Entering the Helix:
Molecular Genetic Testing

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Many patients and physicians erroneously believe ordering genetic tests is as straight forward as ordering a complete blood cell count. However, genetic testing is not yet as technologically advanced as suggested by popular culture. While molecular diagnostics have improved exponentially over the past two decades, we still do not have reliable testing for many genetic conditions. Patients, families, and even physicians may be surprised to know that, in some cases, we cannot necessarily distinguish between genetic and non-genetic disease.

What questions should I ask?

Physicians should ask five questions before submitting for a genetics test (Table 1).

1. Has the relevant clinical information been confirmed?

The accuracy of molecular testing hinges upon the accuracy of the family member's diagnosis. It is, therefore, important to obtain records on all relevant family members to confirm the reported diagnosis.

Sally's Case

During a routine appointment with her family physician, Sally mentions several of her relatives have had cancer and that she's worried about her risk. The physician refers Sally to the local genetics clinic for the "genetic cancer test".

According to Sally, her sister had ovarian cancer at age 25, her mother had breast cancer at 42, and her grandmother died of ovarian cancer at 65.

While her family history is very suspicious for breast and ovarian cancer syndrome (warranting genetic testing for breast cancer [BRCA] 1 and 2 mutations), if records show Sally's sister had cervical cancer, her mother underwent surgery for a benign breast nodule, and her grandmother died of uterine cancer, then her history is not at all suggestive of a familial cancer syndrome.[1]

2. Is molecular testing available?

Online Mendelian Inheritance in Man (OMIM™), an online catalogue of human genes and genetic disorders, contains descriptions of 15,110 known genetic conditions.[2]

Genetests, the Web-based directory of genetic testing funded by the National Institute of Health, indicates that testing is available for 1,029 different genetic disorders.[3] Therefore, molecular testing is available for only 7% of known genetic disorders.

Table 1

Questions to ask before ordering a molecular test

1. Has the relevant clinical information been confirmed?
2. Is molecular testing available?
3. Is molecular testing the best option?
4. What is the sensitivity of this test?
5. Have all the pre-test counselling issues been addressed?

Clinical testing is available for 680 of the 1,029 diseases listed on Genetests.[3] However, testing may only be available in selected molecular laboratories. Funding may not cover tests sent to an out-of-province laboratory.

Testing for the remaining 349 diseases is available on a research-only basis. While some research laboratories don't release results, others recommend research results be confirmed in a clinical laboratory prior to offering testing to other relatives. Thus, it can take years to obtain results. Regardless of the drawbacks, when there is no other way to offer testing for at-risk relatives, research testing may be the only option.

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3. *Is molecular testing the best option?*

Biochemistry can be a relatively inexpensive and efficient way to diagnose some conditions.[4] In other cases, genetic testing is not required to confirm the diagnosis. For example, the clinical finding of multiple colonic polyps or multiple cysts in the kidneys of an adult patient, especially with a consistent family history, is diagnostic of familial polyposis coli[5] and adult polycystic kidney disease,[6] respectively.

4. *What is the specificity and sensitivity of molecular testing?*

Table 2 outlines factors which affect the accuracy of molecular testing.

The sensitivity of molecular testing for BRCA1 and 2 mutations varies depending on the technique(s) used. The most exhaustive testing for BRCA 1 and 2 mutations has a sensitivity of 95%.[7] However, only 30% of breast and ovarian cancer syndrome is associated with BRCA1 or BRCA2 mutations.

The specificity of all molecular testing should be 100%; only known pathogenic mutations are reported as positive, thereby eliminating the risk of false positives. Mutations of unknown significance are reported as such, rather than as a positive result.[8]

5. *Have all the pre-test counselling issues been addressed?*

The sensitivity and specificity of the genetic test should be discussed in every case. If the testing does not detect a mutation, is that result negative or uninformative? In the case of BRCA1 and 2 testing, a mutation will be detected 30% of the time. In the 70% of cases where no mutation will be detected, the finding is negative for BRCA1 and 2

(with 95% confidence), but uninformative for other causes of hereditary breast and ovarian cancer.[7] Studies have shown uninformative results may be misinterpreted by the patient and physicians, creating a source of great anxiety.[8]

The potential social and family implications need to be acknowledged, including the potential for discrimination (*i.e.*, insurance, employment, immigration, *etc.*,) on the basis of genetic risk status. When a genetic test identifies a predisposition to genetic disease, it should be clearly distinguished from a diagnosis of current illness.[9]

When testing is done to confirm a clinical diagnosis, there may be little direct benefit for the patient as treatment may not be effective or even available. Genetic testing may be offered to an affected individual only to enable other at-risk relatives to undergo testing. The availability, accuracy, and implications of carrier testing and prenatal diagnosis should also be fully explained.

Table 2

Factors affecting the accuracy of molecular testing

1. The number and characteristics of the gene(s) of interest

Characteristics such as genetic heterogeneity, phenocopies, and mosaicism can make molecular testing for single gene disorders complicated.

2. The molecular features of the disorder

Most currently diagnosable conditions are single-gene disorders inherited in Mendelian patterns. Most adult-onset disorders are multi-factorial in nature.

3. The type and number of mutations

Some types of mutations are readily revealed, while others are difficult to detect or undetectable using routine genetic tests.

4. The molecular testing technique

Molecular techniques screen for mutations in a variety of ways and are each suited to detect different types of mutations (*e.g.*, point mutation, small or large deletion, insertion or duplication).

Interpreting the results

If molecular testing identifies a mutation not previously reported, it may not immediately be clear whether the sequence change is disease-causing or a normal variation. Further testing, involving multiple family members, may be required to further define the mutation.

Molecular testing is available for only 7% of the 15,110 known genetic disorders.

In most genetic disorders, a causative mutation does not yield black and white information about symptom manifestation. When a condition has reduced penetrance, not all individuals develop clinical symptoms of the disease. With variable expressivity, age of onset and severity cannot be predicted.[5]

When a genetic test has a high accuracy, people can be tested for carrier status without reference to the family results.[9] In contrast, with a lower accuracy test, the results are informative only when interpreted in conjunction with family results.[9] In these cases, once a mutation is identified in a family member, subsequent testing of other family members is straightforward.[8] If there is no identifiable mutation in the affected family member(s), carrier testing cannot be offered to anyone.

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One of the difficult challenges in using genetic testing is the constantly changing knowledge base. There are a wide variety of resources available, not the least of which are local genetic clinics.

Our increasing knowledge of the genetics of disease is bringing the discipline into the medical mainstream and the public arena. Since the scope of genetic testing is expanding to include tests which assess the genetic risk of common diseases, such as cancer and cardiovascular disease, there is an increasing need for physicians to understand more complicated genetic phenomena and the implications of genetic testing. CME

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Take-home message

- The accuracy of molecular testing hinges upon the accuracy of the family member's diagnosis.
- When genetic testing does identify a predisposition to genetic disease, it should be clearly distinguished from a diagnosis of current illness to avoid potential family and social implications (*i.e.,* discrimination).
- When molecular testing identifies a previously unreported mutation, testing of multiple family members may be necessary to further define the mutation.
- Genetic tests with high accuracy can test for carrier status without reference to the family results, but lower acurracy test results are informative only when interpreted in conjunction with family results.