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Identification of five new families strengthens the link between childhood choroid plexus carcinoma and germline *TP53* mutations

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Abstract

We present five families of paediatric patients suffering from choroid plexus carcinoma in which we found germline *TP53* mutations. Only one of the families conformed to the criteria of Li–Fraumeni syndrome and only three (including the Li–Fraumeni syndrome family) met the Chompret criteria for germline *TP53* mutation testing. In the remaining two families no family history of cancer was identified and/or the parents of the patient were shown not to carry the mutation. Our results give further support to the notion that the occurrence of this rare paediatric tumour, especially in combination with a positive family history of cancer, but possibly also without any family history, may be an indicator of a germline *TP53* mutation. The identification of this genetic defect has important consequences for cancer prevention and treatment in affected families.

Keywords: Choroid plexus carcinoma; Childhood brain tumours; *TP53* germline mutations; Li–Fraumeni syndrome; Genetic counselling; Turner syndrome

1. Introduction

Choroid plexus tumours are very rare brain tumours with a total incidence of 0.3 cases per million per year, accounting for 0.4–0.8% of all brain tumours and for approximately 1% of paediatric brain tumours [1,2]. A recent analysis of 566 patients has shown that choroid

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plexus tumours are diagnosed at all ages from the foetal period to late senescence and most typically in early childhood. Although the median age at diagnosis is 3.5 years for all choroid plexus tumours, the age is strongly correlated with tumour location: 1.5 years of age for tumours located in the lateral or the third ventricle, but 22.5 and 35.5 years of age for tumours in the fourth ventricle and cerebellopontine angle, respectively [1]. Although the histological classification is sometimes ambiguous, two types of choroid plexus tumours can be distinguished, choroid plexus carcinoma and papilloma. The survival rate of patients with papilloma is significantly higher, but both types of choroid plexus tumours can metastasise and progression from papilloma to carcinoma has also been reported [1,3]. Interestingly, although the incidence ratio of paediatric choroid plexus carcinoma and papilloma is approximately 1 to 4 [4], the incidence of choroid plexus carcinoma among 225 congenital brain tumours was only 0.4% and that of choroid plexus papilloma was 7.1% [3].

Choroid plexus tumours have been found in rhabdoid predisposition syndrome, a familial cancer syndrome caused by germline mutations in the *SNF5* gene [5]. Choroid plexus papillomas have also been reported to be associated with Aicardi syndrome [6]. Most significantly, however, choroid plexus tumours are associated with Li–Fraumeni syndrome or with germline mutations in the *TP53* gene. The first indication for this came from the observation of an affected child in a family with a pedigree characteristic of Li–Fraumeni syndrome, followed by the identification of six other cases in the literature [7]. Since then, additional evidence has accumulated to support this link. There are reports in the literature of choroid plexus tumours in 12 families with germline *TP53* mutations and in two families with unknown *TP53* status but with clustering of cancer suggestive of Li–Fraumeni syndrome [8–18].

In this report, we present five new families of patients with childhood choroid plexus carcinomas carrying germline mutations in the *TP53* gene. This observation strengthens the link between childhood choroid plexus carcinoma and germline *TP53* mutations, with important consequences for the patient and his or her relatives.

2. Materials and methods

2.1. Patients

The patients and/or their parents or relatives were offered medical care and genetic counselling in four centres: Charles University Second Medical School and University Hospital Motol, Prague (families 1, 4, and 5); Thomayer University Hospital, Prague (family 2); The Children's Hospital, Denver, USA (family 3); University Hospital Ostrava, Ostrava (family 4).

Patient 1-III.4 (family 1) presented at the age of 8 years with headaches, nausea, diplopia, and right abducens palsy. A head CT scan demonstrated a 5×4 cm lesion in the left lateral ventricle. The tumour, a choroid plexus carcinoma, grade III, was completely resected, and the patient was treated with chemotherapy and radiotherapy postoperatively. At the age of 12 years he complained of nasal obstruction and bleeding. MRI and rhinoepipharyngoscopy revealed a vascular tumour filling the right maxillar sinus, extending into the nasal cavity and ethmoids. The tumour, a malignant haemangioendothelioma, was radically resected and the patient was treated for 1 year with immunotherapy. At present the boy is 16, and he is without any clinical signs of the disease. His mother died in her early thirties from breast cancer. Otherwise the family had no positive history of cancer (Fig. 1).

Patient 2-III.1 (family 2) asked for genetic counselling because of a remarkable family history of malignant tumours. Both of his brothers died from cancer, one from a choroid plexus carcinoma at the age of 2 years and the other from a metastatic liposarcoma at the age of 26 years. His father died from colon cancer at the age of 34 years, and both paternal grandparents suffered from early onset cancer as well (Fig. 1).

Patient 3-II.1 (family 3) presented at the age of 11 months with loss of milestones, vomiting, rhinorrhea, weight loss and somnolence. A head CT demonstrated a 6 cm lesion filling the right lateral ventricle and causing hydrocephalus. MRI of the head and spine confirmed, in addition to a ventricular mass, brainstem oedema and multiple nodular metastatic lesions in the thoracic, lumbar and sacral spine. The patient was subjected to endoscopic third ventriculostomy, biopsy and broviac placement. Pathology showed choroid plexus carcinoma. Because of the poor prognosis the decision was made to pursue no therapy. The patient was discharged with hospice care and died within several weeks. There was no history of cancer in the family and more distant relatives of this patient (Fig. 1).

Patient 4-IV.1 (family 4) presented at the age of 1 year with vomiting, malaise, and somnolence, and was diagnosed with choroid plexus carcinoma filling the left lateral ventricle and measuring $3 \times 3 \times 4$ cm. She was treated by incomplete tumour resection and successive chemotherapy and radiotherapy to the ventricles at the age of 2 years. She finished the treatment in complete remission, but 6 years later an isolated metastasis of the tumour in posterior brain fossa was identified and radically resected. No other treatment was administered and she is disease-free 3 years from the recurrence. Other symptoms, such as growth impairment, mental retardation, hypogonadism, somatic stigmatisation, myopia, strabismus, autoimmune thyroiditis and skin pigmenta-

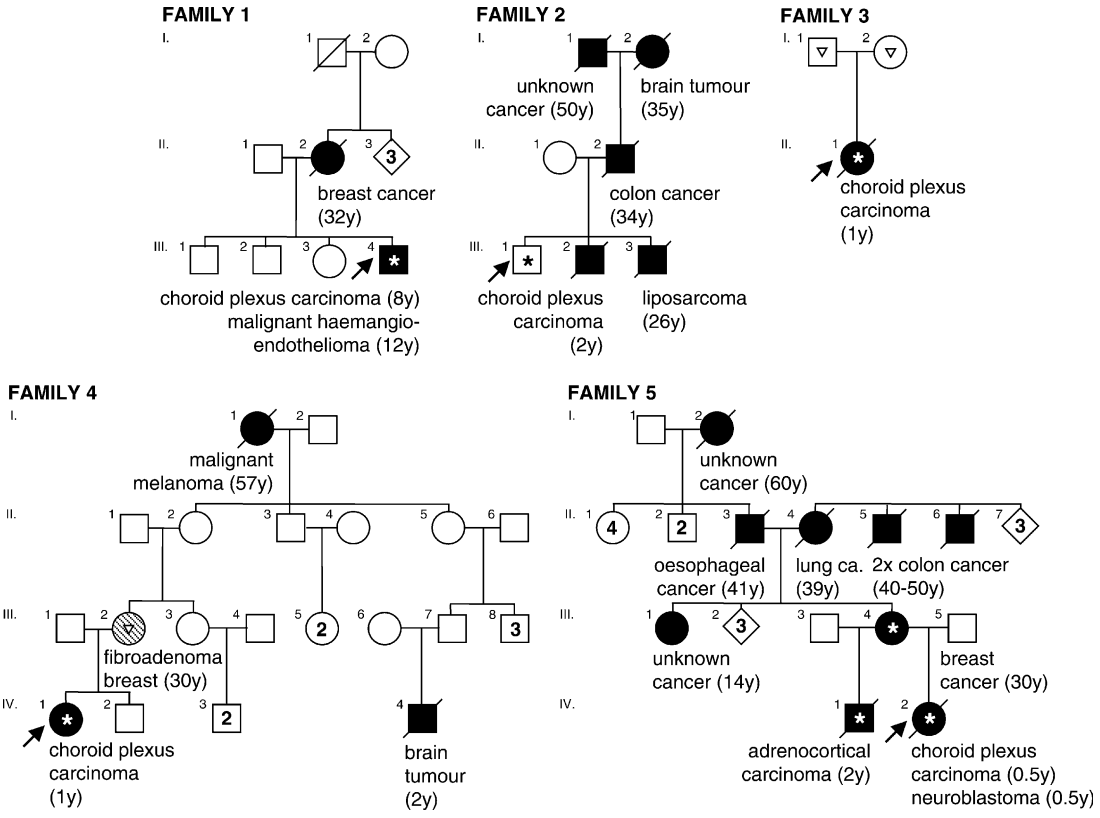

Fig. 1. Pedigrees of families with choroid plexus carcinomas. The tumour type and age at onset are shown for each affected relative. Arrows indicate probands, stars mark individuals in whom a germline *TP53* mutation was found by molecular analysis. Open triangles mark individuals tested negatively for the mutation.

tion led to the diagnosis of Turner syndrome at the age of 10 years. The karyotype confirmed X-monosomy. Her second cousin died from a rhabdoid brain tumour at the age of 2 years, and grandmother's mother suffered from malignant melanoma. The mother of the patient is followed up for breast fibroadenoma (Fig. 1).

Patient 5-IV.2 (family 5) died at seven months of age from excessive bleeding during surgery of a choroid plexus carcinoma localised in the left lateral ventricle. In addition, another tumour, a neuroblastoma in the left adrenal gland, was identified at autopsy in this patient. Her brother was diagnosed at the age of 2 years with adrenocortical carcinoma. The mother of these two siblings suffered from breast carcinoma at the age of 30, and several other cancers were identified among her relatives (Fig. 1).

2.2. TP53 mutation screening and analysis, p53 protein immunohistochemistry

After obtaining informed consent, exons 5–9 of the *TP53* gene of the family members were analysed by direct sequencing of PCR products from blood lymphocyte DNA, and the p53 protein was analysed in tumours by immunohistochemistry as described [11]. Previous reports of identical germline and somatic *TP53* mutations were identified in the Database of Germline *TP53* Mutations (http://www.lf2.cuni.cz/projects/germline_mut_p53.htm [19]) and The p53 Database (http://p53.curie.fr/index.html [20]), respectively. The evolutionary conservation of amino acid residues affected by mutations was assessed by multiple alignments of p53 and p53-related sequences from several animal species using Clustal (http://www.ebi.ac.uk/clustalw/index.html) and Boxshade (http://www.ch.embnet.org/software/BOX_form.html). The amino acid sequences were retrieved from the NCBI protein database (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Protein) or from the JGI Ciona Database (http://genome.jgi-psf.org/ciona4/ciona4.home.html).

3. Results

All individuals who were tested for germline *TP53* mutations are indicated in Fig. 1, and the mutations identified are described in detail in Fig. 2 and Table 1. All of the mutations were missense mutations leading to amino acid substitutions. Because they affected highly conserved residues in conserved regions III to V of the DNA-binding domain of the human p53 protein (Fig. 3), these mutations were likely to be

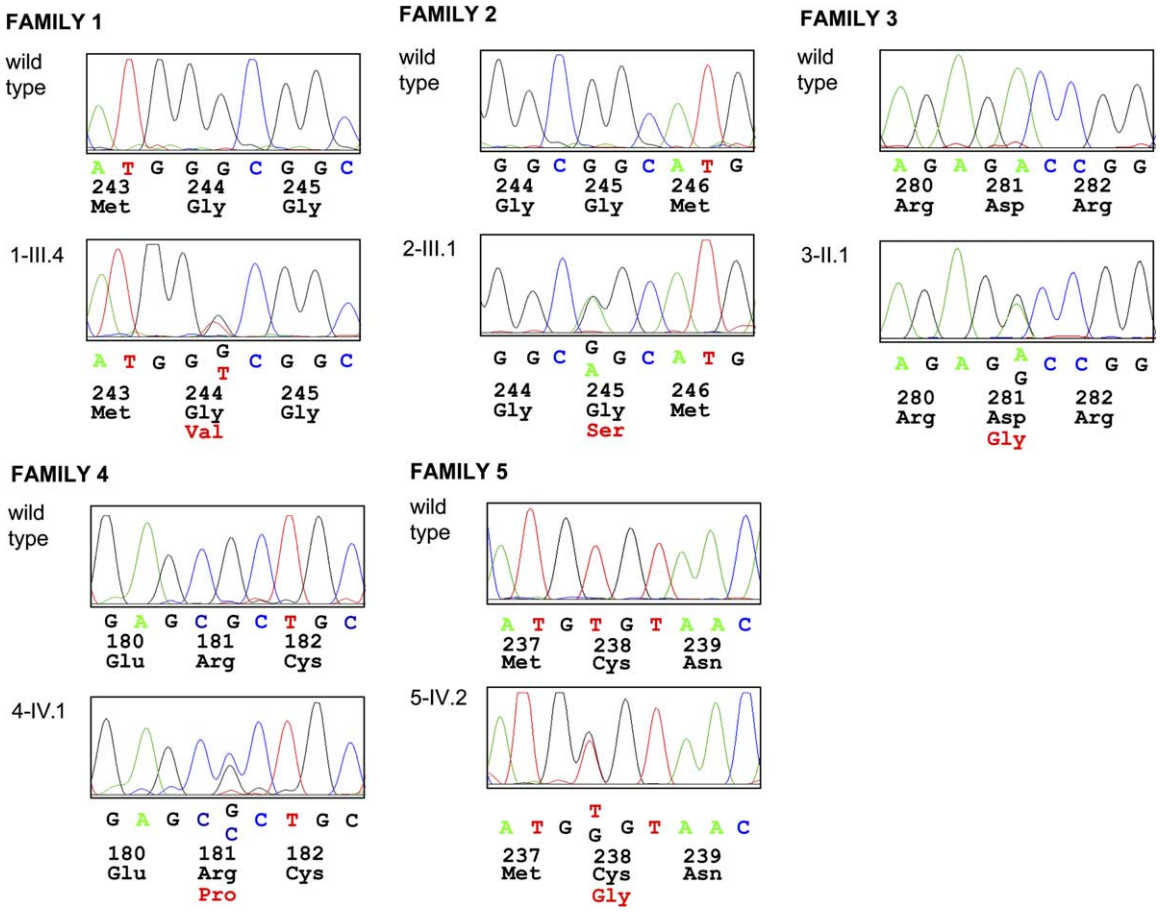

Fig. 2. Germline TP53 mutations identified in families of patients with choroid plexus carcinomas. Sequence electrophoreograms of the heterozygous carriers are compared to wild type sequences.

pathogenic. With the exception of the recurrent codon 245 mutation in family 2, these mutations have not been reported in the germline before, although for three of them another amino acid substitution was described in the same codon [19] (Table 1). However, all of the mutations have been repeatedly reported among somatic mutations in human tumours [20] (not shown).

The analysis of family structure, tumour spectra, and ages at tumour onset (Fig. 1) showed that only one of the families (family 2) conformed to the criteria of the

Table 1
Germline *TP53* mutations identified in five families with childhood choroid plexus carcinomas

Family	Clinical criteria	Exon	Codon	Nucleotide change	Amino acid change	Previous reports of germline mutations in these codons [19]
1	Ch	7	244	GGC to GTC	Gly to Val	Novel mutation
2	LFS, Ch	7	245	GGC to AGC	Gly to Ser	Recurrent: 10 × GGC to AGC 1 × GGC to TGC (Cys) 1 × GGC to GAC (Asp) 1 × GGC to GTC (Val)
3	–	8	281	GAC to GGC	Asp to Gly	Novel mutation 1 × GAC to GTC (Val)
4	FH	5	181	CGC to CCC	Arg to Pro	Novel mutation 1 × CGC to CAC (His) 1 × CGC to TGC (Cys) 1 × CGC to CTC (Leu)
5	LFL, Ch	7	238	TGT to GGT	Cys to Gly	Novel mutation 1 × TGT to TAT (Tyr)

Clinical criteria: LFS, Li–Fraumeni syndrome [21]; LFL, Li–Fraumeni-like [22]; Ch, Chompret criteria [23]; FH, weakly positive family history of cancer; –, no family history of cancer.

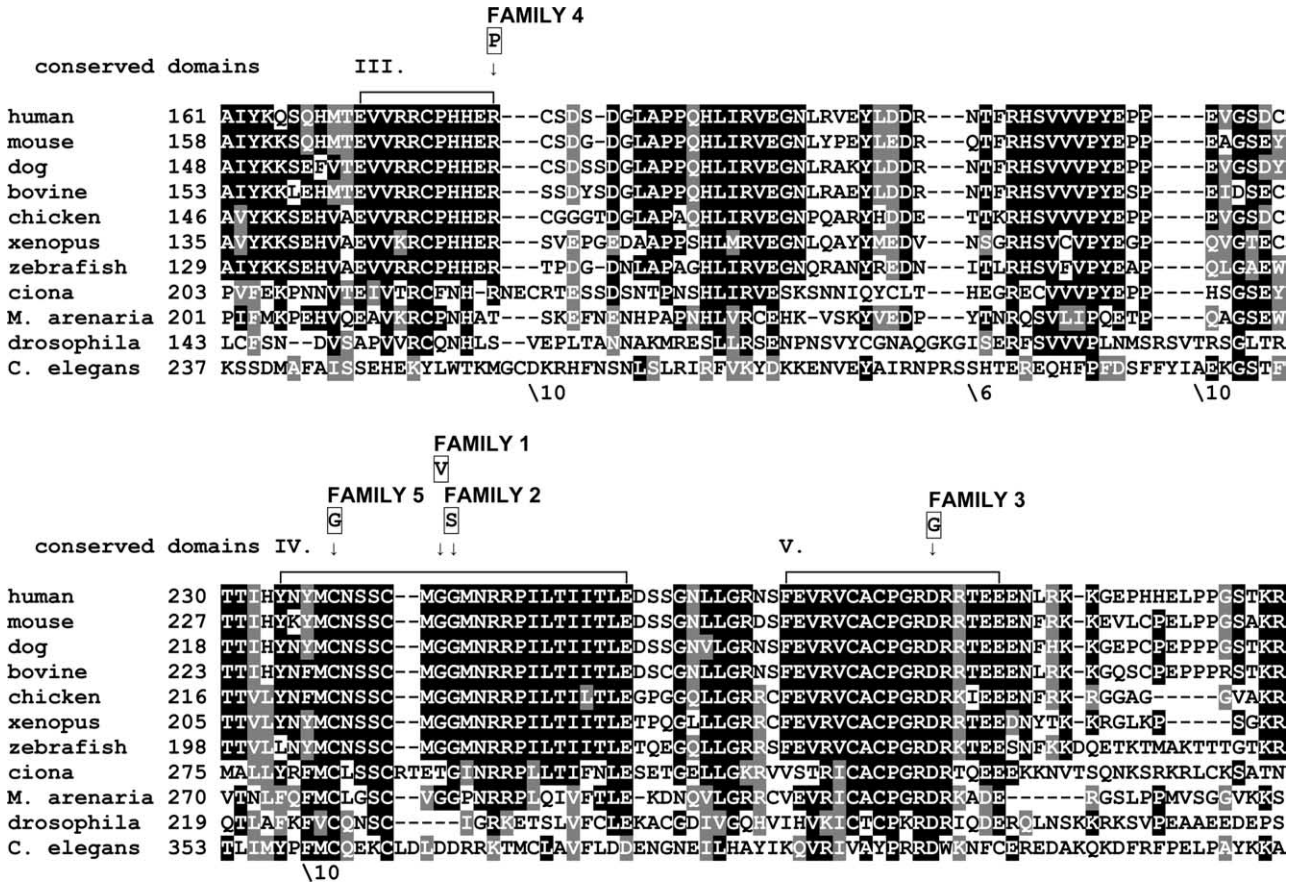

Fig. 3. Alignment of amino acid sequences of p53 and p53-related proteins from multiple species. Residues conserved in more than 50% of species are shaded in black, similar residues in grey, dashes indicate gaps. The *C. elegans* homologue contains multiple insertions of several residues indicated by numbers below the sequence. All mutations described in this paper reside in conserved domains III to V of the p53 protein and affect residues which are conserved at least in all vertebrate species. The following sequences were compared: p53_human, p53_mouse, p53_canfa (dog), p53_bovin, p53_chick, p53_xenla (xenopus), p53_brare (zebrafish), ci0100137503 (ciona), AAF67733 (Mya arenaria, a bivalve mollusc), AAF40427 (drosophila), and NP_492262 (*C. elegans*).

Li–Fraumeni syndrome [21], one family (family 5) conformed to the criteria of the Li–Fraumeni-like syndrome [22], and only three of the families (families 1, 2, and 5) met the criteria for *TP53* mutation testing recommended by Chompret *et al.* [23]. Of the remaining two families, only family 4 had a positive history of cancer in the maternal branch of the family, but the DNA analysis showed that the mother of the patient 4-IV.1 was not a carrier of the mutation. The DNA of the father was not available for study. A similar situation was observed in family 3, where although the patient 3-II.1 was shown to carry a germline mutation, both of her parents were found to be negative for the mutation, and there was no family history of cancer among other relatives. Provided there was no non-paternity, this patient had to be a carrier of a *de novo* mutation.

In addition to blood leukocyte DNA, three tumours from the affected individuals were available for analysis. The malignant haemangioendothelioma of patient 1-III.4 retained heterozygosity of the *TP53* gene at the site of the germline mutation, but immunohistochemical examination of the p53 protein showed a strongly positive nuclear reaction in virtually all tumour cells, while normal cells were negative. On the contrary, both the choroid plexus carcinoma and the neuroblastoma of patient 5-IV.2 displayed loss of the wild type *TP53* allele. The choroid plexus tumour showed strong p53 staining in vast majority of tumour cell nuclei, the neuroblastoma was not tested (data not shown).

4. Discussion

The identification of additional five families with paediatric patients with choroid plexus carcinoma and germline *TP53* mutation adds further credence to the notion that this very rare type of brain tumour may be an indicator of a germline *TP53* mutation. This is especially significant when the tumour is present in an individual with even a weakly positive family history of cancer. Obtaining a complete family history should therefore be done with all patients suffering from this

tumour, and a genetic consultation and *TP53* mutation testing should be discussed. It is important to stress, however, that two of the current five families did not meet any clinical criteria used in decision making for *TP53* gene testing (Li–Fraumeni syndrome, Li–Fraumeni-like syndrome, or the Chompret criteria [21–23]). In one of these families the mother of the proband had a weak family history of cancer but did not carry the germline *TP53* mutation. The DNA of the father could not be analysed but there was no history of cancer in the paternal branch of the family. In the other family there was no history of cancer, and both parents did not carry the mutation found in their affected daughter. This may be indicative of *de novo* germline *TP53* mutations, similar to that described in another paediatric patient suffering from choroid plexus carcinoma [14] reported in the literature among the 12 cases of this tumour in germline *TP53* mutation carriers [8–16]. At least two other patients from this series had no family history of cancer [15,16]. *TP53* gene testing may therefore be considered in all patients with choroid plexus carcinoma. This is certainly going to be affected on the one hand by the availability, insurance, and other issues and on the other hand by the rarity of this tumour. The two largest centres participating in this study, the children hospitals in Prague and Denver, both serving a population of approximately 5 million, have offered care to 8 patients each during the last 9 years.

The association of childhood choroid plexus carcinoma with germline *TP53* mutations can have important consequences for the relatives of the patients who can be at increased risk of cancer. Due to age-specific risks of developing various types of cancer, the mothers of patients with these tumours may have, for example, a very high risk of breast cancer, because many of them are in their late twenties/early thirties. The association of choroid plexus carcinoma with germline *TP53* mutations can also have important practical implications for treatment of the patients. Although radiotherapy is generally recommended after gross total resection of choroid plexus carcinoma, at least in adolescents and adults [1], the increased risk of induced secondary malignancies when a germline *TP53* mutation is present must be considered [24]. The malignant haemangioendothelioma of patient 1-III.4 described in this study was likely to be radiation-induced.

Some choroid plexus papillomas and almost all choroid plexus carcinomas show immunohistochemical positivity for p53 [25], supporting the role of the p53 protein in the development of this tumour. In germline *TP53* mutation carriers, the choroid plexus carcinomas may exhibit p53 protein accumulation despite the absence of loss of heterozygosity at the site of the germline *TP53* mutation, and this may be associated with the expression of SV-40 T-antigen in these tumours [15]. However, the exact mechanism of predisposition of the germline *TP53* mutation carriers to this rare tumour remains to be elucidated. In this context, it is of interest that the *TP53* gene is strongly expressed in the inner lining of the brain ventricles during normal mouse embryogenesis and that a subset of p53-deficient mouse embryos develops defects in neural tube formation [26,27]. It is tempting to speculate that the preponderance of development of choroid plexus tumours prenatally or in early childhood can be associated with considerable structural rebuilding observed in the choroid plexus in the embryonal and foetal period [28]. Gross structural changes are observed during pre- and neonatal development also in adrenal cortex [29], which is another preferential site of tumourigenesis in early childhood in germline *TP53* mutation carriers [30]. It is possible that these rebuilding processes may be critical for unmasking of possible negative effects of constitutional haploinsufficiency of the *TP53* gene.

Conflict of interest statement

None declared.

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