Screening for Germ Line p53 Mutations in Children with Malignant Tumors and a Family History of Cancer


Abstract

We have undertaken a routine investigation of the p53 status for all the children treated at our institution either affected by multiple tumors or whose family displays at least one second degree relative or less, affected by cancer before the age of 45 years. We report here on the first set of ten such families, eight of which were identified through a proband with sarcoma. p53 exons 5 to 8 have been sequenced following polymerase chain reaction amplification performed on DNA isolated from total blood. A missense mutation affecting codons 248, 273, and 282 was identified in three families. The mutation was inherited in these three families and was detected in unaffected members. In seven families no mutation was detected in exons 5 to 8.

Introduction

The occurrence of organ-specific malignancies affecting several relatives within a family is an indication of an underlying genetic susceptibility. Such a hereditary condition is well established for retinoblastoma and some colon cancers and the gene and the mutations responsible for the respective susceptibility to cancer have been identified (1, 2). A locus associated with inherited breast and ovarian cancer has also been identified by linkage analysis (3, 4). Familial aggregations of cancers affecting multiple organs also point to a genetic predisposition. LFS3 is a well documented example of such a condition (5, 6) in which affected relatives develop diverse malignancies including breast cancers, sarcomas, brain tumors, leukemias, and adrenocortical carcinomas. Families affected by this syndrome display a proband diagnosed with sarcoma before the age of 45 years, a first degree relative with cancer before the age of 45 years, and another first or second degree relative with either a sarcoma diagnosed at any age or any cancer diagnosed under the age of 45 years (5). Germ line mutations of the p53 gene have been reported in affected individuals belonging to LFS or other familial aggregations (7–15). The p53 gene is also a frequent target for genetic alterations in sporadic human tumors (16–18). In most of the cases, both alleles are altered, one by point mutation and the other due to allelic loss. This suggests that the suppression of p53-associated biological activities is essential for malignant transformation.

In order to evaluate the contribution of a genetic predisposition in childhood cancer, we are reconstructing the family history of all the children treated for any malignant tumor in the Department of Pediatric Oncology at the Institut Gustave Roussy in Villejuif since 1960 is now routinely investigated. Among the 900 or so families which have been interviewed during the past 18 months, 130 pedigrees have been identified because of the occurrence of either of the following conditions: (a) at least one other case of cancer affecting a first or second degree relative or a first cousin before the age of 45 years, associated with the proband’s cancer; (b) multiple primary cancers in the proband regardless of the family history. Clinical records have been appropriately verified for all affected relatives. Blood samples were obtained from the proband, his/her parents, and all the available family members.

Materials and Methods

Families. The family history of cancer of all children under 18 years, diagnosed with any malignancy (proband) and treated in the Department of Pediatric Oncology at the Institut Gustave Roussy in Villejuif since 1960 is now routinely investigated. Among the 900 or so families which have been interviewed during the past 18 months, 130 pedigrees have been identified because of the occurrence of either of the following conditions: (a) at least one other case of cancer affecting a first or second degree relative or a first cousin before the age of 45 years, associated with the proband’s cancer; (b) multiple primary cancers in the proband regardless of the family history. Clinical records have been appropriately verified for all affected relatives. Blood samples were obtained from the proband, his/her parents, and all the available family members.

Mutation Analysis in p53 Gene. The strategy used for p53 gene sequence analysis is essentially similar to that described by Malkin et al. (7). Briefly, the region encompassing exon 5 to exon 8 was amplified from DNA isolated from total blood by polymerase chain reaction as a 1.7-kilobase fragment defined by the following couple of primers:

5' ATGGG-AATCTCCCTGCGCTAACAAGAT-3'
5' TATAAGGATC-CGTTGTTAGGGCTCTCCCTT-3'

The amplified fragment was cloned in a pGEM3Z(−) vector and exons 5, 6, 7, and 8 sequenced using specific primers. Four independent amplification reactions were pooled and six plasmid inserts sequenced.

In some cases, direct sequencing was performed on the amplified 1.7-kilobase fragment using the same set of exon-specific primers and the reagent kit commercially available from BRL.

Results

Our aim is to carry out germ line p53 mutation screening in all the probands belonging to a familial aggregation of cancers fulfilling the above-mentioned criteria. Our attention has been focused on a first set of ten families displaying multiple cases of cancer, eight identified through a proband with sarcoma. The characteristics of the ten families studied in this report are presented in Table 1. They do not necessarily represent the overall pattern of cancer aggregations expected to emerge from the selection procedure. A germ-line mutation of the p53 gene was detected in three of the ten patients included in this study (Table 2). The pedigree of each of the three families is presented in Fig. 1.
GERM LINE p53 MUTATIONS AND CHILDHOOD CANCER

Table 1 Clinical features of the families screened for the p53 mutations

<table>
<thead>
<tr>
<th>Family</th>
<th>Tumor type (age at diagnosis)</th>
<th>Second tumor (age at diagnosis)</th>
<th>Cancer in relatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>RMS (7yr)</td>
<td>OS (15 yr)</td>
<td>M</td>
</tr>
<tr>
<td>7b</td>
<td>RMS (16 yr)</td>
<td>OS (15 yr)</td>
<td>B</td>
</tr>
<tr>
<td>37b</td>
<td>RMS (9 yr)</td>
<td>OS (20 yr)</td>
<td>M</td>
</tr>
<tr>
<td>57b</td>
<td>NE (1 yr)</td>
<td>OS (14 yr)</td>
<td>S</td>
</tr>
<tr>
<td>144</td>
<td>OS (16 yr)</td>
<td>OS (21 yr)</td>
<td>F</td>
</tr>
<tr>
<td>151</td>
<td>RMS (2 yr)</td>
<td>OS (20 yr)</td>
<td>PGM</td>
</tr>
<tr>
<td>153b</td>
<td>NB (5 yr)</td>
<td>OS (20 yr)</td>
<td>M</td>
</tr>
<tr>
<td>231</td>
<td>RMS (1 yr)</td>
<td>OS (20 yr)</td>
<td>S</td>
</tr>
<tr>
<td>332b</td>
<td>RMS (7 yr)</td>
<td>OS (14 yr)</td>
<td>M</td>
</tr>
<tr>
<td>867</td>
<td>RMS (14 yr)</td>
<td>OS (21 yr)</td>
<td>M</td>
</tr>
</tbody>
</table>

Table 2 p53 germ-line mutations in three patients with a familial history of cancer

<table>
<thead>
<tr>
<th>Family</th>
<th>Exon</th>
<th>Codon</th>
<th>Base substitution</th>
<th>Amino acid substitution</th>
</tr>
</thead>
<tbody>
<tr>
<td>151</td>
<td>7</td>
<td>248</td>
<td>CGG to TGG</td>
<td>Arg to Trp</td>
</tr>
<tr>
<td>231</td>
<td>8</td>
<td>273</td>
<td>CGT to GTG</td>
<td>Arg to Gly</td>
</tr>
<tr>
<td>867</td>
<td>8</td>
<td>282</td>
<td>CGG to TGG</td>
<td>Arg to Trp</td>
</tr>
</tbody>
</table>

In family 151, the analysis of germ line p53 in the proband demonstrated the presence of both wild-type and mutant alleles. A missense mutation was observed at position 248 in exon 7 with the substitution of a tryptophan for an arginine. The mutant allele is of maternal origin and has also been transmitted to the proband’s brother who is in good health at the age of 19 years.

In family 231, the proband carries one p53 allele with a missense mutation at position 273 in exon 8 where glycine is substituted for an arginine. The proband’s sister, affected by acute lymphoblastic leukemia, also carries the same mutated allele. Rather unexpectedly, considering the three cases of cancer in the proband’s mother’s family, the mutation is not carried by the mother but is transmitted through the father who is healthy at the age of 41 years.

In family 867, a germ line p53 mutation was identified in the proband. This mutation has been transmitted by his father who is in good health and in his mid-50s. The proband’s brother and mother are homozygous for the wild-type p53 allele. No blood sample was available from the proband’s sister. The mutation is a CpG transition at position 282 in exon 8 where a tryptophan is substituted for an arginine.

In the other seven families, no mutation was detected in the region spanning exons 5 to 8.

Discussion

We have searched for germ line p53 mutations in ten cancer-prone families of children treated in the Department of Pediatric Oncology at the Institut Gustave Roussy in Villejuif. As pointed out previously, these ten familial cases were selected from the 900 or so cancer cases but they are not claimed to be representative of the pattern of cancer aggregations expected to emerge from our ongoing survey, particularly because 8 of them were ascertained through a proband with sarcoma. Nevertheless they enlarge the panel of families investigated for germ line p53 mutations significantly.

Four of these families display cancer aggregations identifiable as LFS (families 7, 231, 332, and 867). Mutations have been detected in two of them (231 and 867). In the other six families the cancer aggregations do not strictly fit LFS criteria but some of the malignancies belong to the LFS syndrome. For example, the association of a rhabdomyosarcoma with a breast cancer in a first degree relative as found in families 4 and 151 can be defined as "incomplete LFS" (19). A mutation was found in one of these families (151). In the other families, it was not possible to establish a clear-cut classification because the tumors observed do not generally belong to the LFS (tongue carcinoma in family 37, non-Hodgkin’s lymphoma in family 57, lung carcinoma in family 144, neuroblastoma in family 153). No mutation was identified in these families.

In family 231, the choriocarcinoma which affected the proband’s mother raises the issues of the origin, the genetic constitution, and ultimately the p53 status of this tumor. It was diagnosed 1 month after the proband’s birth and therefore is likely to be a gestational choriocarcinoma. Since this type of trophoblastic disease carries paternal genetic information (20), it is tempting to postulate that the paternal mutant p53 allele might be involved in the genesis of this particular choriocarcinoma. Unfortunately, it has not been possible so far to characterize the p53 status of this tumor.

The first reports of screening for p53 mutations in the LFS (7–9), suggested that these mutations might represent a major molecular event underlying the predisposition to cancer in this syndrome. However, Santibáñez-Koref et al. (10) later reported the absence of a germ line mutation in the p53 gene at exon 7 in six families with this syndrome. Our observation of two negative LFS families supports the hypothesis which assumes the existence of alternative genetic events in the genesis of this syndrome. However, it should be emphasized that our investigation has been restricted to exons 5 to 8, and it is possible that the "negative" families might carry mutations in the uninvestigated part of the p53 gene. Exon 7 undoubtedly represents a
Fig. 1. Pedigree of families carrying p53 mutations. Arrow, proband. Filled symbols, individuals with cancer before 45 years of age; gray symbols, individuals affected after 45 years of age. The type of cancer is indicated, followed by the age at diagnosis (under bracket). BC, breast cancer; RMS, rhabdomyosarcoma; OS, osteosarcoma; ALL, acute lymphoblastic leukemia; CC, choriocarcinoma; TC, thyroid carcinoma; wt, wild-type p53 allele; mu, mutant p53 allele.

Three distinct mutations have been observed which affect codons 248, 273, and 282, respectively, located in the conserved domain IV (248) or V (273, 282) of the p53 gene. These sites are not unprecedented targets for germ line mutations. They have been reported in the LFS (7–15) and in multiple primary malignancies syndrome (14). In the case of codon 248 and 282, the amino acid substitutions described here (tryptophan for arginine in both cases) are identical to those already reported. They have been shown to inactivate p53 biological activities (21). By contrast the mutation detected in family 231 on codon 273 where an arginine was replaced by a glycine is thus far unique and its biological consequences are unknown.

There are unaffected mutation carriers in each of the three families: individual II2 (19 years old) in family 151; individual II2 (41 years old) in family 231; individual II2 (41 years old) in family 867.
old) in family 231, and individual II3 (mid-50s) in family 867. The existence of unaffected mutation carriers both here and in previous studies (7, 9) raises the question of evaluating the risk of cancer for mutation carriers according to age. This is one of the issues addressed in our ongoing investigation. Because of the likelihood of genetic heterogeneity, it will be of great interest to compare our estimated risk with the value obtained by Lustbader et al. (22) by segregation analysis of families selected on probands with soft tissue sarcomas (60% by the age of 45 years and nearly 95% by the age of 70 years).

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References

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