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Specific Genomic Aberrations Predict Survival, But Low Mutation Rate in Cancer Hot Spots, in Clear Cell Renal Cell Carcinoma

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Abstract: Detailed genetic profiling of clear cell renal cell carcinoma (ccRCC) has revealed genomic regions commonly affected by structural changes and a general genetic heterogeneity. *VHL* and *PBRM1*, both located at chromosome 3p, are 2 major genes mutated at high frequency but apart from these aberrations, the mutational landscape in ccRCC is largely undefined. Potential prognostic information given by the genomic changes appears to depend on the particular cohort studied. We analyzed a Swedish ccRCC cohort of 74 patients and found common changes (loss or gain occurring in >20% of the tumors) in 12 chromosomal regions (1p, 3p, 3q, 5q, 6q, 7p, 7q 8p, 9p, 9q, 10q, and 14q). A poor outcome was associated with gain of 7q and losses on 9p, 9q, and 14q. These aberrations were more frequent in metastasized tumors, suggesting alterations of genes important for tumor progression. Sequencing of 48 genes implicated in cancer revealed that only *VHL*, *TP53*, and *PTEN* were mutated at a noticeable frequency (51%, 9%, and 9%, respectively). Shorter relative telomere length (RTL) has been associated with loss of specific chromosomal regions in ccRCC tumors, but we could not verify this finding. However, a significantly lower tumor/nontumor (T/N) RTL ratio was detected for tumors with losses in 4q or 9p. In conclusion, poor outcome in ccRCC was associated with gain of 7q and loss on 9p, 9q, and 14q, whereas the mutation rate overall was low in a screen of cancer-associated genes.

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Renal cell carcinoma (RCC) is an aggressive disease for which 20% to 30% of the patients have distant metastases at diagnosis. About 80% of all RCCs are represented by the clear cell type (ccRCC), characterized by allelic loss of chromosome 3p and inactivation of the tumor suppressor von Hippel-Lindau (*VHL*) gene on 3p25.3.[1] *VHL* was first identified in families where affected individuals were predisposed to develop a variety of neoplasms and RCC among others.[2] In familial RCC, as well as in sporadic ccRCC, it is common that one *VHL* allele is deleted, whereas the remaining allele has acquired an inactivating aberration rendering a complete loss of functional protein.[1] It has been speculated that additional tumor suppressors are present on chromosome 3p as loss of this arm is a frequent event in ccRCC and other tumors. Recently, mutations in *PBRM1* (3p21.1), *BAP1* (3p21.31), and *SETD2* (3p21.31) have been reported in 40%, 14%, and 3%, respectively, in ccRCCs.[3–6] Mutations in *UTX*, *JARID1C*, *MLL*, *MLL2*, and *MLL4* are also found at a low frequency, pointing to the importance of factors involved in histone and chromatin modulation.[3] The PI(3)K/AKT pathway is additionally emerging as a key player in ccRCC tumorigenesis.[7]

ccRCC demonstrates substantial genetic heterogeneity with large chromosomal regions affected by losses or gains. Loss on chromosomes 1p, 3p, 4q, 6q, 8p, 9p, 9q 10q, and 14q, and gain on 1q, 2q, 5p, 5q, 7q, 8q, 12p, and 20q are frequently reported.[8–13] These alterations can potentially give prognostic information. Monzon et al[11] reported gain of chromosome 8q and loss of 14q to be associated with decreased survival time. Loss of chromosomes 4p, 9p, and 14q were coupled to a poor prognosis, whereas loss of 3p was associated to a better prognosis in a study including 246 patients.[14] Further, loss on chromosomes 1p, 4, 9, 13q, and 14q and gain on 1q, 3q, and 8q were collectively recognized as genomic events coupled to decreased survival,[8] whereas loss on chromosomes 9p and 9q and gain on 7q and 20q were

correlated with poor survival.[15] Moreover, loss of chromosomes 19, 20, and 22 were associated to poor survival in a study by Antonelli et al.[16] Together, these studies indicate that the prognostic information given by different genomic alterations to a large extent appears to be cohort-dependent and more studies are needed to clarify genetic factors of more general importance for tumor progression.

To define the genetic constitution of our ccRCC cohort we genotyped the tumors using high-resolution genome-wide single-nucleotide polymorphism (SNP) arrays and detected alterations were correlated to stage and survival time. In a commercially available panel containing 48 genes, implicated to be altered in cancer, we aimed to find RCC-specific mutations by sequencing at high mean depths.

We and others have shown that telomeres in ccRCC are shorter compared with normal tissue.[17–19] Short telomeres are known to cause genetic instability and have been reported in ccRCC to be coupled to loss on specific chromosome arms.[20] We tested if relative telomere length (RTL) in our tumors was associated with loss of specific chromosomal regions.

MATERIALS AND METHODS

Patients

The patients were nephrectomized with histologically verified ccRCC at the Department of Urology, Umeå University Hospital, Umeå, Sweden. In total, 74 patients with sporadic ccRCC, diagnosed between 2001 and 2008, were included in the study (see Table 1 for clinical characteristics). Routine staging procedures included physical examination and computerized tomography of the abdomen and chest. Staging was performed according to the 2002 tumor-node-metastasis (TNM) classification system.[21] RCC type was defined according to the Heidelberg consensus conference.[22] Follow-up data were available for all patients. Of these, 31 had died of the disease, 10 of other causes, and 33 patients were alive with a median survival of 80 months (range, 57 to 127 mo). The study was approved by the regional ethical review board in Umeå (Dnr 07-071M), and each patient participated after providing informed and signed consent.

TABLE 1. Clinical Characteristics of the Tumors, n = 74

Sex	
Male	43
Female	31
Age (y)	
Male	67 (44-87)
Female	69 (49-84)
Follow-up time (mo)	65.5 (0-127)
Tumor diameter (mm)	80 (16-180)
TNM stage	
I	23
II	10
III	16
IV	25
Grade	
1	3
2	27
3	34
4	10

For age, follow-up time, and tumor diameter median values are shown with range shown within brackets. Grade is represented by morphological grade.

Samples

Samples from tumors and tumor-free kidney cortex obtained immediately after extirpation were snap-frozen in liquid nitrogen. Blood samples were collected before therapy, and DNA was extracted from fresh-frozen tumor, kidney cortex, and buffy coats using a BioRobot M48 Workstation with MagAttract technology as described elsewhere (Qiagen Inc., Valencia, CA).

SNP Array

Genotyping of 74 tumors was performed using the HumanCytoSNP-12 v2.1 beadchip arrays, including approximately 300,000 SNPs, according to manufacturer's protocol (Illumina Inc., San Diego, CA). For 22 of the 74 tumors, paired kidney cortex and peripheral blood samples were also genotyped. The signals were imaged on a BeadArray Reader and analyzed with Genome Studio v1.8 (Illumina Inc.). Log R ratios (LRR) and B allele frequencies (BAF) were used to identify regions of loss and gain. The LRR is a normalized measure of total signal intensity, meaning that LRR values decrease (LRR < 0) when a genomic region is lost and increase (LRR > 0) when a genomic region is gained. BAF is a measure of the allelic intensity ratio and BAF values cluster around 0, 0.5, and 1 for AA, AB, and BB genotypes, respectively. In heterogenous tumor tissues, a gain or loss will result in a split of the AB cluster. The SNP data set was deposited into the Gene Expression Omnibus database and is accessible through accession number GSE30460.

Mutation Analysis

Mutation screening was performed on 36 of these RCCs using a TruSeq Amplicon cancer panel (Illumina Inc.) targeting 48 genes implicated in cancer (Supplementary Table 1, Supplemental Digital Content 1, http://links.lww.com/AIMM/A47). Sequencing libraries were made according to manufacturer's protocol and sequenced on a MiSeq (Illumina Inc.) at an average depth of 218x. Corresponding kidney cortex from 5 tumors and peripheral blood from 2 geographically matched healthy volunteers were additionally included. The sequences were processed through MiSeq Reporter (Illumina Inc.) using a banded Smith-Waterman algorithm for alignment and Somatic Variant Caller for variant calling. The variants were subsequently visualized in Amplicon Viewer (Illumina Inc.). Nonsynonymous variants were detected using R[23] with genome assembly GRCh37. Variants found in dbSNP (database SNP, NCBI), volunteers, and the paired kidney cortex samples were filtered from the data set. The sequence data were uploaded to the Sequence Read Archive and is accessible through accession

number PRJEB3971. *VHL* sequencing of its 3 exons and adjacent intronic sequences was performed using primer pairs that have been previously published.[24] Polymerase chain reaction (PCR) amplification (with annealing at 58°C for all amplicons) and sequence analysis were performed as described elsewhere.[25] PCR reactions for exon 1 additionally contained 5% dimethyl sulfoxide.

Telomere Length Measurements

RTL was assessed by real-time PCR according to the method described by Cawthon[26] and has been published previously by us.[27] Telomere-to-Single copy gene (T/S) values for the samples were divided with the T/S value of a reference DNA included in each assay, generating RTL values. Samples were available for 70 tumors and corresponding kidney cortex tissues. Tumor/nontumor (T/N) RTL ratios were generated by dividing tumor RTL with normal kidney cortex RTL. Tumors with loss of >10% of a chromosome arm were categorized as the "loss" group when testing for differences in distribution of RTL ratios. This categorization was carried out according to Chen and colleagues as our intention was to test if we could replicate their findings.

Statistical Analyses

PASW statistics 18 (IBM, New York, NY) was used for statistical analyses. Survival analysis was performed using the Kaplan-Meier with the log-rank test. Cancer specific survival was defined as the time (in months) between the date of diagnosis to ccRCC-specific death or to the date of last follow-up (March 2012). Cross tabulation with the Pearson χ^2 tests were used to evaluate differences in distribution between tumor groups with and without chromosomal changes in relation to TNM stage, Fuhrman grade, and metastatic stage. Pearson χ^2 tests were also used to test for difference between patients with and without *VHL* mutations in relation to metastatic stage and the Fuhrman grade. The Kruskal-Wallis test was used to check for differences in number of altered chromosomes and number of mutations between TNM stage groups I-III and IV. Multivariate analysis with the Cox regression model including the chromosomal aberrations, TNM stage, and Fuhrman grade used the backward conditional method to test if the chromosomal aberrations could prognosticate the patients. Differences in distribution of RTL ratios between tumor groups with and without chromosomal changes were analyzed with the Mann-Whitney *U* test.

RESULTS

Characterization of Genomic Aberrations in ccRCC

We observed large chromosomal aberrations in 70 of 74 ccRCCs. The breakpoints of the aberrations could be defined with great precision because of the high resolution of the SNP array. Three tumors showed no genomic changes (denoted by arrows in Fig. 1). In one tumor, a region of 2 Mbp demonstrated a gain in copy number on chromosome 7q, also seen in the corresponding kidney cortex sample. Apart from that, no genomic change was detected in the 22 paired kidney cortex tissues or blood samples, confirming the dissection of histologically nontumorous kidney cortex tissues.

We identified 12 regions with common changes, defined as occurring in >20% of the ccRCCs. Loss of genetic material was found on chromosomes 1p (26%), 3p (88%), 3q (31%), 6q (28%), 8p (27%), 9p (24%), 9q (28%), 10q (22%), and 14q (39%). Gain of genetic material was detected on chromosome 5q (50%), 7p (26%), and 7q (26%). Minimum region of overlap ranged from 2.2 M base pairs to whole chromosomes (Table 2). A larger number of altered chromosomes was found in tumors with distant metastases (M1) compared with

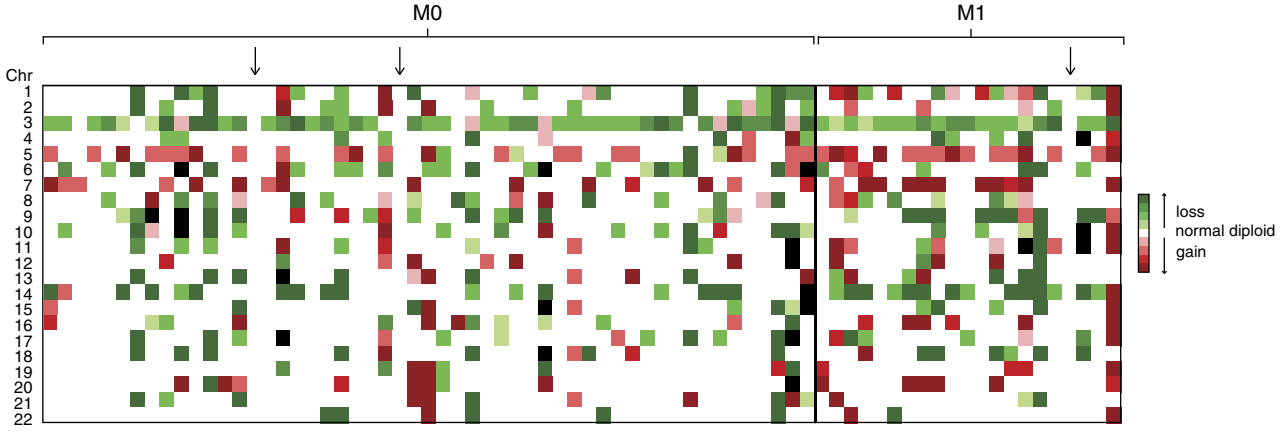

FIGURE 1. Heat map of tumors showing chromosomes affected by a genetic change. M0 (nonmetastatic) tumors are shown in the left pane and M1 (metastatic) in the right. The color gradients going from light green/red to dark green/red represents the extent of loss/gain on each chromosome. The darkest shading equals whole chromosome loss/gain and the palest shading represent more loss than gain/more gain than loss. Black color indicates undefined change. Arrows denote 3 tumors that showed no genomic alteration.

TABLE 2. Common Changes Detected in the ccRCC Tumors

Chromosome, Type of Change	% of Tumors	Minimum Region	Size (Mb)
1p, loss	26	25,334,666-32,100,024	6.8
3p, loss	88	tel-46,765,401	46.8
3q, loss	31	cen-97,267,562	2.2
5q, gain	50	172,012,494-tel	8.6
6q, loss	28	152,878,353-tel	17.9
7p, gain	26	Whole arm	57.4
7q, gain	26	Whole arm	97.6
8p, loss	27	12,653,559-27,797,474	15.1
9p, loss	24	tel-28,013,465	28.0
9q, loss	28	130,719,998-138,443,132	7.7
10q, loss	22	85,932,576-90,524,678	4.6
14q, loss	39	69,560,017-91,189,113	21.6

ccRCC indicates clear cell renal cell carcinoma.

nonmetastatic (M0) cases ($P = 0.04$) (Figure 1). No tumor had >8 common genomic changes.

Association of the Chromosomal Aberrations to Metastatic Stage and Survival Time

The Kaplan-Meier analysis with disease-specific survival as endpoint showed that TNM stage group III had shorter survival time than stage group I and II ($P = 0.006$). Stage group IV had further shorter survival time than stage group III ($P = 1e^{-6}$) (Supplementary Figure 1a, Supplemental Digital Content 2, http://links.lww.com/AIMM/A48). Grade 4 tumors presented shorter survival time than grade 3 ($P = 0.0003$), whereas no difference was found between grade 3 and 2 or grade 2 and 1 (Supplementary Figure 1b, Supplemental Digital Content 2, http://links.lww.com/AIMM/A48). The Kaplan-Meier analysis further showed significantly shorter survival for patients with tumors containing gain of the 7q region, or loss of the 9p, 9q, or 14q regions (log-rank $P = 0.008$, 0.0004, 0.0003, and 0.001, respectively) compared with cases without these aberrations (Figs. 2A–D). Losses of 1p, 3p, 3q, 6q, 8p, or 10q or gains of the 5q or 7p regions were not coupled to survival time.

Pearson χ^2 tests reached the level of significance for chromosomes 5q, 7p, 7q, 9p, 9q, and 14q when testing for differences in the distribution of genomic changes between M0 and M1 tumors (Table 3). The more advanced tumors (M1) demonstrated an accumulation of genetic changes compared with M0 tumors ($P < 0.00001$) (Table 3). Loss of genetic material on chromosome 3p is known to be an early event in ccRCC. Concordantly, we found no difference in distribution of 3p loss between M0 and M1 tumors ($P = 0.6$). The 7q, 9p, 9q, and 14q regions were also found more often in high TNM stage groups (III and IV) than in low stage groups (I and II) (Table 4). Furthermore, the 9p and 9q regions were found more often in grade 3 and 4 than in grade 1 and 2 tumors (Table 4).

The Multivariate Cox regression analysis using stepwise elimination showed that loss of the 9p region was an independent prognostic factor ($P = 0.04$) in patients without metastases, when including TNM stage, gain of 7q, losses of 9p, 9q, and 14q regions, respectively. When grade (1 + 2) versus (3 + 4) was included in the multivariate analysis (Table 5), loss on 9p presented borderline significance together with TNM stage. When including all patients, only TNM stage and tumor grade remained as independent significant factors ($P = 0.00002$ and 0.03, respectively), whereas loss of 9p lost its prognostic information ($P = 0.08$).

Sequencing of Cancer Hot Spot Genes

Deep sequencing of 48 cancer related genes revealed rather few mutations in the 36 ccRCCs tumors. One tumor was excluded from the analysis because of incorrect calling by the software. This could possibly be explained by poor DNA quality and/or the flexibility in the variant caller to allow for very low–frequency mutations. Nonsynonymous mutations were detected in *APC*, *CDKN2A*, *FGFR3*, *GNAS*, *IDH1*, *KIT*, *KRAS*, *PTEN*, *SMARCB1*, *SMO*, *TP53*, and *VHL* (Fig. 3). Mutations in *PTEN* and *TP53* were found in 3 (9%) tumors each, and mutations in *VHL* were found in 11 (31%) ccRCCs. No other gene showed mutations in >6% of the tumors. Three tumors lacking large genomic alterations are marked with arrows in Figure 3. Two of them showed no mutations at all and the third presented a mutation in *VHL*. The *VHL* mutations detected with the cancer panel were verified by the Sanger sequencing (see further below). There was no difference in the number of mutations in the M0 group compared with the M1 RCCs ($P = 0.5$) detected by this commercial NGS gene panel.

VHL Mutation Status

Tumors were defined as *VHL*-mutated if one allele was deleted and the second allele contained a nonsynonymous or frame-shift mutation. Mutations in *VHL* were detected in 37 of 72 (51%) analyzed ccRCCs, all of which were *VHL* mutation negative in corresponding kidney cortex or blood samples by sequencing. Eleven tumors were *VHL* wild type. There was no difference in survival time, stage, or Fuhrman grade based on *VHL* mutation status ($P = 0.7$, 0.4, and 0.1, respectively).

Association to Telomere Length

No significant associations were observed between short tumor RTL and loss of chromosomal regions, although a borderline significant P (0.06) was found for chromosome 3p. There were, however, significant differences for 2 of 13 regions when including tumor-to-nontumor (T/N) RTL ratio as a parameter. Significantly, lower T/N RTL ratios were detected for patients with loss on 4q and 9p ($P = 0.02$ and 0.05, respectively).

DISCUSSION

Gain of the q arm of chromosome 7 and losses on 9p, 9q, and 14q, each associated significantly to short survival in the present study. In total, we identified 12 common (occurring in >20% of the investigated ccRCCs) genomic regions on chromosomes 1p, 3p, 3q, 5q, 6q, 7p, 7q, 8p, 9p, 9q, 10q, and 14q with structural changes. Alterations

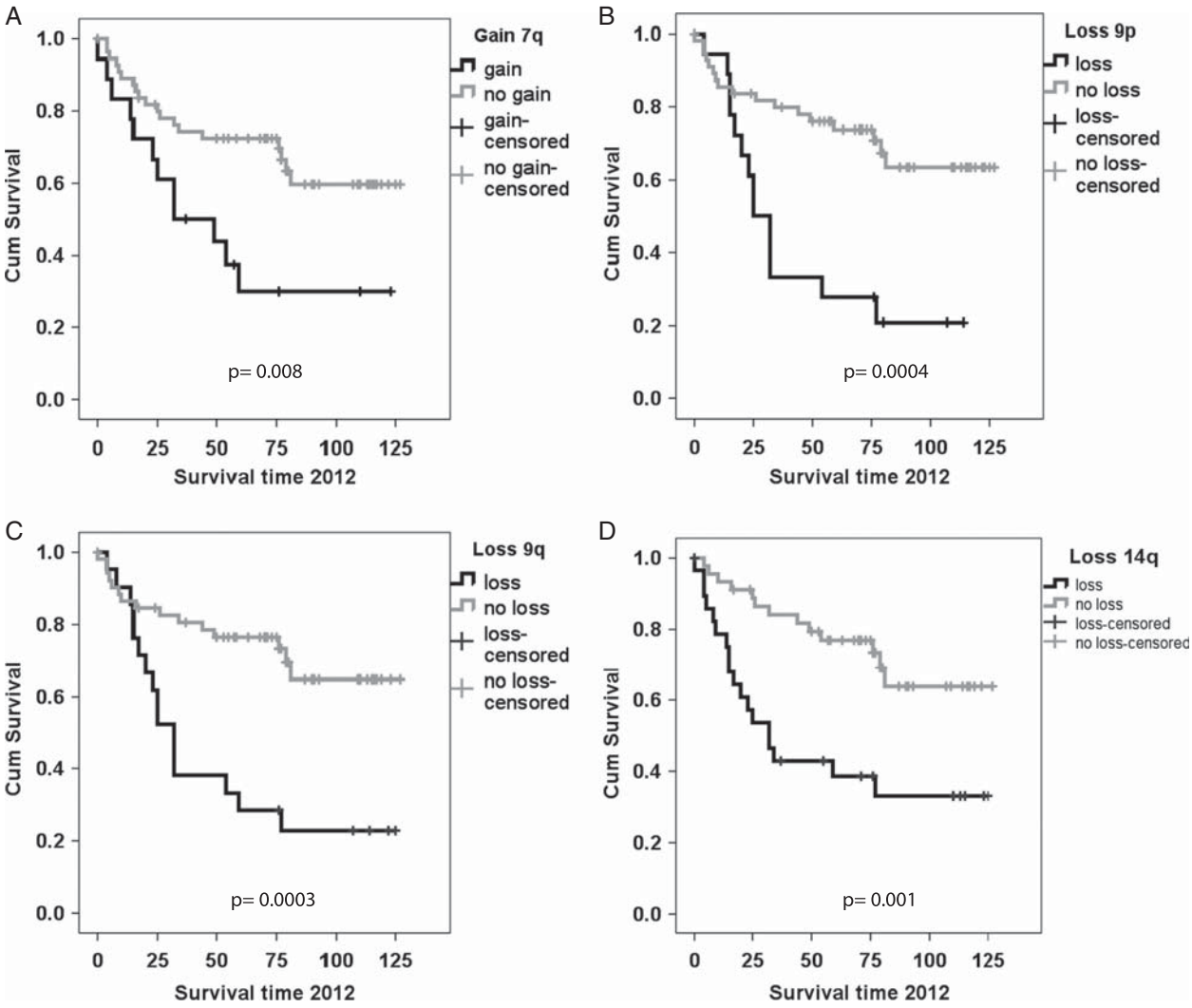

FIGURE 2. The Kaplan-Meier analysis with disease-specific survival as endpoint in relation to genomic alterations in the 7q, 9p, 9q, and 14q regions. Survival time is shown in months and log-rank *P*-values are presented. A–D, Tumors containing the given genomic alteration showed a significantly decreased survival time.

affecting these regions have been shown in previous investigations on ccRCC, but there is no overall consistent pattern in the literature.[10,12,13,20] There was a large variation in our ccRCCs, showing a spectrum from no altered chromosome in 3 cases to 1 tumor with changes on 17 chromosomes.

The associations found for specific chromosomal aberrations are in line with previously published studies also using genome wide approaches, especially concerning chromosomes 9p and 14q (Table 6), indicating these regions to be consistently important for ccRCC progression. We also found the same regions to be more

TABLE 3. Distribution of Genomic Aberrations in Relation to Metastatic Stage, n = 74

		5q		7p		7q		9p		9q		14q		ΣAberrations	
	n	n+	n−	n+	n−	n+	n−	n+	n−	n+	n−	n+	n−	n+	n−
M0	53	22	31	10	43	8	45	8	45	10	43	15	38	73	245
M1	21	15	6	9	12	10	11	10	11	10	11	14	7	68	58
P		0.02		0.03		0.003		0.003		0.004		0.002		< 0.00001	

Statistical analysis of differences between groups used the Pearson χ^2 analysis. Number of patients with genomic aberrations and without is showed as n+ versus n−. M0 indicates nonmetastatic tumors; M1, metastatic tumors.

TABLE 4. Distribution of Genomic Aberrations in Relation to TNM Stage and Fuhrman Grade, n = 74

		7q		9p		9q		14q	
	n	n+	n−	n+	n−	n+	n−	n+	n−
TNM stage I + II	37	5	32	5	32	6	31	7	30
TNM stage III + IV	37	13	24	13	24	15	22	22	15
P		0.03		0.03		0.02		0.0004	
Grade 1 + 2	30	6	24	2	28	3	27	9	21
Grade 3 + 4	44	12	32	16	28	18	26	20	24
P		0.5		0.003		0.004		0.2	

Statistical analysis of differences between groups used the Pearson χ^2 analysis. Number of patients with genomic aberrations and without is showed as n+ versus n−.

often affected in metastasized tumors, further strengthening this notion. For other genomic aberrations in ccRCC, the literature is heterogenous with different regions implicated for survival in different cohorts. One contradictory finding is , for example, gain on 5q reported as associated with longer survival,[29,31] whereas we found this aberration to be more frequent in the group of metastatic ccRCCs. The background for the various inconsistencies regarding survival is unclear but can be because of differences in study populations, treatment protocols, or the methodologies used for genomic analysis.

The *VHL* mutation status did not correlate to survival, TNM stage, or the Fuhrman grade. Our data confirm results in previous studies[32–34] and further strengthens the belief that deletion of chromosome 3p and VHL inactivation is an initiation event in the tumorigenesis of ccRCC. *VHL* can also be silenced by methylation, which could explain why only 51% of the cases presented mutations compared with rates up to 80%, which has been published.[35] Next to *VHL,* only *PBRM1* has been shown to be frequently mutated in ccRCC, a gene not present in the cancer panel used in this study. We used a commercially available sequencing panel to investigate the presence of mutations in genes commonly reported in cancer. Of the genes analyzed only *PTEN* and *TP53*, beside *VHL*, showed mutations to an apparent extent with mutations in >9% of the tumors. Mutations in *PTEN* and *TP53* have been reported in ccRCC at rates of 7% and 4% to 14%, respectively.[36–38] An interesting finding in our ccRCCs with *TP53* or *PTEN* mutations was that they also had the second allele deleted, implying that no functional protein was produced. This is likely to have been of importance for tumor progression in these particular patients.

Overall, the mutation rate was low in our screen of 48 cancer-associated genes. For instance, only one mutation was found in the gene encoding SMARCB1, which is a subunit of the SWI/SNF complex. As PBRM1 is a part of SWI/SNF, it could be speculated that *SMARCB1* would also present mutations to a significant extent. *CDKN2A*, located at 9p21, was mutated in only one

TABLE 5. Multivariate Cox Analyses of the 7q, 9p, 9q, and 14q Regions in Nonmetastatic Patients, n = 52

			95% CI for Odds Ratio	
	P	Odds Ratio	Lower	Upper
(a) TNM stage, tumor grade, and chromosomal aberrations in 7q, 9p, 9q, and 14q				
Step 1				
TNM (I + II) vs. (III + IV)	0.02	5.17	1.26	21.12
Grade (1 + 2) vs. (3 + 4)	0.05	0.19	0.04	1.00
Gain 7q	0.3	2.61	0.48	14.16
Loss 9p	0.3	2.58	0.39	17.22
Loss 9q	0.7	1.53	0.22	10.62
Loss 14q	0.4	0.53	0.12	2.46
Step 4				
TNM (I + II) vs. (III + IV)	0.02	4.20	1.22	14.40
Grade (1 + 2) vs. (3 + 4)	0.07	0.24	0.05	1.14
Loss 9p	0.05	3.58	1.00	12.86
(b) TNM stage, chromosomal aberrations in 7q, 9p, 9q, and 14q				
Step 1				
TNM (I + II) vs. (III + IV)	0.05	3.36	1.00	11.25
Gain 7q	0.4	1.81	0.39	8.32
Loss 9p	0.5	2.03	0.28	14.66
Loss 9q	0.4	2.08	0.31	14.18
Loss 14q	0.9	0.87	0.21	3.70
Step 4				
TNM (I + II) vs. (III + IV)	0.04	3.50	1.06	11.52
Loss 9p	0.04	3.57	1.03	12.30

Backward conditional Cox regression analysis of factors predicting survival in patients without distant metastases (M0, n = 52) including (a) and (b).

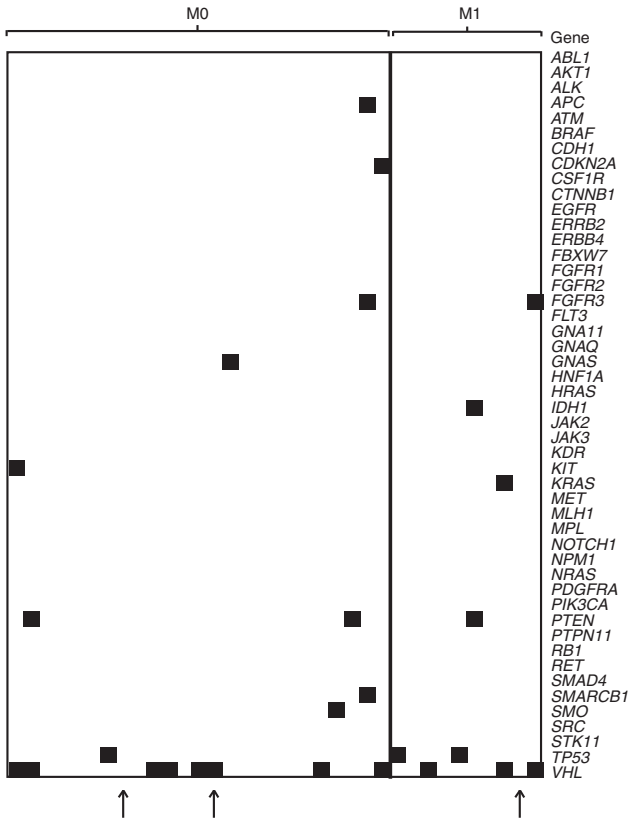

FIGURE 3. Nonsynonymous and frame-shift mutations found in 48 sequenced genes. M0 (nonmetastatic) tumors are shown in the left panel and M1 (metastatic) in the right. Black squares represent a detected mutation. Arrows denote the 3 tumors that showed no genomic alteration in the single-nucleotide polymorphism (SNP) array.

ccRCC, despite that lack of p16 expression has been reported in 53% of RCCs in another study.[39] For *FGFR3*, in contrast, we found mutations in 2 of 35, whereas no mutation was detected in a screen of 101 ccRCCs.[40] Thirty-six of the 48 genes screened did not show any nonsynonymous or frame-shift mutations at all, and the mutations detected were comparable between the M0 and M1 groups, suggesting that genes associated with tumor progression in ccRCC were absent in this panel.

Loss on 9p is the most common structural change with impact on survival in ccRCC. The 28 Mbp region on 9p deleted in our cohort contains *PTPRD*, *CDKN2B*, and *TUSC1*, all of which have been reported mutated and/or inactivated in several tumor types.[41–44] Screening of these genes may reveal inactivating mutations also in ccRCC. Preliminary gene expression data for our tumors further showed that *TUSC1* mRNA is down-regulated in tumors with allelic loss of *TUSC1*, $P = 0.00002$ (data not shown).

Two genes (*JAK2* and *CDKN2A*) in the cancer panel are located within the altered 9p region showing prognostic significance in multivariate analysis. However, only 1 mutation was demonstrated (in *CDKN2A*), indicating that aberrant expression of these genes did not contribute to the outcome of the patients. However, hypermethylation of *CDKN2A* is known to occur in RCC[39] showing that other regulatory mechanisms for gene expression might occur. Three tumors, with typical ccRCC appearance by histology, showed no large genomic alterations in the SNP arrays and only 1 harbored a *VHL* mutation. These 3 ccRCCs represent different grades (3 to 4), stages (I to IV), and had different survival times (26, 80, and 117 mo). It is likely that these RCCs contain genomic aberrations that remained undetected with the approaches used. DNA methylation of genes located within chromosome 7, the 9p, and 9q regions are reported in RCC[45] and gain in copy number per se is known to increase the expression of genes in ccRCC and copy-number loss decreases the expression.[9,13] Together, this suggests that more factors besides mutations have impact on the survival seen for our patients.

Short telomeres cause genetic instability and may inflate the amount of chromosome losses or gains. Chen et al[20] reported shorter telomeres in tumors with loss on chromosome arms 1p, 2q, 3p, 4q, 6p, 6q, 9p, 9q, 10q, 17p,

TABLE 6. Genome Wide Studies Presenting Association of Specific Chromosomal Changes to Survival in ccRCC

References	Study Size (n)	Method	Chromosomal Change Associated With Shorter Survival Time	Chromosomal Change Associated With Longer Survival Time
This study	74	SNP array	Gain 7q Loss 9p, 9q, 14q	
Girgis et al[28]	154	SNP array (meta-analysis)	Gain chr12 Loss 1p, 4, 9, 13q, 14q, 18	
Monzon et al[11]	85	SNP array	Gain 8q Loss 14q	
Sanjmyatav et al[15]	53	CGH array	Gain 7q, 20q Gain/loss 12, 16 Loss 9p, 9q	
Antonelli et al[16]	131	G-banding	Loss 19, 20, 22	
Klatte et al[14]	246	G-banding	Loss 4p, 9p, 14q	Loss 3p
Arai et al[8]	51	CGH array	Gain 1q, 3q, 8q Loss 1p, 4, 9, 13q, 14q	
Gunawan et al[29]	104	G-banding		Gain 5q
Moch et al[30]	37	CGH array	Loss 9p	

18p, 18q, and 22q. It can be hypothesized that deletions on 4q and 9p affect genes of importance for telomere maintenance as loss of these regions were found to be associated with short RTL by Chen and colleagues and with low T/N RTL ratio in the present study.

In conclusion, our study showed an association between structural chromosomal changes and the clinical course in patients with ccRCC. In total, 12 chromosomal regions, commonly affected by genetic changes, were identified in the tumors. Decreased survival time for patients with ccRCC was significantly associated to gain of 7q and loss on 9p, 9q, or 14q, respectively, highlighting the inconsistent findings between different ccRCC cohorts. Of 48 cancer implicated genes evaluated, only *VHL*, *PTEN*, and *TP53* showed mutations to an apparent extent.

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