Association Study of the G-Protein β3 Subunit C825T Polymorphism with Disease Progression an Overall Survival in Patients with Head and Neck Squamous Cell Carcinoma

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Abstract

The T-allele of a common C825T single nucleotide polymorphism (SNP) in the gene *GNB3*, encoding the G3 subunit of heterotrimeric G-proteins, is associated with a truncated form of the G3 protein that imparts a greater signaling capacity than the alternative C-allele encoding a nontruncated protein. We analyzed the C825T-allele status with regard to disease progression in patients with head and neck squamous cell carcinoma (HNSCC). The prognostic value of the SNP was evaluated in an unselected series of 341 patients treated with curative intent for HNSCC including all tumor stages with different therapeutic regimens. Genotype analysis was done by Pyrosequencing using DNA from paraffin-embedded tissue samples. Genotypes were correlated with relapse-free and overall survival. Proportions of 5-year relapse-free intervals were 62% for CC, 60% for TC, and 42% for TT genotypes. Kaplan-Meier curves revealed a significant genotype-dependent relapse-free interval (P = 0.036). In multivariate analysis with stage, localization, grade, gender, and smoking habits as covariates, *GNB3* 825T homozygous patients displayed a higher risk for relapse than C825 homozygous patients (TT versus CC, hazard ratio; 95% confidence interval, 1.4-4.8; P = 0.002). The same genotype effect was found for overall survival, TT genotypes were at higher risk for death compared with CC genotypes (hazard ratio, 2.6; 95% confidence interval, 1.6-4.3; P < 0.001), and 5-year survival proportions were 60% for CC, 52% for TC, and 33% for TT. The *GNB3* C825T SNP thus represents a host derived prognostic marker in HNSCC, which allows identifying high-risk patients, which could benefit from novel and/or more aggressive therapeutic regimes. (Cancer Epidemiol Biomarkers Prev 2008; 17(11):3203–7)

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most prevalent neoplasm in the world. Worldwide annually, ~500,000 cases are reported to be diagnosed with HNSCC (1). HNSCC develops in the squamous epithelial cells of the upper aerodigestive tract and is found in the oral cavity, oropharynx, hypopharynx, and larynx. Due to advances in our understanding and novel treatment modalities, the 5-year survival rates of HNSCC have improved to some extent, varying from site to site, but still the therapeutic outcome in HNSCC represents an oncological challenge.

Treatment options of HNSCC include surgery, radiotherapy, conventional chemoradiotherapy, radiotherapy with new target drugs or a combination of these modalities. Both the therapeutic approach and the patients' prognosis are currently determined by tumor volume and tumor stage. However, neither the biological behavior nor the response to therapy can be sufficiently predicted by these factors (2). Thus, additional variables would be most desirable to optimize the prediction of tumor behavior enabling a more individualized therapeutic approach.

G-protein βγ subunits are of major importance for cell migration on stimulation of G-protein–coupled receptors.

We have shown that the 825T-allele of the *GNB3* single nucleotide polymorphism (SNP) is associated with the occurrence of an alternative splice variant called Gβ3s (3). G-protein β subunits belong to the family of ''propeller proteins'' and consist of seven regular protein domains, each coding for one propeller blade. The splice variant Gβ3s lacks the equivalent of one propeller domain. Despite this structural deletion, Gβ3s is functionally active and has been associated with an increased signal transduction (3, 4). The enhanced signal transduction is correlated with increased chemotaxis as shown for migrating cells from 825T-allele carriers (5, 6). In carriers of the *GNB3* 825T-allele, stimulation with chemokines or autocoids leads to enhanced chemotaxis in Gβ3s-expressing neutrophils and lymphocytes (6, 7). Our group recently showed that in bladder cancer tumor cells, Gβ3s is expressed exclusively in patients carrying an 825T-allele, whereas wild-type Gβ3 was associated with the homozygous CC

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genotype (8). Moreover, 825T allele carriers showed a shorter time to metastasis compared with carriers of the homozygous CC genotype (8).

The aim of the present study was to investigate whether the C825T SNP of the *GNB3* gene is related to the clinical outcome in patients with HNSCC. Genotyping of this SNP may offer the opportunity to define subgroups of HNSCC patients who may benefit from novel therapeutic options.

Materials and Methods

The study was strictly done according to the Declaration of Helsinki and approved by the local Ethics Committee of the University Hospital of Essen.

Patients. The study group consisted of 341 patients (340 Caucasians, 1 Asian) with HNSCC diagnosed and treated between 1995 and 2001 at the West German Cancer Center Essen. Relevant clinico-pathologic data [American Joint Committee on Cancer (AJCC) and tumor-node-metastasis tumor stage, tumor site, histologic grading, therapeutic regimens, 5-y follow-up, relapse, and cause of death] were extracted from the patients' files or collected by telephone interviews with the patients or their family doctors. HNSCC cases that had been defined as palliative by the West German Cancer Center Essen interdisciplinary tumor board (the vast majority classified as tumor stage IVC) were excluded from this study.

Genotyping. For genotyping of the C825T polymorphism of the *GNB3* gene, genomic DNA was isolated from several 10- to 20-μm-thick sections (overall weight not exceeding 25 mg) from routinely processed paraffin blocks. Genotypes of the *GNB3* polymorphism were determined by Pyrosequencing as described (9). Genotyping was done blinded, with the genotyping laboratory being unaware of any clinical data.

Healthy Blood Donors. The control group consisted of 255 age- and sex-matched healthy Caucasian individuals who were randomly recruited at the local Institute of Transfusion Medicine, University Hospital of Essen. The control sample consisted of 161 males and 94 females, and the mean age was 56.7 ± 4.4 y. Details of this control group have been published previously (10).

Statistical Analysis and Presentation of Data. The genotyping results of the present study were correlated with overall survival (OS; defined as time from first diagnosis to death or loss of follow-up) and time to relapse (defined as time from last day of therapy to day of diagnosis of tumor relapse). Kaplan-Meier plots and the log-rank test for trend were used to retrospectively evaluate the relationship between tumor localization and *GNB3* genotypes. Log-rank tests for *GNB3* genotypes were adjusted for tumor localization. Multivariate models of clinical follow up were established using clinical and pathologic variables known as predictors of prognosis including the genotypes of the C825T polymorphism. A backward stepwise Cox proportional hazard model was used to calculate hazard ratios, 95% confidence interval, and *P* values (11). Contingency tables and the Pearson's χ^2 test were used to compare categorical variables using *GNB3* genotypes as indicated. Because the *GNB3* polymorphism displays a gene-dose effect (3, 12), a linear ANOVA was used for comparison of parametric continuous variables where appropriate. Nonparametric linear variables were compared using the Kruskal-Wallis test. Differences were regarded significant at a *P* value of <0.05. All statistical analyses was done using SPSS 15.0 (SPSS). Continuous variables are given as means ± SD.

Table 1. C 825 T genotype distribution

	Total	CC	CT	TT	*P*
n (%) total	341	170 (49.8)	144 (42.2)	27 (7.9)	
Oral cavity	20	5 (25.0)	12 (60.0)	3 (15.0)	
Oropharynx	108	64 (59.3)	35 (32.4)	9 (8.3)	
Hypopharynx	49	23 (46.9)	25 (51.0)	1 (2.0)	
Larynx	150	73 (48.7)	66 (44.0)	11 (7.3)	
Multiple sites	14	5 (35.7)	6 (42.9)	3 (21.4)	0.033
Mean age (y ± SD)	61.8 ± 12.1	61.2 ± 10.7	62.1 ± 13.8	63.6 ± 10.7	0.315
Median follow up, mo (range)	61 (3-143)	65 (3-128)	57 (6-143)	38 (6-97)	0.018*
Gender (m/f)	286/55	137/33	129/15	20/7	0.496
Smoking, *n* (%)	321 (90.0)	152 (49.5)	131 (42.7)	24 (7.8)	0.724
Pack years	46.6 ± 22.5	45.4 ± 21.2	46.2 ± 23.2	56.0 ± 25.7	0.101
AJCC					
I	53	22 (41.5)	27 (50.9)	4 (7.5)	
II	50	25 (50.0)	21 (42.0)	4 (8.0)	
III	55	27 (49.1)	22 (40.0)	6 (10.9)	
IVA	157	82 (52.2)	62 (39.5)	13 (8.3)	
IVB	23	12 (52.2)	11 (47.8)	0 (0)	
IVC	3	2 (66.7)	1 (33.3)	0 (0)	0.866
Grade					
1	21	8 (38.1)	11 (52.4)	2 (9.5)	
2	246	116 (47.2)	109 (44.3)	21 (8.5)	
3-4	74	46 (62.2)	24 (32.4)	4 (5.4)	0.170

NOTE: C825T genotype distribution with regard to tumor site, demographic characteristics, primary therapy, tumor stage (AJCC), and histologic grading in 341 patients with HNSCC.
*Kruskal Wallis Test.

Results

C825T Genotype Distributions and Correlation with Clinical Data. Demographic characteristics, genotype, and clinical data at the time of first diagnosis for the whole case group are displayed in Table 1. The mean age of the 341 patients (286 males, 55 females) was 61.8 ± 12.1 years, and median follow-up time from the time of primary therapy on was 61 months (range, 3-143 months). The frequency of the T allele in the patient group was 0.29, and this distribution was compatible with the Hardy-Weinberg equilibrium.

Both genotype distributions and allele frequencies of the 341 patients were comparable with those of healthy white blood donors, thus arguing against an association of C825T genotypes with an increased susceptibility for HNSCC.

We found no genotype association with patients' age and AJCC tumor stage at time of first diagnosis. A lack of association was also observed with the primary therapy (Table 1). However, genotypes were significantly associated with different tumor localization. In the group of hypopharynx patients, only 1 patient (2%) with the TT genotype was found, whereas in the whole group, the frequency of the TT genotype was 7.9% ($P = 0.033$; Table 1).

C825T Genotype and Clinical Follow up. During follow up, 135 patients (39.6%) experienced a relapse of the disease and 153 (44.9%) died. To confirm that our sample was representative for patients with HNSCC, Kaplan-Meier curves were calculated for relapse-free and OS depending on AJCC stage (data not shown) and anatomic subsite (Fig. 1A and B). Relapse-free survival and OS were significantly related both to the AJCC tumor stage ($P < 0.001$) and tumor localization ($P < 0.0001$), and computed values were compatible with published data (13).

Proportions of 5-year relapse-free intervals were 62% for CC, 60% for TC, and 42% for TT genotypes (Fig. 2A). Additionally, a significant genotype-dependent treatment-free interval with an apparent gene-dose effect was observed (Fig. 2A; $P = 0.036$): *GNB3* 825T homozygous patients displayed a higher risk for disease progression than C825 homozygous patients (TT versus CC, 2.6; 95% confidence interval, 1.4-4.8; $P = 0.002$) with TC genotypes showing intermediate values.

A similar genotype effect was found for OS (Kaplan-Meier curve; Fig. 2B), again displaying a gene-dose effect ($P = 0.008$; Fig. 2B). TT genotypes were at higher risk for death compared with CC genotypes (hazard ratio, 2.6; 95% confidence interval, 1.6-4.3; $P = <0.001$), and 5-year survival proportions were 60% for CC, 52% for TC, and 33% for TT. As we observed a significant association of *GNB3* genotypes with tumor localization (Table 1), we investigated whether genotypes of the C825T polymorphism showed an interaction with HNSCC tumor localization or whether they were independently associated with disease progression and OS. Cox proportional hazard models including predictors of prognosis revealed that the C825T *GNB3* SNP represents an independent risk factor for tumor progression (Table 2) and death (Table 3) in HNSCC. Hazard ratios for patients with TT genotype were 2.6 for relapse-free survival as well as for OS compared with homozygous 825C allele carriers.

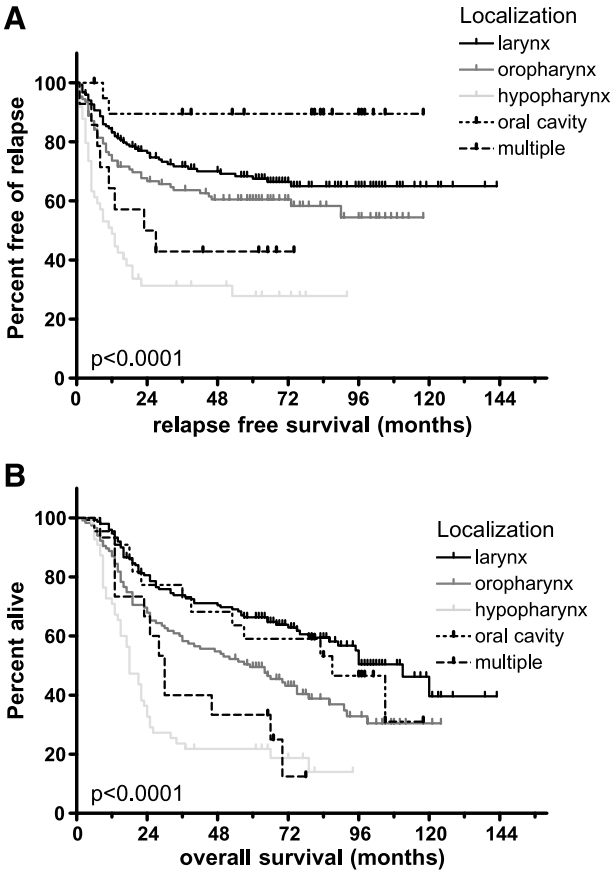

Figure 1. A and **B.** Relapse-free survival (**A**) and OS (**B**) Kaplan-Meier curves for 341 patients with HNSCC based on the anatomic subsite. *P* values are given for log-rank tests.

Discussion

The results of the present study on a large series of HNSCC show that (*a*) the *GNB3* C825T SNP does not represent a causative risk factor and (*b*) homozygous carriers of the 825T allele show a significantly decreased relapse-free and OS. In comparison to some other large series (13-15), proportions of the HNSCC tumor sites were similar within our series of 341 patients with the oral cavity being slightly underrepresented and larynx tending to be overrepresented. This might be explained by the individual therapeutic focus of our department. Interestingly, within the subgroup of the hypopharynx being associated with unfavorable clinical course, the TT genotype carriers were even underrepresented (only 1 of 27).

Why tumor localization was found to be significantly associated with the genotypes remains elusive, anyhow, Cox proportional hazard models including predictors of prognosis revealed the C825T polymorphism as an independent risk factor for tumor progression and death in HNSCC.

Our findings are in accordance with previous data obtained in patients with bladder carcinoma (8). However, in patients suffering from chronic lymphatic lymphoma, homozygous CC carriers were found to be

associated with a high relapse rate (16). Krippl et al. (17) described that carriers of the T-allele display a significantly longer metastasis-free survival in patients with low-grade breast carcinoma. In another study of this group (24), the TT genotype was associated with a significantly decreased risk for bone metastasis in breast cancer.

These results indicate that the biological effect of the C825T SNP of the GNB3 gene are mediated by different signaling pathways in different tumor types. Stimulation with chemokine stromal-derived factor 1α resulted in enhanced migration of human T lymphocytes in 825T-allele carriers (5). Very recently, it has been shown that stromal-derived factor 1α influences the migration and invasion of HNSCC cells (18, 19) via the nuclear factor-κB signaling pathway (19) by binding to the CXCR4 receptor, which also belongs to the family of G-protein–coupled receptors. It is thus suggested that 825T-allele carriers respond more sensitively to chemokine stimulation, which may explain the observed tendency for earlier tumor progression in HNSCC patients carrying the T allele. The decreased risk for the development of bone metastasis in homozygous TT carrying breast cancer patients was associated with a putative role of the β3-subunit in the nuclear factor-κB ligand RANKL/Osteoprotegerin signaling components via G-protein–coupled receptors (20). Another signaling pathway that is apparently linked to G-protein–coupled receptors is the epidermal growth factor receptor pathway; crosstalk between G-protein–coupled receptors and epidermal growth factor receptor has recently been shown to substantially contribute to growth and invasion of HNSCC (21, 22). The various signaling pathways could also explain a different susceptibility to the applied treatment modalities such as radiosusceptibility or chemosusceptibility and therefore affect the clinical outcome. Treatment substantially differs between HNSCC and breast carcinoma, for which chemotherapy represents a powerful therapeutic tool. This point could further help to explain for the contradictive role of C825T in HNSCC and breast carcinoma.

However, due to the multiple G-protein–linked signaling pathways, the exact mode of action in terms of the prognostic effect of the 825T allele remains rather speculative but at the same time represents an interesting field for further investigations.

Conclusion. The results of the present study suggest that the C825T SNP of the *GNB3* gene represents a host-derived prognostic marker in HNSCC. As shown in univariate and multivariate analysis, particularly patients carrying the homozygous TT genotype are at a

Table 2. Multivariate analysis for relapse

Variable	Hazard ratio confidence interval (95%)	*P*
AJCC		
I	1*	
II	0.9 (0.4-2.0)	0.820
III	1.1 (0.5-2.3)	0.742
IVA	1.5 (0.8-2.8)	0.234
IVB+IVC	2.6 (1.2-5.9)	0.020
Tumor localization		
Larynx	1*	
Oropharynx	1.1 (0.7-1.8)	0.578
Hypopharynx	2.4 (1.4-4.1)	0.001
Oral cavity	0.2 (0.6-1.1)	0.072
Multiple sites	1.5 (0.7-3.4)	0.305
Gender		
Female	1*	
Male	2.1 (1.1-3.7)	0.015
Smoking		
No	1*	
Yes	1.5 (0.7-3.1)	0.320
Age	0.9 (0.9-1.0)	0.054
Grade		
1	1*	
2	3.8 (0.9-15.8)	0.062
3-4	3.0 (0.7-12.8)	0.143
GNB3		
CC	1*	
CT	1.1 (0.8-1.6)	0.507
TT	2.6 (1.4-4.8)	0.002

NOTE: Multivariate Cox proportional hazard model for relapse in 341 patients with HNSCC.
*Reference.

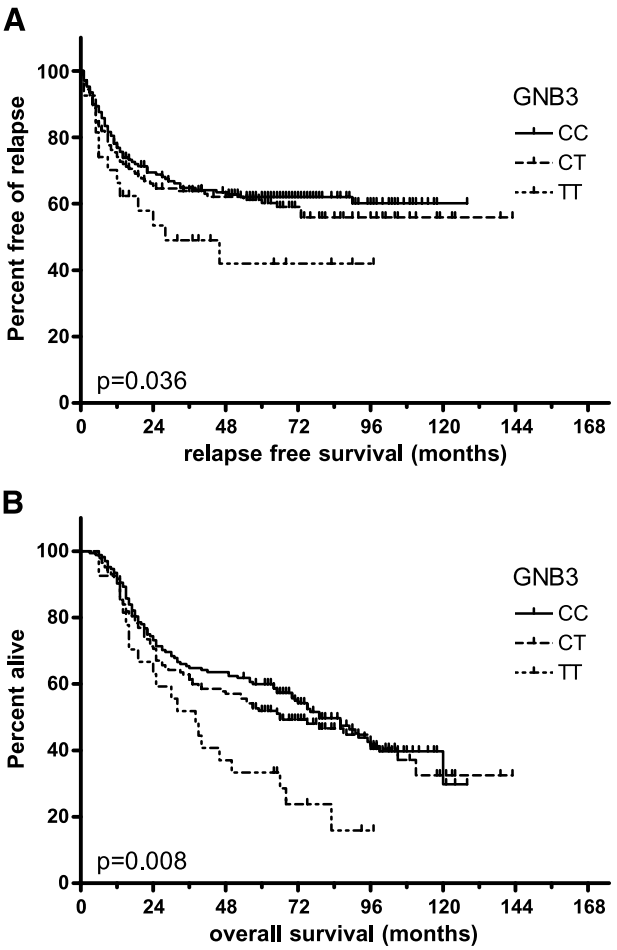

Figure 2. A and **B.** Relapse-free survival (**A**) and OS (**B**) Kaplan-Meier curves for 341 patients with HNSCC based on C825T genotypes. *P* values are derived from log-rank tests for trend and are adjusted for tumor localization.

Table 3. Multivariate analysis for death

	Hazard ratio confidence interval (95%)	*P*
AJCC		
I	1*	
II	1.5 (0.8-3.0)	0.254
III	2.3 (1.2-4.4)	0.010
IVA	3.5 (1.9-6.2)	<0.001
IVB+IVC	4.8 (2.2-10.6)	<0.001
Tumor localization		
Larynx	1*	
Oropharynx	1.4 (1.0-2.1)	0.080
Hypopharynx	3.2 (2.0-5.2)	<0.001
Oral cavity	1.3 (0.6-2.6)	0.427
Multiple sites	1.8 (0.9-3.6)	0.091
Gender		
Female	1*	
Male	2.5 (1.5-4.2)	<0.001
Smoking		
No	1*	
Yes	1.5 (0.8-2.1)	0.171
Age	1.1 (1.0-1.1)	0.078
Grade		
1	1*	
2	1.2 (0.6-2.6)	0.563
3-4	1.0 (0.4-2.2)	0.910
GNB3		
CC	1*	
CT	1.1 (0.8-1.4)	0.586
TT	2.6 (1.6-4.3)	<0.001

NOTE: Multivariate Cox proportional hazard model for death in 341 patients with HNSCC.
*Reference.

significantly higher risk both for tumor relapse and death. Determination of the genotype of the C825T SNP can thus be proposed as a potential biomarker to guide a more individualized therapy in HNSCC patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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