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Familial Hepatoblastoma and *APC* gene mutations: renewed call for molecular research

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Abstract

Recent findings have increased our understanding of the molecular mechanisms involved in the pathogenesis of hepatoblastoma and their relationship to the molecular pathology of familial adenomatous polyposis (FAP). Here, we describe hepatoblastoma in siblings who share a gene mutation for FAP inherited from their father. This observation confirms the link between these diseases and has implications for future molecular research. We also raise the question; should other members of 'at-risk' families be screened following a new diagnosis of either hepatoblastoma or FAP?

Keywords: Hepatoblastoma; Hepatoblastoma and adenomatous polyposis coli gene; Familial adenomatous polyposis; *APC* gene mutation; Family screening

1. Introduction

Hepatoblastoma is an embryonal liver tumour that typically affects children aged less than 3 years and is more common in boys [1]. It is rare—comprising less than 5% of abdominal cancers in the paediatric population—but is, nevertheless, the most common malignant liver tumour in children. Inherited genetic factors contribute to at least some cases, for example, hepatoblastoma has been associated with chromosomal alterations, with mutations of the adenomatous polyposis coli (*APC*) gene and with various diseases/syndromes including glycogen storage diseases, Beckwith–Wiedemann (BWS) and Simpson Golabi Behmel syndromes. However, the molecular pathogenesis of hepatoblastoma is still poorly understood.

There have been six reports of familial hepatoblastomas during the last 30 years [2–7], and links have been established both between hepatoblastoma and familial adenomatous polyposis (FAP) [8–11], and between FAP and the presence of congenital hypertrophy of the retinal pigment epithelium (CHRPE) [8,12]. We report here the novel finding of a family (Family B) in which hepatoblastoma developed in siblings in association with a FAP gene mutation and CHRPE-positivity.

2. Family case report

2.1. Child 1

The elder brother (II-2 in Family B pedigree, Fig. 1) presented in December 1996, aged 7 months, with an enlarged abdomen due to a right-sided tumour. Imaging revealed a primary hepatic mass that displaced the midline liver structures to the left, and extended exophytically from the right liver lobe to the right lower abdomen and multiple metastatic lesions throughout the left lobe of the liver, but no lung metastases. Serum alpha-fetoprotein (AFP) level was 200 000 KU/l and a

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liver biopsy confirmed this tumour to be a 'fetal type' hepatoblastoma. No family history of FAP was noted at the time.

He was managed according to the contemporary Societe Internationale d'Oncologie Pediatrique (SIOP)[13] protocol; SIOPEL II as a high-risk group patient because all four sections of the liver were involved by tumour. After receiving chemotherapy, there was an impressive tumour response enabling a right hemihepatectomy, followed by further chemotherapy as per protocol. Unfortunately the tumour recurred in 1998 in the caudate lobe of the liver associated with rising serum AFP. He underwent further hepatic resection in March 1999 (resection of segment 4 and caudate lobe). Histology of the resected tumour confirmed 'fetal type' hepatoblastoma with minimal pleomorphism. Immunohistological stains of the resected tissue were positive for AFP and cytogenetic studies of tumour cells revealed a normal male karyotype and no chromosomal aberrations.

Since then, he has done well and is now (May 2003) 6 years old with no evidence of relapse and a normal serum AFP level.

2.2. Child 2

His brother (II-3 in Fig. 1) subsequently presented in November 2001, also aged 7 months, with abdominal fullness secondary to hepatomegaly. Investigation revealed an elevated serum AFP level and computerised tomography (CT) scan demonstrated a large solitary median (segments 4, 5 and 8) mass within the liver, but no lung metastases. Biopsy confirmed the diagnosis of hepatoblastoma (fetal type) and cytogenetic study of the biopsy revealed a 46XY normal male karyotype.

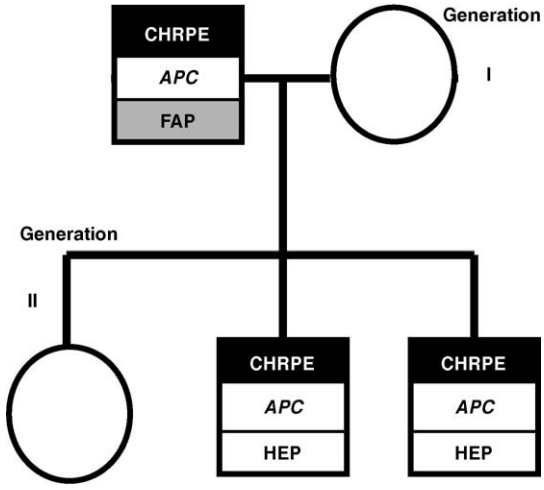

Fig. 1. B Family tree (parents and three children) with hepatoblastoma cases (HEP), congenital hypertrophy of the retinal pigment epithalium (CHRPE) positivity (black filling), adenomatous polyposis coli (*APC*) gene mutation and total colectomy for familial adenomatous polyposis (FAP, grey filling). There was no history of colon disease prior to generation I.

He was treated according to the SIOPEL III protocol (standard risk), with four courses of cisplatin monotherapy prior to surgery. There was a good response with progressive reduction in both the tumour size and serum AFP levels. He underwent a left extended hepatectomy at 11 months of age and the tumour was successfully resected with clear resection margins. He is currently doing well 10 months after surgery (May 2003), with a normal AFP level.

2.3. Family B investigations (Fig. 1)

In view of the family history of hepatoblastoma, fundoscopy was performed in Case 2 and congenital hypertrophy of the retinal pigment epithelium (CHRPE) was identified. On further questioning, the father admitted a history of bloody diarrhoea, which he had attributed to a previous enteric urinary diversion, the consequence of treatment of a previous urethral structure. He then proceeded to have a colonoscopy that revealed polyposis and established the diagnosis of FAP; he has since had a total colectomy. Multiple adenomatous polyps were identified but no malignancy was found. Both brothers remain asymptomatic, but will have close gastrointestinal follow-up including colonoscopy from 10 years of age. Ophthalmological review, constitutional karyotyping and *APC* gene mutation analysis was arranged for the immediate family.

Ophthalmological review revealed CHRPE in the father and both brothers (cases 1 and 2), but not in the mother or sister. Karyotype was normal in all five members of the family, but a truncating (nonsense) mutation S770X, consistent with the diagnosis of FAP, was identified in exon 15(B) of the *APC* gene in the father and in both brothers (cases 1 and 2). The mother and sister were negative for known mutations of the *APC* gene.

3. Discussion

There have been only five reports of hepatoblastoma occurring in siblings during the last 3 decades [2–5,7] (Table 1). An association with FAP was identified in only one of these families and in that case hepatoblastomas developed in identical twins. De Chadarevian and colleagues [6] recently reported another interesting FAP family in which one boy developed a hepatoblastoma and his brother had multiple adenomas and a well-differentiated hepatocellular carcinoma. No mutations were identified in the *APC* gene in this family. Instead, the FAP phenotype occurred as a result of a chromosome rearrangement (inversion 5q21 to 5q31.3), involving the *APC* gene locus inherited from their father and grandfather. The family we report here is therefore unique in showing co-inheritance of two characteristic

Table 1
Literature review: hepatic neoplasia in siblings and related genetic background

[Ref.]	Year	Tumour type[a]	Sibling characteristics	Phenotypic background	Inheritance from	CHRPE-positivity	*APC* gene mutation	Genetic abnormality
[3]	1969	2 HBL	Sisters	–	–	–	–	–
[4]	1977	2 HBL	Brother/sister	–	–	–	–	–
[7]	1987	2 HBL	Brother/sister	Glycogen storage disease	–	–	–	–
[2]	1989	2 HBL	Brother/sister	–	–	–	–	–
[5]	1990	2 HBL	Identical male twins	Presumed FAP	Grandmother/mother	Mother only	–	–
[6]	2002	1 HBL 1 Ad/HCCA	Brothers	FAP	Grandfather/father	–	Not found	5q21 to 5q31.3 inversion
This series	*2003*	*2 HBL*	*Brothers*	*FAP*	*Father*	*Father and two brothers*	*Father and two brothers*	*S770X mutation in exon 15(B)*

[a] HBL, hepatoblastoma; Ad/HCCA, multiple adenomas with features of well differentiated hepatocarcinoma; CHRPE, congenital hypertrophy of the retinal pigment epithelium; APC, adenomatous polyposis coli; FAP, familial adenomatous polyposis.

features of FAP—*APC* gene mutation and CHRPE-positivity—and familial hepatoblastoma. It is also interesting to note that genetic transmission was through the male line, as was the case for the chromosome rearrangement reported by De Chadarevian and colleagues [6].

The link between hepatoblastoma and FAP is well established [9,10,14,15] and the incidence of FAP in hepatoblastoma patients is approximately 100 times that in the general population. There are also reports of survivors of hepatoblastomas developing adenomatous lesions and these patients may also exhibit CHRPE [1]. These findings support the suggestion that specific gene mutations may lead to a transmissible risk of hepatoblastoma. A handful of reports have mentioned familial hepatoblastoma associated with FAP in other family members, but this is the first family where hepatoblastoma occurred in two brothers who had inherited an *APC* gene mutation and CHRPE-positivity. Giardello and colleagues investigated a select group of families with FAP and hepatoblastoma. They found that hepatoblastoma was associated with *APC* mutations in the 5′ end of the gene, but the site of the mutation could not be used to predict the development of hepatoblastoma in other FAP pedigrees [16].

There has been much progress in our understanding of the development of liver neoplasia, and especially hepatoblastoma, during the last decade [17–22]. A major function of the *APC* gene is the downregulation of beta-catenin, a transcription-activating protein with oncogenic potential. *APC* gene mutations can alter this 'tumour suppressor' function with an increased risk of tumorigenesis in various organs. The crucial role of beta-catenin mutations in the development of hepatic malignancy in general, and hepatoblastoma in particular, is now well recognised [18,19]. Sporadic hepatoblastoma seems to be associated with germ-line rather than genomic *APC* gene mutations in non-FAP families (48 and 69% of cases in the reports by Koch and colleagues and Oda and colleagues, respectively [21,22]). In addition, chromosomal translocations, without identifiable *APC* gene mutations, have been found in the tumour cells in a number of hepatoblastoma cases [6,17]. The molecular mechanisms underlying these abnormalities are unknown.

Despite these advances in our understanding of the aetiology of liver tumorigenesis, the precise molecular pathogenesis of hepatoblastoma is still incomplete and more studies are needed. Hepatoblastoma is the most frequently occurring liver tumour in children, with 10–20 new cases/year in the United Kingdom (UK) and around 100 new cases/year in Europe [13]. A number of potentially fruitful areas of molecular research have been suggested [17–19,22]. This paper renews the call for further molecular genetic studies of families with FAP in order to provide further valuable information on mutations in the *APC* gene that may be associated with hepatoblastoma [23].

In the light of this report, the question again arises—'Should all patients with hepatoblastoma and their families be screened for FAP, and *vice versa*?' [1,5,9,24,25]. If so, what method should be used? It has been estimated that, in the UK, only one case in 20 of hepatoblastoma is associated with FAP [26]. The real incidence may be considerably higher as no precise local data are available [1] and studies from Japan have shown *APC* gene alterations [21] in the tumour cells in up to 69% of sporadic hepatoblastoma cases.

CHRPE can be present in some normal individuals [8] but is the commonest extracolonic manifestation of FAP and an early marker for this disease (60% of patients with FAP have CHRPE). CHRPE appears as a well-demarcated grey-brown to black round or oval lesions in the retinae of affected individuals [12]. Ophthalmoscopic screening for CHRPE is neither fully sensitive nor specific, but is a direct, non-invasive and inexpensive test, and is easy to perform on a routine

basis. Multiple or bilateral CHRPE lesions are considered to be indicative of FAP and correlate with the position of the mutations in the *APC* gene [8]. If the index case, in a family with FAP, has CHRPE then screening for CHRPE has been successfully used, by us and others, as an early clinical marker in 'at-risk' patients [8]. In the family reported here, the discovery of CHRPE in the second hepatoblastoma case led to the detection of multiple colonic adenomas (FAP) in the father, fortunately before he developed colonic malignancy. If systematic screening of the family had been carried out following the diagnosis of the first hepatoblastoma case, the father would not have been left 'at risk' for so long. Thus, screening for FAP should be considered in every family with hepatoblastoma. The converse, i.e. that infants in families with FAP should be screened for hepatoblastoma has already been proposed [1,24,25]. Relative cancer mortality is significantly higher in young children from FAP families, largely due to the increased incidence of hepatoblastoma [25].

In other cancer predisposition syndromes, such as the BWS, screening for early tumour detection is of potential benefit. A retrospective case control analysis has shown that the mean tumour stage at diagnosis of Wilms' tumour (WT) in a group of patients with BWS/hemi-hypertrophy who underwent 4-monthly abdominal ultrasonography was significantly lower than in a similarily affected group who were not screened [27]. The lower stage at diagnosis in the screened group is consistent with earlier diagnosis. This resulted in less intense curative therapy, with its reduced risk of damaging 'late effects'. It also affords the potential for limited surgery with partial nephrectomy [27]. However, whether screening children with BWS creates a survival advantage, reduces late effects or is cost-effective still needs to be addressed in a large prospective international study.

In families 'at risk' of developing hepatoblastoma, regular clinical examination with serum AFP level monitoring in infants and small children could be recommended and such screening would be relatively simple. Earlier diagnosis of hepatoblastoma would have a major impact on management and outcome, because it may lead to the discovery of smaller tumours, which could be considered for primary resection with low surgical morbidity [13]. If the histology was favourable, chemotherapy might not be needed in such cases [28]. In this group of patients, survival rates close to 100% can be achieved, but currently less than 10% of new cases are diagnosed at that stage. Around half of newly-diagnosed hepatoblastomas are already advanced (SIOP stages III and IV) and their outcome, despite advances in chemotherapy and surgery, are worse. Liver transplantation, which is sometimes necessary to cure the disease [29], would be needed less often if an earlier diagnosis was made.

It is important for paediatricians and oncologists to know that, although prospective screening in 'at-risk' families is not currently recommended, contrary advice is available to the public on the Internet (http://www.coloncancer.org/hccr_files/hccrtemp.htm or www.naspgn.org/PDF.ahnen.pdf). As a result of screening, there is a risk of overdiagnosis of benign lesions such as hemangioma—a relatively common tumour of infancy. In addition, physiologically raised levels of serum AFP may be misinterpreted, with potentially disastrous consequences [30]. This is of particular importance in BWS, as the serum AFP in these children may be higher that the 'normal' physiological range and the normal rate of fall significantly slower [31].

Recognising that CHRPE may be absent in some FAP kindreds raises the question as to whether alternative screening methods, such as molecular studies, are feasible. With adequate support and funding, the latter could first be done as part of a prospective national study that could, in turn, fuel further molecular research. In that setting, families with familial hepatoblastoma and especially those with both hepatoblastoma and FAP, would be of great importance to researchers. Whether systematic molecular screening in 'at-risk' families is useful will only be answered by such studies.

In conclusion, the findings in this unusual family confirm previously reported links between FAP and *APC* gene mutations, CHRPE and hepatoblastoma. In families in which more than one child develops hepatoblastoma, we propose the case for systematic screening via clinical history with or without ophthalmological review, colonoscopy and/or molecular studies. We also raise the question as to whether screening for hepatoblastoma may be justified in infants born into known AFP families using serial serum AFP monitoring and abdominal ultrasonography.

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