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Editorial

Which Retinoblastoma Patients Should be Screened for *RB1* Mutations?

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BEFORE THE cloning of the retinoblastoma predisposition gene (*RB1*), the only screening option available for the majority of 'at risk' children was regular ocular examination under anaesthetic from birth to 3 years of age. Anyone with a relative (including cousins) who had retinoblastoma was considered to be at risk. These examinations were essential since the best chances for survival, low morbidity and retaining some vision in the affected eyes, depended on detecting tumours early. With the significant advances in our understanding of the molecular pathology of retinoblastoma over the past few years, this intense clinical screening programme should be unnecessary for the majority of children who have been shown molecularly not to carry the *RB1* mutation which is present in their affected relative. Consequently, valuable resources can then be directed more appropriately to the children at very high risk.

It has been 12 years since the *RB1* gene was cloned and sequenced [1], making it possible for at risk individuals to undergo molecular genetic screening to establish whether or not they carry a predisposing mutation. The advantages are clear: if the result is negative, the children do not need to undergo repeated and costly screening. If the result is positive, the appropriate timely counselling and/or examination can achieve early treatment of tumours. Although the published frequencies vary, 10% of probands have a family history of retinoblastoma, 40–45% carry new germline mutations and the other 40–45% develop tumours because of somatically acquired mutations. Conventional karyotyping reveals only the 3–5% of patients who have deletions or translocations involving the *RB1* gene and so the majority of *RB1* mutations are not detectable by karyotype.

Where there is a family history of retinoblastoma the benefits of genetic testing are clear. Furthermore, with only a few unusual exceptions [2], all members of the family would be expected to carry the same germline mutation. The investment in the first instance, therefore, is well justified since all subsequent testing of family members would then only involve a single PCR or sequencing reaction. In these cases, however, it is usually not necessary to find the mutation, since there are a large number of linked polymorphisms available which can approximate carrier status, although some errors can occur [3]. For the more common non-familial cases, where the question from the patient is inevitably, 'Are my children at risk?', linkage is not an option. It is now well established that bilaterally affected, non-familial cases represent individuals with *de novo* germline mutations. Multiple tumours in both eyes simply do not occur by chance! All of these individuals will benefit greatly by the identification of their precise *RB1* mutation and a rigorously tested triage of approaches to find these mutations has now been established [4].

In contrast to the bilaterally affected individuals, the unilateral non-familial cases are generally non-hereditary, although it has been estimated that approximately 2% do carry germline mutations [5]. The very important question here is, ''which 2%?'' From studies such as the one presented by Zajaczek and associates in this issue of the *European Journal of Cancer* ([6]; pp. 1919–1921) we get a deeper insight into this question. It is probably the unilateral cases with early onset disease which are more likely to carry germline mutations. These studies support previous observations from a smaller series of patients [7] and confirm the prediction from Knudson's hypothesis [8] that, in addition to the multifocal development of tumours, early onset of a tumour may also be an important indicator of genetic predisposition. To this point, it has not been feasible or practical to screen unilaterally affected individuals since, on average, only 1:50 of them would carry a mutation. From two independent reports, it now appears that analysing the early onset cases, at least, might be reasonable. However, how early is early? In one study [6] 12 months was chosen as the cut-off, which was within the mean age range of onset for bilaterally (hereditary) affected cases. Zajaczek and associates found one mutation in a patient who apparently presented at the age of 18 months, but all of the others presented much earlier.

This paper also raises the important issue of economic justification for testing. It is the size and structure of the *RB1* gene that makes testing expensive. Mutation testing for *RB1*

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has been routinely performed in laboratories with a long-term research interest in the biology and genetics of retinoblastoma [9–12], and mutation studies became a natural extension of this work. Now that this type of study is no longer research, most laboratories have stopped testing, leaving a vacuum where the patients will suffer if alternatives are not forthcoming.

The familial cases and the bilateral cases must have a germline mutation and only one gene, *RB1*, has been implicated. It has been estimated that at least 90% of mutations in these cases can be identified from studies of blood, using a combination of approaches [4]. In the unilateral non-familial cases, this is not so clear-cut; if no mutation is detected in blood, did we just miss it? The most certain and efficient way to provide useful mutation information to unilaterally affected patients is to find the *RB1* mutations that led to the tumour. However, sufficient DNA must be available from the retinoblastoma tumour to conduct an extensive series of tests, which requires that tumour DNA be prepared from fresh tumour after surgery, before the tumour is fixed for pathological examination. Most unilaterally affected persons have not had this opportunity. This uncertainty, in turn, raises the question of the role of continued ophthalmological screening in the face of molecular studies. Clearly, where the precise mutation is known, ophthalmological screening is not necessary for any relative who does not carry this mutation. When no *RB1* mutation is found in the affected child, regular eye examinations for the relatives are still warranted. In the final analysis, however, molecular testing should be able to establish whether or not ophthalmological screening is necessary in up to 60% of families.

In an ideal world genetic testing would be freely accessible for everyone who wanted it. In its various guises throughout the world, 'Managed Health Care' demands cost-effectiveness. Since molecular identification of the mutation in the *RB1* gene in an affected child was shown in 1996 [4] to be significantly cheaper than the conventional clinical examinations of children at risk in the family, it is puzzling why there is any hesitation to endorse and fund the cheaper and better care for retinoblastoma families. Indeed, many healthcare providers in the U.S.A. have paid for molecular testing on a case-by-case basis. More efficient would be the universal adoption of this preferred route.

In what has been a relatively short period, therefore, the impact of molecular testing for retinoblastoma has re-defined optimal clinical management for this tumour. The benefits of screening have clearly been shown in terms of cost [4] and the relief for those who do not carry their family's *RB1* mutation. The challenge now is to ensure that this message gets through to those responsible for the clinical management of retinoblastoma families, so that scarce resources can be concentrated on those who need them.

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