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Letters

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No Association Between the Presence of a Constitutional *RB1* Gene Mutation and Age in 68 Patients with Isolated Unilateral Retinoblastoma

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THE RESULTS presented in two recent reports in the *European Journal of Cancer* [1, 2] indicated that patients with a diagnosis of isolated unilateral retinoblastoma at a young age are more likely to carry constitutional mutations in the *RB1* gene than patients diagnosed at an older age. The identification of *RB1* mutations in patients with unilateral tumours is important as it helps to reduce significantly the number of infant relatives that require clinical surveillance for retinoblastoma [3]. However, efforts to identify mutations in constitutional DNA are often in vain because only 2–12% of these patients are heterozygous for an oncogenic *RB1* gene mutation. Therefore, if constitutional mutations are, in fact, more prevalent among patients with an early age at diagnosis, resources for mutation detection could be focused. Extending a previous study, we recently completed mutation analysis in 68 patients [4, 5], and here report the age distribution in these patients. Our results do not suggest that constitutional mutations are more prevalent among patients with early presentation of unilateral retinoblastoma.

Genotyping of intragenic polymorphic loci, Southern blot hybridisation, single stranded DNA conformation polymorphism (SSCP) and heteroduplex analysis, as well as sequence analysis, were used to identify *RB1* gene mutations in tumours and peripheral blood DNA from 81 patients with isolated unilateral retinoblastoma [4, 5]. Mutation analysis was successful in tumours of 68 (84%) patients. In 6 of these patients (9%), an *RB1* gene mutation was also identified in DNA from constitutional cells. Currently, none of these patients have developed a tumour in the other eye (length of follow-up 30–103 months, mean 60.6). These data were used to identify a possible association between age at diagnosis and the presence of a constitutional *RB1* gene mutation. Following the arguments proposed by Kato and colleagues [6], the age at the time of operation was analysed instead of age at diagnosis. As all patients were treated by enucleation shortly after diagnosis of retinoblastoma, the absolute difference between age at diagnosis and operation was negligible. The proportion of patients not yet operated upon was plotted as a function of time applying the Kaplan–Meier method (Figure 1). A comparison of the curves with the log-rank test (JMP version 3.1.6 software for the Macintosh, SAS Institute, Cary, North Carolina, U.S.A.) showed no significant difference in age at operation between patients with a constitutional mutation and those in whom neither of the two mutations identified in the tumour was detected in DNA from peripheral blood leucocytes.

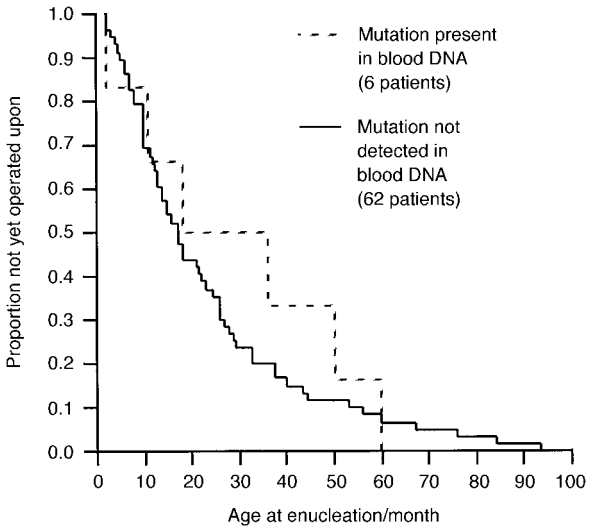

Figure 1. Kaplan–Meier analysis of age at operation comparing those with and without a constitutional *RB1* mutation.

Our results are not in accordance with the findings presented in two recent reports in the *European Journal of Cancer*. Cowell and Cragg [2] identified constitutional *RB1* gene mutations in 2 of 3 patients treated under the age of 12 months and speculated that patients with early presentation are more likely to carry constitutional mutations. In our series, 22 (32%) of 68 patients were treated under the age of 12 months. Only 2 (9%) of these patients had a constitutional mutation. The prevalence of mutations among the remaining 48 patients was almost identical (4 of 48; 8%). Zajaczek and colleagues [1] identified *RB1* gene mutations in 4 of 17 patients with isolated unilateral retinoblastoma. Age at diagnosis in these patients ranged from 5 to 18 months. They inferred that age at diagnosis may be a major factor in identifying patients with a constitutional mutation. In our series, age at operation in the 6 patients with a constitutional mutation ranged from 2 to 60 months. Only half of them were treated under the age of 18 months. In conclusion, our data do not suggest that patients with diagnosis and treatment of isolated unilateral retinoblastoma at an early age are more likely to carry a constitutional *RB1* gene mutation than older patients. Consequently, it seems unjustified to assume that these patients have an increased risk of hereditary retinoblastoma or should be given a higher priority for genetic screening.

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Response from S. Zajączek and J. Lubiński

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DOCTORS LOHMANN and Horsthemke summarised published and unpublished results from their laboratory, suggesting that there is no association between the presence of constitutional *Rb-1* mutations and age at tumour diagnosis in patients with sporadic unilateral retinoblastoma (SURB) [1, 2] which is not in agreement with our [3] and Cowell and Cragg's results presented in the *European Journal of Cancer* [4].

The occurrence of *Rb-1* mutations in clinically well-characterised cases of SURB has not been studied intensively. Series of reported consecutive cases of SURB with described age at diagnosis include only 68 cases studied by Lohmann and colleagues and 16 cases studied by our centre [1–3]. Altogether, the number of constitutional mutations in both series is small and include 9 cases diagnosed by Lohmann and colleagues and 3 cases from our studies (1 patient with mutations reported in our paper [3] developed a second eye tumour after 35 months of follow-up (data not shown), thus we excluded him). In such a situation it is obvious that the discussed problem cannot be solved until further investigations are conducted.

Comparing our and Dr Lohmann's studies, we note one very important difference—we identified mutations by complete 'exon by exon' sequencing and Lohmann and colleagues used pre-selection techniques which detect DNA abnormalities with a lower sensitivity. This is of particular importance for the detection of point mutations [5, 6]. In our experience, in cases of SURB the proportion of single base substitutions may be higher.

Thus, further studies on the correlation between the occurrence of *Rb-1* constitutional mutations and the age of tumour diagnosis on larger series studied by full sequencing are needed.

At present, no definitive conclusion on the discussed problem is possible, but our suggestion of the existence of an association between the presence of germline *Rb-1* mutations and early onset of retinoblastomas is supported additionally by the findings of Cowell and Cragg [4] and, what is most important, by the two-hit Knudson hypothesis, which has been verified as appropriate for many other types of hereditary malignancies.

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