A novel TP53 splicing mutation in a Li–Fraumeni syndrome family: a patient with Wilms’ tumour is not a mutation carrier

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Summary We report a Li–Fraumeni syndrome family in which we have detected a splice acceptor mutation in intron 3 of TP53. The mutation affects one of the invariant residues at the splice acceptor site, as a result of which two aberrant transcripts are produced. A child with Wilms’ tumour aged 3 years in this family was shown not to be a mutation carrier.

Keywords: TP53; Li–Fraumeni syndrome; splicing; Wilms’ tumour

Li–Fraumeni syndrome (LFS) is a rare autosomal dominant disorder in which there is greatly increased cancer susceptibility. LFS is characterized by bone and soft-tissue sarcomas, early-onset breast cancer, brain tumours, leukaemia and childhood adrenal-cortical tumours (Li et al. 1988), and a proportion of LFS families show germline transmission of a mutant TP53 gene. We have identified an LFS family in which there is a number of tumours typical of the syndrome, and we have screened for a germline TP53 mutation. The identification of a TP53 mutation in this family (Varley et al. 1997) has allowed us to exclude a number of cancer-affected individuals in the family from linkage to a germline TP53 mutation, including a child with Wilms’ tumour.

RESULTS AND DISCUSSION

Screening of the TP53 gene detected a point mutation at the splice acceptor site of intron 3 in both V-3 and his mother, but not in his father. The mutation affects one of the invariant residues at the splice acceptor site (ag/TC → aa/TC), and is predicted to perturb splicing of intron 3. We have previously reported the mutation in this family as part of a larger study of LFS families (Varley et al. 1997), but in the present report we have carried out more detailed analysis of a larger number of family members and determined the effect of the mutation on splicing. To confirm that splicing was abnormal, we carried out RT-PCR analysis on RNA extracted from lymphocytes from the proband and his mother using primers within exons 2/3 and 5 or 5/6. In addition to a fragment of the expected size, two more products were seen (data not shown), one approximately 20 bp smaller than the expected and the other approximately 280 bp smaller. These products were gel purified and sequenced directly. The smallest fragment corresponded to a product in which there was splicing between the splice donor site of intron 3 and the splice acceptor site of intron 4, generating a product in which exon 4 was completely absent (Figure 2A). This transcript is predicted to result in a protein product with an in-frame deletion of codons 132–125. The transcript which was 20 bp smaller than normal was the result of aberrant splicing between the splice donor of intron 3 and a cryptic splice acceptor site within exon 4 (Figure 2B). The hypothetical protein product of this transcript would have an in-frame stop codon at the equivalent of position 37, with four novel amino acids at the carboxy terminus. As judged by the relative levels of each of the splice products in the RT-PCR reaction, the wild-type allele was expressed at approximately the same level as the two aberrant products, however we made no effort to carry out quantitative analysis.

This mutation has not been reported as a germline mutation in any other family, nor indeed has an identical mutation been detected as a somatic event in a tumour. A survey of the database of TP53 mutations (Hollstein et al. 1996) found three intron 3 splice acceptor mutations, none of which is identical to that reported in this present study (Takahashi et al. 1990; Suzuki et al.
1992; Lai et al. 1993) but all of which result in skipping of exon 4. None of these mutations, however, results in the use of the cryptic splice site reported in family 86. The cryptic splice site shows good homology with the consensus splice acceptor sequence, and its usage would result in the translation of a truncated TP53 product with an altered C-terminus. Both this product and the product of the exon 4-skipped transcript would be predicted to be non-functional.

DNA samples were available from a number of other family members, all of whom were negative for the splice acceptor mutation except for one individual with a meningal sarcoma aged 29 (IV-9, see Figure 1). A number of cancer-affected individuals in this family did not have the mutation, including III-1 (ovarian carcinoma aged 58), III-16 (epidermoid carcinoma of the cervix aged 43), IV-1 (CIN III aged 36) and IV-3 (Wilms’ tumour aged 3). This result is particularly interesting as previous studies have indicated that Wilms’ tumours may occur at an increased frequency in patients with Li–Fraumeni syndrome (Hartley et al. 1993). There is only one report of a Wilms’ tumour patient with a germline TP53 mutation (Bardeesy et al. 1994), in a family in which there are two cases of Wilms’ tumour. However, in that family, the Wilms’ tumour is unlikely to be due to the TP53 mutation because the mutation was inherited from the mother, with the history of Wilms’ tumour on the paternal side. The child’s mother had a strong personal history of cancer at a young age, including a glioma. We have not detected a germline mutation in any Li–Fraumeni syndrome family in which there is a Wilms’ tumour (Varley et al. 1997) except the family described in this present report, and the individual with Wilms’ was not a mutation carrier. Although there is still evidence for a familial aggregation of Wilms’ tumours and other tumours characteristic of LFS (Li et al. 1988; Hartley et al. 1993), it appears likely from our own studies, including the present report, that this clustering is not due to the presence of a germline TP53 mutation. The ages of onset and types of tumours in III-1, III-16 and IV-1 are not typical of LFS, and we have confirmed that none of these tumours are associated with the germline TP53 mutation. However, the presence of the mutation in two affected individuals (IV-9 and V-3) confirms that the mutation is causative of the cancer phenotype in this family. This type of detailed study strongly reinforces the need to genotype as many individuals as possible in such families to reach valid conclusions about the spectrum of tumours related to inheritance of germline TP53 mutations.
Although the mutation reported in family 86 affects one of the invariant residues of the splice acceptor sequence and is, therefore, predicted to completely abolish correct splicing from the mutant allele, we felt it necessary to demonstrate abnormal splicing before offering the family a predictive test. Although we may have been overcautious in this case, it is strongly recommended that novel mutations, or those in which the functional significance is unclear, are evaluated as fully as possible before being used in predictive testing. The evaluation may take the form of testing in one of the functional assays currently available or, in the case of splicing mutations, analysis of the transcripts produced.

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