Predictive Gene Testing For Retinoblastoma: Still Far From Routine Application.

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Abstract

Predictive gene testing for a few rare cancers are already in clinical use. Retinoblastoma, a rare childhood tumor affecting one or both eyes of infants and little children, is one of the first tumors for which predictive gene testing has been made available. However, an unbiased view of the application of predictive gene testing to retinoblastoma diagnosis and prevention, allows room for ethical questions that go far beyond the conventional ethical issues raised by the application of predictive gene testing to cancer. It is the opinion of the author that the widespread application of genetic testing to cancer must fulfill specific requirements such as specificity, sensitivity, repeatability, and clinical usefulness. Unfortunately, predictive gene testing, as applied to retinoblastoma diagnosis and prevention, does not yet fulfill all these requirements, and further investigation aimed at improving the quality of the standards of the currently available tests, is strongly required.

INTRODUCTION

The extreme reductionism of modern medicine and biology has led many to look at genes as the only cause for cancer, even if the evidence in favour of their direct role, is still weak. A good example of this is represented by the Rb1 gene, i.e.: the gene "responsible" for retinoblastoma; although much has been learned about its structure and function, over more than thirty years of investigations, only now we realize that: ". the precise role of Rb1 loss in the development of retinoblastoma remains unclear"[1] Moreover, researchers in the field of cancer biology are becoming aware that not only cancer derives from the accumulation of several gene mutations[2], but also that cancer cells are not "islands", and they cannot be investigated out of their natural context, i.e.: the tissues, with the normal surrounding cells, the extra-cellular milieu, the stroma, etc.[3] Also, studies on microsatellite instability, mutator phenotypes, genome instability[4], aneuploidy[5,6], gene methylation[7], etc., are presently disclosing to researchers in the field of oncology, a new, dynamic and extremely complex picture of interactions between the cell genome and the environment.

DISCUSSION

With these emerging new trends in cancer genetics, does the routine application of Predictive Gene Testing (PGT) for cancers such as retinoblastoma make any sense? PGT for retinoblastoma has become, in the opinion of some Authors, an integral part of the contemporary management of retinoblastoma[8], and in some countries, PGT for retinoblastoma is routinely offered to the public as either linkage analysis or direct DNA sequencing[9] It is evident that the old idea of diagnosing or predicting the risk of cancer by looking for a single alteration in a single gene, is now largely overcome by the evidence that cancer is a far more complex process. We are presently investigating the methylation profile of some key genes in tumor development, other than the Rb1, such as CSP8 and RASSF1A[10] in retinoblastoma, and our preliminary results (data not shown) seem to indicate that methylation of these genes may play a role in the genesis of the disease. However, in the era of the Human Genome Project and the expanding business of biotechnology companies, the hunt for a "responsible gene" has been surely more rewarding and gratifying than understanding the presumably complex interactions between the cell genome and the environment, and among different genes within the cell genome. The resistance of supporters of the clinical usefulness of the routine application of PGT in retinoblastoma, to this new evidence, is still strong and not surprising. But the arguments against the routine application of PGT in retinoblastoma go beyond the above concepts regarding cancer aetiology and pathogenesis.

An articulated body of regulations concerning genetic testing, has been formulated, since 1997, by the National

Human Genome Research Institute. It encompasses a series of procedures aimed at ensuring the development of safe and effective genetic tests. In this regard, the task force, created by the NIH has defined safety and effectiveness to encompass not only the validity and utility of genetic tests, but also their delivery in laboratories of assured quality, and their appropriate use by health care providers and consumers[11]. Similar recommendations had been previously formulated by the American Society of Clinical Oncology. This has not prevented PGT for retinoblastoma from being applied as a clinical routine, although it doesn't meet the requirements of safety and effectiveness expressed by both the NIH Task Force and the ASCO statements[12]. The American Society of Clinical Oncology (ASCO) recommended that genetic testing for cancer predisposition should be offered only when: 1) the person has a strong family history of cancer or very early age of onset of the disease; 2) the test can be adequately interpreted; and 3) the results of the test will influence the medical management of the patient or family member.

Requisite 1) is generally fulfilled in retinoblastoma, since the affected population is made of young children. However, requisite 3) is not fulfilled since therapeutic choices, in retinoblastoma, highly depend on clinical considerations, rather than genetic evaluation. Furthermore, requisite 2) is far from being fulfilled, at least in retinoblastoma, since a great deal of variability is implicated in the techniques commonly used for PGT as currently applied to this disease.

PGT as applied to retinoblastoma has become a rather generic term to define a number of different approaches to the molecular diagnosis of this disease, encompassing heterogeneous techniques such as: linkage analysis[13, 14], heteroduplex[15], and SSCP mutation screening analysis[16], two-dimensional gene scanning (TDSG)[17, 18], and direct DNA sequencing[19].

This heterogeneous approach, is not helpful in the perspective of the routine application of PGT to retinoblastoma or any other cancer, where standardization, and quality control represent the minimal essential requirements.

To further complicate this picture, it has to be outlined that all the techniques used in genetic testing, including the Polymerase Chain Reaction (PCR), although widely used, are difficult to standardize and show a great deal of variation from lab to lab. A high rate of variability in PGT, is to be expected, given the variability implicated in the Polymerase Chain Reaction (PCR) [20], the most widely used ,molecular technique in genetic testing. Also, further variability depends on the complexity and number of the mutation screening techniques currently used in PGT[21], their apparent low sensitivity[22] and their dependence on PCR. Finally, no improvement of standards is to be expected by the application of direct DNA sequencing[23], not only because it depends on the same variables implicated in PCR, but also because it is neither enough sensitive[24], nor specific for retinoblastoma, given the pleiotropic activity of the Rb1 gene[25].

Can such an amount of variability be accepted in routine genetic testing? All physicians involved in the field, should require that PGT criteria and methods be standardized as in other laboratory tests applied to the clinical routine. Therefore, in order to achieve this goal, a few relevant questions have to be answered in advance, such as:

1. Is the test sensitive enough? I.e.: does it allow the detection of all "positive" cases?

2. Is it specific enough? I.e.: does it allow exclusion of all "negative" cases?

3. Is it repeatable? I.e. can it be reproduced by different people in different labs and circumstances?

4. Is it valuable for clinical purposes? I.e.: is the therapeutic decision influenced by the results of the test?

5. Can it be adequately interpreted? I.e.: does it give unequivocal responses?

We believe that, for now, the answer to all these questions, in the case of PCR-based PGT in retinoblastoma is "no". We therefore recommend that the use of PCR-based PGT for retinoblastoma be confined to the field of research until its sensitivity, specificity, and repeatability are demonstrated with the standards of Quality Assessment (QA) and Control (QC), and Good Laboratory Practice (GLP). Once these necessary standards are applied to the test, further investigation of its real clinical usefulness will be of crucial importance before its routine application to the diagnosis or

prevention of retinoblastoma.

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