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ORIGINAL ARTICLE

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Characterization of a familial RCC-associated t(2;3)(q33;q21) chromosome translocation

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Abstract A Polish family was identified in which multifocal clear cell renal carcinoma segregated with a balanced constitutional chromosome translocation, t(2;3)(q33;q21), similar to the renal cell cancer-associated t(2;3)(q35;q21) reported in a Dutch family. Bacterial artificial chromosome (BAC) contigs encompassing the 2q and 3q breakpoints were constructed and BACs crossing the breakpoints were partially sequenced. All known regional markers, genes, and expressed sequence tags (ESTs) were mapped relative to the contigs, as well as to the breakpoint sequences. Two single ESTs mapped within the 2q breakpoint BAC, whereas the repeat-rich 3q breakpoint region was gene poor. Physical mapping suggested that the 3q break was in 3q13, possibly near the border with 3q21. Physical mapping illustrated that the 2q break was closely telomeric to the 2q31 *FRA2G* site, consistent with the G-band assignment. Characterization of full-length cDNAs for the ESTs near the 2q break will determine if a gene(s) is altered by this familial translocation.

Key words Clear cell RCC · Familial renal cancer · Constitutional chromosome translocation · *FRA2G* · BAC contigs · Positional cloning

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Introduction

Renal cell cancer (RCC) is a relatively rare, heterogeneous neoplasm, comprising several histological subtypes. The clear cell variant, representing more than 70% of all RCCs, is most prevalent and the most unpredictable in terms of clinical management of patients (Eble 1997). By light microscopy, it is characterized by abundant clear cytoplasm and is thought to arise from proximal tubule epithelium. Understanding molecular events in carcinogenesis of kidney tumors could improve their classification, detection, and treatment. It is generally believed that initiation and progression of renal cell cancer is a multistep process that results from accumulation of genetic and phenotypic changes. Although RCC occurs mostly as a sporadic neoplasm, it is estimated that up to 4% of cases are inherited (Eble 1997). The hallmarks of hereditary RCCs are that they occur at an early age, develop in several close relatives in different generations within the family, and are usually bilateral and multifocal. Through analysis of genes altered in familial RCCs, progress has been made in the characterization of genes that cause kidney cancer.

In the 1980s, cytogenetic and molecular studies demonstrated deletions on the short arm of chromsome 3 in RCCs (Zbar et al. 1987; for review, Kok et al. 1997) and it is now known that clear cell RCCs invariably show loss of multiple regions of chromosome 3p (Lubinski et al. 1994; Druck et al. 1995; Hadaczek et al. 1996; van den Berg and Buys 1997). Regions typically include 3p25 (harboring the *VHL* gene), 3p21, 3p14 (harboring the *FHIT* gene), and 3p12. Familial mutations in the *VHL* gene predispose to clear cell RCC and other neoplasms (Latif et al. 1993; Gnarra et al. 1994), and genetic alterations leading to loss of wild-type Vhl protein have been observed in the majority of sporadic clear cell RCCs (Gnarra et al. 1994). Brauch et al. (2000) have recently shown that inactivation of the *VHL* gene in sporadic clear cell RCCs correlates with large tumor size. It is possible that the ability to characterize combinations of 3p genes and other suppressor genes lost in clear cell RCCs will lead to prognostically useful tumor suppressor profiles

that will improve clinical management. One copy of the *FHIT* gene at 3p14.2 (Ohta et al. 1996; Huebner et al. 1998) is inactivated by a constitutonal familial clear cell RCC-associated t(3;8) translocation (Li et al. 1993), and Fhit protein expression is lost or reduced in the majority of clear cell RCCs (Hadaczek et al. 1998; Hadaczek et al. 1999; Eyzaguirre et al. 1999); complete loss of Fhit expression is most frequent in low-grade clear cell RCCs (Hadaczek et al. 1998; Eyzaguirre et al. 1999). Thus, characterization of genes altered by other RCC-associated constitutional translocations may lead to the discovery of novel tumor suppressor genes or oncogenes relevant to diagnosis, prognosis, or treatment of RCC.

At least two other reported familial germline translocations predispose to clear cell renal cancer. In the first family, a constitutional translocation t(3;6)(p13;q25) was observed in three consecutive generations (Kovacs et al. 1989). As yet, only one member of this family developed bilateral renal cell cancer. More recently, a constitutional translocation t(2;3)(q35;q21) was reported in several Dutch family members over three generations, including four patients with renal cell cancers (Koolen et al. 1998; Bodmer et al. 1998; Geurts van Kessel et al. 1999). Interestingly, at the same time, we identified a family in Poland transmitting a similar constitutional translocation t(2;3)(q33;q21) (Zajaczek et al. 1999). This balanced chromosomal aberration was found in two living brothers with early-onset clear cell renal cancer. Records of five other patients with kidney cancers were noted in two earlier generations in the pedigree of this family.

Occurrence of two families with similar cytogenetic alterations and tumor phenotypes suggest that the long arm of chromosome 2 and/or chromosome 3 harbor a gene(s) involved in the process of tumorigenesis of some clear cell renal cancers. To identify candidate genes, we implemented positional cloning of genomic loci surrounding the affected chromosomal regions in translocation t(2;3)(q33;q21). Here we describe results of physical mapping of the breakpoint regions and preliminary results of cloning of new genes.

Several common fragile sites (*FRA2G*,2H, 2I) have been described (Glover et al. 1984) in the vicinity of the 2q33 region. To elucidate their possible role in the pathogenesis of kidney cancers in our familial cases, we also localized a common fragile site in relation to the breakpoint on the long arm of chromosome 2.

Materials and methods

The t(2;3) family

The family came to the attention of investigators at the Hereditary Cancer Center, Szczecin, Poland because two brothers presented with clear cell renal cancers at the ages of 45 and 38 years. The brothers were individuals III:14 and III:16 in the pedigree shown in Fig. 1. The cancers in the family occured earlier than the average sporadic clear cell renal carcinoma, were multifocal, and were in some patients bilateral. Peripheral blood lymphocytes and fibroblasts from the proband, his sons, and brother were cultured using standard methods. Metaphase slides were routinely prepared, stained according to G and R banding methods, and analyzed at the 550–800 band level. In the proband and his brother, the translocation t(2;3)(q33;q21) was found in peripheral blood lymphocytes as well as in fibroblasts (Fig. 2). Both healthy sons of the proband were karyotypically normal. DNA and RNA were prepared using standard protocols from the normal lymphocytes of the brothers. Lymphoblasts from individual III:14 were Epstein–Barr virus transformed and fibroblasts from both brothers were established in tissue culture.

Chromosome walking with human YAC and BAC clones

Human yeast artifical chromosome (YAC) clones were selected based on searches of the Whitehead Institute for Biomedical Research database (www.genome.wi.mit.edu)

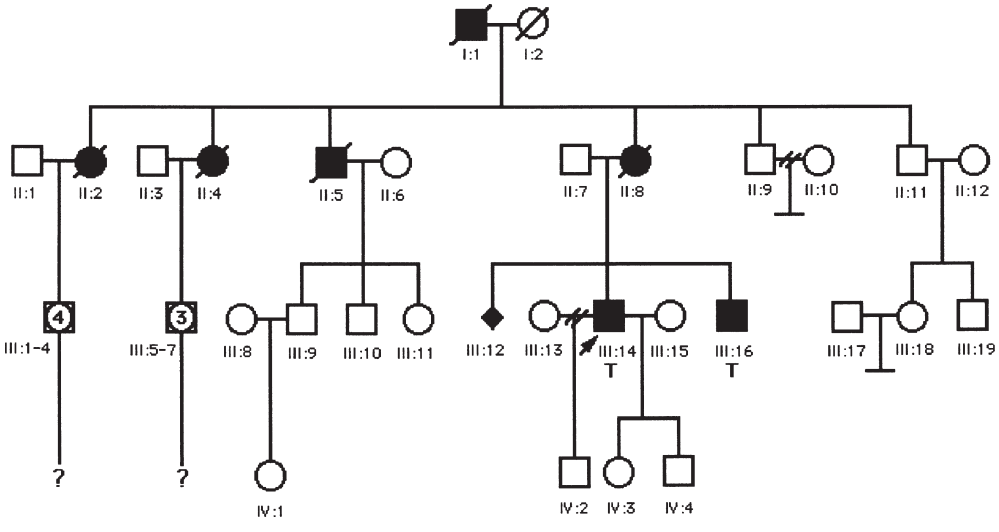

Fig. 1. Pedigree of the family with multiple cases of renal cell carcinomas. *Solid shapes* indicate family members with renal cell cancer (RCC). T is a proven carrier of the t(2;3)(q33;q21). *I:1*, RCC, died at age 51 years; *II:2*, RCC, died at age 51; *II:4*, RCC, died at age 51; *II:5*, bilateral RCC, died at age 51; *II:8*, RCC, onset at age 63, died at age 64; *III:14*, RCC, onset at age 45; *III:16*, RCC, onset at age 38, epilepsy

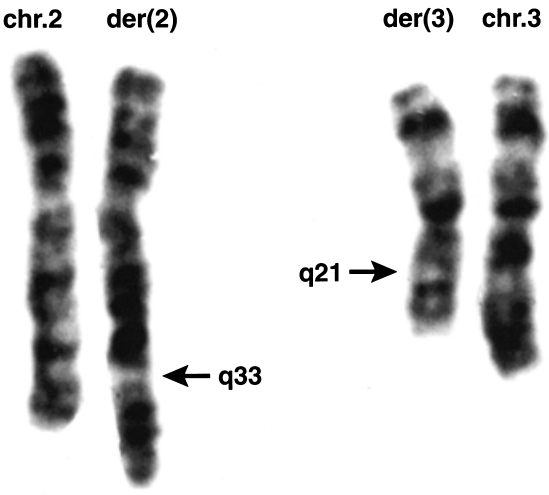

Fig. 2. Lymphocyte-derived partial karyotype of patient III:14. Illustration of normal chromosomes (*chr*) 2 and 3 and the translocation t(2;3) derivatives (*der*). The breakpoints are seen within bands 2q33 and 3q21 on the derivative 2 and derivative 3 chromosomes

and obtained from Research Genetics. Precise localization and characterization of breakpoint regions was done using the chromosomal fluorescent in situ hybridization (FISH) technique with human YAC and bacterial artifical chromosome (BAC) clones as described elsewhere (Wang et al. 1993). Briefly, YAC genomic DNA was isolated using a standard protocol with zymolase. One to two micrograms of YAC (DNA) was nick-translated with biotin (GIBCO BioNick Labeling System, Rockville, MD, USA), denatured, and suppressed with 15μg of COT-1 DNA for 20–30min at 37°C and then hybridized to metaphase spreads obtained from peripheral blood lymphocytes of one patient carrying the t(2;3) translocation. Signal detection was enhanced using the amplification method with fluorescein-avidin D and biotinylated anti-avidin D antibody (Vector Laboratories, Burlingame, CA, USA). Images were captured by cooled coupled device (CCD) camera and analyzed using IP Lab software.

Based on karyotypic data from the patients, initially a few YAC clones spanning or flanking cytogenetic bands 2q33 and 3q21 were selected from the YAC-contig database (Whitehead Institute for Biomedical Research). A total of 14 YAC clones for chromosome 3q and 16 YACs for chromosome 2q were tested by FISH analysis to find YACs that would hybridize to both derivative chromosomes. Using database information, genomic markers within the YACs covering the translocation breakpoints were selected for further analyses. Additional markers were generated from YAC-insert ends by inverse polymerase chain reaction (PCR) (Inoue et al. 1997). Using all available markers, the human CITB/C BAC library (Research Genetics, Huntsville, AL, USA) was screened by PCR amplification (Inoue et al. 1997). Positive clones were grown in 500ml of Luria broth (LB) media and DNA was isolated with Qiagen Plasmid Maxi or Qiagen Large Construct Kits (Valencia, CA, USA). The BAC ends were sequenced using vector-specific T7 or M13 reverse primers, new sequence tagged site (STS) primer pairs were designed from these sequences using computer program Oligo 4.0 (National Biosciences, Plymouth, MN, USA), and the next rounds of BAC library screening were performed. Selected BAC clones were also tested by FISH on the t(2;3) lymphoblast metaphases.

Southern blotting

DNA from normal and familial lymphoblastoid cell lines was extracted using standard methods. For Southern blotting, 5μg of DNA was digested in a 25-μl reaction containing 1X restriction buffer, 5mM spermidine, and 25U restriction enzyme (*Eco*RI, *Bam*HI, and *Eco*RV). The DNA was electrophoresed on 0.8% agarose gels, denatured, neutralized, and blotted to nylon membrane. The probe was labeled with [α-^{32}P]deoxycytidine triphosphate by random priming. Hybridization was overnight at 42°C in 50% formamide, 5X Denhardt's solution, 1% sodium dodecyl sulfate (SDS) and 0.1mg/ml salmon sperm DNA. Hybridized filters were washed in 2 × sodium chloride/sodium citrate (SSC), 0.1% SDS for 15min; 0.1 × SSC, 0.1% SDS for 15min; and then at 65°C for 30in. After the washes, the membranes were exposed to X-ray film.

Sequencing of BAC clones

BAC DNA of the clone 181L24 crossing the breakpoint region on chromosome 3q was randomly sheared by sonication, and DNA preparations with a peak size of 2–3kb were used to produce plasmid libraries for shotgun sequencing, as described previously (Inoue et al. 1997). Recombinant plasmids were isolated by Qiagen Robot 9600 and sequencing reactions were performed using BigDye chemistry (Applied Biosystems, Foster City, CA, USA). Reaction products were electrophoresed on the 377 DNA sequencer (Applied Biosystems). Two hundred eighty of these sequences have been analyzed and aligned into several small contigs using the Sequencher package (Gene Codes, Ann Arbor, MI, USA). For sequencing of the BAC clone 470J8 covering the breakpoint locus on chromosome 2q, the DNA was partially digested with *Sau* 3AI restriction enzyme and 1–2kbp fragments were excised from the agarose gel and ligated into the pUC18 vector. Two hundred seventy sequences have been aligned. All sequences have been used in BLAST searches of relevant databases, including expressed sequence tag (EST) databases.

Mapping of genes, STSs, and ESTs to t(2;3) breakpoint regions

Several known genes suspected to lie in close proximity to breakpoints were directly tested using genomic amplimers on YAC and/or BAC templates. EST data were obtained from GeneMap (www. ncbi.nlm.nih.gov/genemap). Radiation hybrid (RH) mapping data for the ESTs and STSs in our regions of interest, and our own PCR amplifications from genomic clones covering the breakpoints, were used to identify the candidate gene sequences for further analysis.

Sequences within BACs representing unique regions were identified by inspection of nucleotide sequences and verified as unique sequences by hybridization to total human DNA on Southern blots. Unique region probes within BACs near or crossing breakpoints were then amplified, labeled, and used as probes on Southern and Northern blots.

Fragile site induction and analysis

Phytohaemagglutinin-stimulated normal peripheral lymphocytes were cultivated for 96 h at 37°C, 5% CO_2 in RPMI 1640 medium supplemented with 15% fetal bovine serum, penicillin–streptomycin (50 mg/ml), and 0.2 mM glutamine. The expression of FRA2q31 was induced, as previously described, with minor modifications (Zimonjic et al. 1997). Aphidicolin (Apc) (0.4 mM) (Sigma, St. Louis, MO, USA), which was dissolved in dimethyl sulfoxide (Wang et al. 1993), or combined Apc and ethanol (0.2%), were added during the last 26 h of culture (Kuwanto et al. 1987). To enhance the expression of fragile sites, we exposed separate cultures to caffeine (2.2 mM) (Sigma) for 6 h before chromosome preparation. Metaphase spreads were prepared after 1 h colcemid (50 μg/ml) treatment by standard KCl hypotonic incubation, acetic acid–methanol fixation and air dry slide preparation. A YAC probe for 2q31 labeled with digoxigenin was used for in situ hybridization. The conditions for FISH, detection of the signal, and digital imaging were previously described (Zimonjic et al. 1995).

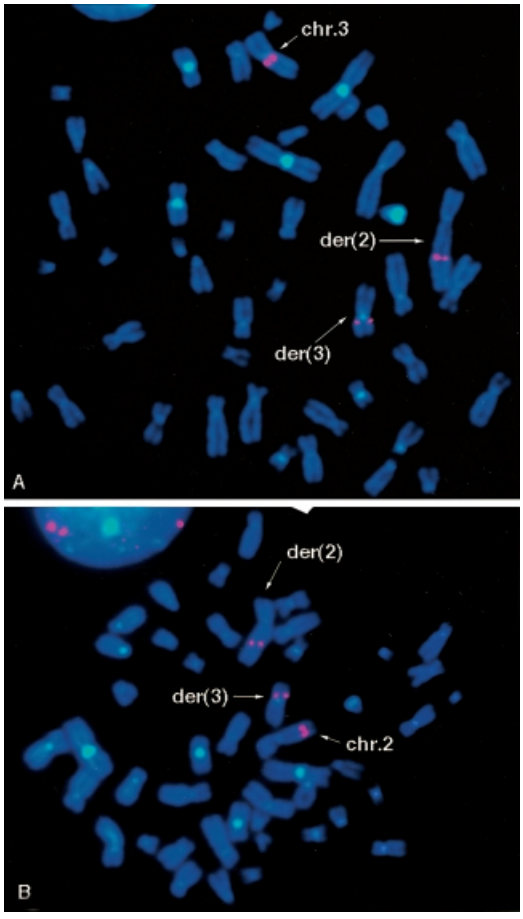

Fig. 3A,B. FISH analysis of lymphocyte-derived metaphases of patient III:14. **A** Hybridization of bacterial artificial chromosome probe 181L24 to normal chromosome 3, der (3) and der (2) indicates that this clone physically covers the breakpoint region on chromosome 3q. **B** Similarly, human yeast artificial chromosome probe 821E6 hybridizing to normal chromosome 2, der (2) and der (3) crosses the break on chromosome 2q

Results

Physical mapping of the chromosome 2q breakpoint region

FISH analyses of t(2;3) metaphase spreads allowed identification of four YAC clones (821E6, 806D10, 879F7, 858D12) that covered the breakpoint region on chromosome 2q (see Fig. 3A for an example and Fig. 4 for the physical map). These clones constitute a part of Whitehead contig WC2.15. Analysis of integrated genomic maps indicates that this region is located in cytogenetic bands 2q31–32. Based on known markers within YAC clones (*D2S103*, WI-4762, WI-7548, and WI-7678) and sequences generated from the region, a BAC contig was delineated (Fig. 4). The location of several BAC clones relative to the breakpoint was verified by FISH. Two BACs (470J8, 6C23) were found to cross the breakpoint.

Genes and ESTs in the 2q breakpoint region

Analysis of two markers, WI-7548 and WI-7678, revealed that they represent ESTs derived from collagen 3A1 and collagen 5A2 genes, respectively. The two collagen genes are contained within a single BAC, 173H4, and map telomeric to the breakpoint on chromosome 2q (Fig. 4). Two other genes, growth differentiation factor 8 (*GDF8*) and *PMS1*, were also localized to the telomeric side of the breakpoint by means of direct PCR testing, using genomic primers.

Markers from the GeneMap interval *D2S2257–D2S115* were delineated relative to collagen genes and other nearby markers using the RH mapping servers. Four of these markers represent genes and were confirmed to be localized in the YAC contig. STS WI-12408 and EST R68572 map to the centromeric side of the breakpoint (Fig. 4) and represent the recently cloned human homolog of the *CED6* gene (Liu et al. 1999; Smits et al. 1999). Two other STSs, A005T33 (hypothetical protein) and stSG13189 (ferroportin gene), are located in the telomeric part of YAC 806D10 (not shown).

About 75 kbp of sequence information in the form of ~30 small contigs was obtained from our large-scale sequencing of BAC 470J8; high throughput genomic sequences AC027781 and AC068111 overlap with a portion of this BAC. Analysis of these contig sequences identified two single EST clones: AI468595 present also in BACs 6C23

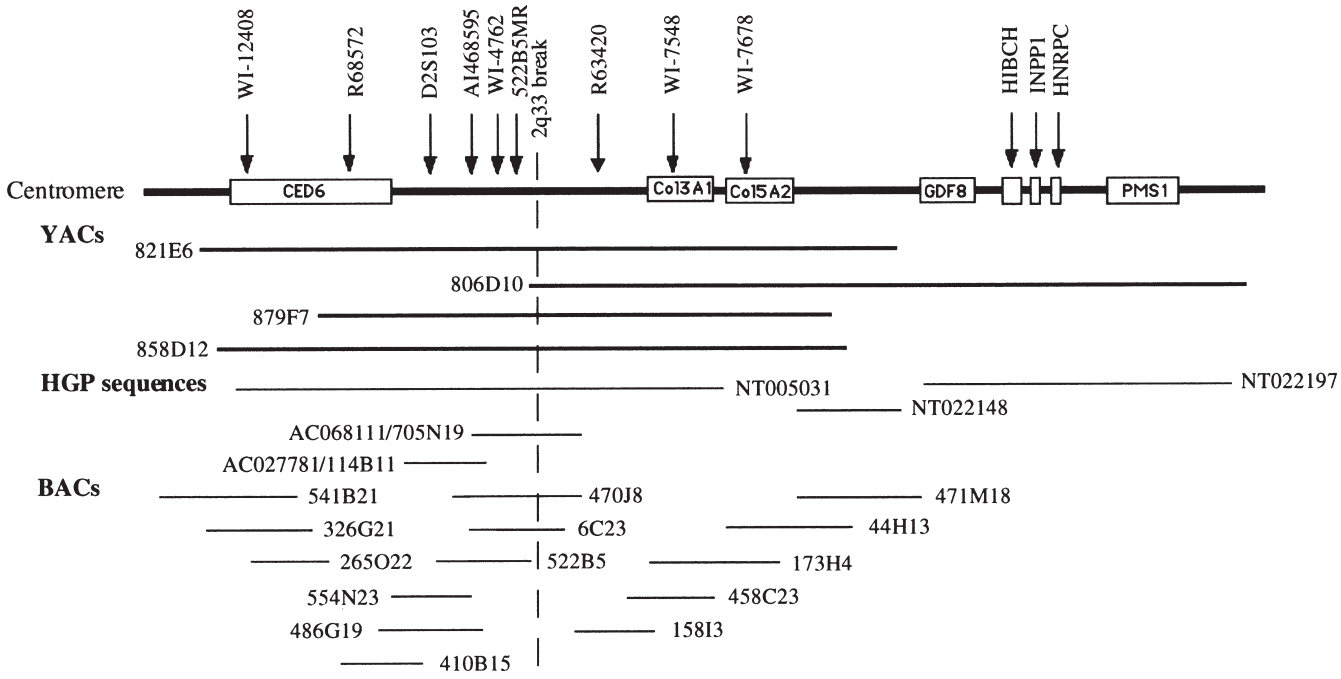

Fig. 4. Organization of the breakpoint locus on chromosome 2q. A scheme of the normal 2q33 region is shown with the genomic region (not to scale) represented by the *top line*, with positions of sequence tagged site (STS) markers, genes, and the t(2;3) translocation break point indicated. *YACs*, human genome project (*HGP*) sequences and *BACs* in the region are also shown. The accession number and Roswell Park BAC address is given for the HGP sequences

and 522B5, and R63420 present also in BAC 158I3, respectively, located centromeric and telemeric to the breakpoint. No gene or protein similarities for these ESTs were found in public databases.

Human Genome Project (HGP) working draft sequence segments NT_005031, NT_022148, and NT_022197 span our breakpoint region. These sequences do not overlap; therefore there are still unsequenced gaps, perhaps quite large, because the *Co15A2* gene is not contained in any HGP sequence. Genes *HIBCH*, *INPP1*, and *HNRPC* were added to our 2q breakpoint contig after examining the NT_022197 sequence. We were unable to find any additional EST or gene candidates after analyzing these sequences.

Primer pairs were prepared from the M13 reverse end sequence of BAC 522B5 and mapped onto the contig to determine relative position to the 2q breakpoint. This STS (522B5MR) was the closest known marker to the translocation. By this strategy we identified, amplified, and labeled a unique region probe that was within a few kilobases of the 2q translocation breakpoint and we were able to detect a rearrangement, as illustrated in Fig. 5.

Physical mapping of the chromosome 3q breakpoint region

Two human YAC clones were mapped to the chromosome 3q break by FISH, 810G6 and 734G2. They constitute Whitehead contig WC3.15. This contig is probably located in the gap between markers D3S2459 and D3S1291. Only two genomic markers were present in this YAC contig,

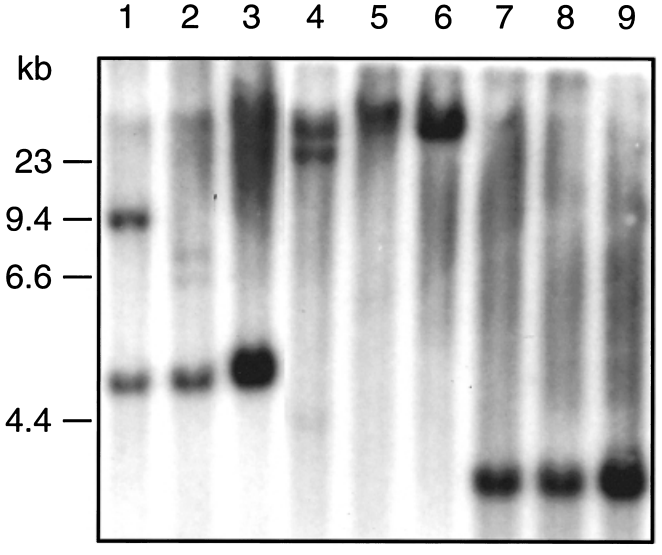

Fig. 5. Detection of a rearranged 2q breakpoint locus by Southern hybridization. A unique region probe from the M13 reverse end of BAC 522B5 was determined to be near the 2q breakpoint by its position within the BAC clone contig. To determine how close it was to the breakpoint, cellular DNAs from normal (*lanes 2*, *3*, *5*, *6* and *8*, *9*) and t(2;3) familial lymphoblastoid cells (*lanes 1*, *4* and *7*) were cut with restriction enzymes *Eco*RI (*lanes 1–3*), *Bam*HI (*lanes 4–6*), and *Eco*RV (*lanes 7–9*), blotted to nylon membrane, and hybridized to the unique region probe, 522B5MR, that was prepared and radiolabeled by polymerase chain reaction amplication using [^{32}P]-deoxycytidine triphosphate. Rearranged hybridizing bands in *lanes 1* and *4* illustrate that the *Eco*RI and *Bam*HI fragments of one 2q allele in the familial lymphoblasts were altered by the translocation, suggesting that the probe is within a few kilobases of the translocation breakpoint

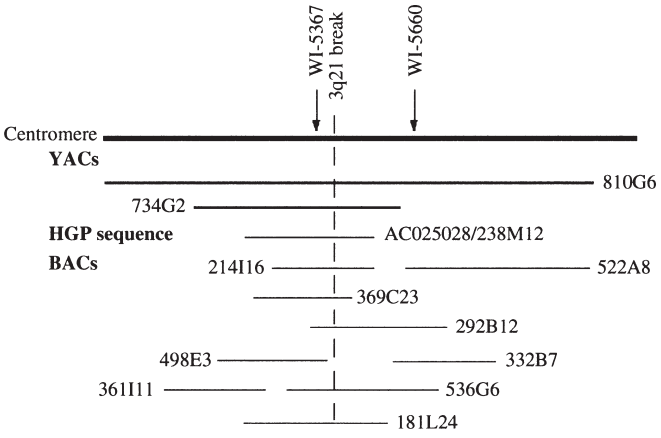

Fig. 6. Organization of the breakpoint locus on chromosome 3q. The normal 3q21 region is shown with the genomic region (not to scale) represented by the *top line* and the position of markers WI-5367 and WI-5660 relative to the t(2;3) translocation breakpoint indicated by *arrows*. YACs crossing the break, human genome project sequence AC025028 (RP11-238M12), and BACs within the region are also shown

WI-5367 and WI-5660. A BAC contig was then constructed (Fig. 6) and several clones were shown to cover the 3q breakpoint by FISH on metaphases from the lymphocytes of one brother with the t(2;3) (Fig. 3B).

Genes and ESTs in the 3q breakpoint region

Using genomic amplimers, we tested 34 markers previously mapped to the GeneMap interval between markers D3S1271 (contig WC3.14) and D3S1291 (contig WC3.16), that is, to the region containing our YAC contig WC3.15. Most of the markers were shown to be located in WC3.14 or WC3.16 contigs with none mapping in YACs covering the 3q breakpoint.

Shotgun sequencing of BAC 181L24 produced ~100 kbp of sequence aligned into more than 30 contigs. BLAST searches with these sequences did not reveal any gene or EST hits. However, HGP working draft sequence AC025028 matched a portion of BAC clone 181L24. BLAST searches identified the presence of a locus with 90% similarity to the gene for reduced nicotinamide adenine dinucleotide-ubiquinone oxidoreductase mlrq subunit with an open reading frame (ORF) of 81 amino acids. Another ORF consisting of 117 amino acids was found in close proximity. At the protein level, this sequence was 96% identical to a series of p150-like proteins. The 3q breakpoint itself is in a highly repetitive region.

Position of common fragile site *FRA2G* in relation to the 2q breakpoint

To determine if the 2q31 fragile site (*FRA2G* or FRA2q31) might be near our 2q breakpoint, the YAC clone crossing the 2q break (821E6) was labeled and hybridized to metaphases exhibiting fragile sites. Metaphase spreads prepared from peripheral lymphocytes of two normal donors, after 26 h treatment with aphidicolin and ethanol or in combination with caffeine, exhibited breaks and gaps at FRA2q31 in 20%–30% of the cells. Although caffeine itself did not increase the frequency of gaps and breaks, it made the fragile sites more evident in the metaphase chromosomes. The majority of lesions involved one chromosome, but in two cells, gaps were observed in both homologs. To obtain a sufficient number of metaphases with informative signal at FRA2q31, two slides from each donor were hybridized with the YAC breakpoint probe. The location of the hybridization signal was assigned in 50 metaphases with DAPI-enhanced G-like banding, exhibiting gaps and breaks at FRA2q31 (Fig. 7). In metaphases with gaps at 2q31, the signal was located within the achromatic region, whereas in metaphases with distinct chromatid breaks and displacement of the chromatids, the symmetrical signal was exclusively located at the telomeric flank of the FRA2q31 (Fig. 7 middle and lower). Thus, the 2q break is telomeric to *FRA2G*, suggesting that this chromosome alteration did not result from alteration at the fragile region, unlike the *FHIT* locus at *FRA3B* (Zimonjic et al. 1997).

Discussion

We have characterized the balanced t(2;3) chromosome translocations that we observed in family members inheriting a predisposition to clear cell renal cancer. In two living brothers of this family who were diagnosed with renal cell cancers, a t(2;3)(q33;q21) constitutional translocation was found, but it is probable, because of the clinical features of the other five RCC cases that developed in close relatives, that the relatives were also carriers of this chromosomal abnormality. Another family segregating t(2;3)(q35;q21) translocations with multiple cases of RCC was reported by investigators in the Netherlands (Koolen et al. 1998; Bodmer et al. 1998; Geurts van Kessel et al. 1999). Based on the cytogenetic findings, the Netherland translocations appeared to be similar to our familial translocation.

Through studies of somatic cell hybrids, Bodmer et al. (1998) showed that marker *D3S1303* was centromeric to the 3q breakpoint in the Dutch family, and *D3S1290* mapped telomeric to it. We physically mapped the 3q break in our familial t(2;3) translocation to the region q13–21. FISH experiments with human YAC clones and estimation of physical distance between marker WI-5367 in our 3q breakpoint region and *D3S1303* in the Dutch 3q breakpoint region, indicate that the Dutch 3q breakpoint is at least 10–20 Mbp telomeric to our 3q break. Somatic cell hybrid analysis also allowed Bodmer et al. to position their 2q breakpoint telomeric to marker *D2S156* (located in Whitehead YAC contig WC 2.11) and centromeric to *D2S172* (Whitehead contig WC2.16). The distance between these markers on Whitehead maps is ~72 cM. We mapped our chromosome 2q break to the q32 cytogenetic locus, in the Whitehead YAC contig WC 2.15, ~24 cM telomeric to the marker *D2S156*, and thus in the middle of the large region to which

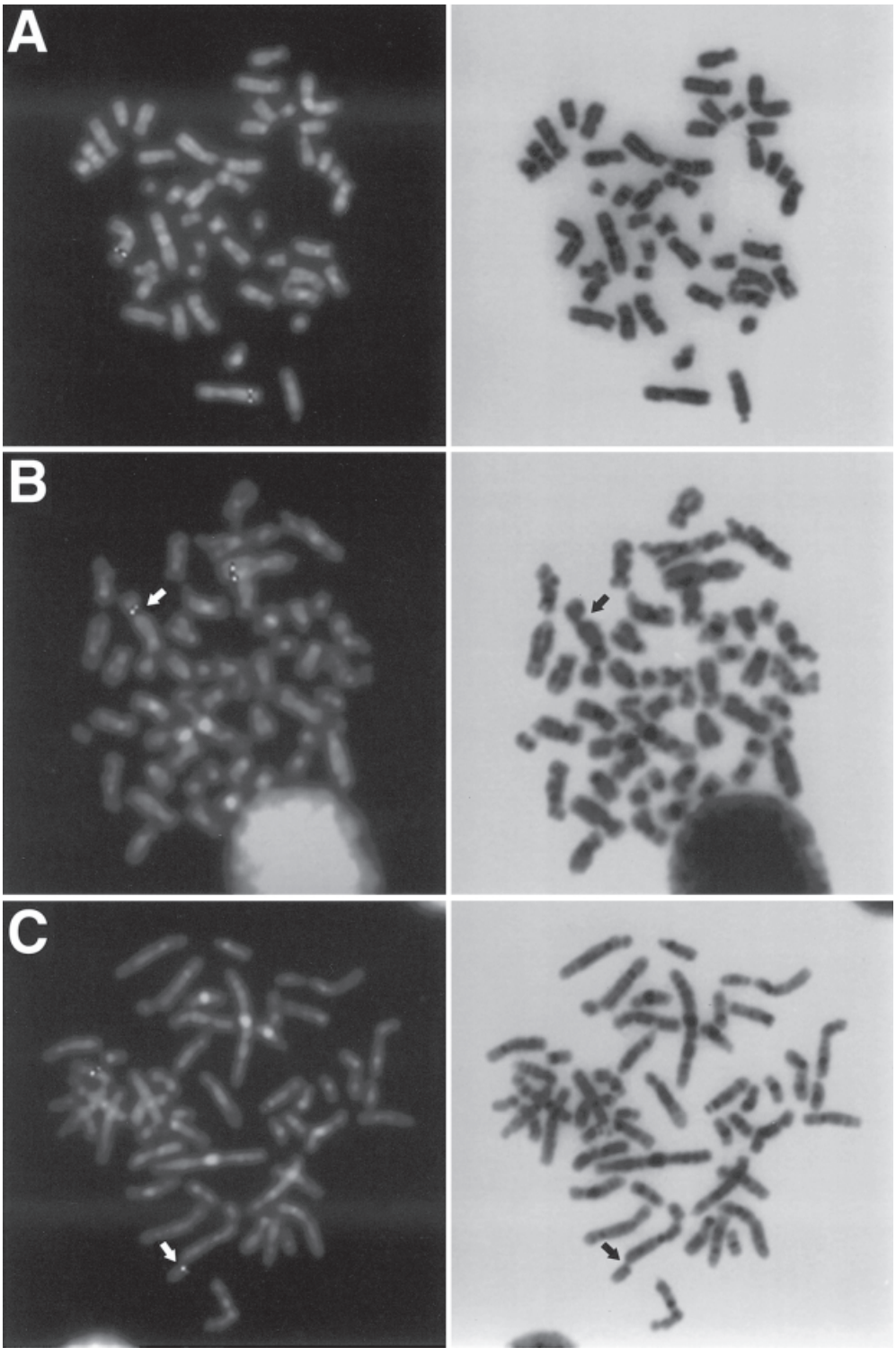

Fig. 7. Position of *FRA2G* at 2q31. Metaphase spreads prepared from a normal donor were hybridized to YAC probe 821E6 labeled with digoxigenin. Fluorescent signal was visualized on DAPI-stained chromosomes (*left panels*) and DAPI-enhanced G-banded chromosomes (*right panels*) for precise localization. *Upper*, a control metaphase without stimulation with aphidicolin. The YAC probe hybridized to the region 2q33. *Middle* and *lower*, two metaphases stimulated with aphidicolin, showing multiple fragile sites on different chromosomes, represented by gaps and displacements of the chromatids; *arrows* indicate the position of FRA2q31. Symmetrical hybridization signal from probe 821E6 is located at the telomeric flank of the *FRA2G* (FRA2q31)

the Netherlands 2q familial break maps. Therefore, although it is possible that the Polish and Dutch 2q breaks are similar, the cytogenetic data suggest they are far apart.

Based on the Dutch t(2;3) familial translocation and the observation that the RCCs from this family had lost the derivative 3 (der 3) chromosome, Bodmer et al. (1998) suggested a route for multistep tumorigenesis in these RCCs, namely, that loss of the der 3 chromosome has allowed loss of one *VHL* locus, as an initiating step, followed by mutation of the second *VHL* allele. This interpretation is supported by the occurrence of different *VHL* mutations in the RCCs of individual family members. This proposal is similar to that put forward by Gnarra et al. (1994) to explain loss of the derivative 8 chromosome, carrying 3p14-3pter, in the t(3;8) familial RCCs, in which *VHL* mutations were also observed. This is an attractive theory because the tumors

would lose one copy of the *VHL* gene, as well as one copy of each of the other putative 3p suppressor genes, including the *FHIT* gene. However, acceptance of this proposal does not preclude a contribution to tumorigenesis of an altered breakpoint gene in these t(2;3) families, a contribution that can only be verified by identification and characterization of breakpoint genes affected by the t(2;3) translocations.

Interestingly, a possible tumor suppressor gene locus at 2q33 has been reported for lung cancer (Kohno et al. 1993). DNA sequences from within this homozygously deleted locus were kindly provided by Dr. Jun Yokota, and we have determined by PCR amplification that these sequences are not within our 2q breakpoint contig (data not shown). Also, through the analysis of translocations in patients with isolated cleft palate, a new locus for this disease was recently revealed at 2q32 telomeric to our 2q breakpoint region (Brewer et al. 1999). The common fragile site at 2q31–32 is closely centromeric to our 2q translocation. The role of this common fragile site in the stimulation of genomic instability in the neighboring chromosomal segments needs further elucidation.

Large-scale sequencing of selected genomic clones crossing translocations on both chromosomes revealed that the breakpoint regions are poor in genes, and rich in repetitive sequences. Currently, we are exploring the EST clones we have mapped near the translocation site on chromosome 2q by isolating full-length cDNA clones and mapping exons relative to chromosome breakpoints. Genes at breakpoints can then be examined for integrity in familial and sporadic renal cancers to determine if they exhibit characteristics of oncogenes, suppressor genes, or caretaker genes.

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