From Bench to Bedside . . . But When?

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*But mousy, thou art no' thy 'lane
In proving foresight might be vain
The best laid schemes o' mice and men gang
aft agley
And leave us nought but grief and pain
For promised joy*

Robert Burns

Prior to September 1994, we physicians in Cancer Family Clinic sat in front of patients who had family histories of breast cancer, prophesying, albeit cautiously, that we would be practicing clinical cancer genetics differently once the first breast cancer susceptibility gene, *BRCA1* was cloned. In the autumn of 1994, *BRCA1* was delivered into an expectant world of scientists, clinical cancer geneticists, patients, and curious onlookers (Miki et al. 1994). In rapid succession, thanks to the Human Genome Project and its fallout technology and information, the second breast cancer susceptibility gene *BRCA2* was mapped and isolated (Wooster et al. 1994, 1995); other genes that may lend susceptibility to breast cancer, *ATM* (ataxia-telangiectasia) and *PTEN* (Cowden syndrome), ensued (Savitsky et al. 1995; Nelen et al. 1996; Liaw et al. 1997). The era of molecular oncology had arrived.

But what makes cutting-edge genetic findings of today clinical practice tomorrow? The most important criterion is benefit or potential benefit to the patient—that genetic tests result in altered clinical management. Other criteria (Table 1) include ease of mutation detection, the majority of people with a specific inherited cancer syndrome contain mutations within the same gene, the mutation analysis allows prediction of cancer risk, and effective surveillance or effective prophylactic procedures are available. Two inherited cancer syndromes illustrate the agony and ecstasy—apologies to Michelangelo—of translating the latest molecular genetic

findings into clinical practice: the hereditary breast cancer (HBC) syndromes and multiple endocrine neoplasia type 2 (MEN 2). As highlighted in a *Lancet* Grand Round convened at the Royal Marsden Hospital, Sutton, UK (Eng et al. 1994), the differences between these syndromes were obvious even before the identification of *BRCA1*.

The Syndromes: MEN 2 vs. HBC

MEN 2 is an inherited cancer syndrome characterized by medullary thyroid carcinoma (MTC) (the most common fatal complication), pheochromocytoma (PC), and hyperparathyroidism (HPT). Depending on the tissues involved and their combination of features, this syndrome is subdivided further into MEN 2A, MEN 2B, and familial medullary thyroid carcinoma (FMTC). Prior to genetic testing, annual biochemical screening for all individuals at risk started at age 6 for MEN 2A and FMTC, and younger for MEN 2B. Like all indirect screening tests, false positives and false negatives occurred.

HBC comprises a group of autosomal dominantly inherited cancer syndromes, including site-specific breast cancer, breast-ovarian cancer syndrome, and site-specific ovarian cancer syndrome. To complicate matters, other HBC syndromes do exist (Table 2), but for purposes of this discussion, only these three will be considered.

Ease of Mutation Detection: *RET* vs. *BRCA1* and *BRCA2*

The *RET* proto-oncogene, encoding a receptor tyrosine kinase, is the susceptibility gene for MEN 2 (Eng 1996). Although *RET* has 21 exons (Myers et al. 1995), germ-line mutations in only one of eight codons encompassed within five exons have thus far been associated with MEN 2 (Eng et al. 1996). Direct se-

quence analysis, differential restriction digestion, single-strand conformation polymorphism (SSCP) with heteroduplex analysis, and denaturing gradient gel electrophoresis (DGGE) are equally accurate and user friendly with a high throughput and reasonable cost (Marsh et al. 1994; Mulligan et al. 1994; Borst et al. 1995; Kambouris et al. 1996).

BRCA1 and *BRCA2* together have a total of 49 exons. Unlike *RET* in MEN 2, germ-line mutations associated with HBC are scattered along the full length of these genes (Breast Cancer Information Core, http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic). Thus, various mutation scanning technologies are employed instead of the gold standard of nucleotide sequencing (Eng and Vijg 1997). The tradeoff of accuracy for high throughput is germane, as the entire discussion about mutation frequency, penetrance, and risk (below) can be obviated if the techniques used in these molecular epidemiologic studies have sensitivities or specificities well below 100%. Some may fear, for example, that pure heteroduplex analysis used in the recent study by Couch et al. (1997) has a low sensitivity. However, it is comforting to note that the mutation frequencies obtained in comparable studies were identical using heteroduplex analysis (Couch et al. 1997) and DGGE (Stoppa-Lyonnet et al. 1997), a technique with virtually 100% accuracy (Eng and Vijg 1997).

A *RET*-MEN 2-like situation, however, does exist for HBC in the Ashkenazi Jewish population, a genetically distinct population of Jews, whose ancestors lived in central and eastern Europe (for review, see Tonin et al. 1996; Streuwing et al. 1997). Three ancient mutations, c.185delAG and c.5382InsC in *BRCA1* and c.6174delT in *BRCA2*, are commonly found in Ashkenazi HBC families. It is relatively easy to test a panel of these mutations by any detec-

tion technique; in the Research Molecular Diagnostics Laboratories of the Dana-Farber Cancer Institute, these are the only *BRCA1* and *BRCA2* mutations that are tested.

Although the detection technology choice is a nonissue in MEN 2, it impacts greatly on HBC genetic testing. Hopefully, in the not too distant future, cost-efficient mutation scanning of several genes in parallel will help (Eng and Vijg 1997).

Germ-Line Mutation Frequencies in MEN 2 and HBC Susceptibility Genes

Germ-line mutations in *RET* occur in >92% of all MEN 2 cases (Eng et al. 1996). *Res ipsa loquitur* (That which is obvious or self-evident). Unlike *RET* testing in MEN 2, 3 years after *BRCA1* isolation and 2 years after *BRCA2* cloning—a long time in molecular genetic terms—we still sit in front of our HBC patients in Cancer Genetics Clinic, but we most certainly cannot offer them *BRCA(X)* testing as a routine clinical service.

Initially, based on linkage analysis with 17q markers flanking the *BRCA1* locus in “large” families with breast and ovarian cancer, *BRCA1* mutations were predicted to account for the majority of HBC families, even as high as 90% (Easton et al. 1993). Similarly, ~50% of large site-specific breast cancer families appeared to be linked to this locus (Hall et al. 1990; Easton et al. 1993). The key word is large, as the original studies were based on the most obvious families, comprising multigenerational families with many affected individuals. In addition, families with ovarian cancer were sought for those studies because it increased the probability of linkage to

17q. More often than not, these do not represent the patient population in our cancer genetics clinics. More recently, taking “all comers” with familial breast cancer, with or without ovarian cancer, two centers (one in France and the other in the U.S.) found ~15% had germ-line *BRCA1* mutations (Couch et al. 1997; Stoppa-Lyonnet et al. 1997).

Can we predict which families represent the 15% with germ-line *BRCA1* mutations, raising the possibility of implementing practical genetic testing for these families? The presence of ovarian cancer seems to increase the chances that there is a *BRCA1* mutation (Couch et al. 1997; Stoppa-Lyonnet et al. 1997), bearing out predictions based on linkage, although not to that great an extent. Instead of the 90% predicted, ~40%–45% of breast-ovarian cancer families have these mutations. Unlike linkage-based estimates using large families, only 7% of the clinic families with breast cancer alone carried a *BRCA1* mutation (Couch et al. 1997). Interestingly, the absolute number of family members affected with breast cancer is not independently predictive of the presence of *BRCA1* mutation.

Partially reflecting the 2% prevalence of the three ancient mutations in the Ashkenazi Jewish population, the clinic-based study by Couch et al. (1997) revealed that belonging to the Ashkenazim increased the probability of detecting a germ-line *BRCA1* mutation whether one was diagnosed with breast cancer under the age of 40 or belonged to a family with breast cancer only or breast and ovarian cancer (26% overall vs. 16% entire study).

An equivalent all comers study has yet to be performed for *BRCA2*, al-

though, doubtless, such analyses are in progress. However, extrapolating from the *BRCA1* experience, clinical cancer geneticists suspect that the *BRCA2* figures will also be lower.

Overall, unlike MEN 2, HBC is genetically heterogeneous. Thus, more predisposition genes can be expected, and recent data support such expectations. Paradoxically, however, when practicing clinical cancer genetics, familiarity can still be presumed in all HBC as a whole. Bayesian calculations are still used to predict inheritance and cancer risk.

Cancer Risk in Mutation-Positive Individuals

Both MEN 2 and HBC are believed to have age-related penetrance (Ponder et al. 1988; Easton et al. 1993). Based on clinical presentation only, 70% of presumed *RET* mutation carriers will develop a sign or symptom by the age of 70 (Easton et al. 1989). Biochemical screening increases the penetrance figures to 95% by the age of 35 (Easton et al. 1989). Hence, in general, carriers of germ-line *RET* mutations have a high risk of developing MTC, PC, or HPT.

Based on the original linkage studies in large, exaggerated families with HBC, risk estimates for breast and/or ovarian cancer reached 85%–90% by the age of 70 and 50% by the age of 50 (Easton et al. 1993). A novel way of studying penetrance “in the field” is to perform a population-based estimate. Of course, this can be done only when the prevalence is reasonable. Such a study, which polled a large group of the Ashkenazim in the Washington, D.C. area, was performed to determine the penetrance of breast, ovarian, and prostate cancer among carriers of one of the three ancient *BRCA1* mutations (Streuwering et al. 1997). The investigators concluded that the risk of developing breast, ovarian, or prostate cancers in mutation carriers by the age of 70 was 56%, 16%, and 16%, respectively. Previous estimates for the general population were as high as 85%, 40%, and 15%, respectively. This latest study, however, calculated penetrance based on questionnaire family histories. Clinical cancer geneticists will tell us

Table 1. Criteria for the Practical Translation of Genetic Testing to Clinical Practice

Ease of mutation detection (for review, see Eng and Vijg 1997)
high sensitivity
no false negatives (100% specificity)
high throughput, i.e., turnaround time consistent with that of a routine clinical laboratory
user friendly
inexpensive (no more than \$150/sample/whole gene)
Majority of mutations in single gene, preferably within a few exons
Ability to predict cancer risk from mutation data
Ability to offer effective surveillance or prophylactic procedure

Table 2. Hereditary Breast Cancer Syndromes

Syndrome	Known susceptibility gene(s)
Site-specific breast cancer	<i>BRCA1</i> , <i>BRCA2</i>
Site-specific ovarian cancer	<i>BRCA1</i>
Breast-ovarian cancer	<i>BRCA1</i> , <i>BRCA2</i>
Cowden syndrome	<i>PTEN</i>
Li-Fraumeni syndrome	<i>p53</i>
HNPCC (Lynch syndrome)	<i>hMSH2</i> , <i>hMLH1</i> , <i>hPMS2</i>

that patient-based family histories, especially of cancers in the abdomen and pelvis, are unreliable. Penetrance is a result of several factors, possibly including the nature and position of a mutation (Gayther et al. 1995, 1997), the modulating effects of other loci, and environmental exposure, all of which we really do not understand well. I suspect that the true penetrance of these three ancient mutations is probably not as high as previous estimates for the general non-Ashkenazim mutation carriers but somewhere in between.

Effective Surveillance and Prophylaxis

It is generally accepted that both prophylactic surgery (prophylactic thyroidectomy) and surveillance for component tumors for those at risk for MEN 2 are effective and life saving (Wells et al. 1994). The standard of care in MEN 2 is to perform routine *RET* mutation analysis as a clinical test. No prophylactic thyroidectomy should be performed without proof of the presence of the family-specific mutation in an at-risk individual.

How about in HBC? Surveillance of the breasts and ovaries in at-risk individuals is recommended—but primarily because none dare to do otherwise. This recommendation is not based on direct evidence, only on extrapolations from population-based screening studies. Multiple studies have shown the benefit of mammography in the female population-at-large over the age of 50. Controversy continues concerning the efficacy of screening younger women. There has never been a population-based study proving that ovarian screening is effective. These negatives are likely attributable to the low prevalence of the disease

being screened in the general population. Many screen women in high-risk families only because the prevalence of cancer in these families is much higher than in the general population; hence, the efficacy of screening should be evident. Hard evidence, however, is still lacking.

What of prophylactic surgery? From limited family studies, such surgery likely decreases the risk of breast and ovarian cancers, but by how much is unknown. It certainly does not decrease the risks to population levels. Obviously, randomized trials are not possible. Hence, to help surmount this problem, decision analysis modeling has been employed (Schrage et al. 1997). Modeled “numbers” are obtained: years gained, lives saved, and so on. However, these sorts of analyses and conclusions must be approached with great caution on the part of the public and clinicians (Healey 1997).

Concluding Remarks

By all counts, *RET* mutation analysis in MEN 2 meets all the criteria outlined in Table 1: No wonder it took only 6 months from publication (Mulligan et al. 1993) to routine clinical testing. But HBC is not MEN 2—so, what do we do now? Sometimes, the best action is . . . inaction. Tongue in cheek aside, however, uncertainty does not mean that we should do nothing: In the long tradition of the art of medicine, it behooves the clinician to practice and advise to the best of his/her ability given the knowledge of the day—and the knowledge grows day by day. The clinician should not view cancer genetics as a strange beast either: The apparently decreasing frequency of *BRCA1* mutations in HBC each time a study is performed is no dif-

ferent from phase I–II chemotherapeutic trials yielding phenomenally high response rates for metastatic cancer only to be dashed by phase III trials. The parallels continue: Patients chosen for phase I–II trials are stringently selected with narrow eligibility criteria just as much as the first studies on HBC targeted the most obvious, large families with multiple-affected individuals. Clinical cancer genetics as a burgeoning field where routine gene testing is performed to affect management decisions is at hand. To help it along, however, more, and not less genome-based research is required.

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