Molecular Genetic Testing for Adult-Onset Disorders: The Evolving Laboratory, Physician, Patient Interface

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Molecular genetics is providing the power to predict and diagnose a variety of diseases. This is an evolving field, with new information being gained at a very rapid rate. Ultimately the molecular tools should be available to prevent and/or cure a number of diseases with genetic etiology. The molecular genetics laboratory is playing a pivotal role in effecting the utilization of this technology. However, molecular genetic testing is in many ways unlike other clinical laboratory testing. The process of molecular genetic testing and counseling is complex. The laboratory offering molecular genetic testing must be able to assess pedigrees, decide the

proper test to perform and provide interpretation of the tests results obtained. Although standards governing the use of high-complexity genetic tests are evolving it is clear that the laboratory offering these tests will have to have on staff personnel trained specifically in molecular genetics. The challenge will be to stay abreast of evolving technology as well as standards and recommendations governing the use of these tests. We will review the process of molecular genetic testing and the issues that must be considered by laboratories who offer these tests. *J. Clin. Lab. Anal.* 11:23–27. ©1997 Wiley-Liss, Inc.

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INTRODUCTION

The development of recombinant DNA technology has provided the tools to more easily identify genes involved in disease states. This technology is being expanded as part of the Human Genome Project whose ultimate goal is to map and sequence the approximate 100,000 human genes (1). Genes involved in disease states are being identified at a rate so rapid that it has become difficult to stay abreast of progress. With the discovery of a given disease gene often a genetic test can be quickly developed. For many disorders a genetic test has been developed even before knowledge of the molecular basis of the mutation is known or treatment available. Thus, there may be issues that have to be resolved in order to determine the best use of the information to diagnose, prevent or treat, if possible, a disease with genetic etiology (2).

The laboratory providing molecular genetic testing at present must be aware not only of new technical developments but also of recommended policies governing the use of molecular genetic tests. There are many factors that laboratory management must consider before reaching a decision to offer a molecular genetic test. Some of the traditional factors are listed in Table 1. However, molecular genetic testing in many ways is unlike any previously offered clinical laboratory testing. There are several additional factors and unresolved issues that a laboratory planning to offer molecular

genetics testing should consider. Since molecular genetic testing has created, in my opinion, a unique laboratory, physician, patient interface, I briefly present the factors and issues in this context. This is not an exhaustive treatment of the subject, but rather a select overview that should provide an indication of how this type of testing is evolving. Since genetic tests that have a foundation in other genetic subdisciplines (i.e., cytogenetic and biochemical tests) have been used for 30 or more years, the focus of the following discussion is on molecular genetic testing. However, some of the issues discussed are relevant to the use of these other genetic tests. In addition, since most of my experience is in dealing with adult-onset disorders, I refer to these as examples.

THE MOLECULAR GENETICS KNOWLEDGE GAP

Knowledge in all subdisciplines of genetics is being gained so fast that there is a longer than usual lag time before practicing physicians are aware and understand how to use mo-

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TABLE 1. Traditional Factors Governing the Offering of Molecular Genetic Tests

Clinical value of the test
Demand for the test
Expected volume of tests requested
Cost of establishing the test
Personnel trained in the technology

lecular genetic testing information. One study, which used a questionnaire to assess knowledge of genetics, revealed that nongeneticist physicians correctly answered an average of 73.9% of the questions, compared to 94.6% for medical geneticists and genetic counselors (3). The physicians who graduated before 1970 had a mean genetic knowledge score of 66.9%. With the current level of training, it does not appear that in the foreseeable future there will be enough trained genetic personnel to handle the volume of genetic testing and counseling (4). Thus, the laboratory who offers molecular genetic testing will be placed in a position of having to support more than usual physicians who order these tests.

The power of molecular genetic testing has not escaped the attention of lay people. Patients who learn from the news media that a particular gene for a disease that they know or perceive is clustering in their family may urge their physician to order the test for them. The concerns of these patients often stimulate them to directly contact the laboratory in order to obtain testing for themselves or family members.

However, for several genetic disorders, for example Huntington's disease and familial breast cancer, national guidelines recommend that pre- and post-test genetic counseling be conducted (5,6). Some physicians may not feel comfortable conducting counseling that involves explaining molecular genetic tests. Therefore, laboratories offering molecular genetic testing have found it useful to establish relationships with medical geneticists or genetic counselors that they can recommend to physicians or to have a genetic counselor on their staff. An in house genetic counselor may be better trained to answer genetic questions from physicians and their staff than the laboratory director or technical personnel. Lastly, the laboratory may benefit by offering molecular genetics continuing education seminars. These are excellent opportunities to learn the extent of the molecular genetic knowledge gap among physicians who may refer specimens for testing and establish a rapport with them.

MOLECULAR GENETIC COMPARED TO TRADITIONAL LABORATORY TESTING

The molecular genetic laboratory differs from the traditional clinical laboratory in many areas. The need to interact with other genetic specialists such as a genetic counselor is just one example of the staffing differences. The technology differences, how the tests are used and interpreted, as well as the standards or lack thereof are briefly discussed.

DNA-Based Technology

Molecular genetic testing is based on recombinant DNA technology. Most of the tests are conducted by restriction fragment length polymorphism (RFLP) analysis or polymerase chain reaction (PCR), or a combination of both. RFLP and PCR technologies are not limited to the molecular genetics laboratory in that they are being used in the blood bank, microbiology and immunology sections of the clinical laboratory as well. Other tests to detect genetic mutations are continuing to evolve. The protein truncation test, which detects mutations that result in premature termination of protein synthesis, has been used to detect mutations in the *APC* and *NFI* tumor suppressor genes and in *BRCA1* (7-9). Automated DNA sequence-based testing to detect mutations that result in various genetic disorders will clearly be the method of choice in future. As variations of these technologies and newer ones emerge, the laboratory director will have to continue to evaluate technical advances and make appropriate decisions that best fits the needs of the molecular genetics laboratory.

Uses of Molecular Genetic Testing

The way in which these technologies are used and the types of information generated are what set molecular genetic testing apart from other clinical laboratory tests. The same method may be used for a variety of purposes. For example, in order to detect expansion of trinucleotide repeats in the gene responsible for myotonic dystrophy one would first assess the number of repeats by PCR (10). If the expansion is greater than can be detected by this method, which would be the case in the congenital form of the disease, one would then resort to an RFLP analysis. Both techniques could also be used to assess variable number of tandem repeats (VNTRs) or short tandem repeats (STRs) in parentage testing (11,12), forensic analysis (12,13) or assessing engraftment of bone marrow (14). Thus, the same methodological approach and equipment could be used for several types of analyses.

In order to determine the risk that an individual will develop a given genetic disorder, one may have to assess several members of their family. The genetic information obtained may have implications for the patient for whom the test was ordered and for family members as well. This represents a major difference between molecular genetic testing and other types of clinical laboratory tests where the results are usually for only one individual. Moreover, genetic information might lead to discrimination by potential employers and life and health insurance providers (15). This possibility emphasizes the need for laboratories to maintain all genetic test results in a system that cannot be accessed by unauthorized users.

Interpretation

The results of molecular genetic tests must be interpreted using methods that are not often used in reporting other clini-

cal laboratory tests. Most clinical laboratory reports state the amount observed for a given biological substance (i.e., 400 mg/dl of glucose) but do not contain a diagnosis. Molecular genetic testing can provide information useful in predicting the probability of disease occurring. For example, women who carry BRCA1 mutations have an 87% cumulative lifetime risk of breast cancer by age 80 years and are also at increased risk of ovarian and colon cancer (16). However, many physicians may not be trained in genetics and cannot appropriately interpret probabilistic genetic test results. Thus, the molecular genetics laboratory report should contain an interpretation of results (17).

Standards

Many clinical laboratories may not have a great deal of experience using recombinant DNA techniques. The Committee on Assessing Genetic Risks recommended that HCFA create a new specialty of clinical genetics into which clinical cytogenetics would be incorporated and to create subspecialties of biochemical and molecular genetics (17). Moreover, the committee called for HCFA and CDC to establish proficiency programs for genetic tests and for the Food and Drug Administration (FDA) to approve all new genetic test kits. Although laboratories operating at present under CLIA88 have to meet certain standards, these do not include standards specific for molecular genetic testing. The American Society for Histocompatibility and Immunogenetics has standards that accredited laboratories must follow governing molecular HLA typing for the purpose of matching tissues or organs for transplantation, disease diagnosis, and parentage testing (18). Likewise, the American Association of Blood Banks have standards that accredited laboratories must follow who perform molecular genetic testing for parentage testing (19). Although the American Society for Human Genetics has been active in issuing policy statements concerning the delivery of clinical genetics services (20), the American College of Medical Genetics in 1993 assumed the role of setting standards. Considering the power of molecular genetic testing and the fact that the results can be used to make decisions concerning one's health as well as reproduction, employment, and insurance, specific standards and mandatory proficiency testing programs will be forthcoming.

PROCESS OF GENETIC TESTING AND COUNSELING

Molecular genetics has captured the imagination of society. The technology is providing the power of presymptomatic diagnosis, which increases the possibility of prevention, treatment, and curing a variety of disorders (21). The proper use of the technology is still being debated (2,4,15,21,22). The clinical laboratory offering molecular genetic testing has to balance the requests of health care providers and patients with the recommendations that are being issued by a number of

lay and professional groups as to which tests can be offered, to whom and at what age (5,6,16,23). In addition, in order to ensure that one adheres to these guidelines and the appropriate decisions regarding genetic testing are made, more information than would normally be required for a laboratory test has to be obtained either by the laboratory or the appropriate genetic specialist. As can be seen in Table 2, there are a number of steps required in order to obtain the information necessary to determine one's risk of developing a disease with genetic etiology. All these steps may not be required for every disorder.

Family History of Disease Information

Before any decision can be made with regard to whether a given individual has a familial disorder, for which a molecular genetic test is available, detailed family history of disease information must be obtained. Several factors must be considered when obtaining the family histories that are beyond the scope of this article. These factors have been discussed in detailed elsewhere (24). Physicians normally do not obtain the detailed family history of disease information needed, although this may change in future. Thus, a genetic specialist or a staff member of the laboratory may have to obtain the information. Someone trained in genetics then has to construct and interpret the pedigree. This often is more of a challenge than might seem. For example, consider breast cancer. There are several genes, each of which could be responsible for familial breast cancer. Most premenopausal familial breast cancer is due to mutations in BRCA1 or BRCA2 (25). In addition, a female carrying a BRCA1 mutation is also at risk of ovarian cancer and colon cancer, while males are at risk of colon and prostate cancer. However, approximately 30% of families with an autosomal dominant pattern of breast will not have mutations in BRCA1 or linkage to BRCA2 (16). There are a number of other genetic syndromes, although rather rare, which are associated with risk of breast cancer (16). As should be obvious, without a good knowledge of the genetics of these cancers and detailed family history information one cannot determine which gene might be mutated in the family and the appropriate genetic test to use in order to assess the mutation and estimate risk of disease.

Pretest Counseling

After it has been determined that a disorder is occurring in a person's family, for which a genetic test is available to

TABLE 2. Process of Genetic Testing

Obtain family history of disease information
Construct and assess pedigree
Conduct genetic counseling
Conduct if appropriate genetic testing
Report genetic test results with interpretation
Conduct genetic counseling
Follow up

refine risk, the physician and their patient have to decide if genetic testing is an option they wish to pursue. As discussed, for some disorders it is recommended that pretest counseling be conducted by someone knowledgeable in the genetics of the disorder prior to making the decision to conduct the test. Post-test counseling is also recommended for some disorders. For some familial disorders, such as breast cancer there should be a team available representing all the specialties that may be needed to answer relevant questions (16). Some physicians may elect to conduct the counseling. As a service to the patient whose physician does not wish to conduct genetic counseling the laboratory should have a genetic specialist on staff who can perform this function or have established a relationship with a trained person to whom patients can be referred.

Determining the Types of Tests to Use

As part of evaluating a pedigree to determine the type of genetic disorder segregating one also has to keep in mind the types of genetic tests available and how the composition of the family may determine whether a given test can be offered. For several genetic disorders the mutation can be assessed by RFLP or PCR, or a combination of both. Even when direct mutation analysis is available one can often use the pedigree to determine which technology to use. For example, suppose blood has been forwarded to the laboratory from a newborn who, based on physical exam and family history, is suspected of having myotonic dystrophy. The family history indicates that the mother began having symptoms at approximately 20 years of age. Given that congenital myotonic dystrophy is passed from the mother and the largest number of CTG repeats have been observed in this form of the disease, PCR would probably not amplify the large repeats and RFLP would be the assay of choice (10).

For other genetic disorders, additional factors must be considered. Again I will use breast cancer as an example. Although BRCA1 has been cloned, the gene is quite large and numerous mutations have been observed (26). The extent of mutations in the population, their distribution in various racial and ethnic groups and the effect of each one on cancer risk is currently being investigated. The lack of this knowledge has led to recommendations that population screening not be conducted and that mutation or linkage analyses only be offered to high-risk families (6,16). In order to conduct linkage analysis a minimum number of affected family members must be available. In some families all the members who have developed breast cancer may not be alive. Since there are several STR loci within and flanking the BRCA1 gene, PCR can be used to determine the genotype at each locus. Therefore, if paraffin tissue blocks from the deceased family member can be obtained from pathology archives, if DNA can be extracted from these blocks and phase set, it may be possible to conduct linkage analysis.

The Report

The content of the report will, in part, be determined by the role the laboratory played in the entire process of genetic testing and counseling. The laboratory director should consider the minimum guidelines for reporting genetic testing results (17,20). I suggest the following information be forwarded back to the physician: a copy of the pedigree with interpretation and/or recommendations, a summary of the genetic pretest counseling session, the results of the genetic tests with interpretation, and a summary of the post test genetic counseling session.

DNA BANKING

The laboratory may consider offering DNA banking. The genes have not been mapped or cloned for many familial disorders. However, considering progress the Human Genome Project has made to date one can optimistically predict that many more disease genes soon will be identified. Individuals who realize that a genetic disorder is in their family, or on the suggestion of a physician, may wish to store DNA from affected individuals. The laboratory should realize that a family history of disease is not static and may change over time. Thus, the laboratory conducting linkage analysis on a family may consider storing the DNA for use should other members of the family elect to be assessed for their risk of developing the disease or for use when the specific gene is cloned or direct mutation analysis becomes available. The possibility of using stored Guthrie cards as a source of DNA for testing purposes has been discussed (27).

Recommendations concerning the operation of DNA data banks have come from many sources (28–30). There is concern with some of the same issues governing genetic testing such as quality assurance, responsibility to clients, who has access to the material, and confidentiality. Although legislation has been passed in several states establishing DNA forensic databases, most of these do not deal with DNA databases for medical purposes (31). This is another area where operating standards and proficiency testing programs in future will likely be mandated under CLIA or other agencies.

PERSONNEL CERTIFICATION, LABORATORY ACCREDITATION

As discussed there are no mandated personnel requirements specific for clinical molecular genetics. The American Society for Human Genetics has recommended minimum qualifications for directors of DNA-based genetic-testing laboratories (32). The Committee on Assessing Genetic Risks has recommended that genetic tests be classified as high complexity and called for regulation of laboratories offering genetic testing (17). The American Board of Medical Genetics (ABMG) certifies training programs and individuals in a number of genetic subdisciplines, including clinical molecular

genetics. No doubt the ABMG will play a strong role in affecting future educational and certification requirements for directors of genetic laboratories. Although it is uncertain what future requirements will be mandated, considering the complexity of genetic testing and interpretation and the sensitive nature of the results they likely will be quite strict.

SUMMARY

The power of molecular genetics offers challenges and opportunities for laboratories who can provide the service. Some of the challenges involve the continuing evolution of the technology and the uncertainty as to when a given genetic test can be used and under what circumstances. In addition, genetic testing is in many ways unlike any other clinical laboratory test. The process of genetic testing and counseling is involved and requires much more educational support of the health care provider than for most other clinical laboratory tests. The regulations that laboratories must meet in order to offer molecular genetic testing also are evolving. These regulations possibly will restrict who can perform and interpret molecular genetic tests. In future molecular genetic knowledge will provide an unprecedented ability to predict, prevent and treat genetic disorders. The challenge to our specialty and society at large will be to ensure that the knowledge is used wisely.

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