, age, lymph node metastasis, distant metastasis, extrathyroidal invasion, angioinvasion, and rs966423, the increased mortality was independently associated with only four factors: age older than 45 years (HR, 11.97; 95% CI, 4.73–30.26; Wald $P = 1.56e-07$), extrathyroidal invasion (HR, 1.8; 95% CI, 1.03–3.16; Wald $P = 0.04$), distant metastasis (HR, 9.24; 95% CI, 4.57–18.66; Wald $P = 5.85e-10$), and rs966423 (HR, 1.89; 95% CI, 1.14–3.13; Wald $P = 0.014$). The association was not significant for sex ($P = 0.674$), lymph nodes metastases ($P = 0.228$), and angioinvasion ($P = 0.054$; Table 3).

Interaction between rs966423 and clinical factors in shaping the overall mortality

Rs966423 modifies the mortality risk associated with several conventional clinicopathologic risk factors, including age, angioinvasion, extrathyroidal invasion, and lymph node metastasis (Table 4). Importantly, the HRs remained statistically significant also after adjustment for age and sex. The differences in overall mortality in patients with the rs966423 risk genotype coexisting with various clinicopathologic factors were reflected in the Kaplan–Meier survival curves (Fig. 2A).

In patients with angioinvasion, the deaths per 1,000 person-years were 17.71 (95% CI, 10.31–28.35) versus 5.33 (95% CI, 2.84–9.11) in TT versus CC/CT patients (adjusted HR, 3.48; 95% CI, 1.67–7.22; log-rank $P < 0.001$]) as compared with 3.09 (95% CI, 2.02–4.53) in angioinvasion-negative CC/CT patients. In patients with extrathyroidal invasion, deaths per 1,000 person-years were 12.26 (95% CI, 7.68–18.56) versus 5.85 (95% CI, 3.71–8.77) in TT versus CC/CT patients (adjusted HR, 2.07 95% CI, 1.15–3.73; log-rank $P = 0.013$) as compared with 2.7 (95% CI, 1.63–4.22) in extrathyroidal invasion–negative CC/CT patients. In patients with lymph node metastasis, deaths per 1,000 person-years were 19.8 (95% CI, 9.5–36.42) versus 5.07 (95% CI, 1.65–11.82) in TT versus CC/CT patients (adjusted HR, 3.47; 95% CI, 1.16–10.4; log-rank $P = 0.018$) as compared with 3.71 (95% CI, 2.58–5.16) in lymph node metastasis–negative CC/CT patients. In patients with stage IV disease, the deaths per 1,000 person-years were 23.22 (95% CI, 12.99–38.29) versus 11.34 (95% CI, 6.35–18.71) in TT versus CC/CT patients (adjusted HR, 2.13; 95% CI, 1.03–4.42; log-rank $P = 0.037$) as compared with 3.05 (95% CI, 2.04–4.38) in stage I to III of CC/CT patients (Table 4, Fig. 2A).

In patients older than 45 years who carry the rs966423 risk genotype (TT), deaths per 1,000 person-years were 11.18 (95% CI, 7.6–15.88) as compared with 6.5 (95% CI, 4.69–8.79) in patients older than 45 years without the risk genotype (adjusted HR, 1.66; 95% CI, 1.04–2.65; log-rank $P = 0.03$; Table 4, Fig. 2B). In patients older than 65 years who carry the rs966423 risk genotype (TT),

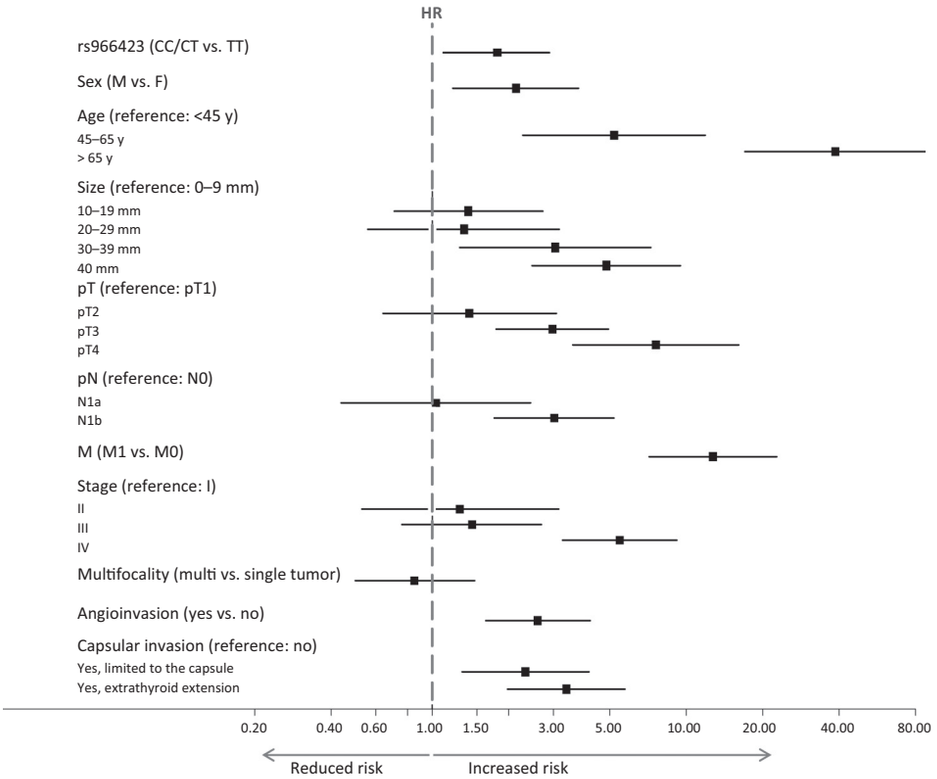


Figure 1.
Stratified analysis of various clinicopathologic factors associated with survival outcome of patients with thyroid cancer. Vertical line represents the HR equal to 1.

deaths per 1,000 person-years were 40.08 (95% CI, 24.13–62.6) as compared with 20.67 (95% CI, 13.11–31.02) in patients older than 65 years without the risk genotype (adjusted HR, 1.89; 95% CI, 1.03–3.47; logrank $P = 0.038$; Table 4, Fig. 2B).

Discussion

Several germline changes have been reported to predispose to DTC, including variants in microRNA-146a, ATM-CHEK2-BRCA1 pathway, TERT gene, and 5 SNPs pinpointed by GWAS study (2–5, 12–15). So far, no study revealed the influence of germline (inherited) variants on the overall mortality of patients with thyroid cancer.

Analyzing the relationship between the 5 GWAS-derived variants and overall mortality of patients with thyroid cancer, we found the association of rs966423 with increased risk of death. As such, this study reveals the first genetic marker allowing for assessment of the clinical outcome of DTC prior to the surgery.

The rs966423 polymorphism was previously associated with thyroid-stimulating hormone levels and genetic predisposition to PTC in different populations (2, 4, 16), but its effect on the clinical outcome of cancer was unknown. The SNP is located within the *DIRC3* gene found to be associated with renal cancer (17), but the data on its biologic role are still lacking. Although our previous study showed that the more common C allele predisposes to thyroid cancer (4), this study revealed that the rare T allele is associated with worse prognosis of patients with thyroid cancer. This is a common observation that different alleles of the same gene are associated with predisposition to cancer (initiation) or its clinical outcome (progression), as most recently shown by Zhao and colleagues (18).

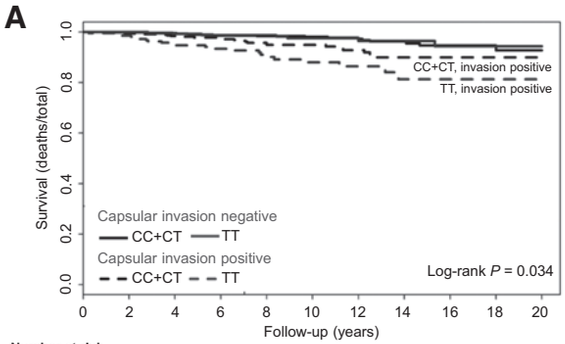
In our cohort of 1,836 patients with DTC, the overall mortality amounted to 4.63% (85 of 1836) and, as expected on the basis of other clinical data, was significantly higher in patients with FTC (6.94%) and insular FTC (35%) compared with patients with PTC (3.9%). We replicated well-documented findings that extrathyroidal invasion, lymph node and distant metastases, as well as angioinvasion are risk factors leading to increased mortality of patients with cancer (19–21). Our results supported also the well-established impact of age on the outcome of thyroid cancer (22). In a study by Jonklaas and colleagues, the authors showed that the risk of death due to PTC was significantly lower in female than in male patients (HR, 0.40; 95% CI, 0.24–0.65, $P < 0.001$) but concluded that this advantage reflected a generalized gender survival advantage seen in the U.S. population (23). Moreover, after adjustment for the disease stage and age, women's outcomes were similar to men's. In our work, analyzing all conventional clinicopathologic risk factors in one multivariate model, we received comparable results. This analysis showed no survival advantage or disadvantage associated with sex; however, such factors as age older than 45 years, extrathyroidal invasion, and distant metastasis were all independently associated with worse

Table 3. Multivariate analysis of genetic and clinicopathologic risk factors

No.	Factor	Wald *P*	HR (95%CI)
1	rs966423 (TT)	0.014	1.89 (1.14–3.13)
2	Sex (M)	0.674	1.17 (0.57–2.38)
3	Age (≥45 y)	1.56E-07	11.97 (4.73–30.26)
4	Angioinvasion	0.054	1.76 (0.99–3.13)
5	Extrathyroidal invasion	0.04	1.8 (1.03–3.16)
6	Lymph node mets (N1b)	0.228	1.56 (0.76–3.2)
7	Distant metastasis (M1)	5.85E-10	9.24 (4.57–18.66)

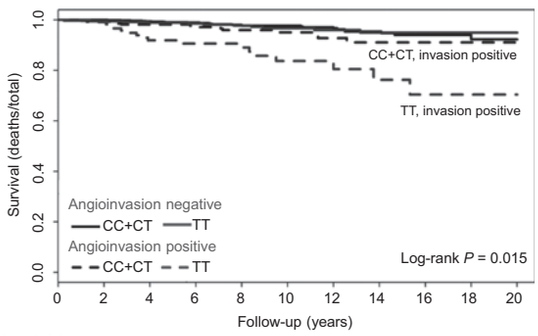
Table 4. Overall mortality, person-years of follow-up, and HRs for rs966423 TT carriers and CT/CC carriers in various clinicopathologic categories

	Mortality, *n*/total (%)					Deaths per 1,000 person-years (95% CI)		Unadjusted		Adjusted	
Category	Overall	rs966423-TT	rs966423-CC/CT	*P*	Person-years follow-up	rs966423-TT	rs966423-CC/CT	Log-rank *P*	HR (95%CI)	Log-rank *P*	HR (95% CI)
All patients	80/1,777 (4.5)	33/518 (6.4)	47/1,259 (3.7)	0.017	16,557 (11,709 ± 4,848)	6.81 (4.69–9.56)	4.01 (2.95–5.34)	0.022	1.67 (1.07–2.61)	0.038	1.6 (1.02–2.49)
Gender											
Female	66/1,586 (4.2)	26/454 (5.7)	40/1,132 (3.5)	0.052	14,913 (10,578 ± 4,335)	6 (3.92–8.79)	3.78 (2.7–5.15)	0.076	1.56 (0.95–2.55)	0.12	1.48 (0.9–2.42)
Male	14/191 (7.3)	7/64 (10.9)	7/127 (5.5)	0.238	1,644 (1,130 ± 514)	13.63 (5.48–28.08)	6.19 (2.49–12.76)	0.126	2.22 (0.78–6.35)	0.115	2.28 (0.79–6.57)
Age_45											
<45	7/741 (0.9)	2/214 (0.9)	5/527 (0.9)	1	7,325 (5,248 ± 2,077)	0.96 (0.12–3.48)	0.95 (0.31–2.22)	0.992	0.99 (0.19–5.11)	0.99	0.99 (0.19–5.1)
≥45	73/1,036 (7)	31/304 (10.2)	42/732 (5.7)	0.016	9,232 (6,460 ± 2,772)	11.18 (7.6–15.88)	6.5 (4.69–8.79)	0.028	1.67 (1.05–2.67)	0.03	1.66 (1.04–2.65)
Age_45_65											
<45	7/741 (0.9)	2/214 (0.9)	5/527 (0.9)	1	7,325 (5,248 ± 2,077)	0.96 (0.12–3.48)	0.95 (0.31–2.22)	0.992	0.99 (0.19–5.11)	0.99	0.99 (0.19–5.1)
45–65	31/825 (3.8)	12/242 (5)	19/583 (3.3)	0.235	7,645 (5,348 ± 2,298)	5.22 (2.7–9.12)	3.55 (2.14–5.55)	0.356	1.4 (0.68–2.9)	0.357	1.41 (0.68–2.91)
>65	42/211 (19.9)	19/62 (30.6)	23/149 (15.4)	0.014	1,586 (1,112.5 ± 474)	40.08 (24.13–62.6)	20.67 (13.11–31.02)	0.035	1.91 (1.04–3.5)	0.038	1.89 (1.03–3.47)
pT											
pT1	28/1,085 (2.6)	9/298 (3)	19/787 (2.4)	0.668	9,514 (6,792 ± 2,722)	3.31 (1.51–6.28)	2.8 (1.68–4.37)	0.775	1.12 (0.51–2.49)	0.78	1.13 (0.49–2.59)
pT2	8/189 (4.2)	2/56 (3.6)	6/133 (4.5)	1	1,777 (1,278 ± 499)	4.01 (0.49–14.48)	4.69 (1.72–10.22)	0.824	0.83 (0.17–4.14)	0.576	0.63 (0.13–3.16)
pT3	29/362 (8)	17/118 (14.4)	12/244 (4.9)	0.003	3,265 (2,237.5 ± 1,028)	16.54 (9.63–26.48)	5.36 (2.77–9.37)	0.002	3.05 (1.46–6.4)	0.01	2.61 (1.23–5.53)
pT4	9/46 (19.6)	3/19 (15.8)	6/27 (22.2)	0.716	404 (241 ± 163)	18.38 (3.79–53.7)	24.9 (9.14–54.21)	0.733	0.79 (0.19–3.17)	0.775	0.81 (0.19–3.42)
pN											
N0	51/1,383 (3.7)	16/375 (4.3)	35/1,008 (3.5)	0.521	13,098 (9,440 ± 3,658)	4.37 (2.5–7.1)	3.71 (2.58–5.16)	0.649	1.15 (0.63–2.07)	0.781	1.09 (0.6–1.98)
N1a	6/171 (3.5)	4/65 (6.2)	2/106 (1.9)	0.202	1,492 (941 ± 551)	7.26 (1.98–18.6)	2.13 (0.26–7.68)	0.145	3.29 (0.6–17.97)	0.148	3.29 (0.6–18.1)
N1b	15/166 (9)	10/59 (16.9)	5/107 (4.7)	0.011	1,492 (987 ± 505)	19.8 (9.5–36.42)	5.07 (1.65–11.82)	0.008	3.84 (1.31–11.24)	0.018	3.47 (1.16–10.4)
pM											
M0	60/1,670 (3.6)	25/479 (5.2)	35/1,191 (2.9)	0.029	15,763 (11,172 ± 4,591)	5.45 (3.52–8.04)	3.13 (2.18–4.36)	0.04	1.7 (1.02–2.84)	0.065	1.62 (0.97–2.71)
M1	15/55 (27.3)	6/21 (28.6)	9/34 (26.5)	1	357 (226 ± 131)	45.86 (16.83–99.82)	39.78 (18.19–75.51)	0.775	1.16 (0.41–3.27)	0.198	1.97 (0.69–5.6)
Capsule											
N	27/1,024 (2.6)	19/750 (2.5)	8/274 (2.9)	0.826	9,780 (7,025.5 ± 2,754)	2.7 (1.63–4.22)	2.9 (1.25–5.72)	0.894	1.06 (0.46–2.42)	0.861	0.92 (0.38–2.22)
T0	20/347 (5.8)	12/234 (5.1)	8/113 (7.1)	0.469	2,989 (2,055 ± 934)	5.84 (3.02–10.2)	8.57 (3.7–16.88)	0.389	1.48 (0.6–3.62)	0.356	1.52 (0.62–3.72)
T1	25/312 (8)	11/211 (5.2)	14/101 (13.9)	0.013	2,739.5 (1,879 ± 861)	5.85 (2.92–10.48)	16.26 (8.89–27.29)	0.007	2.83 (1.28–6.24)	0.011	2.73 (1.22–6.11)
Angioinvasion											
N	38/1,253 (3)	12/362 (3.3)	26/891 (2.9)	0.718	11,939 (8,401 ± 3,538)	3.39 (1.75–5.92)	3.09 (2.02–4.53)	0.806	1.09 (0.55–2.16)	0.881	0.95 (0.47–1.93)
T	30/394 (7.6)	17/117 (14.5)	13/277 (4.7)	0.001	3,401 (2,441 ± 960)	17.71 (10.31–28.35)	5.33 (2.84–9.11)	0.001	3.28 (1.59–6.76)	0	3.48 (1.67–7.22)
Multifocality											
s	55/1,235 (4.5)	21/370 (5.7)	34/865 (3.9)	0.178	11,479 (8,065 ± 3,414)	6.15 (3.81–9.4)	4.22 (2.92–5.89)	0.157	1.48 (0.86–2.55)	0.161	1.48 (0.85–2.56)
m	17/459 (3.7)	8/125 (6.4)	9/334 (2.7)	0.091	3,921 (2,828 ± 1,093)	7.32 (3.16–14.42)	3.18 (1.46–6.04)	0.169	1.97 (0.73–5.3)	0.29	1.7 (0.63–4.61)
Size, mm											
0–9	15/606 (2.5)	6/153 (3.9)	9/453 (2)	0.226	5,052 (3,752 ± 1,300)	4.62 (1.69–10.05)	2.4 (1.1–4.55)	0.222	1.88 (0.67–5.29)	0.142	2.17 (0.75–6.26)
10–19	18/544 (3.3)	9/173 (5.2)	9/371 (2.4)	0.121	4,770 (3,187 ± 1,583)	5.69 (2.6–10.79)	2.82 (1.29–5.36)	0.235	1.77 (0.68–4.59)	0.216	1.81 (0.7–4.72)
20–29	7/212 (3.3)	0/59 (0)	7/153 (4.6)	0.194	2,016 (1,450 ± 566)	0 (0–6.52)	4.83 (1.94–9.94)	0.095	0 (0-Inf)	0.063	0 (0-Inf)
30–39	8/95 (8.4)	4/35 (11.4)	4/60 (6.7)	0.461	861 (571 ± 290)	13.78 (3.75–35.28)	7 (1.91–17.93)	0.304	2.04 (0.51–8.18)	0.342	1.94 (0.48–7.78)
>40	21/153 (13.7)	10/48 (20.8)	11/105 (10.5)	0.126	1,387 (991 ± 396)	25.26 (12.11–46.46)	11.1 (5.54–19.86)	0.064	2.2 (0.93–5.19)	0.2	1.75 (0.74–4.16)
Stage											
I	24/956 (2.5)	8/251 (3.2)	16/705 (2.3)	0.481	8,437 (6,120 ± 2,317)	3.45 (1.49–6.8)	2.61 (1.49–4.25)	0.632	1.23 (0.52–2.89)	0.722	1.18 (0.48–2.89)
II	6/151 (4)	1/45 (2.2)	5/106 (4.7)	0.67	1,465 (1,042 ± 423)	2.37 (0.06–13.18)	4.8 (1.56–11.2)	0.484	0.47 (0.06–4.05)	0.384	0.4 (0.05–3.42)
III	16/374 (4.3)	8/124 (6.5)	8/250 (3.2)	0.175	3,461 (2,346 ± 1,114)	7.18 (3.1–14.15)	3.41 (1.47–6.72)	0.137	2.07 (0.78–5.52)	0.208	1.87 (0.7–5.02)
IV	30/224 (13.4)	15/77 (19.5)	15/147 (10.2)	0.064	1,968 (1,322 ± 646)	23.22 (12.99–38.29)	11.34 (6.35–18.71)	0.05	2.02 (0.99–4.14)	0.037	2.13 (1.03–4.42)



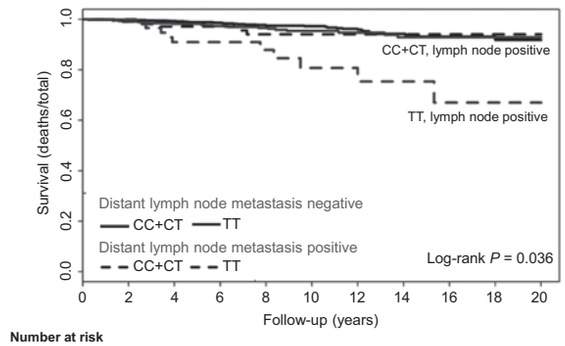
Number at risk

Thyroid capsule invasion negative											
TT	274	274	251	211	173	128	94	64	37	12	6
CC+CT	750	748	667	518	424	306	212	133	83	49	23
Thyroid capsule invasion positive											
TT	214	211	179	134	104	74	47	27	14	7	2
CC+CT	445	443	397	296	243	164	107	56	33	18	6



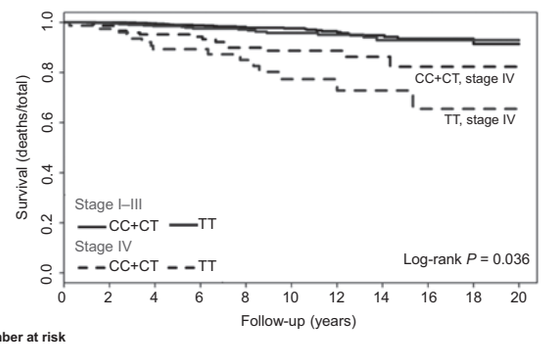
Number at risk

Angioinvasion negative											
TT	362	360	335	275	217	164	116	74	44	14	6
CC+CT	891	891	811	627	514	367	252	150	96	54	24
Angioinvasion positive											
TT	117	116	88	68	59	38	26	17	7	5	1
CC+CT	277	274	237	187	154	104	65	38	19	11	4



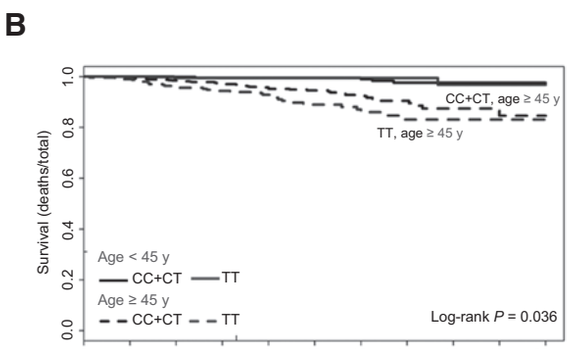
Number at risk

Distant lymph node metastasis negative											
TT	440	437	391	322	257	189	134	87	51	20	9
CC+CT	1,114	1,111	992	764	631	444	307	191	125	72	32
Distant lymph node metastasis positive											
TT	59	59	47	34	28	21	15	11	6	3	3
CC+CT	107	106	96	71	57	46	31	20	10	7	3



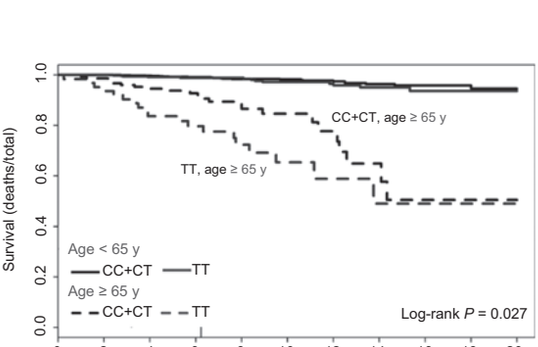
Number at risk

Stage I–III											
TT	304	301	263	214	165	115	84	50	30	13	7
CC+CT	732	729	651	495	386	261	172	100	54	31	13
Stage IV											
TT	77	75	62	47	37	26	17	12	7	4	3
CC+CT	147	145	127	96	79	62	41	25	12	7	3



Number at risk

Age < 45 y											
TT	214	214	188	149	126	99	68	50	29	12	6
CC+CT	527	526	466	362	322	245	179	118	87	50	24
Age ≥ 45 y											
TT	304	301	263	214	165	115	84	50	30	13	7
CC+CT	732	729	651	495	386	261	172	100	54	31	13



Number at risk

Age < 65 y											
TT	456	456	403	325	263	199	143	95	56	24	12
CC+CT	1,110	1,108	991	768	646	474	331	208	137	78	36
Age ≥ 65 y											
TT	62	59	48	38	28	15	9	5	3	1	1
CC+CT	149	147	126	89	62	32	20	10	4	3	1

Figure 2.
Kaplan–Meier survival curves of the interaction of rs966423 variant with clinicopathological risk factors (A) and age (B) in affecting overall survival of patients with thyroid cancer. In all panels, follow-up time is truncated at 20 years. In each panel, *P* values are from the log-rank test.

prognosis. In the same analysis, we obtained positive results for the rs966423 variant, for which the overall mortality in TT-carriers was almost doubled (6.4% vs. 3.7%) compared with patients who were heterozygous or homozygous for the C allele (Fig. 1). In addition, the variant showed a significant additive interaction with several conventional risk factors including angioinvasion, lymph node metastasis, extrathyroidal invasion, and stage IV of the disease in tailoring the overall mortality of patients with thyroid cancer. For example, in patients with angioinvasion or distant lymph node metastasis, deaths per 1,000 person-years were 3.3- or 3.8-fold higher, respectively, in TT-carriers as than in CC/CT-carriers. Of note, all the HRs remained statistically significant also after adjustment for age and gender.

Interestingly, the analysis revealed that the overall mortality rates do not differ between patients with stage I, II, and III of thyroid cancer. It is only stage IV that significantly increases mortality by the mode of 4.3-fold compared with patients with lower stages of cancer. This observation is consistent with a number of other long-term observations indicating that staging is not an ideal indication of patients' survival (24).

The main strength of this study is a large number of analyzed patients who were treated in the same comprehensive cancer center, thus the overall outcome is free from the inter-center bias resulting from different treatment approaches. Also, all the samples were genotyped in a single experiment, thus the results are fully comparable. The presented results are therefore obtained in a coherent and fully controlled study. The most important finding of the study is the impact of rs966423 on the mortality of patients with thyroid cancer. Of note, although the C allele was associated with increased risk for PTC development, it is the T variant that doubles the overall mortality among patients with thyroid cancer.

The main limitation of the study is lack of validation cohort. Unfortunately, the study of the overall survival in cancers with low mortality requires especially large cohort with long enough observation time, and such cohort of patients with DTC is not available.

To our knowledge, the rs966423 variant is the only genetic risk marker whose clinical significance is still valid after adjustment for sex and age at diagnosis, as well as for lymph node metastasis, extrathyroidal invasion, angioinvasion, and distant metastasis in multivariant analysis. Contrary to *BRAF* mutation and other somatic changes putatively associated with patients' mortality, the status of germline rs966423 variant is known before the surgery and might be used in management of mortality risk by means of modification of therapy, nevertheless it requires further investigation in large prospective studies before it is ready for clinical application.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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