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Research Article

Deletion of 2q21 Characterizes a Small Subgroup of Aggressive ERG-Negative Prostate Cancers

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Abstract

Purpose: Deletions of 2q21 are known to occur rarely in prostate cancer but the clinical relevance is still unclear.

Methods: To evaluate the clinical relevance of this deletion, a tissue microarray including more than 12,000 prostate cancers with clinical follow-up data was analyzed by dual labeling in-situ hybridization employing probes for 2q21 and centromere 2.

Results: Deletions of 2q21 were found in 9% of 5,945 analyzable cancers. 2q21 deletions were linked to unfavorable tumor phenotype, including advanced tumor stage ($p=0.0032$), high Gleason score ($p<0.0001$), increased cell proliferation ($p<0.0001$), elevated preoperative PSA levels ($p<0.0001$), and early PSA recurrence ($p=0.0002$). 2q21 deletions were more frequent in ERG-negative than in ERG-positive cancers. Only 6% of ERG-positive cancers were 2q21-deleted, while 12% of ERG-negative cancers harbored 2q21 deletions. Separate analyses of ERG-positive and ERG-negative cancers revealed that all associations between 2q21 deletions and unfavorable tumor phenotype or prognosis were driven by ERG-negative cancers. In ERG-negative cancers - but not in ERG-positive tumors or in the entire patient cohort - the prognostic impact of 2q deletions was independent from established pre- and postoperative prognostic parameters ($p\leq0.05$).

Conclusion: In summary, our findings identify 2q deletion as an independent prognostic marker in the subgroup of ERG-negative prostate cancers. As FISH is precise analysis method delivering yes/no answers, it appears possible that 2q21 deletions may become a component for future multiparametric prognostic tests panels.

Keywords: 2q deletion; ERG; Prostate cancer; Tissue mircoarray

Abbreviations: aCGH: Array-Based Comparative Genomic Hybridization; CGH: Comparative Genomic Hybridization; cT: Clinical Tumor Stage; FISH: Fluorescence In-Situ Hybridization; IHC: Immunohistochemistry; Ki67LI: Ki67 Labeling Index; LOH: Loss of Heterozygosity; NGS: Next Generation Sequencing; pN: Pathological Lymph Node Status; PSA: Prostate-Specific Antigen; pT: Pathological Tumor Stage; TMA: Tissue Microarray

Introduction

Prostate cancer is the second most common cancer worldwide with nearly 1,3 million diagnosed cases in 2018 and the fifth leading cause of cancer death [1]. Most prostate tumors grow slowly and may need minimal or even no treatment. However, some cancers are highly aggressive, spread quickly, and treatment is essential [2-4]. Established pretreatment prognostic parameters such as Gleason grade and tumor extent on biopsies, are statistically powerful but not always sufficient for optimal individual treatment decisions. As screening strategies identify prostate cancers already

at early stages of the disease, it becomes increasingly important to avoid overtreatment of patients with less aggressive disease [1]. Accordingly, it will be important to establish molecular markers enabling distinction between indolent and aggressive forms of the disease. Deletions of variable size at multiple chromosomal loci are a hallmark of prostate cancer. Frequently deleted regions for example include 5q21 (CHD1), 6q, 8p, 10q23 (PTEN), 16q, and 17p13 (TP53). Studies have shown that deletions in these regions occur in 10% to 40% of all prostate cancers and that all of them are strongly associated with an aggressive phenotype [5-12]. Deletions of chromosome 2q have been reported to occur in 2% to 42% in studies examining 20 to 622 prostate cancers by Array-Based Comparative Genomic Hybridization (aCGH), classical CGH, and Loss Of Heterozygosity (LOH) analysis [11-20]. Published data mapping 2q deletions described a small commonly deleted region on 2q21 [12,21-23]. Only one study analyzed the prognostic impact of 2q deletions but found no relationship to patient prognosis in a series of 20 prostate cancers [16]. To clarify the clinical relevance of 2q deletion in prostate cancer, a prostate cancer Tissue Microarray (TMA) with more than 12,000 prostate cancers with available follow-up data was analyzed by Fluorescence In-Situ Hybridization (FISH) using probes for 2q21 and centromere 2.

Materials and Methods

Patients. Radical prostatectomy specimens were available from 12,427 patients, undergoing surgery between 1992 and 2012 at the Department of Urology and the Martini Clinic at the University Medical Center Hamburg-Eppendorf. Histo-pathological data was retrieved from the patient files, including tumor stage, nodal stage and stage of the resection margin. In addition to the traditional Gleason categories, "quantitative" Gleason grading was performed as described before [24]. Follow-up data were available for a total of 11,665 patients with a median follow-up of 36 months (range: 1 to 241 months; Table 1). Prostate-Specific Antigen (PSA) values were measured following surgery and PSA recurrence was defined as the time point when postoperative PSA was at least 0.2 ng/ml and increasing at subsequent measurements. All prostate specimens were diagnosed according to a standard procedure, including complete embedding of the entire prostate for histological analysis [25]. The TMA manufacturing process was described earlier in detail [26]. In short, one 0.6 mm core was taken from a representative tissue block from each patient. The molecular database attached to this TMA includes data on ERG expression in 10,678 [27,28], ERG rearrangement by FISH analysis in 7,099 (extended from [27,28]), and cell proliferation measured by Ki67 labeling index (Ki67LI) in 7,008 (extended from [29]) cancers.

	No. of patients (%)	
	Study cohort on TMA (n=12427)	**Biochemical relapse among categories**
Follow-up (mo)		
n	11665 (93.9%)	2769 (23.7%)
Mean	48.9	-
Median	36.4	-
Age (y)		
≤50	334 (2.7%)	81 (24.3%)
51-59	3061 (24.8%)	705 (23.0%)
60-69	7188 (58.2%)	1610 (22.4%)
≥70	1761 (14.3%)	370 (21.0%)
Pre-operative PSA (ng/ml)		
<4	1585 (12.9%)	242 (15.3%)
4-10	7480 (60.9%)	1355 (18.1%)
10-20	2412 (19.6%)	737 (30.6%)
>20	812 (6.6%)	397 (48.9%)
pT category (AJCC 2002)		

pT2	8187 (66.2%)	1095 (13.4%)
pT3a	2660 (21.5%)	817 (30.7%)
pT3b	1465 (11.8%)	796 (54.3%)
pT4	63 (0.5%)	51 (81.0%)
Gleason grade		
≤3+3	2848 (22.9%)	234 (8.2%)
3+4	6679 (53.8%)	1240 (18.6%)
3+4 Tert.5	433 (3.5%)	115 (26.6%)
4+3	1210 (9.7%)	576 (47.6%)
4+3 Tert.5	646 (5.2%)	317 (49.1%)
≥4+4	596 (4.8%)	348 (58.4%)
pN category		
pN0	6970 (91.0%)	1636 (23.5%)
pN+	693 (9.0%)	393 (56.7%)
Resection margin status		
Negative	9990 (81.9%)	1848 (18.5%)
Positive	2211 (18.1%)	853 (38.6%)
NOTE: Numbers do not always add up to 12427 in the different categories because of cases with missing data. Abbreviation: AJCC, American Joint Committee on Cancer.		

Table 1: Patient Cohort.

Fluorescence in-situ hybridization. Four micrometer TMA sections were used for FISH. For proteolytic slide pretreatment, a commercial kit was used (paraffin pretreatment reagent kit; Abbott, Chicago, USA) TMA sections were deparaffinized, air-dried, and dehydrated in 70%, 85%, and 100% ethanol, followed by denaturation for 5 min at 74˚C in 70% formamide 2x SSC solution. The FISH probe set consisted of a spectrum-green labeled 2q21 (*CCNT2*) probe (made from a mixture of BAC RP11-355K12 and BAC RP11-383L16), and a commercial spectrum-orange labeled centromere 2 probe (#06J36-027; Abbott, Chicago, USA) as a reference. Hybridization was overnight at 37˚C in a humidified chamber. Slides were subsequently washed and counterstained with 0.2µmol/L 4'-6-diamidino-2-phenylindole in antifade solution. Stained slides were manually interpreted with an epifluorescence microscope, and the predominant FISH signal numbers were recorded in each tissue spot as follow: Homozygous 2q21 deletion: complete absence of 2q21 FISH probe signals in ≥60% of tumor nuclei, with the presence of one or two 2q21 FISH signals in adjacent normal cells. Tissue spots with a lack of 2q21 signals in all (tumor and normal cells) or lack of any normal cells as an internal control for successful hybridization of the 2q21 probe were excluded from analysis. Heterozygous 2q21 deletion: presence of fewer 2q21 signals than centromere 2 probe signals of ≥60% tumor nuclei (Figure 1). These thresholds were based on a previous study [10].

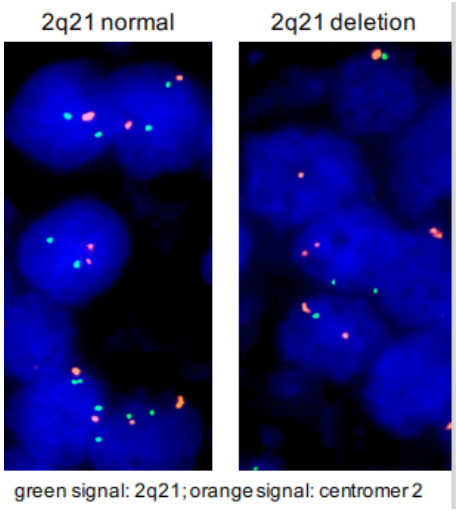

Figure 1: Example of 2q21 FISH findings.

Statistics: For statistical analysis, the JMP 14.0 software (SAS Institute Inc., NC, USA) was used. Contingency tables and Chi-square (Likelihood) tests were utilized to study the relationship between 2q21 deletion and categorical clinico-pathological variables. Kaplan Meier curves were generated for PSA recurrence free survival. The log-Rank test was applied to test the significance of differences between stratified survival functions. Cox proportional hazards regression analysis was performed to test the statistical independence and significance between pathological, molecular, and clinical variables.

Results

Technical aspects. 5,945 of 12,427 (47.8%) cancers were analyzable in 2q21 FISH. Analysis of the remaining 6,482 tumors was not successful because of weak or absent 2q21 and centromere 2 signals, lack of unequivocal tumor cells in the tissue spot, or missing tissue spots on the TMA section.

2q21 deletion and *TMPRSS2:ERG fusion*. Data on *TMPRSS2:ERG fusion* status obtained by FISH were available from 4,458 and by immunohistochemistry (IHC) from 5,208 tumors with evaluable 2q21 data. Data on both ERG FISH and IHC were available from 4,289 cancers, and an identical result was found in 4,121 (96%). Deletions of 2q21 were significantly linked to absence of the *TMPRSS2:ERG fusion*. 2q21 deletions were seen in 11.5% (IHC) and 11.9% (FISH) of cancers without *TMPRSS2:ERG fusion*, but found in only 6.3% (IHC) and 6.1% (FISH) of cancers with *TMPRSS2:ERG fusion* ($p<0.0001$ each; Figure 2). 2q21 deletion and cancer phenotype**.** 2q21 deletion was always heterozygous and found in 8.6% (n=514) of 5,945 analyzable prostate cancers. 2q21 deletions were strongly linked to adverse tumor features (Table 2), including advanced tumor stage ($p=0.0032$), high Gleason grade ($p<0.0001$), and increased preoperative PSA levels ($p<0.0001$). All statistical associations with unfavorable phenotype were still found in ERG-negative cancers ($p\leq0.02$), whereas in ERG-positive cancers only the Gleason grade ($p=0.0032$) was associated with 2q21 deletions (Table 2). 2q21 deletions were also linked to increased cell proliferation ($p<0.0001$; Table 3). This was again more prominent in ERG-negative ($p<0.0001$) than in ERG-positive cancers ($p=0.0333$).

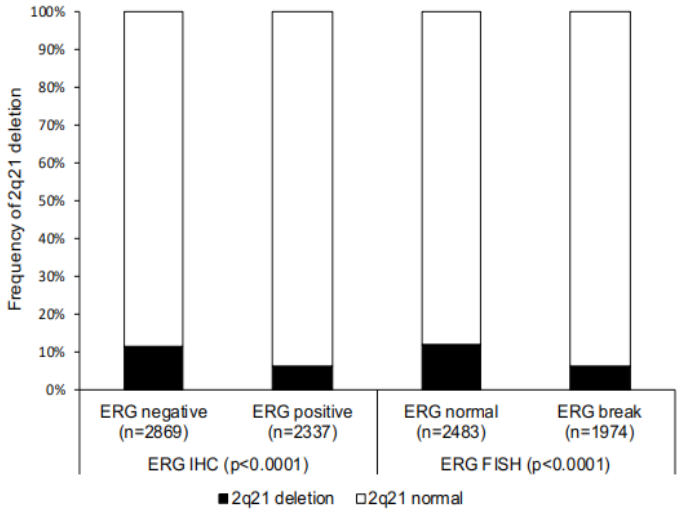

Figure 2: 2q21 deletion and *TMPRSS2:ERG fusion*.

	All cancers			ERG-negative cancers			ERG-positive cancers		
	n	**2q21 deletion (%)**	**p value**	**n**	**2q21 deletion (%)**	**p value**	**n**	**2q21 deletion (%)**	**p value**
	5945	8.6		2869	11.5		2337	6.3	
Tumor stage									
pT2	3781	7.7	0.0032	1907	10.2	0.0119	1352	5.6	0.1306
pT3a	1336	10.5		578	14.4		629	8.0	
pT3b-4	806	10.0		379	13.5		347	6.1	
Gleason grade									
≤3+3	1281	4.5	<0.0001	588	5.4	<0.0001	450	3.8	0.0354
3+4	3030	8.3		1440	10.7		1270	6.1	
3+4 Tert. 5	223	9.0		125	12.0		79	6.3	
4+3	560	13.9		293	19.1		217	9.2	
4+3 Tert. 5	346	14.5		172	18.0		144	10.4	
≥4+4	276	16.3		152	22.4		88	6.8	
Lymph node metastasis									
N0	3267	9.8	0.5636	1634	13.0	0.7797	1296	6.8	0.1436
N+	384	8.9		174	13.8		173	4.1	
PSA Level (ng/µl)									
<4	726	6.8	<0.0001	304	9.5	0.0078	315	6.0	0.2824
4-10	3536	7.8		1690	10.2		1403	5.8	
10-20	1197	11.3		634	14.8		434	7.1	
>20	415	12.1		217	14.3		155	9.7	
Surgical margin									
negative	4681	8.5	0.4148	2261	11.6	0.5475	1816	5.9	0.1787
positive	1147	9.2		559	10.7		473	7.6	

Table 2: 2q21 deletion and prostate cancer phenotype.

	2q21	numbers	Ki67LI (mean±SD)
all cancers (p<0.0001)	normal	3193	3.0±0.05
	deletion	340	4.1±0.1
ERG-negative cancers (p<0.0001)	normal	1633	2.8±0.1
	deletion	231	4.3±0.2
ERG-positive cancers (p=0.0333)	normal	1499	3.1±0.1
	deletion	106	3.6±0.2

Table 3: 2q21 deletion and cancer cell proliferation.

2q21 deletion and patient prognosis. Deletions of 2q21 were significantly associated with early biochemical recurrence in the subset of 5,322 cancers with available follow-up data (p=0.0002; Figure 3). This association was driven by the subset of ERG-negative cancers (p<0.0001), while 2q21 deletions lacked prognostic relevance in ERG-positive cancers (p=0.8548). However, 2q21 deletions lacked prognostic significance in subgroups of identical traditional or quantitative Gleason grade (Figure 4). This also applied to the subgroup of ERG-negative cancers (Figure 5).

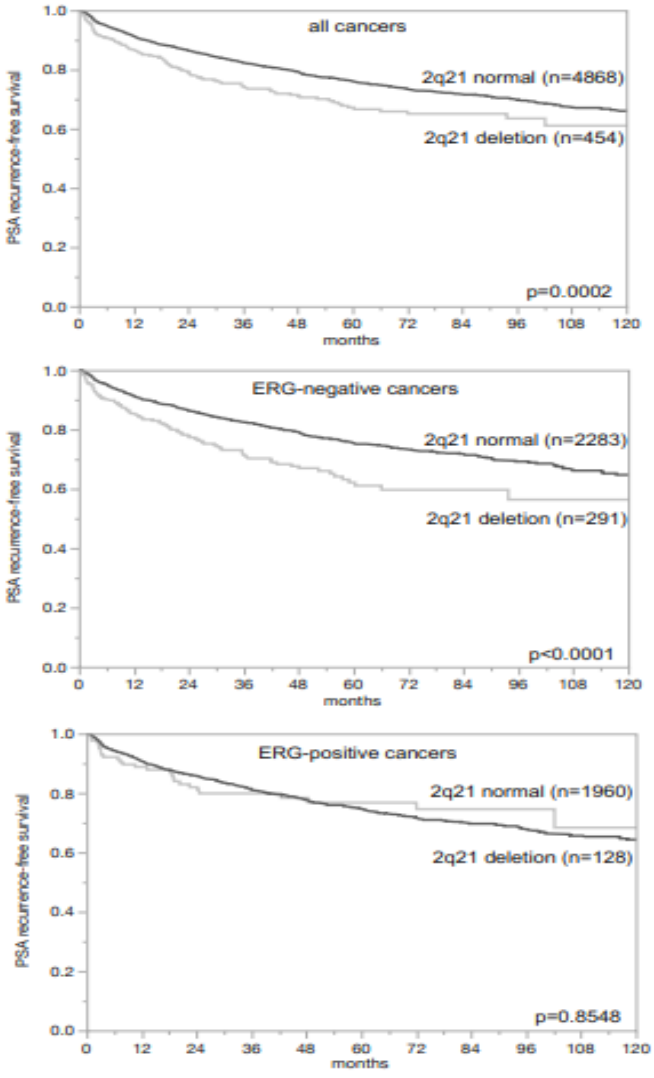

Figure 3: 2q21 deletion and patient prognosis.

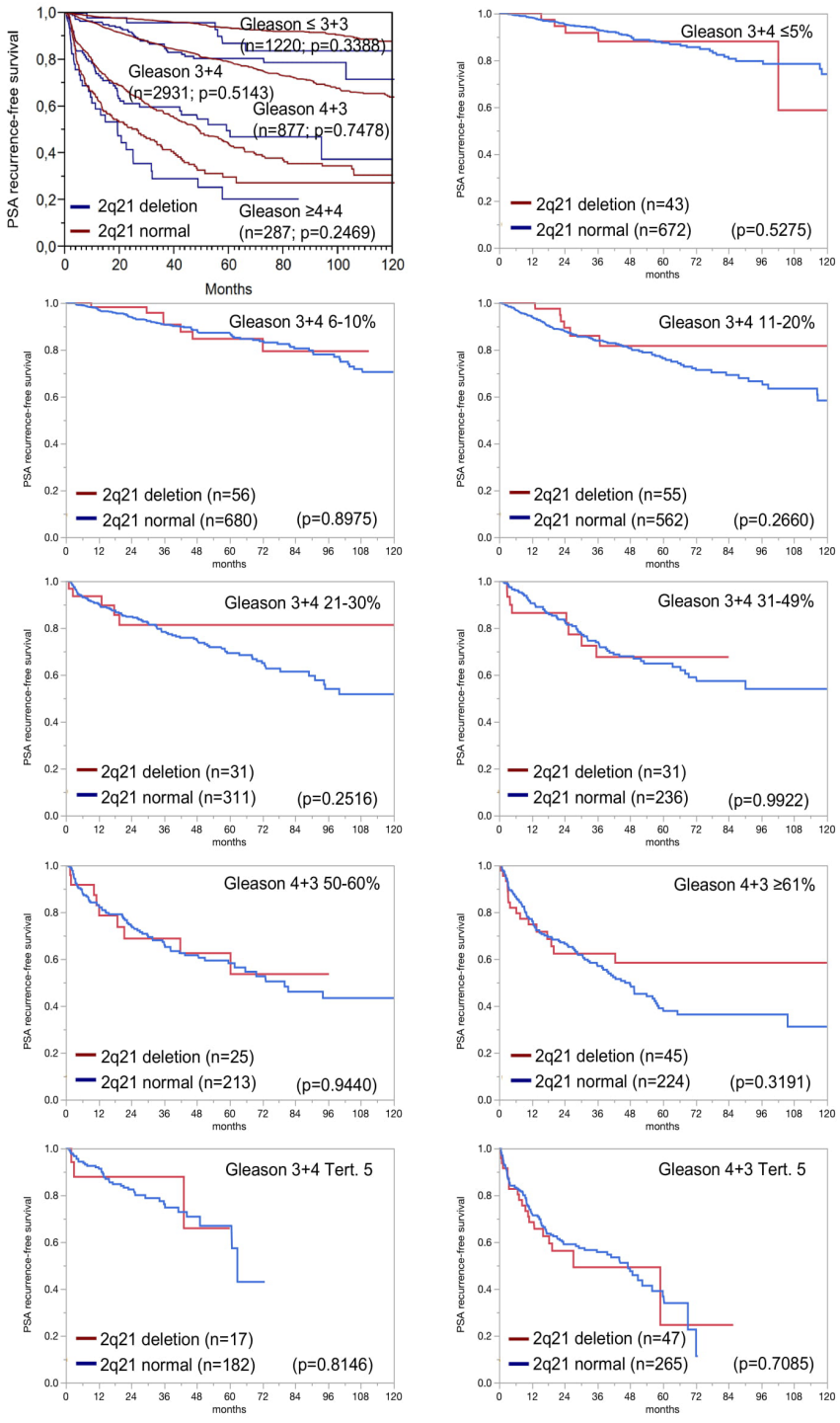

Figure 4: 2q deletion and patient prognosis in tumors with identical classical and quantitative Gleason grade groups.

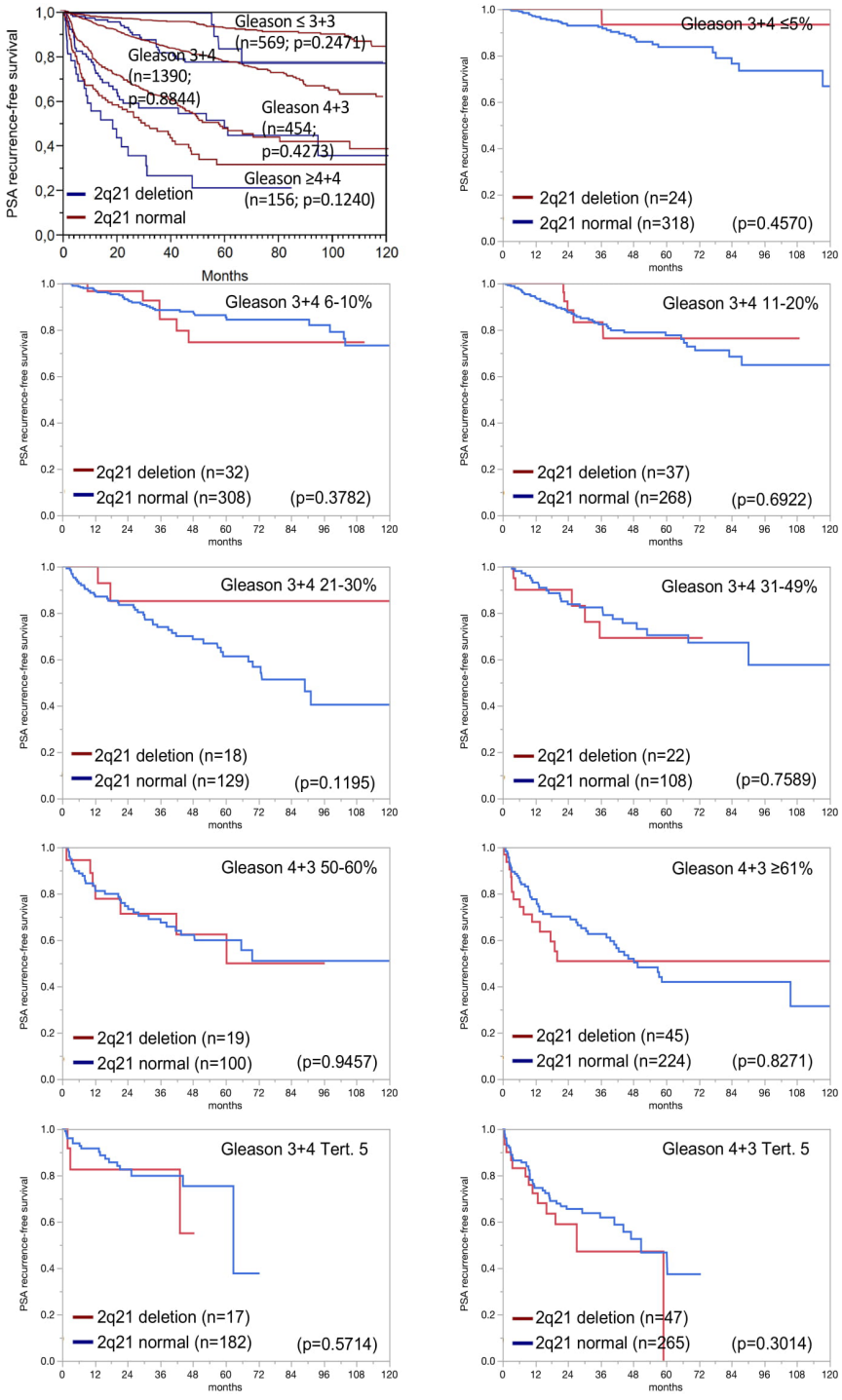

Figure 5: 2q deletion and patient prognosis in ERG-negative tumors with identical classical and quantitative Gleason grade groups.

Multivariate analyses. The prognostic relevance of 2q21 deletion was further assessed in four different multivariate analyses, including established pre- and postoperative prognostic parameters. Scenario 1 investigated the postoperatively available prognostic parameters pathological tumor stage (pT), pathological lymph node status (pN), surgical margin status, preoperative PSA value and prostatectomy Gleason grade. In scenario 2, nodal metastasis was excluded from the postoperatively available set of data, because lymph node dissection is not standardized and preferably applied in high-risk cancers, which may introduce a statistical bias. The next two scenarios were to model the preoperative situation to the best possible extent. Scenario 3 included deletion status of 2q21, preoperative PSA value, clinical tumor stage (cT) and Gleason grade obtained on the prostatectomy specimen. It is of note, that postoperative determination of a tumors Gleason grade is usually "better" than the preoperatively determined Gleason grade (subjected to sampling errors and consequently under-grading in more than one third of cases). Therefore, in scenario 4, the preoperative Gleason grade obtained on the original biopsy was combined with preoperative PSA value, cT and 2q21 deletion status. For all scenarios, 2q21 deletion predicted PSA recurrence independently in the subgroup of ERG-negative cancers ($p \leq 0.05$; Table 4).

	Scenario	analyzable (n)	p -value							
			preoperative PSA-Level	pT Stage	cT Stage	Gleason-grade prostatectomy	Gleason grade biopsy	N-Stage	R-Status	2q21 deletion
all cancers	1	3152	<0.0001	<0.0001		<0.0001		<0.0001	0.0714	0.4567
	2	5176	<0.0001	<0.0001		<0.0001			<0.0001	0.6102
	3	5115	<0.0001		<0.0001	<0.0001				0.7661
	4	5038	<0.0001		<0.0001		<0.0001			0.356
ERG-negative cancers	1	1577	<0.0001	<0.0001		<0.0001		0.0002	0.0497	0.0483
	2	2504	<0.0001	<0.0001		<0.0001			0.0023	0.0564
	3	2497	<0.0001		<0.0001	<0.0001				0.0201
	4	2468	<0.0001		<0.0001		<0.0001			0.0285
ERG-positive cancers	1	1262	0.0052	<0.0001		<0.0001		0.1062	0.9173	0.1113
	2	2032	0.001	<0.0001		<0.0001			0.2303	0.2309
	3	1983	<0.0001		<0.0001	<0.0001				0.2813
	4	1943	<0.0001		0.0001		<0.0001			0.2295

Table 4: Multivariate analysis.

Discussion

Our study shows that 2q deletions characterize a small subgroup of aggressive ERG-negative prostate cancers.

A FISH probe targeting *CCNT2* was used for 2q deletion analysis, because this gene is located in the center of the recurrently deleted 2q21 region that was thoroughly mapped in previous CGH, and aCGH studies [12,21-23]. The proportion of 2q21 deleted prostate cancers was 9% in our FISH analysis. This was lower than in earlier studies describing 2q21 deletions in 2%-42% by CGH [11,13-15,17], 29%-40% by aCGH [20,16,12,19], and 29% by LOH analysis [18]. The TCGA database containing data obtained by Next Generation Sequencing (NGS) describe 2q21 deletions in 14% of 818 analyzed prostate cancers [30]. It is of note, that FISH represents the gold standard for deletion detection. FISH enables a precise cell by cell analysis of the copy number of genomic regions of interest. FISH is not dependent on the presence and quantity of inflammatory or stroma cells. Some "false deletions" can, however, be assumed in FISH analyses as some signals are always lost due to truncated cell nuclei that are incompletely represented on a tissue slide measuring only 3-4 µm in thickness. A rigid cut-off of 60% of tumor cells having less 2q21 than centromere 2 signals was thus requested in this project to define 2q21 deleted tumors. This is based on the assumption that relevant intratumoral heterogeneity will not occur within a TMA spot measuring 0.6mm in diameter. In an earlier study, we had found a 100% concordance between FISH and array CGH data for identifying *PTEN* deletions using this definition for deletion [10].

Deletions of 2q21 were significantly linked to an adverse prostate cancer phenotype. The one previous study interrogating the prognostic role of 2q deletions only involved 20 cancers and could not find a link to poor outcome [16]. That deletions of 2q21 are related to poor patient prognosis is not surprising, however. Using the same prostate cancer TMA as in this study, our group had earlier investigated deletions of 3p13 [22], 5q21 [5], 6q15 [8], 8p21 [6], PTEN [10], 12p13 [31], 13q14 [32], 16q23 [9], TP53 [7] and 18q24 [33] and found that all of them were significantly linked to unfavorable tumor phenotype and poor prognosis. As for 2q21, a clear-cut target tumor suppressor gene of which inactivation drives tumor progression and poor prognosis is not known for

most of these deletions. It is assumed, that haplo-insufficiency is the most likely cause for the link between all these deletions and patient outcome. That a lack of one allele can contribute to tumor development even in case of an intact second allele has for example been shown for $p27^{Kip1}$, *TP53, DMP1, NF1*, and *PTEN* (reviewed in [34]). It is thus expected, that adequate function of many more genes requires presence of two intact gene copies [35]. Putative tumor suppressor genes on 2q for example include *FHL2* (2q12.2) [36], *RAB6C* (2q21.1) [37], *MCM6* (2q21.3) [38], *ZRNB3* (2q21.3) [39], *RPRM* (2q23.3) [40], *RIF1* (2q23.3) [41], *STK17B* (2q32.3) [42], *TMEFF2* (2q32.3) [43], *CASP8* (2q33.1) [44], *CD28* (2q32.2) [45], and *BOK* (2q37.3) [46]. Most likely, deletions result in cancer relevant dysfunctions of multiple genes. The larger a deletion is, the higher the number of potentially haplo-insufficient genes with a potential tumor suppressive role. Accordingly, it was shown for some chromosomal loci that prognosis deteriorates with increasing deletion length [47].

The *TMPRSS2:ERG* fusion represents the most common genetic alteration in prostate cancer and occurs in approximately 50% of tumors [27]. As a result of this gene fusion the transcription factor *ERG* comes under control of the androgen regulated *TMPRSS2* promoter and is expressed in prostate cancer cells [48]. The fusion results in overexpression of the transcription factor ERG which by itself has no prognostic relevance [27]. However, ERG modulates more than 1,600 genes in prostate epithelial cells [49,50]. Thus, the ERG status modifies the cellular microenvironment which might modify the prognostic impact of other molecular features as well as the likelihood of secondary events to occur. This particularly applies for chromosomal deletions most of which show a predilection for either ERG-positive or ERG-negative cancers. For example, *PTEN* and 3p deletions are linked to ERG positivity [10,12,22] and 5q and 6q deletions are tight to ERG negativity [5,8,51]. For 5q21 deletions, it was found that a reduced expression of Chromodomain-Helicase-DNA-Binding Protein 1 (CHD1) located at 5q21 abrogate androgen signaling and therefore impedes development of the *TMPRSS2:ERG* fusion [5]. It is possible that a similarly mechanism also exist for 2q deleted genes. The mechanisms for this are unknown. There are, however, 2q genes known to play a role in the regulation of the androgen receptor signaling such as *FHL2* and IL-1ß at 2q14 [52,53].

The prognostic role of 2q deletions was completely limited to ERG-negative cancers. In this subgroup, however, the influence on prognosis was strong and independent of established prognostic parameters irrespective of whether preoperatively or postoperatively available parameters were included into statistical analyses. This suggest a potential clinical utility of 2q deletion analysis in ERG-negative cancer. Multiple other molecular features have earlier shown to be prognostic either in ERG-positive [22,54,55] or in ERG-negative [56-58] prostate cancers but not in both molecularly defined subgroups. This observation limits the feasibility of one molecular test for prostate cancer prognosis that is equally applicable to all patients. That 2q deletions did not show prognostic impact in ERG-negative cancers with identical traditional and quantitative Gleason grade demonstrates the strength of the Gleason grading and shows how difficult it is for a molecular prognostic parameter to outperform tumor morphology. The Gleason score is the strongest preoperatively available prognostic parameter. However, Gleason grading suffers from interobserver variability of up to 40%, even between expert pathologists [59]. pT and pN category are not decisive for initial treatment decisions, because they can only be determined after surgery and depend on the thoroughness of the macroscopic and microscopic analysis [60]. Future prognostic biomarkers for prostate cancer must not only be independent of currently established factors but better reproducible and thus more reliable. In principle, FISH analysis is optimally suited for diagnostic testing as it provides unequivocal yes/no answers. A future prognostic FISH test - in particular if designed for ERG-negative cancers - may include a probe for 2q21 together with other frequently deleted loci.

Conclusion

In summary, the results of our study identify 2q21 deletion as a rare aberration in prostate cancer that preferably occurs in ERG-negative cancers and which is strongly linked to patient outcome in this patient subgroup.

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