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RB1 Gene Mutations in Peripheral Blood DNA of Patients with Isolated Unilateral Retinoblastoma

To the Editor:

Two recent reports in this *Journal* (Lohmann et al. 1997; Sippel et al. 1998) indicated that the proportion of patients with isolated unilateral retinoblastoma who carry RB1 gene mutations in constitutional cells is higher than estimated previously (Vogel 1979; Draper et al. 1992). Mutation analysis in patients with unilateral tumors is important because it helps to significantly reduce the number of infant relatives who require clinical surveillance for retinoblastoma (Gallie 1997). Moreover, molecular investigation of these patients can identify carriers of mutations associated with incomplete penetrance and reduced expressivity and thus can extend our knowledge of the genotype-phenotype correlation (Gallie 1997; Lohmann et al. 1997). We have now analyzed additional tumors and have found that the frequency of constitutional mutations in patients with isolated unilateral retinoblastoma is not as high as indicated by our previous study (Lohmann et al. 1997).

Forty-two retinoblastomas that showed loss of constitutional heterozygosity (LOH) at the intragenic loci RBi2 (Toguchida et al. 1993) or RB1.20 (Yandell et al. 1989) were available for mutation analysis. Twenty-one of these tumors had been part of a previous study but were not analyzed for small mutations at that time (Lohmann et al. 1997). We analyzed the methylation status at the 5′ end of the RB1 gene by Southern blot analysis, using the methylation-sensitive enzymes *Bss*HII and *Sac*II as described elsewhere (Greger et al. 1994). Hypermethylation was identified in tumors from six patients. We performed SSCP to screen for small mutations, using a method reported elsewhere (Lohmann et al. 1996). Single base substitutions, including 17 transitions at CpG-dinucleotides, and small length alterations were identified in 24 and 3 tumors, respectively. To identify mutations in the remaining 10 tumors, we sequenced all 27 exons and the promoter region of the RB1 gene. However, no small mutation was identified.

In all, mutations were identified in tumors from 32 (76%) of 42 patients (RB1 gene mutation database). In the tumor of one patient (M6485), a missense base change (c.929G→A, E310G) in exon 9 and a nonsense mutation in exon 15 (c.1399C→T, R467X) were identified in addition to LOH. The missense base change was also present in peripheral blood DNA of this patient. Further investigation showed that this variant RB1 allele was inherited from the father and is, at least, carried by four adult relatives who are unaffected by retinoblastoma. The sequence variant, which, to our knowledge, has not been reported before, is expected to alter an amino acid located N-terminal of the pocket domains A and B (Hu et al. 1990). Only a few reported missense mutations with putative oncogenic effect are located outside the regions that code for these domains (RB1 gene mutation database). Considering that the tumor of patient M6485 also shows a somatic nonsense mutation and LOH, the missense base change is probably a neutral polymorphism and has not contributed to tumorigenesis. However, detailed analyses are required, to demonstrate that this sequence variant does not change the functional properties of the Rb protein (Bremner et al. 1997; Otterson et al. 1997).

In our previous study, we detected small RB1 gene mutations in leukocyte DNA from 6 (17%) of 36 patients with isolated unilateral tumors (Lohmann et al. 1997). In the present study, none of the bona fide oncogenic mutations identified in tumors was also detected in corresponding peripheral blood DNA by direct sequencing of PCR products. Therefore, when the data presented here are included, the proportion of patients with mutations in leukocyte DNA drops to 6 (9%) of 68. Because of mutational mosaicism (Lohmann et al. 1997; Sippel et al. 1998), this figure underestimates the true prevalence of constitutional RB1 gene mutations in patients with isolated unilateral retinoblastoma. However, in almost all patients with isolated unilateral retinoblastoma who have affected children, the mutation is readily detectable in peripheral blood DNA (Sippel et al. 1998; authors' unpublished data). It is reasonable to

assume, therefore, that the proportion of unilaterally affected patients with mutations in leukocyte DNA approximates the prevalence of hereditary retinoblastoma among patients with isolated unilateral tumors. The percentage obtained from our conjoint studies is now in accord with a previous estimate by Vogel (1979) that was based on epidemiological data.

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Electronic-Database Information

URL for data in this article is as follows:

RB1 gene mutation database, http://home.kamp.net/home/dr.lohmann

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TDT Clarification

To the Editor:

A potentially misleading statement occurs in the invited editorial "The TDT and other family-based tests for linkage disequilibrium and association" by Spielman and Ewens (59:983–989), published in the November 1996 issue of the *Journal*. We wish to make the following clarification.

In discussing some issues that arise when the transmission/disequilibrium test (TDT) is used in families where genotype data are unavailable for one parent, we noted the finding of Curtis and Sham (1995) that, when there is a single affected offspring who is homozygous for an allele present in the available (heterozygous) parent, the TDT gives a biased result and should not be used. We then stated that "[w]hen there is more than one offspring in the sibship, it sometimes will be possible to deduce that the unavailable parent [is also heterozygous], and, in these cases, we may proceed as though this [reconstructed] genotype were known directly" (Spielman and Ewens 1996, p. 987).

We should have emphasized that this claim assumes that the reconstruction is done from the genotypes of unaffected offspring. A bias will usually arise in the TDT statistic if the reconstruction uses, in whole or in part, genotype data from affected offspring whose genotypes are then used in the TDT. A bias can also arise when both parental genotypes are reconstructed from the genotypes of the offspring.

The bias resulting from reconstruction occurs for a reason different from that noted by Curtis and Sham