Lack of Evidence for Predictive and Prognostic Value of Cyclin D1 Gene Polymorphism *CCND1* G870A for Oral Squamous Cell Carcinoma

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Abstract. *Background/Aim: Cyclin D1 gene (CCND1) has a G to A polymorphism at the splice donor site of exon 4, position 870. The A allele codes for a truncated variant, cyclin D1b, which may have higher transforming activity. Data regarding the predictive and prognostic value of the CCND1 G870A polymorphism in tumors are controversial. We aimed to examine this polymorphism in patients with oral carcinoma. Materials and Methods: Genotyping of CCND1 G870A was determined by means of direct sequencing in 83 patients with oral carcinomas and in 102 healthy controls. Association with clinical outcomes was evaluated statistically. Results: We failed to find any significant association of CCND1 G870A with risk of oral carcinomas in this German population, with clinical and pathological features of the tumours or with overall survival of the patients. Conclusion: Our results suggest that CCND1 G870A has no, or only very limited, predictive and prognostic value for oral carcinoma.*

More than 90% of oral malignant neoplasms are squamous cell carcinomas (OSCC), which are among the ten most frequent malignancies in humans and this entity is the eighth leading cause of cancer-related death worldwide (1). Development and progress of OSCC is a multistep process involving various factors, while individual genetic susceptibility is also believed to play an important role. Growing knowledge regarding molecules that control the cell cycle and apoptosis is expected to contribute to the identification of new therapy targets. Cyclin D1, a 45-kDa protein encoded by the cyclin D1 gene (*CCND1*) on 11q13, is one such molecule. Cyclins are a group of proteins that play a key role in the control and regulation of the cell cycle. They are able to build complexes with cyclin-dependent kinases (CDKs) and regulate their activity (2). The activation of the kinase function is then followed by a cascade of protein phosphorylations that promotes the transition through the cell-cycle checkpoint from the G_1 phase into the S phase (3). Expression of cyclin D1 has been reported to be a strong predictor for the clinical outcome of OSCC (4), and amplification of *CCND1* was found significantly more frequently in patients with OSCC than in healthy controls (5), suggesting the predictive and prognostic value of this molecule and its potential as a therapeutic target. However, other studies failed to confirm such associations (6).

CCND1 has a G to A polymorphism at nucleotide position 870, which is the splice donor site of exon 4 (7). The G allele represents the conserved consensus splice donor site, while the A allele is not recognized in the splicing process, leading to altered transcriptions resulting in the production of a truncated protein. This truncated variant, cyclin D1b, does not have the part which is required for export from the nucleus into other cell localizations and therefore has higher transforming activity (8). A predictive and prognostic value of the *CCND1* G870A polymorphism has been reported by some studies while not by others (9-11).

Since the previous findings were of explorative nature, we typed *CCND1* G870A in a total of 83 oral carcinoma patients of our own collective and 102 healthy controls, and evaluated its predictive and prognostic value.

Materials and Methods

Patients and clinical data. This study was approved by the local Ethics Committee of the Chamber of Physicians in Hamburg, Germany. A written informed consent was obtained from all patients for use of their peripheral blood samples. For this study,

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Table I. *Allelic frequency of the cyclin D1 (CCND1) G870A polymorphism in patients with oral squamous cell carcinoma (OSCC) and in healthy controls.*

		CCND1 G870 genotype		
Group	No. of cases	AA	AG	GG
OSCC patients	83	15 (18%)	55 (66%)	13 (16%)
Healthy controls	102	26 (26%)	56 (55%)	20 (20%)
Odds ratio (95% confidence interval)		1.0	1.7 (0.8-3.6)	1.1 (0.4-2.9)
p-Value			0.16	0.80

Table II. *Clinicopathological characteristics of patients with oral squamous cell carcinoma (OSCC) by cyclin D1 (CCND1) G870A genotypes. Staging according to TNM system (13).*

		CCND1 G870A genotype			Correlation
Clinicopathological characteristics	No. of cases	AA	AG	GG	*p*-Value
Total	83	15 (18%)	55 (66%)	13 (16%)	
Male	54	11	34	9	
Female	29	4	21	4	
Tumour staging					0.78
1	34	7	21	6	
2	23	3	17	3	
3	8	1	7	0	
4	18	4	10	4	
Lymph node staging					0.39
0	50	11	33	6	
1	18	2	14	2	
2	14	2	7	5	
3	1	0	1	0	

83 patients with OSCC who underwent surgery at the Department for Oral and Maxillofacial Surgery at the University Medical Center Hamburg-Eppendorf between May 1989 and September 2005, and 102 cancer-free individuals who were randomly selected from an anonymized database, were chosen retrospectively. The mean age of the OSCC patients was 60 years, while that of the controls was not obtained. OSCC was confirmed by histological evaluation in all cases. The resection margins were tumour-free on histological examination of the surgical specimen and no patient had evidence of distant metastasis. Lymph node status at the time of diagnosis was evaluated by ultrasound, computed tomography and magnetic resonance tomography imaging, or acquired by histological determination following surgery. Tumour stage and grade were re-classified according to the most recent TNM classification of the International Union against Cancer (UICC). Local relapse, lymph node or distant metastases were considered as events in Kaplan-Meier relapse-free survival analysis. The overall follow-up period ranged from 2 to 230 months, with a median of 20 months.

Genotyping of the CCND1 G870A polymorphism. Genomic DNA was isolated from peripheral blood of patients and controls using a QIAamp Blood Tissue Kit (Qiagen, Hilden, Germany). The *CCND1* G870A polymorphism in exon 4 was determined by sequencing the 234-bp fragment amplified by polymerase chain reaction (PCR) using primers GTGAAGTTCATTTCCAATCC and TTGGCAC CAGCCTCGGCATTTC. Sequencing was carried out using a BigDye Terminator Sequencing Kit (Perkin-Elmer, Foster City, USA) and a nested primer, CATTTCCAATCCGCCCTCCA.

Statistical analysis. For statistical analysis, SPSS® (Version 13.0, IBM, Armonk, NY, USA) for Windows® (Microsoft Corp., Richmond, WA, USA) was used. Survival of patients were plotted on a Kaplan-Meier curve and analyzed using the log-rank test. Cox regression analysis was used for multivariate analysis to assess the independent influence of the *CCND1* G870A polymorphism simultaneously with other covariates. The value of *p* for statistical significance was set to less than 0.05.

Results

The genotype distribution of *CCND1* G870A of the 83 patients with OSCC did not differ significantly from those of the 102 healthy controls (Table I). For both groups, heterozygous G870A was the most frequent genotype.

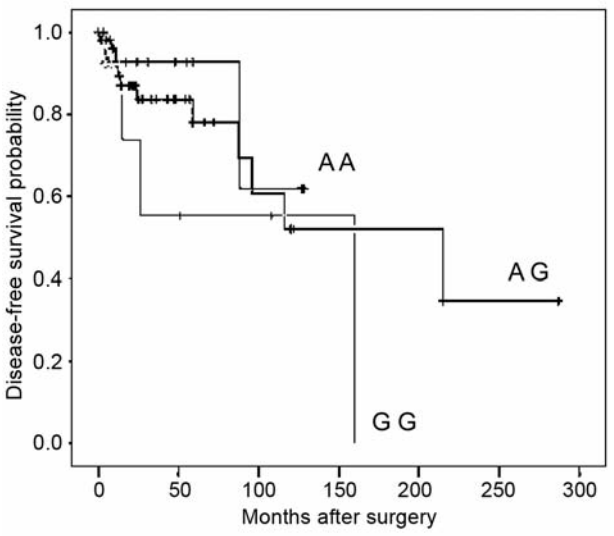

Figure 1. *Kaplan-Meier survival curves of the three patient groups, each with one of the three cyclin D1 (CCND1) G870 genotypes.*

No correlation was found between the *CCND1* G870A genotypes and tumour invasion depth, lymph node metastasis, histological grading (Table II), and patient survival (Figure 1).

Discussion

Results of studies addressing the possible predictive value of the *CCND1* G870A polymorphism for oral carcinomas are controversial (11). In the present study, we did not find any evidence for any predictive or prognostic value of the *CCND1* G870A polymorphism for patients with OSCC. The increasing number of negative findings may suggest that CCND1 does not play a major role in oral carcinomas, or transforming activity of CCND1 is independent of this polymorphism, or the effect of the polymorphism is so subtle so that it cannot be detected in such a study, with a moderate sample size of 83.

Our results are confirmed by one recent meta-analysis on the impact of G870A polymorphism and oral carcinoma risk (11), but are in contrast to the results of a second analysis of the same kind (9). Both groups retrieved original research data from public databases published predominantly in the English language (9, 11). Both studies, even though applying different inclusion criteria for evaluation of original study data, and therefore had only partial overlap of eligible studies, applied consistent analytic tools to explore the impact of *CCND1* G870A polymorphism on the risk of oral cancer. Zhou *et al*. (11) excluded any association of ethnicity and G870A polymorphism and oral cancer. However, Wang *et al*. (9) revealed the association between the GG genotype and oral cancer in Asians with respect to both the homozygote comparison and the dominant genetic model. These authors concluded that the *CCND1* G870A polymorphism was associated with increased risk of developing oral squamous cell carcinoma in an Asian population (9). Our study is based on patients of Caucasian origin that were referred to the oral and maxillofacial Department from local centers of the Hamburg area. Therefore, the ethnic impact in *CCND1* G870A polymorphism on oral cancer should be investigated in further studies.

Another study revealed the impact of treatment on survival dependent of genotype in head and neck SCC (Zhong *et al*. 2011). Whereas in stage III-IV head and neck SCC (HNSCC) treated with radiation survival was indistinguishable between *CCND1* 870G homozygotes and patients with at least one 870A allele, those who were not treated with radiation, however, showed better overall survival in *CCND1* 870G homozygotes than in patients with at least one 870A allele (12). However, this study did not discriminate the different sites of origin contributing to the umbrella term "HNSCC" and therefore did not take into account the noteworthy differences in etiology, pathogenesis, and prognosis (12).

Negative findings are generally less valued by researchers, journals and readers. However, they are just as important as positive ones since selectively publishing positive results leads to severe publication bias. We, therefore, believe that our negative finding in this study contributes to a balanced and reliable dataset regarding the role of *CCND1* G870A polymorphism in OSCC.

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