Screening hCHK2 for Mutations

Bell et al. (1) reported mutations in the hCHK2 gene in families with classical Li-Fraumeni syndrome (LFS) and in Li-Fraumeni-like (LFL) families. We have screened genomic DNA for mutations in this gene in one individual from each of 11 LFS families and 25 LFL families, using primers designed with reference to GenBank accession number AL117330. Five of these individuals had what at first appeared to be a single base pair deletion at nucleotide 1422 (1422delT); four out of 80 normal control individuals were also found to have a similar sequence variant.

In view of the presence of other sequence variants in all samples, we realized that we may have been amplifying at least two homologous fragments of the gene that include exons 11 through 14 and that have very similar but not identical sequences to the gene.

Database searches using the NCBI BLAST server (2, 3) revealed six homologous fragments that are located on chromosomes 7, 10, 15, 16, 22, and X and that between them encompass exons 10 through 14 of the gene and share 95 to 98% homology. By subcloning polymerase chain reaction (PCR) products of a sample from a normal control individual who had the delT variation (PCR) products of a sample from a normal control individual who had the delT variation and 25 LFL families, using primers designed with reference to GenBank accession number AL117330. Five of these individuals had what at first appeared to be a single base pair deletion at nucleotide 1422 (1422delT); four out of 80 normal control individuals were also found to have a similar sequence variant. In view of the presence of other sequence variants in all samples, we realized that we may have been amplifying at least two homologous fragments of the gene that include exons 11 through 14 and that have very similar but not identical sequences to the gene.

Database searches using the NCBI BLAST server (2, 3) revealed six homologous fragments that are located on chromosomes 7, 10, 15, 16, 22, and X and that between them encompass exons 10 through 14 of the gene and share 95 to 98% homology. By subcloning polymerase chain reaction (PCR) products of a sample from a normal control individual who had the delT variant and subsequently sequencing the separate alleles, we found that the delT was in a homologous fragment which had exactly the same sequence as the copy on chromosome 15 except for the delT (4). There was also evidence of two other copies that are not in the database. The sequence data in the BLAST “htgs” database (corresponding to unfinished sequences from the Human Genome Project), which includes the chromosome 15 homologous fragment, are unconfirmed and subject to change, so we verified the presence of this fragment in a chromosome 15 somatic cell hybrid (5). Because about 5% of the normal controls screened had the sequence variant, we conclude that this variant is a polymorphism in the homologous fragment present on chromosome 15.

Bell et al. (1) also reported the 1422delT variant in a sample from an individual within an LFL family. The individual had multiple colon polyps, colorectal cancer, and bilateral ocular melanomas, which are not among the typical cancers seen in LFS families. The high level of homology in the introns between the original copy and the homologous fragment (92.5%) suggests that Bell et al. may also have been amplifying the homologous fragment and that these cancers may not be attributable to mutations in the hCHK2 gene. The presence of several homologous fragments of the gene has implications for mutation screening in this region of the gene using genomic DNA.

References
4. GenBank accession number ACO18963.

Response: Sodha et al. note that recently deposited human genome sequence data reveal duplication of the 3' terminal exons and introns of the hCHK2 gene on chromosomes 7, 10, 15, 16, and X. Further mapping studies show additional 3' gene fragments on chromosomes 2, 13, and Y. These partial gene sequences are highly conserved with that of the parental gene on chromosome 22, but they are not expressed. PCR-based analysis using genomic DNA templates leads to variable amplification of these hCHK2-related sequences, which can be distinguished from each other by the presence of nucleotide variations.

We have reexamined case DF593, an archivial case of LFS variant for which only genomic DNA was available, and confirm that the 1422delT mutation is indeed present on the chromosome 15 sequence. Sodha et al. have now shown that mutation to be a rare polymorphism in the population. In contrast, the other two reported LFS cases with germ-line mutations in hCHK2 were identified using both reverse transcriptase-PCR analysis of the hCHK2 transcript and genomic DNA sequencing. Examination of the sequence variants surrounding the 1100delC mutation, another focus of our report (1), confirms that this mutation is present in the functional hCHK2 gene on chromosome 22. The T470C mutation within the forkhead homology-associated (FHA) domain is also present in the transcribed gene, and is located within exon 3, which is not duplicated.

We agree with Sodha et al. that the discovery of partial 3' duplications of the hCHK2 gene complicates mutation analysis based on genomic DNA. Sequence variants identified in these 3' exons may originate from nonfunctional alleles, while genuine mutations present within the hCHK2 transcript may not be readily confirmed by genomic DNA analysis. In fact, our initial attempts at detecting loss of heterozygosity (LOH) for the 1100delC mutation within a formalin-fixed tumor specimen were complicated by the co-amplification of the derivative hCHK2 chromosomes. Analysis of the chromosome 22-derived sequences, however, now shows the 1100delC mutation in 11 of 13 independent clones, which suggests LOH.

The multiple duplications of highly conserved hCHK2 genomic fragments are highly unusual, but similar genetic events may become apparent as the full human genome sequence is made available.
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