

## Letter to the Editor

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### Retinoblastoma: The evidence *does* matter

Sir,

We are responding to a letter that appeared in your recent issue [1], which contains a number of major inaccuracies regarding retinoblastoma. Similar speculations and misleading conclusions can be found in two previous papers by the same first author [2,3]. We feel that we can provide enough evidence and expertise from our own work to challenge such misconstructions.

We provide genetic testing for mutations in the *RB1* gene (MIM 180200) through a not-for-profit corporation, Retinoblastoma Solutions. Patient samples are sent to us from around the world (22 countries, 6 continents) and to date we have served over 1080 unique retinoblastoma families. Our own and our collaborators' research has contributed to the understanding of the mechanisms of retinoblastoma, and cancer in general.

Mastrangelo and colleagues state that “. . . in a **large number** of bilateral cases, no mutations [of the *RB1* gene] can be found [. . .] even with the most advanced molecular techniques” [1] (emphasis ours). We do not agree. In 95% (414/436) of the bilateral cases referred to us, a constitutional *RB1* mutation is found. There are known categories of mutations which our methods currently cannot detect. The small proportion of remaining cases is predicted to carry mutations in the *RB1* gene such as damaging translocations, deep intronic mutations, or presently undetectable mosaic mutations. The estimated proportion of currently undetected mosaic mutations alone is predicted to account for the missing 5% of mutations in the blood of persons with bilateral retinoblastoma [4]. In addition we have found mutations in both alleles of 93% (381/408) of retinoblastoma tumors from unilaterally affected patients. This very high sensitivity to find the *RB1* mutation in each family has provided key information for families that not only assists with early (including prenatal) diagnosis resulting in vastly improved vision outcomes, but also decreases the overall cost of health care and improves quality of life by reducing the inten-

sity of surveillance examinations for child relatives of the proband who are proven to not carry the proband's *RB1* mutation. In a recent article [2], Mastrangelo and colleagues state that “[. . .] the mutation rate within the newly diagnosed cases of retinoblastoma, is more likely to approach the 50% more recently reported by Nichols et al.”. This represents a distortion of the results presented in the referenced article. Nichols et al. [5] found *RB1* mutations in blood samples from 91% (77/85) of patients with bilateral retinoblastoma, 70% (7/10) of familial unilateral patients and 7% (6/85) of patients with sporadic unilateral retinoblastoma. The 50% sensitivity quoted by Mastrangelo et al. is only achieved by ignoring the accepted biological differences between the three categories and treating them as one homogeneous set.

Mastrangelo and colleagues state that their “personal series of retinoblastoma survivors (unpublished data), clearly show [. . .] 50% affected children in the offspring of unilateral retinoblastoma survivors”. This is a very unusual series of unilateral patients. It has long been noted that between 2–6% of all offspring of patients with sporadic unilateral retinoblastoma will develop retinoblastoma [6–8]. Our data affirm the 6% risk, since 13% (53/408) of unilaterally affected patients carry in their blood one of the *RB1* mutations found in the tumor, putting them into the hereditary category with up to 50% risk to each of their offspring to develop retinoblastoma. Of the 87% of unilaterally affected individuals shown to not carry either of the tumor *RB1* mutations in blood, none have reported a child with retinoblastoma. We would be pleased to examine (for *RB1* mutations) the blood of the unilateral patients and their offspring with retinoblastoma that Dr. Mastrangelo describes. We predict some will be heterozygous for *RB1* mutations and almost 30% of these will be mosaic for an *RB1* mutation that will be heterozygous in their offspring, consistent with Knudson's two-hit model.

We have shown that loss of both alleles of *RB1* initiates non-proliferative retinomas (benign retinal tumors), a predicted precursor to retinoblastoma [9–11].

This is also consistent with Knudson's original prediction that mutation of both alleles (M1 and M2) of the predisposing gene (*RB1*) is essential but not necessarily sufficient for development of retinoblastoma. Indeed, many of these progressive changes (M3 to Mn) are defined now by careful molecular analysis [12]. To date, no change is more common in retinoblastoma tumors than *RB1* mutations. Furthermore numerous mouse models of retinoblastoma confirm that *RB1* mutation is necessary for retinoblastoma development (for review see [13]).

Mastrangelo and colleagues' recent articles [1–3] suggest that there is a widespread disregard for evidence in the study of the genetics and mechanisms of retinoblastoma. This is certainly not the case. We hope that the evidence that we present, based on rigorous scientific study of large data sets, will reinforce for all readers with a critical eye that *RB1* mutations initiate retinoblastoma tumors.

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