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Disruption of the APC gene by t(5;7) translocation in a Turcot family

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Turcot syndrome (TS) refers to the combination of colorectal polyps and primary tumours of the central nervous system. TS is a heterogeneous genetic condition due to APC and/or mismatch repair germline mutations. When APC is involved the vast majority of mutations are truncating, but in approximately 20%–30% of patients with familial polyposis no germline mutation can be found. A 30-year-old Caucasian woman with a positive pedigree for TS was referred to our Genetic Counselling Service. She was negative for APC and MUTYH but showed a reciprocal balanced translocation t(5;7)(q22;p15) at chromosome analysis. FISH analysis using specific BAC probes demonstrated that 5q22 breakpoint disrupted the APC gene. Transcript analysis by MLPA and digital PCR revealed that the cytogenetic rearrangement involving the 3′ end of the APC gene caused a defective expression of a truncated transcript. This result allowed cytogenetic analysis to be offered to all the other family members and segregation analysis clearly demonstrated that all the carriers were affected, whereas non-carriers did not have the polyposis. A cytogenetic approach permitted the identification of the mutation-causing disease in this family, and the segregation analysis together with the transcript study supported the pathogenetic role of this mutation. Karyotype analysis was used as a predictive test in all members of this family. This family suggests that clinically positive TS and FAP cases, which test negative with standard molecular analysis, could be easily and cost-effectively resolved by a classical and molecular cytogenetic approach.

Keywords Turcot syndrome, APC, conventional karyotype, MLPA, FISH, digital PCR

Introduction

Turcot syndrome (TS) refers to the combination of colorectal polyps and primary tumours of the central nervous system (ORPHA 99818). TS is a heterogeneous genetic condition due to APC and/or mismatch repair (MMR) germline mutations. Usually TS patients showing APC mutations are characterized by classical familial polyposis, with an autosomal pattern of inheritance; instead, MMR related TS patients present multiple colonic polyps and an autosomal recessive pattern of inheritance. TS due to APC mutations is a clinical variant of FAP in which the most common brain tumour is medulloblastoma, but other types of brain tumour have also been described (OMIM) (1).

Focusing on the APC gene, more than 300 different types of mutations are recognized today as a cause of classical FAP and clinical variants. The vast majority of mutations (84%) are truncating, including small deletions, small insertions and nonsense mutations. Missense mutations (3%) and large genomic deletions involving the 5q21 region (13%) have also been reported. In clinically diagnosed FAP patients, standard sequencing and MLPA show a detection rate of APC mutations near to 75%–80% of tested cases. In a small subset of FAP patients showing extraintestinal manifestations such as mental retardation, dysmorphic features or developmental delay, cytogenetic 5q deletion was diagnosed (2–9).

Overall, in approximately 20%–30% of FAP patients, no germline mutation can be found, although the success rate

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is improving with extensive testing. In these families, genetic testing is not helpful. Therefore clinical diagnosis and systematic surveillance are mandatory for all first-degree relatives.

Here we describe a TS family in which standard APC testing proved to be ineffective in identifying the disease-causing mutation and in which a conventional and molecular cytogenetic approach allowed the identification of a previously undiagnosed rearrangement of the APC gene.

Patients and methods

Proband and family history

The patient (IV-I; Figure 1A) was referred to the Genetic Counselling Service at the age of 30 when, after a clinical diagnosis of diffuse colorectal polyposis, she underwent a subtotal colectomy with ileorectal anastomosis. The patient reported that her father (III-1) had had colorectal polyposis and had died from colorectal cancer. Her uncle (III-2) had had diffuse colorectal polyposis and had undergone a subtotal colectomy at the age of 48. After a few months, the patient's uncle had presented with a grade III astrocytoma of the left frontal lobe, from which he died six months later. Subjects IV-5, IV-8 and III-4 were also affected by diffuse colorectal polyposis. Histological examination of all available polyps from the proband and other family members (III-2, III-4, IV-5, IV-8) showed them to be tubular adenomas with low, moderate and high grades of dysplasia. In all patients with polyposis, osteomas and congenital hypertrophy of the retinal pigment epithelium (CHRPE) were excluded.

Subjects II-1 and II-3 both died from colorectal cancer at ages 35 and 42 respectively. The family history was negative for other significant pathologic conditions.

Informed consent to use their DNA and clinical data for research purposes was provided by each individual within the counselling session, following the procedures dictated by the local Ethical Committee.

Genetic testing for APC and MUTYH was offered to the proband (IV-1), but no mutations were found in these genes, neither by direct sequencing nor by MLPA approach using the SALSA P043 APC MLPA kit (MRC-Holland, Amsterdam, The Netherlands).

Considering the genetic heterogeneity of TS, we also performed somatic analysis on the astrocytoma and in the adenomas in order to assess an MMR deficiency. However, both immunohistochemical studies of MLH1, MSH2, MSH6 and PMS2 proteins and MSI analysis excluded MMR defects in all these lesions.

Cytogenetic analysis

Chromosome conventional analysis was performed from peripheral blood lymphocyte cultures using the standard method (10). Slides were QFQ-banded and analysed according the recommendation of ISCN 2013 (11). FISH analysis was performed on metaphase spread from blood lymphocytes as previously described (12). A bacterial artificial chromosome (BAC) contig for 5q22.2 was selected to cover the region containing the entire APC locus (112,707,504–112,846,238; Ensembl, http://www.ensembl.org/). The presence of APC exon sequences in the three overlapping BAC probes was checked using SALSA PO43 APC MLPA kit. FISH analysis on normal metaphases with all probes was performed to verify the physical mapping and to exclude chimeric signals. The selected BAC included: RP11-56P2 (cAPC1) encompassing promoter region and exons 1–8; RP11-107C15 (cAPC2) encompassing exons 1–15 and RP11-248F15 (cAPC3) encompassing exons 12–15 (BlueGnome, Technogenetics). Dual-colour FISH experiments were set-up using green labelled cAPC1 co-hybridized with red labelled cAPC2, and green labelled cAPC2 co-hybridized with red labelled cAPC3.

DNA and RNA extraction

Genomic DNA was extracted from whole blood using DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany). The astrocytoma and polyps were manually microdissected each from three 8-μM sections of formalin fixed and paraffin embedded tissues after xylene/ethanol dewaxing. DNA extraction was performed using QIamp DNA FFPE tissue kit (Qiagen). Total RNA was extracted from whole blood using PAX gene Blood RNA kit according to the manufacturer protocols (PreAnalytix, Qiagen).

MMR protein immunohistochemistry and MSI analysis

The immunohistochemical study of MMR proteins was performed as previously described (13).

Microsatellite analysis was carried out using a pentaplex panel of monomorphic mononucleotide repeats as already described (14).

Figure 1 A. Pedigree of the patient. The arrow indicates the proband. The age of diagnosis is indicated next to the site of each tumour. Carriers of the translocation are indicated by "t(5;7)," while normal karyotypes are indicated by "Norm". B. Dual fluorescence in situ hybridization using bacterial artificial chromosome probes for mapping the rearrangement of the APC gene. cAPC1 green labelled and cAPC2 red labelled: a single red signal on the der7 chromosome and a fused signal on der5 are visible in the left panel. cAPC2 green labelled and cAPC3 red labelled: a fused signal on der7 and a single green signal on der5 are visible on the right. C. Electropherogram profiles showing the results of APC MLPA on cDNA relative to exons 6–10. A diminished dosage of exons 8, 9 and 10 compared to exons 6 and 7 is evident in the proband profile. On the contrary, the non-carrier subject's profile (IV-4) was comparable to that of control. RFU, Relative Fluorescent Units. D. Chart showing digital PCR results on APC transcripts. In subject carriers of t(5;7), IV-1, IV-5 and IV-8, the exon 8–9 transcript is about half of the exon 6–7 transcript. In non carriers (IV-4 and IV-9) the dosage of the two transcripts is similar. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)