Association of a novel constitutional translocation t(1q;3q) with familial renal cell carcinoma

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Abstract

Four cases of late onset clear cell renal cell carcinoma (RCC), a case of gastric cancer, and a case of exocrine pancreatic cancer were identified in a Japanese family. In order to elucidate the underlying mechanism for tumorigenesis in this family, extensive genetic studies were performed including routine and spectral karyotyping (SKY), fluorescence in situ hybridisation (FISH), comparative genomic hybridisation (CGH), loss of heterozygosity studies (LOH), and *VHL* mutation analysis. A germline translocation t(1;3)(q32-q41;q13-q21) was identified by karyotyping in five members of the family including all three RCC cases tested. The translocation was refined to t(1;3)(q32;q13.3) by FISH analysis using locus specific genomic clones, and the two breakpoints were mapped to a 5 cM region in 3q13.3 and a 3.6 cM region in 1q32. Both CGH and allelotyping using microsatellite markers showed loss of the derivative chromosome 3 carrying a 1q segment in the three familial RCCs analysed. Additional chromosomal imbalances were identified by CGH, including amplifications of chromosomes 5 and 7 and loss of 8p and 9. No germline *VHL* mutation was found but two different somatic mutations, a splice (IVS1-2A>C) and a frameshift (726delG), were identified in two RCCs from the same patient confirming their distinct origin.Taken together, these results firmly support a three step model for tumorigenesis in this family. A constitutional translocation t(1q;3q) increased the susceptibility to loss of the derivative chromosome 3 which is then followed by somatic mutations of the RCC related tumour suppressor gene *VHL* located in the remaining copy of chromosome 3.

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Several distinct forms of adult hereditary renal tumours have been described.[1] The most common one is von Hippel-Lindau disease (VHL), which is an autosomal dominant cancer syndrome characterised by clear cell renal cell carcinoma (RCC), retinal angioma, cerebellar haemangioblastoma, phaeochromocytoma, and endocrine pancreatic tumour. Based on positional cloning, the VHL gene was mapped to 3p25-p26 and subsequently cloned.[2] A second form of hereditary renal tumours is hereditary papillary RCC, which was found to be associated with active mutations of the *c-MET* proto-oncogene located in 7q31.[3] Recently, we described another form of familial clear cell RCC, which is not associated with VHL or with constitutional chromosomal abnormalitites.[4] Other forms of hereditary renal tumours include the Birt-Hogg-Dube syndrome,[5] tuberous sclerosis,[6] hyperparathyroidism-jaw tumour syndrome,[7] and familial oncocytoma.[8]

Cohen *et al*[9] described a family with 10 cases of clear cell RCC that were associated with a constitutional t(3;8)(p14;q24) translocation. It has long been thought that the genes at these breakpoints, especially that in 3p, are responsible for the phenotype. To date, both breakpoint genes have been identified but there is no evidence showing their involvement in the tumorigenesis of this family.[10 11] Several subsequent studies showed that the tumours from this family showed loss of the derivative chromosome carrying the 3p segment, and some of them also carried *VHL* mutations in the remaining copy of chromosome 3.[3 12 13] Based on these findings, a three step model for tumorigenesis in this (3;8) translocation family was proposed in the following order: (1) the constitutional 3p translocation, (2) the loss of the derivative chromosome carrying the 3p segment in kidney cells, and (3) the somatic mutations in RCC related genes such as *VHL*. This model was recently supported by studies of a Dutch t(2;3)(q35;q21) family with four cases of clear cell RCC in which all three steps were shown, although the chromosome 3 breakpoint was physically distinct from that of the first family.[14]

We describe here a previously unreported Japanese kindred with familial clear cell RCC in which several genetic studies have been performed to elucidate its underlying aetiology.

Materials and methods

A previously unreported Japanese family with a history of renal cell carcinoma (fig 1) was studied by reviewing their clinical data and histology of their tumours. Informed consent was obtained from II.2, II.3, II.9, III.3, III.4, III.6, and IV.1 for cytogenetic and molecular genetic studies. In addition, the following kidney tumours were studied: one from case II.8, two from case III.3 (one from the second operation on the right kidney and one from the

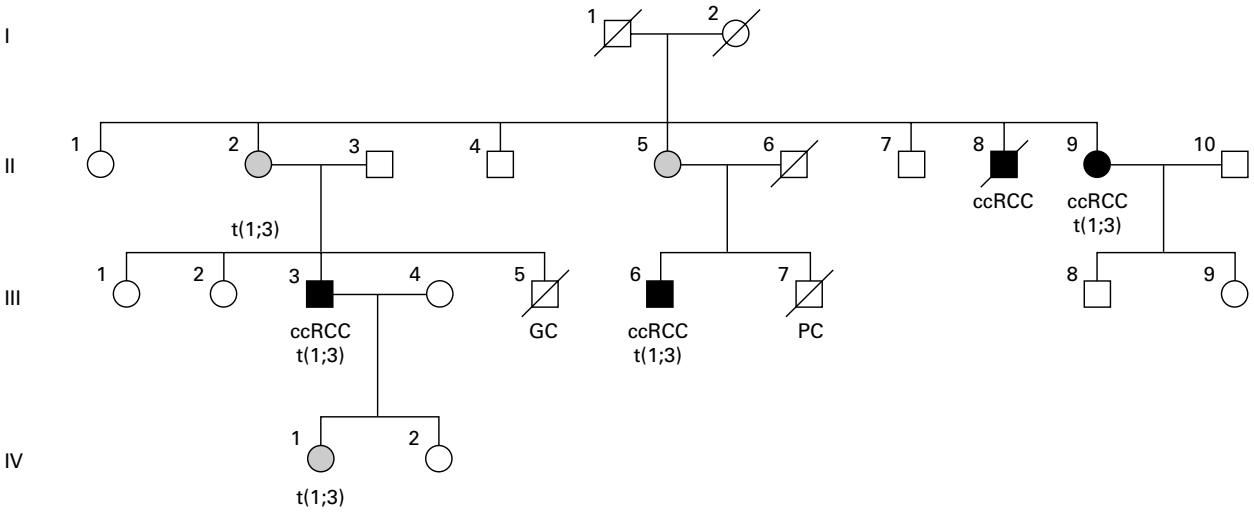

Figure 1 Pedigree of family with inherited clear cell RCC. Filled symbols indicate affected and empty symbols at present unaffected family members. The obligate carriers II.2, II.5, and IV.1 are hatched. Subjects carrying the constitutional translocation are indicated by t(1q;3q). ccRCC = clear cell RCC, GC = gastric cancer, PC = pancreatic cancer.

third operation on the left kidney), and one from case III.6. The tumour samples were used for DNA extraction and subsequent analysis by comparative genomic hybridisation, loss of heterozygosity, and sequencing of the *VHL* gene.

KARYOTYPING

Routine karyotyping was performed in these subjects as previously described.[15] The clonality criteria and the description of karyotypes followed the recommendations of the International System for Human Cytogenetic Nomenclature (ISCN, 1995).[16]

SPECTRAL KARYOTYPING (SKY)

EBV transformed lymphoblastoid cell lines were established from II.2 and III.3. Spectral karyotyping was performed according to the protocol included in the SKY kit (Applied Spectral Imaging, Migdal Haemek, Israel) on metaphase slides to look for any subtle cytogenetic abnormality. Image acquisitions were performed using a SD200 Spectracube system (ASI) mounted on a Zeiss Axioskop microscope with a custom designed optical filter (SKY-1, Chroma Technology, Brattleboro, VT, USA).

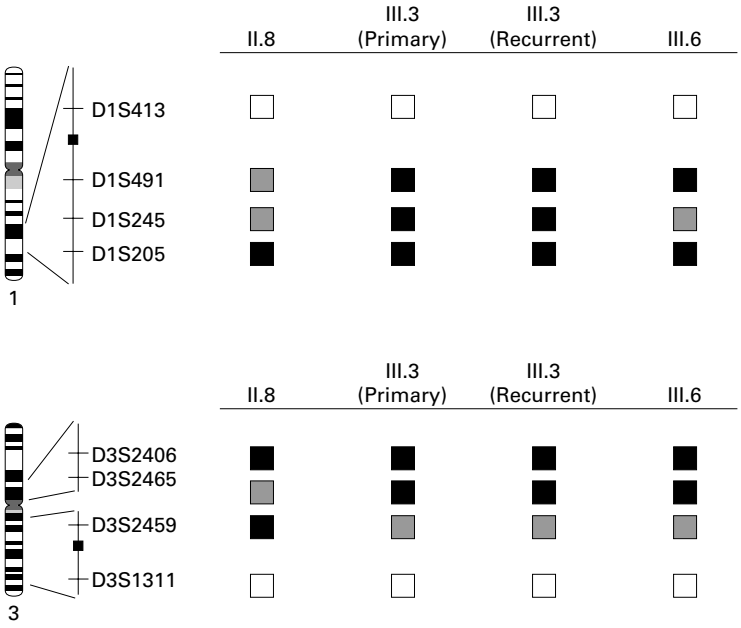

Figure 2 Summary of the results from LOH analyses of the four clear cell RCCs. The microsatellite markers typed are listed in order from centromere to telomere and their physical locations are indicated next to ideograms of chromosomes 1 and 3. Filled symbols indicate LOH, white symbols denote retention of heterozygosity, and grey symbols indicate uninformative loci, that is, constitutional homozygosity.

FLUORESCENCE IN SITU HYBRIDISATION (FISH)

The following BAC clones from BACPAC Resource Center (Children's Hospital, Oakland Research Institute) were used: 196b7 (1q31), 70g20 (1q32.1), 219p13 (1q31-q32), 45f21 (1q32.2), 104a2 (1q32.2), 124a11 (1q32), and 149c8 (1q41) from 1q, and 129g16 (3q11.2), 5k13 (3q13.2), 214a5 (3q13.3), 165b13 (3q13.3), 217p4 (3q13.3-q21), and 59j16 (3q21) from 3q. DNA from the BAC clones was isolated according to the specification of the supplier (www.chori.org/bacpac/.-framebpmini.htm). FISH was performed as previously described[17] and the results were analysed using a Zeiss Axioplan 2 (Carl Zeiss Jena Gmbh, Jena, Germany) epifluorescence microscope and a Sensys charge couple device (CCD) camera (Photometrics, Tuscon, AR, USA) connected to an IPLab Spectrum 10 Workstation (Signal Analytics Corporation, Vienna, VA, USA).

COMPARATIVE GENOMIC HYBRIDISATION (CGH)

CGH was carried out and analysed as previously described.[18–20] Green to red ratios >1.20 were considered as gains and ratios <0.80 were scored as losses of genetic material. Heterochromatic regions in the centromeric and paracentromeric parts of some chromosomes, the short arm of the acrocentric chromosomes, and the Y chromosome were not included in the evaluation.

LOSS OF HETEROZYGOSITY STUDIES (LOH)

Matched pairs of blood and tumour DNA samples were genotyped for nine polymorphic

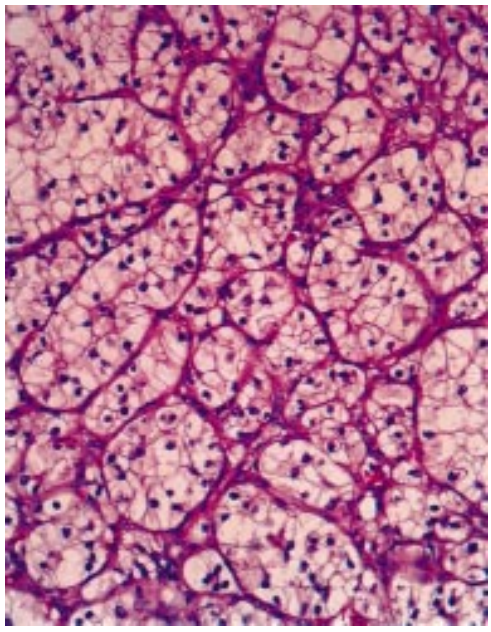

Figure 3 Microphotograph showing the clear cell pattern of the right RCC from patient III.3 after haematoxylin-eosin staining.

microsatellite markers (fig 2) including D3S2406, D3S2465, D3S2459, D3S1311, D1S413, D1S491, D1S245, and D1S205. Both radioactive and non-radioactive (fluorescent) methods of detection were performed as previously described.[7 14] LOH was confirmed when the ratio of allele intensity of the tumour DNA to normal DNA was 50% or less.

DIRECT SEQUENCING OF THE *VHL* GENE

Direct sequencing of the *VHL* gene was performed on constitutional DNA and tumour DNA from patients II.8, III.3 (primary and recurrent tumour DNA), and III.6. The primers were: 1F (5'-CGAAGAGTAC GGCCCT GAAG AAGAC) and 1R (5'-CAGTACCCT GGATGTGTCC TGCCTC) for covering exon 1, 2F (5'-AGACGAGG TTTCAC CACG TTAGC) and 2R (5'-GTCCTCTATC CTGTACTTAC CAC) for exon 2, and 3F (5'-CTGAGACCCT AGTCTGCCAC TGAG GAT) and 3R (5'-CAAAAGCTG AGAT GAAAC AGTGTAAGT) for exon 3. The PCR products were run in a cycle sequencing reaction using the ABI PRISM BigDye Primer cycle sequencing ready reaction kit (Applied Biosystems, PE Corp, Foster City, CA) and the products were run on the automated sequencer ABI 377 (Applied Biosystems, PE Corp, Foster City, CA).

Results

The family pedigree is shown in fig 1. II.8, a 70 year old male, was diagnosed with advanced renal cancer and skeletal metastasis (T2NXM1) in 1993. Left nephrectomy was performed and the tumour was histopathologically diagnosed as a moderately infiltrative clear cell RCC (grade 2) (fig 3). Alpha-interferon therapy was given postoperatively

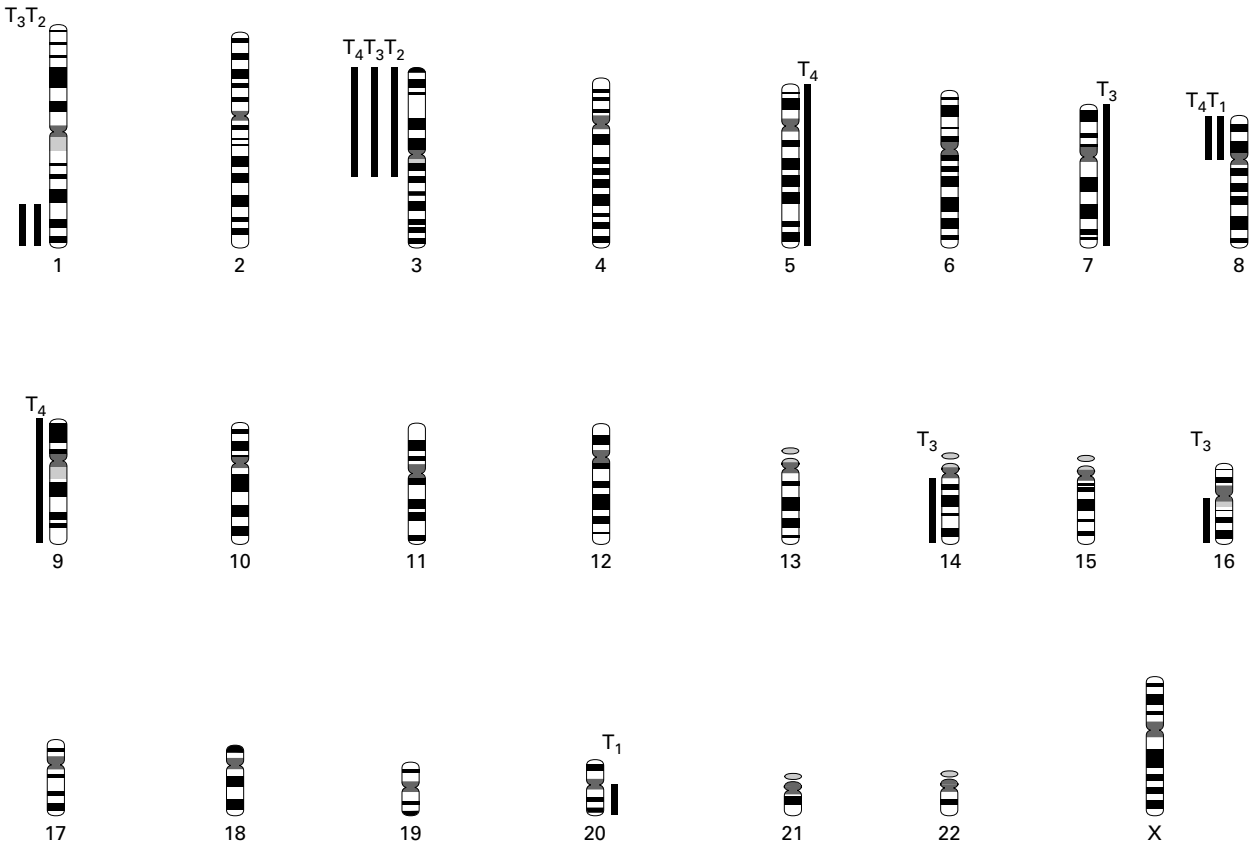

Figure 4 Summary of the numerical chromosomal imbalances detected by CGH in the four RCCs (T1-T4). Each line represents one alteration detected in one tumour, with losses illustrated to the left and gains to the right of the ideograms. T_1 denotes the tumour from case II.8, T_2 is the right and T_3 the left tumour from case III.3, and T_4 is tumour from case III.6.

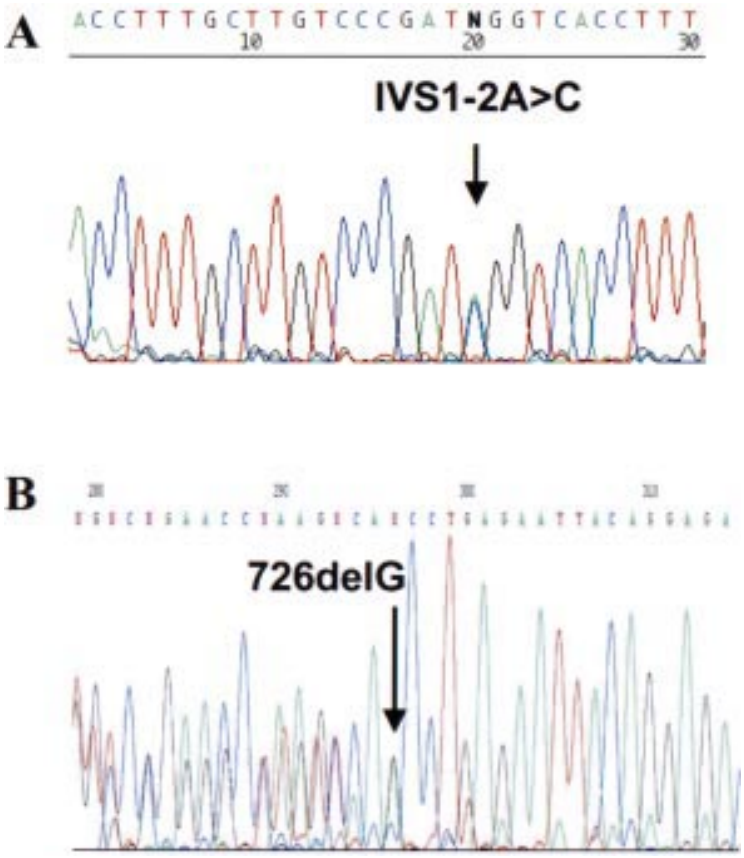

Figure 5 Two somatic VHL mutations detected by direct sequencing (Accession No L15409) in two distinct tumours from the same patient (III.3) including the splice mutation (forward strand) in the right tumour, IVS1-2A>C (A) and the frameshift mutation (reverse strand) in the left tumour, 726delG (B).

but he died six months later. II.9 was a female who was diagnosed with advanced left renal cancer with multiple lung metastases (T4NXM1) at the age of 79 in 1997. Because of the advanced stage of the disease, no surgery was performed. She was treated by left renal arterial embolisation followed by treatment with alpha-interferon. She died a year later.

In the next generation, III.3 was diagnosed with bilateral multiple renal tumours (T2N0M0) at the age of 56 in 1994. He had a left partial nephrectomy removing three tumours with a diameter of 5 cm, 8 mm, and 8 mm, respectively. Histopathological examination confirmed a grade 1, non-infiltrative, clear cell RCC. One month later, he underwent a right radical nephrectomy, which showed a tumour with identical histology to those of the tumours on the left. In 1998, a left 2 cm renal tumour of identical histology was found. The patient is currently being followed up. The fourth affected member is III.6 who was diagnosed with a localised left renal tumour (T2N0M0) at the age of 62 in 1997. Left radical nephrectomy was performed which showed a moderately infiltrative clear cell RCC (grade 2). Follow up so far has shown no evidence of recurrence or metastasis. There were two other family members who died from non-renal cancer. III.5 died of gastric cancer at the age of 49 and III.7 died of exocrine pancreatic cancer at the age of 60.

A constitutional translocation t(1;3)(q32-q41;q13-q21) was found by G banding in all three affected cases tested (fig 1). In addition, two unaffected translocation carriers were identified (II.2 and IV.1), who are obligate gene carriers and are at risk of developing the disease. Similarly, II.5, who was not karyotyped, is also an obligate carrier since her affected son, III.6, carried the 1;3 translocation. The translocation was confirmed by spectral karyotyping, but no additional subtle cytogenetic abnormalities were found. In order to refine the subchromosomal regions involved in the breakpoints, FISH mapping using BAC clones from chromosomes 1 and 3 was performed. Based on these analyses the translocation was established as t(1;3)(q32;q13.3): (1) the 1q breakpoint refined to a 3.6 cM region between clones 219p13 in 1q31-q32 and 45f21 in 1q32.2, and (2) the 3q breakpoint to a 5 cM region between clones 214a5 in 3q13.3 and 165b13 in 3q13.3 (data not shown).

Loss of heterozygosity and comparative genomic hybridisation showed the loss of the derivative chromosome 3 carrying the 1q segment in all four tumours analysed. The LOH results using microsatellite markers are summarised in fig 2, and the CGH results are illustrated in fig 4. Furthermore, a splice VHL mutation, IVS1-2A>C, and a frameshift mutation, 726delG, were detected in the right and left tumours from III.3, respectively (fig 5).

Discussion

Overall, our genetic studies support the three step model for translocation associated RCC tumorigenesis (fig 6). Kovacs and Kung[21] first proposed that the chromosome 3 translocation

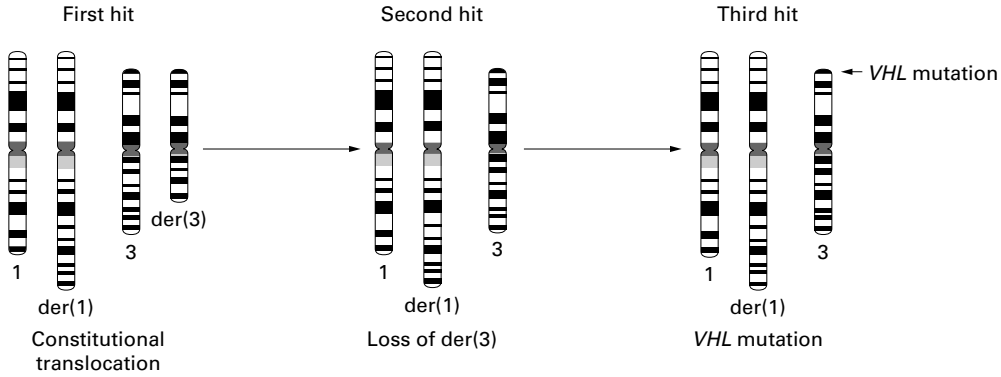

Figure 6 Schematic illustration of the three step model for tumorigenesis of RCC in the present family.

Table 1 Renal cell carcinoma associated with germline chromosome 3 translocations

Family	Translocation	Breakpoint gene	No of RCC patients	Refs
1	t(3;4)(p13;p16)	Unknown	1	22
2	t(3;6)(p13;q25)	Unknown	1	23
3	t(3;8)(p14;q24)	*FHIT* and *TRC8*	10	9
4	t(3;6)(q12;q15)	Unknown	3	22
5	t(3;12)(q13;q24)	Unknown	1	24
6	t(1;3)(q32;q13.3)	Unknown	4	This study
7	t(2;3)(p35;q21)	Unknown	4	25

could lead to a random non-disjunctional loss of the derivative chromosome that carries the 3p segment in which RCC related tumour suppressor genes such as *VHL* are located. In agreement with previous studies,[3 14] we detected the loss of the derivative chromosome 3 in the tumours. The demonstration of the two different *VHL* somatic mutations (IVS1-2A>C and 726delG) in two RCCs from the same patient (III.3) confirmed the third step. The failure to identify any *VHL* mutation in two other tumours can be attributed to several factors. The mutation may reside in a *VHL* regulatory region or involve a large deletion, which was not investigated because of the lack of tumour DNA. It is also possible that the *VHL* gene may be hypermethylated. Alternatively, somatic mutations of as yet unidentified RCC related genes in 3p may be involved.

To date, different germline chromosome 3 translocations have been described in RCC patients (table 1)[9 22–25]: three of them were found in single RCC patients and another three in multiple affected members with RCC. The present study showed a novel chromosome 3 translocation locus, which is associated with multiple RCC cases. Naturally, one would speculate that chromosome 3 translocations per se, irrespective of their loci, would lead to the second and third steps of tumorigenesis (fig 6). However, whether the breakpoint genes, especially those located on chromosome 3, play a pathophysiological role in their tumorigenesis cannot be confirmed. To date, only the *FHIT* gene from the 3p14 breakpoint has been identified. Despite many studies, it remains controversial if the gene is functionally involved in RCC tumorigenesis.[26 27] Therefore, it will be worthwhile to isolate these other chromosome 3 breakpoint genes so that we can examine their involvement in RCC tumorigenesis and in the mean time compare their function with that of the *FHIT* gene. We have narrowed our 1q and 3q breakpoints to a 3.6 cM and a 5 cM region, respectively, and further positional cloning is currently under way.

Clinically, there are features in this family that are similar to those of two other previously reported families.[9 25] First, there is reduced penetrance with two obligate translocation carriers (II.2 and II.5, fig 1) who lived to their eighties without any evidence of malignancy. Second, non-RCC malignancies have been found. In these two families, thyroid cancer and bladder cancer were found and, in our family, pancreatic cancer and gastric cancer were found although the patients could not be confirmed to have the constitutional translocation since they had both died before the present study. The ages of presentation of the RCC cases are relatively high in contrast with VHL cases, which are characterised by Knudson's two step process. This may be caused by the additional step involved that allows the kidney tumours in this family to develop.

The CGH results are interesting as they imply the involvement of additional chromosomal regions containing RCC related genes. One such region is chromosome 8p which is lost in two tumours, and which has been previously implicated in non-papillary RCC. Other abnormalities including loss of chromosome 9 and amplification of chromosome 5 have been frequently reported in RCC suggesting a role for these regions in RCC tumorigenesis.[28]

In summary, we describe here the clinical characteristics of a Japanese RCC family and present the underlying genetic findings that may explain its tumorigenesis.

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