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INVITED EDITORIAL
Predictive Testing for Retinoblastoma Comes of Age

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From research in human genetic diseases we are deriving the tools to predict disease before it has occurred, making it possible to use our resources to maintain health rather than to treat disease. Huge effort has been put forth to map, clone, and analyze genes that cause human disease, in order to achieve precisely this advantage. The research methods and approaches required are now relatively standard. However, the process to incorporate this new knowledge of disease genes into clinical management of families and into routine health care is not so clear.

The rare tumor retinoblastoma has repeatedly been a model that has revealed principles that subsequently were recognized to apply to much more common diseases: the mechanism of cancer induction common in many human tumors, loss of heterozygosity (LOH), was discovered in retinoblastoma (Cavenee et al. 1983; Godbout et al. 1983); analysis of age at diagnosis of retinoblastoma tumors predicted the existence of tumor-suppressor genes (Knudson 1971); and the retinoblastoma gene (RB1) was the first tumor-suppressor gene to be cloned (Friend et al. 1986) and shown to be mutant in patients (Dunn et al. 1988). The biology of the protein product of RB1 has ignited the field of cell-cycle regulation, so that much more literature now refers to the retinoblastoma protein than to the disease.

In this issue of the *Journal,* Lohmann et al. (1997) provide a thorough analysis of RB1 in a subset of probands with retinoblastoma and show that the technology for practical identification of these mutations in families is ready for implementation in health care. They studied the tumors of unilaterally affected retinoblastoma patients who have no relatives with retinoblastoma. Of the mutations presumed to be present on both alleles, 81% were found. Previous studies used inefficient research-laboratory technology to identify unique mutations in the large, 27-exon RB1 gene (Blanquet et al. 1995). Lohmann et al. used a more efficient strategy, screening every exon by a multiplex PCR-based assay to detect any size change in the genomic fragments, followed by heteroduplex analysis and sequencing. In addition to strongly supporting the hypothesis that all retinoblastoma tumors have mutations in both alleles of RB1, this success rate indicates that RB1-mutation identification is ready for implementation in health care.

Previous studies, which counted affected offspring of unilateral patients, estimated risks, ranging from 15% (Bonaïti-Pellie and Briard-Guillemot 1981) to 2% (Draper et al. 1992), that unilateral patients would carry a germ-line mutation in RB1. By their careful, precise molecular studies of RB1, identifying both RB1 mutations present in the tumor and then checking the blood of the patients specifically for those mutations, Lohmann et al. show a convincing RB1 germ-line mutation in 17%.

The types of mutations help to explain the reduced estimate derived from family studies, compared with molecular studies. Mosaicism for the molecular mutation was demonstrated in 2 patients and was suspected in 1 other, of 39 patients studied. Mosaicism for an RB1 mutation would result in both reduced liklihood of developing a retinoblastoma tumor and more unilateral and unaffected carriers. Two patients with deletions encompassing undefined genes adjacent to RB1 are included in this study of unilateral patients; such mutations are known to result in reduced numbers of tumors, presumably because LOH does not lead to tumorigenesis, since the cell cannot survive loss of the adjacent gene(s).

In 29 (74%) of 39 patients, information was obtained that was useful for the families and that significantly reduced the number of infant relatives that would require surveillance for retinoblastoma tumors. Economic comparison of conventional and molecular management for families in which the proband had bilateral retinoblastoma has shown a fivefold advantage of the molecular approach (Noorani et al. 1996). If no RB1 molecular information is available, all siblings, nieces and nephews, first cousins, and offspring of retinoblastoma pa-

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tients conventionally have repeated clinical examination of the retina, some under general anaesthetic, during the first 4 years of life, in order to find small retinoblastoma tumors when early treatment can prevent blindness and death. This results in a significant expenditure, inconvenience, morbidity, and exposure to risk. On the other hand, if the proband's mutation is identified in a retinoblastoma family, only the few carrying the mutation require the clinical surveillance.

Lohmann et al. specifically study and discuss RB1-mutation identification for isolated unilateral retinoblastoma patients, using the tumor as a starting point. Since such children might be the first evidence of a low-penetrance RB1 mutation in the family, it could be argued that molecular studies are especially valuable for unilateral patients; otherwise, infant relatives might not be screened at all, because of the conventional wisdom suggesting no risk, thereby allowing tumors to grow unheeded, as noted in the report of a large low-penetrance retinoblastoma family (Bremner et al. 1997).

Practical testing of unilateral patients requires that tumor be available in sufficient quantity and quality to allow identifcation of the RB1 mutations, which can then be specifically tested for in blood, as described by Lohmann et al. Identification of RB1 germ-line mutations in bilateral patients has an even greater advantage in healthcare, since 100% are predicted to have a mutation detectable in blood. The technology for RB1-mutation detection described by Lohmann et al. is good but could be more efficient; for example, if multiplex PCR is performed quantitatively, the copy number of exons can be detected. As more RB1 individual mutations are identified, the recurrent mutations due to genomic structure become apparent and suggest a exon-testing order that will make the overall strategy more efficient. Such valuable information will be best utilized when all identified human RB1 mutations are available over the Internet. Several Internet databases do contain RB1 mutations from patients, but uniform terminology, entry of mutations, and access to this information has not yet been established.

At present, RB1-mutation identification remains in the research laboratory, as described by Lohmann et al. This is suboptimal, for several reasons: research laboratories are not set up to provide adequate quality control for patient samples; economy of scale is not achieved; and intellectual and financial resources are annexed from research endeavors. In addition, availability is limited by access to the researcher and the research lab. However, particularly for rare diseases and hot areas where knowledge is rapidly advancing, the researcher has the best perspective from which to understand appropriate application to patients.

We need a new creative solution to get the most out of the investment that has been made in identifying human disease genes such as RB1. A new kind of partnership between research, innovative entrepreneurial activities, and clinical management could accomplish this goal. Since the scientist best understands the biology of the particular gene and disease, continued involvement is critical to best ensure appropriate application to patient care. The business world provides opportunities to keep at the innovative-technology forefront and to achieve economic benefits of efficiency and scale. Clinical-service providers can define the necessary quality-control/patient-care interface—for example, genetic counseling—that is required to make the test appropriately available. These needs have become obvious for RB1 testing.

Retinoblastoma is a rare disease, and the best test yet available is difficult and much more expensive than established simple biochemical tests. The economic benefit of RB1-mutation identification is seen only when careful comparative economic studies recognize how expensive is the conventional care for these families. Despite all the evidence, however, uninformed individuals continue to expect the molecular results to be done free of charge, as "research" or for less than cost, and they fail to understand the significant clinical advantage of the tests. For each diseased gene, prior to full implementation into clinical care the specific test must be shown to be valid, sensitive, and clinically relevant and to reduce health-care expenses; when these criteria are fulfilled, we need a *process* for implementation.

Once molecular testing is truly embraced in the clinical care of families, disease prediction will have very significant effects on the impact of that disease. Again, retinoblastoma is an excellent example: not only does the molecular strategy save health-care dollars, but infants and children without the mutation can avoid invasive procedures, and those who do have a germ-line RB1 mutation can be promptly treated. The more common diseases for which a similar case can be made are numerous. At last our resources and efforts will deliver "health," through prevention and early management of disease, which will replace the much more expensive treatment of end-stage illness.

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