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An Alternative Route for Multistep Tumorigenesis in a Novel Case of Hereditary Renal Cell Cancer and a t(2;3)(q35;q21) Chromosome Translocation

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Summary

Through allele-segregation and loss-of-heterozygosity analyses, we demonstrated loss of the translocation-derivative chromosome 3 in five independent renal cell tumors of the clear-cell type, obtained from three members of a family in which a constitutional t(2;3)(q35;q21) was encountered. In addition, analysis of the von Hippel–Lindau gene, *VHL,* revealed distinct insertion, deletion, and substitution mutations in four of the five tumors tested. On the basis of these results, we conclude that, in this familial case, an alternative route for renal cell carcinoma development is implied. In contrast to the first hit in the generally accepted two-hit tumor-suppressor model proposed by Knudson, the familial translocation in this case may act as a primary oncogenic event leading to (nondisjunctional) loss of the der(3) chromosome harboring the *VHL* tumor-suppressor gene. The risk of developing renal cell cancer may be correlated directly with the extent of somatic (kidney) mosaicism resulting from this loss.

Introduction

Renal cell carcinomas (RCCs) represent 85% of all primary renal neoplasms and, together with nephroblastoma, have been studied frequently at both the cytogenetic and the molecular level (Meloni et al. 1992; van den Berg and Buys 1997). They constitute a heterogeneous group of tumors that can be divided into different subtypes, including the papillary and the nonpapillary types (Meloni et al. 1992; Motzer et al. 1996; van den Berg et al. 1997*b*). Papillary RCCs are characterized by a combination of gains of chromosomes 7 and 17 and loss of the Y chromosome. In addition, t(X;1)(p11;q21) has repeatedly been encountered in a subset of these tumors (Meloni et al. 1992). We and others recently found that this translocation results in a fusion of the transcription factor *TFE3* gene, on the X chromosome, to a novel gene, designated *PRCC,* on chromosome 1 (Sidhar et al 1996; Weterman et al. 1996*a,* 1996*b*). In addition, mutations in the *MET* proto-oncogene on chromosome 7 were detected in the germ lines of affected members of papillary RCC families and in a subset of sporadic papillary RCCs (Schmidt et al. 1997).

Nonpapillary RCCs are found mostly in the sporadic form. Loss of heterozygosity (LOH) analysis of these RCCs revealed that allelic losses predominantly occur in the chromosome 3p21 region, together with losses in either 3p25, 3p13-14, or both (van den Berg and Buys 1997). These results indicated that the corresponding regions on 3p may harbor tumor-suppressor genes. One of these putative tumor-suppressor genes is the von Hippel–Lindau (VHL) gene, *VHL,* located on 3p25. Hereditary nonpapillary RCC is rare, is often bilateral and/or multifocal in nature, and has a relatively early onset (Meloni et al. 1992; Stein and Stein 1995; Motzer et al. 1996). These hereditary RCCs may develop as a consequence of VHL disease, in which the *VHL* gene is inactivated by mutation and/or hypermethylation (Gnarra et al. 1994; Herman et al. 1994). Somatic mutations and LOH of the *VHL* gene were also found in primary sporadic RCCs (Foster et al. 1994; Gnarra et al. 1994, 1995; Shuin et al. 1994; Zhuang et al. 1996).

Until recently, only two familial RCC cases with balanced chromosomal translocations had been encountered. In the first family, a constitutional t(3;8)(p14;q24) was found in several family members, including 10 RCC patients, over four generations (Cohen et al. 1979; Li et

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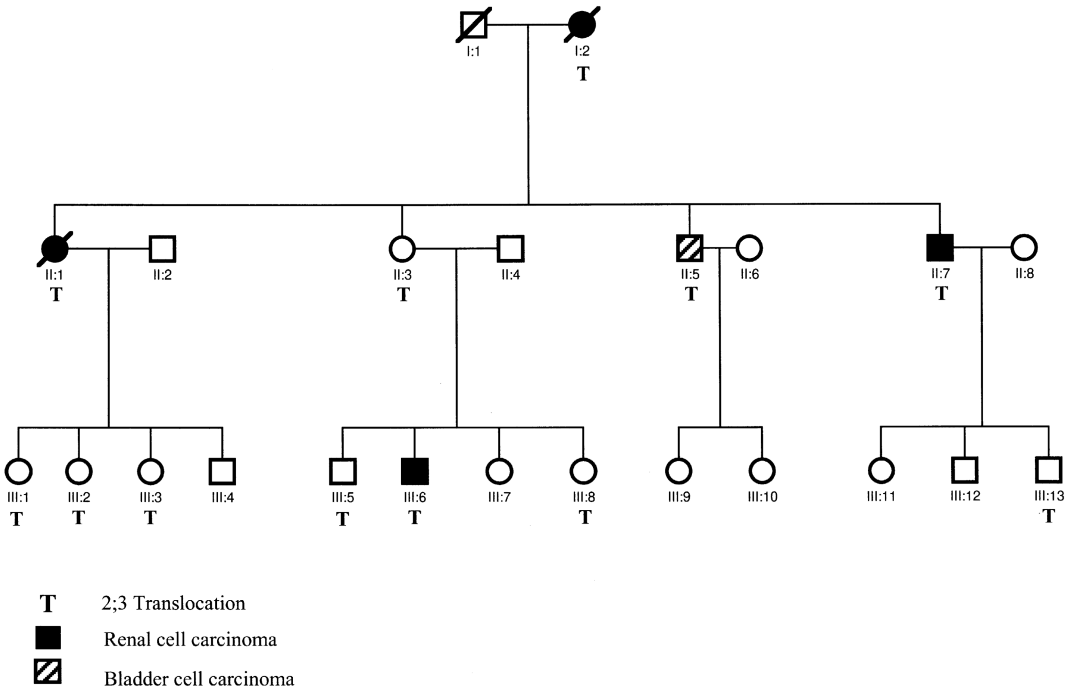

Figure 1 Pedigree of the renal cancer family

al. 1993). In the second family, a constitutional t(3;6)(p13;q25) was found in three consecutive generations. As yet, only the oldest member of this latter family has developed multiple bilateral RCCs (Kovacs et al. 1989). In addition, a single sporadic RCC case has been reported, carrying a constitutional t(3;12)(q13;q24) (Kovacs and Hoene 1988). In both these familial and sporadic RCC cases, the translocation-derived chromosome containing the distal 3p segment was found to be lost in the tumor tissues (Kovacs and Hoene 1988; Kovacs et al. 1989; Li et al. 1993; Kok et al. 1997), which supports a pivotal role for this chromosomal segment in nonpapillary RCC causation. The chromosome 3 breakpoints are different in all three cases. If these translocations disrupt genes, therefore, they will involve different genes. At present, only one of these genes—*FHIT,* which is involved in the (3;8) translocation and maps to 3p14—has been identified (Ohta et al. 1996). However, the exact role of this gene in cancer causation remains to be resolved (van den Berg et al. 1996, 1997*a*).

Recently, we identified a new familial case of RCC (Koolen et al. 1998). Four patients, over three generations, developed nonpapillary RCCs, and one patient was diagnosed with a squamous bladder carcinoma. Cytogenetic analysis revealed that all these patients (and several unaffected members) carried a balanced t(2;3)(q35;q21) translocation. To elucidate the role of this novel chromosome 3 translocation in RCC development, we performed allele-segregation, LOH, and mutation analyses of various normal tissues and primary tumor samples.

Material and Methods

Patient Material and Somatic Cell Hybrids

Paraffin-embedded tumor samples were available (with informed consent) from patients II:1, II:7, and III:6 (fig. 1). From patient II:7, three different samples were obtained. One of these samples was isolated from a tumor in the right kidney, which was removed after RCC had been diagnosed in 1984, and two were isolated from the left kidney after partial nephrectomy and multifocal RCC diagnosis in 1995 (Koolen et al. 1998). Before DNA extraction, tumor-containing areas were selected via microscopic inspection. Wherever possible, adjacent normal renal tissues were included as controls.

Blood samples from all living members of the RCC family (except III:7) were used (with informed consent) to generate lymphocytic cell lines after in vitro Epstein-Barr virus (EBV) transformation. Somatic cell hybrids were isolated after fusion of thymidine kinase–deficient Chinese hamster A3 cells with t(2;3)-positive lymphocytes of patient II:7, following hypoxanthine-aminopterine-thymidine selection, as described elsewhere (Geurts van Kessel et al. 1983). Approximately 80 independent clones were isolated, and their human chro-

mosomal constitution was established through karyotyping and PCR screening with chromosome 2– and chromosome 3–specific primer sets (see below). A3KE-11C was chosen as a derivative chromosome 2 (der[2]) hybrid, A3KE-1D as a der(3) hybrid, and A3KE-34B and A3KE-1A as normal chromosome 2– and chromosome 3–containing hybrids, respectively. No other chromosome 2 or chromosome 3 material was found to be present in these selected hybrid lines.

DNA Isolation and PCR Analysis

Cell line–derived genomic DNAs were isolated by means of proteinase K/SDS treatment followed by phenol/chloroform extraction and ethanol precipitation. DNA extractions from selected parts of paraffin-embedded tumor samples were performed with a lysis buffer and protein-precipitation solution (Puregene, Gentra Systems).

PCR was performed in a volume of 25 μl containing 10 mM Tris-HCl, pH 9.0; 50 mM KCl; 1.5 mM $MgCl_2$; 200 μM each nucleotide; 50 ng each primer; 0.25 U *Taq* polymerase (Thermoperfect Integro); ~100 ng genomic DNA; and 0.1 μl fluorescent-labeled dUTP R110 or R6G (Applied Biosystems, Perkin Elmer). Samples were covered with mineral oil and were amplified for 35 cycles. Each cycle included denaturation at 94°C for 30 s, annealing at temperatures 5°–10°C below the melting temperature of the primers for 45 s, and elongation at 72°C for 1 min. In the first cycle, an extra denaturation step of 2 min was included, and, in the last cycle, elongation was extended for 5 min. The fluorescent-labeled PCR products were analyzed by use of 6% polyacrylamide gels (Sequagel-6, National Diagnostics), an ABI 373A apparatus (Applied Biosystems, Perkin Elmer), and the GENESCAN ANALYSIS software package (Applied Biosystems, Perkin Elmer).

The microsatellite markers used in this study were *D2S162* (Genome Database [GDB] accession number 188450), *D2S144* (GDB 188238), *D2S139* (GDB 188182), *D2S156* (GDB 188377), and *D2S172* (GDB 188529), from chromosome 2, and *D3S1038* (GDB 188715), *D3S1481* (GDB 197992), *D3S1303* (GDB 188447), and *D3S1290* (GDB 188268), from chromosome 3 (Smith et al. 1995; Spurr et al. 1996; van den Berg et al. 1996). These markers were selected from the consensus map generated at the Fifth International Chromosome 3 Workshop (Smith et al. 1995).

Sequence Analysis

For sequence analysis of the *VHL* gene, five different primer sets were used: K54 (5′ GAA ATA CAG TAA CGA GTT GGC CTA GC 3′)/G7R72 (5′ ACC TCG GCC TCG TCC CAG T 3′); G7F27 (5′ CCC GGG TGG TCT GGA TCG 3′)/G7R297 (5′ ATA CGG GCA GCA CGA CGC 3′); VHL-22-1/VHL-7 (GDB 375133); G712F2 (5′ CCT TTG CTT GTC CCG ATA GGT CA 3′)/G713R1 (5′ ACA TCA GGC AAA AAT TGA GAA CTG G 3′); and G713FX4 (5′ CCT TGT ACT GAG ACC CTA GTC TGC CAC T 3′)/G7R727 (5′ CAA GAC TCA TCA GTA CCA TCA AAA GGT 3′). DNA sequences were analyzed by use of the Ready Reaction Dye Terminator Cycle Sequencing kit (Applied Biosystems, Perkin Elmer) and the automated DNA sequencer ABI 373A (Applied Biosystems, Perkin Elmer).

Results

Renal Cell–Cancer Family and t(2;3)(q35;q21)

A family in which four different patients over three generations had developed nonpapillary RCC of the clear-cell type (fig. 1) was referred to our clinic. Cytogenetic analysis revealed that two of the patients (II:7 and III:6) carried a constitutional balanced t(2;3)(q35;q21) translocation (fig. 2*A*). In the two other RCC cases (I:2 and II:1), carriership of the translocation could be deduced through cytogenetic analysis of the patients' children (Koolen et al. 1998). Furthermore, one squamous bladder cell carcinoma was diagnosed in the t(2;3)(q35;q21)-positive patient II:5. The (2;3) translocation was also observed in eight other family members tested (Koolen et al. 1998). Through genealogic investigation, two additional family branches (27 first- and second-degree relatives of both I:1 and I:2) could be identified, but no translocation carriers were detected. This finding suggested that the t(2;3)(q35;q21) most likely occurred de novo in patient I:2. In this branch of the family, the tumors developed at an earlier age than that at which sporadic renal cancers normally develop, and, in some cases, they were bilateral and/or multifocal in nature (Koolen et al. 1998).

Segregation of Chromosome 2 and 3 Markers

The microsatellite markers used in this study and their relative positions on chromosomes 2 and 3 are shown in figure 2*B*. The chromosome 3 markers *D3S1038* and *D3S1481* are located near two of the putative RCC-associated tumor-suppressor loci in the regions 3p25 (*VHL*) and 3p14 (*FHIT*), respectively (van den Berg et al. 1997*b*). Somatic cell hybrid analysis using the II:7-derived lines A3KE-34B, A3KE-11C, A3KE-1A, and A3KE-1D revealed that *D3S1303* is located proximal to the t(2;3)(q35;q21) breakpoint on chromosome 3 and that *D3S1290* maps distal to it (figs. 2*B* and 3). Similarly, on chromosome 2, the marker *D2S172* could be positioned distal to the breakpoint, whereas the other markers (*D2S162, D2S144, D2S139,* and *D2S156*) were mapped proximal to it.

In addition, by use of the same hybrid-cell panel, the

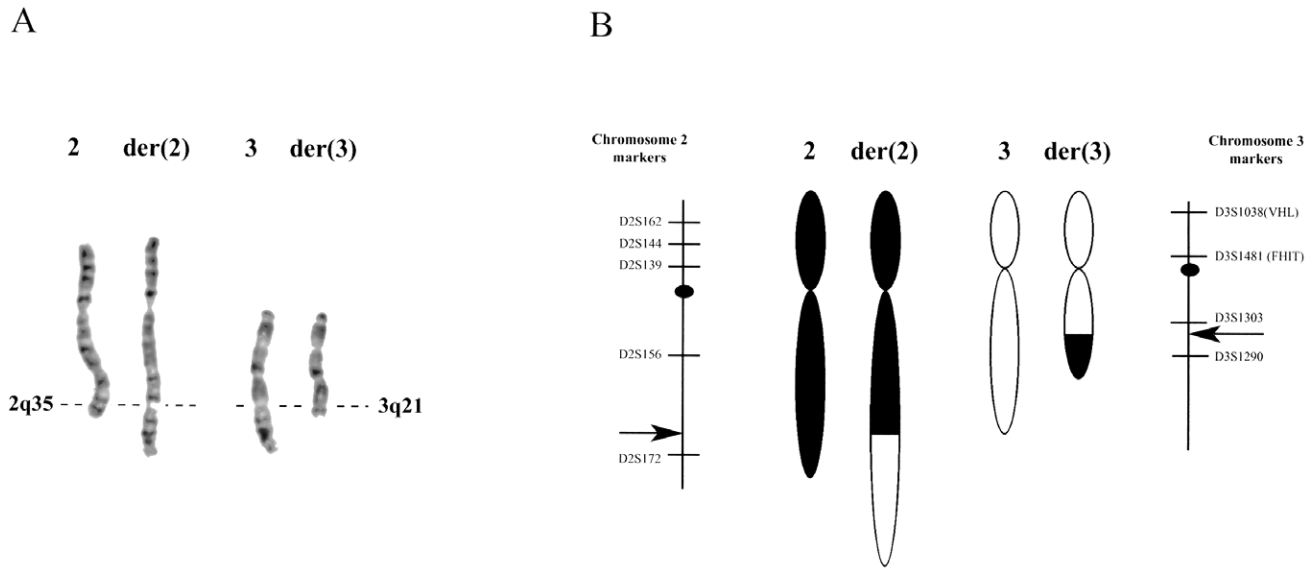

Figure 2 *A,* Lymphocyte-derived partial karyotype of patient II:7, showing the normal chromosomes 2 and 3 and the translocation derivatives der(2) and der(3). The chromosomal breakpoints are located within bands 2q35 and 3q21, respectively. *B,* Schematic overview of both normal and derivative chromosomes 2 and 3 and the relative positions of the different microsatellite markers that were used in this study. The positions of the translocation breakpoints are marked by arrows.

maternal or paternal origin of the different microsatellite alleles on the normal chromosomes 2 and 3 and their respective translocation derivatives could be deduced from patient II:7, assuming that RCC patient I:2 was also a translocation carrier (fig. 3). This microsatellite analysis was extended to all other family members by means of EBV-transformed lymphocytic cell lines and/or patient-derived normal kidney tissues (data not shown). This information was subsequently used for interpretation of the LOH data obtained from the different tumor samples.

Tumor-Associated Loss of the der(3) Chromosome

For LOH analysis, tumor areas were selected by microscopy and were subsequently isolated from paraffin sections by microdissection. After DNA extraction, the chromosome 2 and chromosome 3 microsatellite patterns of the tumor samples were compared with those of the normal control kidney tissues and/or corresponding lymphocytic cell lines. In figure 3, some examples of such an LOH analysis are shown. One of the tumors isolated from patient II:7 (T95.1) was assayed for chromosome 2 markers *D2S139* and *D2S172* and chromosome 3 markers *D3S1303* and *D3S1290,* located proximal and distal to the t(2;3)(q35;q21) breakpoints on chromosomes 2 and 3, respectively (fig. 2*B*). Microsatellite profiles from tumor tissue, normal tissue, and II:7-derived somatic cell hybrids were compared. The alleles located on der(3) (*D2S172:*279 and *D3S1303:* 209) exhibited a significant loss in the tumor tissue as compared with the normal tissue and/or the somatic cell hybrids (marked by LOH in fig. 3). Such losses can best be explained by a loss of the der(3) chromosome in the original tumor. No complete LOH was observed in the tumor DNA, which suggests that the tumor sample was still contaminated with normal cells in spite of microdissection prior to DNA extraction. No LOH was observed for the microsatellite markers *D2S139* and *D3S1290,* located proximal to the breakpoint on chromosome 2 and distal to the breakpoint on chromosome 3, respectively (fig. 3). This result may indicate that both the normal copies of chromosomes 2 and 3 and the translocation-derived der(2) chromosome were retained in the original tumor sample. Similar results were obtained with all other (informative) markers and tumors included in this study. A summary of these data is provided in table 1. After combining the chromosome 2 and 3 segregation data (above) with the LOH data from patients II:1 and III:6 (table 1), we conclude that in these cases, too, the der(3) chromosome must be lost in the tumors.

VHL *Gene Mutations in the RCCs*

On the basis of the observed loss of the der(3) chromosome and, as a consequence, the loss of one copy of the *VHL* gene in the different tumor samples examined within this family, we performed mutation analysis on the remaining *VHL* gene in the tumor-derived DNAs by

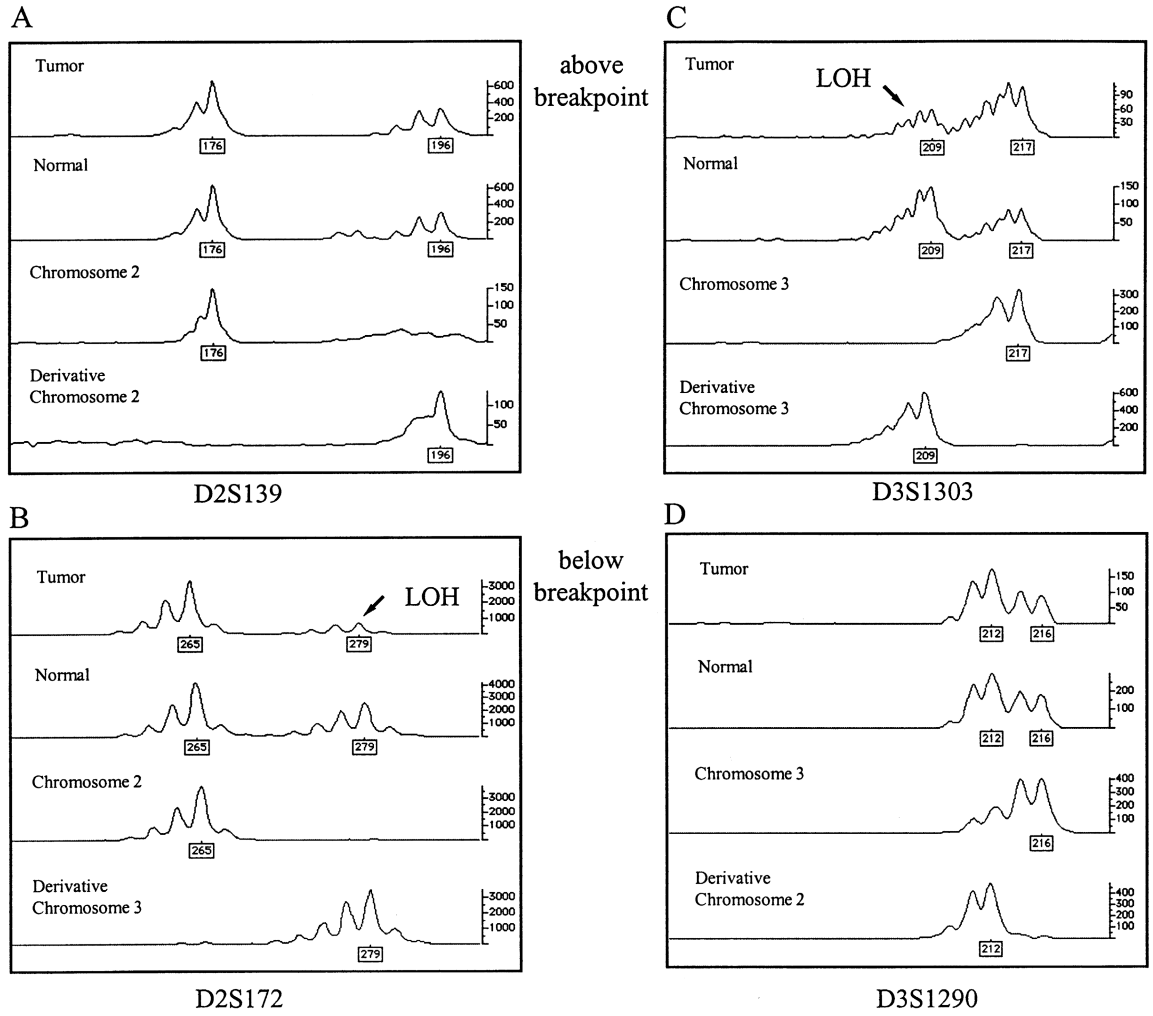

Figure 3 Tumor T95.1 (patient II:7)–derived allele plots of four different markers located on chromosomes 2 and 3. Allele patterns of the tumor tissue were compared with those of normal tissue and II:7-derived somatic cell hybrids containing chromosome 2, chromosome 3, and the respective translocation derivatives. *A*, Marker *D2S139*, located proximal to the breakpoint on chromosome 2. *B*, Marker *D2S172*, located distal to the breakpoint on chromosome 2. *C*, Marker *D3S1303*, located proximal to the breakpoint on chromosome 3. *D*, Marker *D3S1290*, located distal to the breakpoint on chromosome 3.

direct sequencing, using exon 1–, exon 2–, and exon 3–specific primer sets. Again, normal kidney–derived and/or lymphocytic cell line–derived DNAs were included as controls (fig. 4). In one of the tumors (T95.1) of patient II:7, isolated from the right kidney in 1995, sequence analysis revealed a single–base-pair insertion at position 535 (535insC) of exon 1. In the second tumor (T95.2) isolated from the same kidney at the same time, a deletion of 10 bp at position 373 (373del10) was observed, again in the first exon of the gene. In the tumor (T84) isolated from the left kidney of this same patient in 1984, no mutations were detected in either of the exons tested. In the RCC of patient II:1, a 19-bp deletion (722del19) was found in the third exon, and, in the tumor of patient III:6, a single–base-pair substitution, P86H(C→A), was observed in, again, the first exon. All the observed *VHL* mutations are located in the mutation–hot-spot regions described in the literature, and they lead to frameshifts and/or substitutions that result in a loss of function of the encoded proteins. The numbering of the nucleotide positions mentioned corresponds to the GenBank accession number L15409 sequence and to the nomenclature described by Beaudet and Tsui (1993).

Discussion

We have detected a novel familial case of renal cell cancer with a t(2;3)(q35;q21) chromosome translocation. Patient II:7 was of particular interest, since three

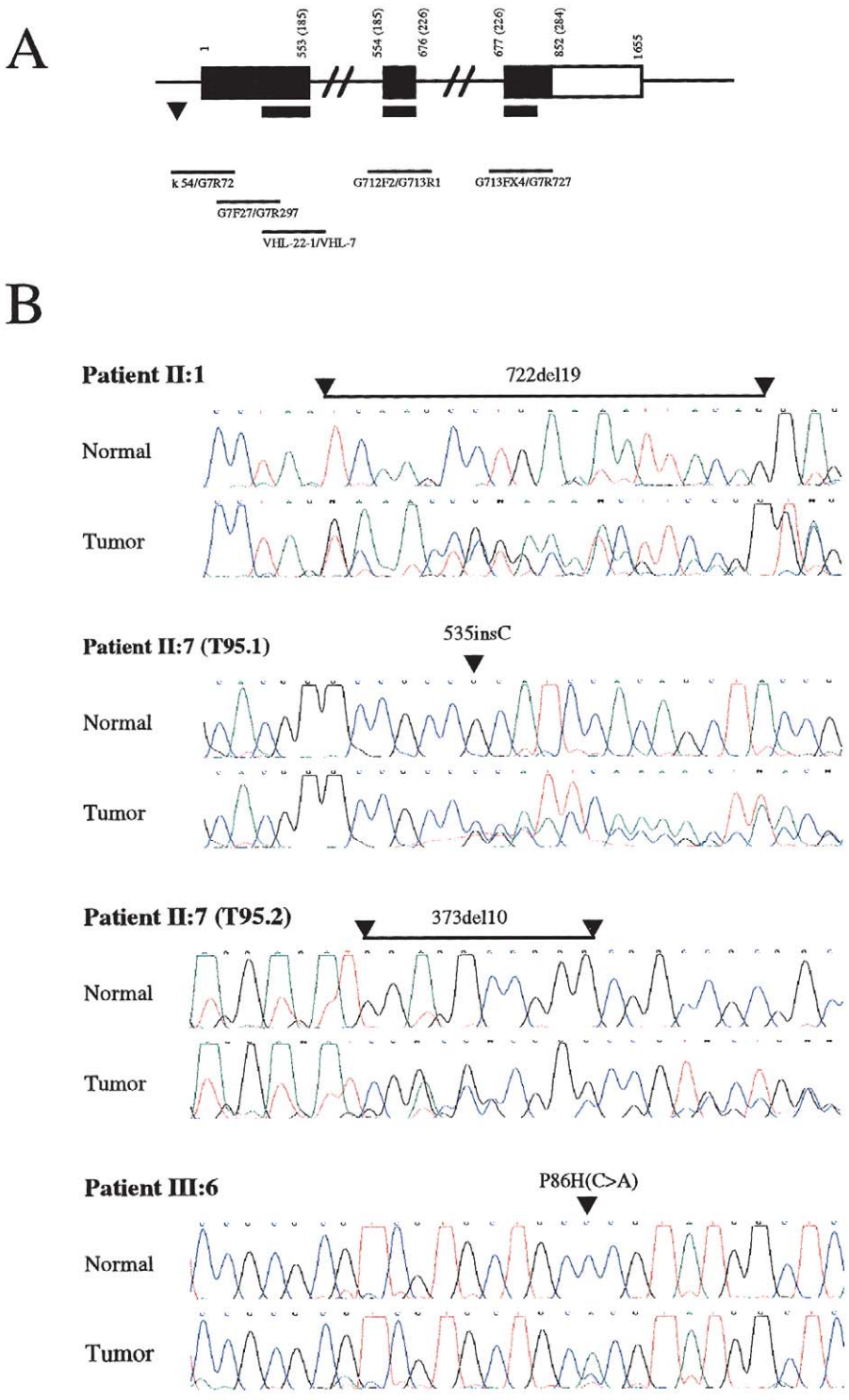

Figure 4 *A*, Schematic representation of the *VHL* gene, including the nucleotide and codon (in parentheses) numbers (above) and the primer sets that were used for mutation analysis (below). The regions underlined with solid bars are known mutation hot spots. *B*, *VHL* gene mutations found in four tumor DNAs of three RCC patients (II:1, II:7, and III:6). Since the tumor samples were contaminated with normal cells, double sequence patterns are seen after the insertion/deletion mutations.

Table 1

Summary of Chromosome 2 and Chromosome 3 LOH Results in Five RCCs from Patients II:1, II:7, and III:6

Chromosome and Polymorphic Marker	RCC in Patient (Tumor)[a]				
	II:1	II:7 (T84)	II:7 (T95.1)	II:7 (T95.2)	III:6
2:					
D2S162	−	−	−	−	−
D2S144	−	−	−	−	−
D2S139	−	−	−	−	−
D2S156	−	−	−	−	−
D2S172	+	+	+	+	+
3:					
D3S1038	+	+	+	+	+
D3S1481	+	+	+	+	+
D3S1303	+	+	+	+	+
D3S1290	−	−	−	−	−

[a] A minus sign (−) indicates LOH negative, and a plus sign (+) indicates LOH positive.

tumors could be obtained independently, from different anatomical locations, at two different occasions, with a time lapse of 11 years (1984–95). Lymphocyte-derived somatic cell–hybrid and microsatellite-marker analyses unambiguously demonstrated that, in all three tumors, the translocation-derived der(3) chromosome was lost. Subsequent segregation and LOH analyses revealed that the der(3) chromosome was also lost in two additional tumors derived from patients II:1 and III:6. Sequence analysis of the *VHL* gene revealed distinct mutations in four of the five tumors tested. All these mutations resulted in a loss of function. These data indicate that the different tumors that were studied developed independently (and not as metastases of a primary tumor—in particular, the three tumors of patient II:7) and that, most likely, in all cases, loss of the der(3) chromosome has occurred as a primary event. As a consequence of this scenario, the different *VHL* gene mutations should be considered as secondary events.

Translocations involving chromosome 3 have frequently been encountered in sporadic RCCs and in some (three, to date) familial RCC cases (Cohen et al. 1979; Kovacs and Hoene 1988; Kovacs et al. 1989; Li et al. 1993). Kovacs and Kung (1991) developed an alternative model for RCC development in which, in contrast to the generally accepted two-hit tumor-suppressor model put forward by Knudson (1993, 1996), familial translocations may act as primary oncogenic events. They proposed that reciprocal translocations involving chromosome 3 could, somatically, result in random nondisjunctional loss of the derivative chromosome carrying the 3p segment harboring putative tumor-suppressor loci such as *VHL* (Kovacs and Frisch 1989; Kovacs and Kung 1991; Kovacs 1993). Subsequent tumor formation would result from mutation of the remaining tumor-suppressor allele. Our results are in full support of this model and provide experimental evidence for it. Nondisjunctional loss may occur in any cell at any stage of (embryonic kidney) development. Accordingly, the risk of developing renal cell cancer may be correlated directly to the extent of somatic mosaicism resulting from this (random) loss. This latter notion nicely explains the relatively wide range in age at RCC onset observed in this family (e.g., patients II:3 and III:6; Koolen et al. 1998).

The proposed nondisjunctional loss may be due to an increased genetic instability, possibly caused by t(2;3)(q35;q21) itself. Therefore, we also searched for microsatellite and/or mitotic anomalies. Microsatellite instability in tumor tissues was not observed for any of the markers tested in this work. Mitotic errors were evaluated in 300 lymphocyte-derived methaphase spreads of three different translocation carriers, but no significant increases in abnormalities could be observed. Other tissues were not investigated.

VHL gene mutations have been reported to occur frequently (in 33%–57% of cases) in sporadic RCCs and in two of the four familial t(3;8)-associated RCCs analyzed (Gnarra et al. 1994; Knudson 1995; Schmidt et al. 1995). All these mutations were found to be located within known *VHL* mutation hot spots (Foster et al. 1994; Gnarra et al. 1994; Zbar et al. 1996; Decker et al. 1997). In one of the current tumors (T84), no mutations could be detected in either of the exons tested. In this case, mutations may still be present in the *VHL* promoter region, or, alternatively, the *VHL* gene may be inactivated in the tumor via hypermethylation (Herman et al. 1994). Obviously, mutations in other putative tumor-suppressor gene(s) on 3p may also be present (van den Berg and Buys 1997). These latter possibilities remain to be tested.

Taken together, our results support the RCC development model of Kovacs and Kung (1991) as an alternative route for multistep tumorigenesis. Currently, we are isolating the t(2;3)(q35;q21) breakpoint to find out whether, analogous to *FHIT,* a gene can be identified at or near the breakpoint in either chromosome 2 or chromosome 3.

Acknowledgments

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Web/Genbank (for von Hippel-Lindau gene, *VHL* [L15409])

Genome Database, http://www.gdb.org/ (for microsatellite markers and primers [188450, 188238, 188182, 188377, 188529, 188715, 197992, 188447, 188268, and 375133])

References

Beaudet AL, Tsui L-C (1993) A suggested nomenclature for designated mutations. Hum Mutat 2:245–248

Cohen A, Li F, Berg S, Marchetto D, Tsai S, Jacobs S, Brown R (1979) Hereditary renal cell carcinoma associated with chromosomal translocation. N Engl J Med 301:592–595

Decker HJH, Weidt EJ, Brieger J (1997) The von Hippel–Lindau tumor suppressor gene, a rare and intriguing disease opening new insight into basic mechanisms of carcinogenesis. Cancer Genet Cytogenet 93:74–83

Foster K, Prowse A, van den Berg A, Fleming S, Hulsbeek MMF, Crossey PA, Richards FM, et al (1994) Somatic mutations of the von Hippel–Lindau disease tumor suppressor gene in non-familial clear cell renal carcinoma. Hum Mol Genet 3:2169–2173

Geurts van Kessel A, Tetteroo PAT, Von dem Borne AEGK, Hagemeijer A, Bootsma D (1983) Expression of human myeloid-associated surface antigens in human-mouse myeloid cell hybrids. Proc Natl Acad Sci USA 80:3748–3752

Gnarra JR, Lerman MI, Zbar B, Linehan WM (1995) Genetics of renal-cell carcinoma and evidence for a critical role for von Hippel–Lindau in renal tumorigenesis. Semin Oncol 22: 3–8

Gnarra JR, Tory K, Weng Y, Schmidt L, Wei MH, Li H, Latif F, et al (1994) Mutations of the *VHL* tumor suppressor gene in renal carcinoma. Nat Genet 7:85–90

Herman JG, Latif F, Weng Y, Lerman MI, Zbar B, Liu S, Samid D, et al (1994) Silencing of the *VHL* tumor-suppressor gene by DNA methylation in renal carcinoma. Proc Natl Acad Sci USA 91:9700–9704

Knudson AG (1993) Antioncogenes and human cancer. Proc Natl Acad Sci USA 90:10914–10921

——— (1995) *VHL* gene mutation and clear-cell renal carcinomas. Cancer J Sci Am 1:180–181

——— (1996) Hereditary cancer: two hits revisited. Cancer Res Clin Oncol 122:135–140

Kok K, Naylor SL, Buys CHCM (1997) Deletions of the short arm of chromosome 3 in solid tumors and the search for suppressor genes. Adv Cancer Res 71:27–92

Koolen MI, Van der Meyden APM, Bodmer D, Eleveld M, Van der Looij E, Brunner H, Smits A, et al (1998) A familial case of renal cell carcinoma and a t(2;3) chromosome translocation. Kidney Int 53:273–275

Kovacs G (1993) Molecular cytogenetics of renal cell tumors. Adv Cancer Res 62:89–124

Kovacs G, Brusa P, De Riese W (1989) Tissue-specific expression of a constitutional 3;6 translocation development of multiple bilateral renal-cell carcinomas. Int J Cancer 43: 422–427

Kovacs G, Frisch S (1989) Clonal chromosome abnormalities in tumor cells from patients with sporadic renal cell carcinomas. Cancer Res 49:651–659

Kovacs G, Hoene E (1988) Loss of der(3) in renal carcinoma cell of a patient with constitutional t(3;12). Hum Genet 78: 148–150

Kovacs G, Kung HF (1991) Nonhomologous chromatid exchange in hereditary and sporadic renal cell carcinomas. Proc Natl Acad Sci USA 88:194–198

Li FP, Decker HH, Zbar B, Stanton VP, Kovacs G, Seizinger BR, Aburatani H, et al (1993) Clinical and genetic studies of renal cell carcinomas in a family with a constitutional chromosome 3;8 translocation: genetics of familial renal carcinoma. Ann Intern Med 18:106–111

Meloni AM, Bridge J, Sandberg AA (1992) Reviews on chromosome studies in urological tumors. J Urol 148:253–265

Motzer RJ, Bander NH, Nanus DM (1996) Renal-cell carcinoma. N Engl J Med 335:865–872

Ohta M, Inoue H, Cottecelli MG, Kastury K, Baffa R, Palazzo J, Siprashvili Z, et al (1996) The *FHIT* gene, spanning the chromosome 3p14.2 fragile site and renal carcinoma–associated t(3;8) breakpoint, is abnormal in digestive tract cancers. Cell 84:587–597

Schmidt L, Duh FM, Chen F, Kishida T, Glenn G, Choyke P, Scherer SW, et al (1997) Germline and somatic mutations in the tyrosine kinase domain of the *MET* proto-oncogene in papillary renal carcinomas. Nat Genet 16:68–73

Schmidt L, Li F, Brown RS, Berg S, Chen F, Wei MH, Tory K, et al (1995) Mechanism of tumorigenesis of renal carcinomas associated with the constitutional chromosome 3;8 translocation. Cancer J Sci Am 1:191–195

Shuin T, Kondo K, Torigoe S, Kishida T, Kubota Y, Hosaka M, Nagashima Y, et al (1994) Frequent somatic mutations and loss of heterozygosity of the von Hippel–Lindau tumor suppressor gene in primary human renal cell carcinomas. Cancer Res 54:2852–2855

Sidhar SK, Clark J, Gill S, Hamoundi R, Crew AJ, Gwilliam R, Ross M, et al (1996) The t(X;1)(p11.2;q21.2) translocation in papillary renal cell carcinoma fuses a novel gene *PRCC* to the *TFE3* transcription factor gene. Hum Mol Genet 5:1333–1338

Smith DI, Glover TW, Gemill RM, Drabkin HA, O'Connell P, Naylor SL (1995) Report of the Fifth International Workshop on Human Chromosome 3 Mapping 1994. Cytogenet Cell Genet 68:125–146

Spurr NK, Bashir R, Bushby K, Cox A, Cox S, Hildebrandt F, Hill N, et al (1996) Report and abstracts of the Fourth International Workshop on Human Chromosome 2 Mapping 1996. Cytogenet Cell Genet 73:255–273

Stein WD, Stein AD (1995) Familial and sporadic human renal cell carcinoma: evidence against a double loss mechanism of carcinogenesis. J Clin Epidemiol 48:767–777

van den Berg A, Buys CHCM (1997) Involvement of multiple loci on chromosome 3 in renal cell cancer development. Genes Chromosom Cancer 19:59–76

van den Berg A, Draaijers TG, Kok K, Timmer T, Van der Veen AY, Veldhuis PMJF, De Leij L, et al (1997*a*) Normal FHIT transcripts in renal cell cancer– and lung cancer–derived cell lines, including a cell line with a homozygous deletion in the FRA3B region. Genes Chromosom Cancer 19:220–227

van den Berg A, Hulsbeek MMF, De Jong D, Kok K, Veldhuis PMJF, Roche J, Buys CHCM (1996) Major role for a 3p21 region and lack of involvement of the t(3;8) breakpoint re-

gion in the development of renal cell carcinoma suggested by loss of heterozygosity analysis. Genes Chromosom Cancer 15:64–72

van den Berg E, Dijkhuizen T, Oosterhuis JW, Geurts van Kessel A, De Jong B, Störkel S (1997*b*) Cytogenetic classification of renal cell cancer. Cancer Genet Cytogenet 95:103–107

Weterman M, Wilbrink M, Geurts van Kessel A (1996*a*) Fusion of the transcription factor *TFE3* gene to a novel gene, *PRCC,* in t(X;1)(p11;q21)-positive papillary renal cell carcinomas. Proc Natl Acad Sci USA 93:15294–15298

Weterman M, Wilbrink M, Janssen I, Janssen H, van den Berg E, Fisher S, Craig I, et al (1996*b*) Molecular cloning of the papillary renal cell carcinoma–associated t(X;1)(p11;q21) breakpoint. Cytogenet Cell Genet 75:2–6

Zbar B, Kishida T, Chen F, Schmidt L, Maher ER, Richards FM, Crossey PA, et al (1996) Germline mutations in the von Hippel–Lindau disease (*VHL*) gene in families from North America, Europe, and Japan. Hum Mutat 8:348–357

Zhuang Z, Gnarra JR, Dudley CF, Zbar B, Linehan WM, Lubensky IA (1996) Detection of von Hippel–Lindau disease gene mutations in paraffin-embedded sporadic renal cell carcinoma specimens. Mod Pathol 9:838–842