There are several steps involved with genetic testing for cancer predisposition. The first step would be to meet with a specialist, such a genetic counselor, who can assess your medical and family history to determine if you would be at a higher chance to carry a genetic change that increases the risk for cancer. This specialist can also order the testing for you, and can answer some questions about insurance procedures.

Samples for genetic testing care collected primarily in two ways: blood or saliva samples. In some cases where a person has had cancer that has spread to the blood or if they have gotten a bone marrow transfusion, other tissue may be required to run the test. The sample is then sent to the lab where they generally run two different types of tests on the genes they are looking at: sequencing and deletion/duplication analysis.

Sequencing

Analyzing genes related to hereditary cancer involves sequencing, or reading through, the letters that make up the gene. Sequencing can help look for spelling mistakes, also called mutations, in the gene that cause it to not work how it should, which increases the risk for cancer. The type(s) of cancer that someone is at increased risk for is determined by which gene the mutation was found in.

Sequencing a gene involves reading the specific spelling of that gene (its DNA sequence) on a machine. The scientists at the lab know what the spelling sequence should look like, and if a spelling error or typo is found, that can mean that the gene is not working how it should. The genes that are linked to hereditary cancer are usually genes that, when they are working properly, help to protect us from cancer. When these genes are damaged, that can leave us more prone to develop certain types of cancer.

Deletion/Duplication Analysis

While sequencing is designed to look for “spelling changes” within the sequence of the DNA letters, deletion and duplication testing looks for large extra or missing pieces involving several letters of DNA that can be missed when DNA sequencing is performed alone. Another way to think about this is to think about spell checking a book. Sequencing would be like skimming the book to look for any misspelled words. A deletion would be like having a whole page being removed, while a duplication would be like having a whole page randomly added to the book. In both cases, the spelling – and therefore the function – of the

gene is affected. However, because with deletions and duplications all of the words are still in the correct order (there are just extra or missing letters), the spell check (sequencing) may not detect this kind of change.

Most laboratory tests now include sequencing and deletion/duplication analysis together in one test. However, if you had testing previously you may not have had deletion/duplication analysis, and therefore may still have some risk of having a genetic change that was not detected. You can talk to a genetic counselor or your ordering provider to determine if you would benefit from any updated genetic testing.

Next Generation Panel Testing

Next generation panel testing is a relatively newer technology that allows the lab to look at more genes in a faster and more cost-effective way than ever before. This is particularly useful for individuals whose personal or family history doesn't look like it could be tied to one specific gene. Before these panel tests were available, if someone's personal or family history of cancer looked like there could be cause by potentially two or three different genes, we typically had to order testing for one gene at a time. Generally we would start with the most likely and work our way down to the least likely, but each gene test can take up to 4-6 weeks, and can cost several thousand dollars.

With the new next generation panel tests, the lab can use one test to look at several different genes at one time. Panel testing results can take a little more time to get back (sometimes up to 12 weeks), and can range in cost, but many of the panel tests today are still over $1000. Even though it can be more time- and cost-effective to do a panel test, some insurances will not cover them. Your medical provider may be able to help you determine if your insurance covers next generation panel testing, or you can also contact your insurance directly.

So typically, if a provider is thinking about more than one gene, it can be more time and cost-effective to do a panel test. One potential drawback to panel testing versus single gene testing is that when the lab looks at multiple genes at one time, there is an increase chance they will get a uncertain result, or a variant of uncertain significance (VUS). VUS's are discussed in more detail below in 'Results'.

Another potential drawback to panel testing is that they generally look at several different genes related to a wide range of types of cancer. Sometimes looking at that many genes can reveal a cancer risk that the patient was not prepared for. An example is a woman who

comes in to get genetic testing for a strong family history of breast cancer who does one of these genetic testing panels and finds out she carries a gene that increases her risk for pancreatic cancer. Her genetic test result in this case didn't explain her family history of breast cancer, but it also told her about an increased risk she carries that she had no idea about.

One last limitation of next generation sequencing panels is that some of them look at genes for which there is no clear information on what types of cancer it increases the risk for, or how much the risk is increased. It can be frustrating to get a result that shows a change in a gene, but to not be able to find out exactly how much your risk is raised. Furthermore, if a patient's insurance does not feel that there is enough information from scientific studies about that gene, they may not cover increased cancer screening.

Next generation panel testing is a newer technology that has much potential. Like all scientific tests, it also has limitations, and it is important for patients to be aware of what those limitations are before they have the test done.

Familial gene mutation testing (Single-Site Testing)

Sequenting, deletion/duplication analysis, and/or panel testing can be used to help try to figure out what the cause of cancer is in the family. Single site testing is done when a harmful gene change that increases the risk for cancer has already been found in a family. Once the specific change has been identified, we can start to offer testing to people in the family who have not yet had cancer to determine whether they also inherited this gene mutation (and share the increased risk for cancer), or whether they did not inherit the gene mutation (and have the same risk for cancer as the general population). Once we determine who is at an increased risk for cancer in the family, we can discuss methods of early detection or prevention to help keep everyone healthy.

Results

The same three possible results that can be seen on a sequencing test may also be seen in a deletion/duplication test:

POSITIVE: A large deletion, duplication, or rearrangement of information in a gene is observed that leads to that gene no longer functioning properly (called a mutation). This person has an increased risk for cancers associated with the gene that the mutation was found.

NEGATIVE: The gene(s) appeared normal; there were no significant large deletions, duplications, or rearrangements detected in the gene(s) that would make it not work properly. It is important to remember that a negative result does not mean that there are NO risks for cancer; all individuals have a lifetime risk of cancer that is not eliminated regardless of the results of a genetic test. Genetic testing is also not perfect. How well a test works can depend on the technology and lab used, as well as your personal and family risk factors. Negative genetic testing is most informative if there has already been a mutation identified in the family (see: single-site testing for a familial mutation). It is important to discuss negative genetic testing results with a genetic counselor who can put those results into context given your family

history.

VARIANT OF UNCERTAIN CLINICAL SIGNIFICANCE (VUS): Sometimes genetic testing can reveal a variant of uncertain significance (VUS). A VUS is a change within the gene that has not been seen enough to know whether it just a normal variation that does not increase the risk for cancer, or whether it is a change that makes the gene not work and does increase the risk for cancer. A VUS is typically put into one of four categories:

- likely benign (not harmful, often called a polymorphism)
- uncertain significance (not enough information to say one way or the other)
- suspected deleterious (some evidence leads us to think the change could be a problem)
- likely deleterious (evidence points to the change likely increasing the risk for cancer)

When a lab comes across a VUS, they try to keep gathering data (looking at research, looking at other families that the VUS was found in, etc.) until they know enough about the VUS to say whether it causes a problem or not. Sometimes this process can take months or even years. When the lab does determine this, generally they let the ordering provider (your doctor or genetic counselor) know, and then the provider passes that information onto the patient. Some clinics recommend that patients who have a VUS periodically check in with them to make sure nothing falls through the cracks. Most of the time, a VUS will be found to be benign. The chance to get a VUS with your genetic testing depends on the gene(s) that are tested, how many genes are tested, and which lab is used. When a VUS is found via genetic testing, recommendations for cancer screening are often made based on the

family history. Testing for the VUS in other family members is also generally not recommended.

How to Decide

Deciding on whether or not to have any genetic test is a very personal decision. Some people choose to perform genetic testing because they feel it will empower them to make informed decisions that will help lower their cancer risks, such as choosing increased surveillance or preventative surgery. Genetic testing gives them information not only for themselves but also for their family members. For some patients who have received a cancer diagnosis, the results of their genetic testing may help them determine what type of treatment they would choose. For example, a woman with breast cancer could be deciding between a lumpectomy (remove the tumor) versus a mastectomy (removal of the breast tissue). She may say that if she carries a gene change that increases her risk to have another breast cancer, that would tip her toward having a mastectomy over the lumpectomy. Some women in this circumstance may also choose to have a prophylactic (preventative) mastectomy of the breast that has not had cancer. Other women who know they carry a gene change that increases the risk for breast cancer may choose to do increased screening (usually annual breast MRI and mammogram) rather than preventative surgery. This decision is very personal and should be discussed with your care team.

Some people do not feel that they are ready to know about a potential hereditary risk for cancer that could be revealed by genetic testing. This can be due to many different reasons, including feeling overwhelmed by other health concerns, feeling that the test results would not affect how they approach their medical care, or not wanting to know about risks to other family members. These are all important issues to consider when deciding about genetic testing for hereditary cancer predisposition.