

Genetic Testing for Neurofibromatosis type 1 and 2
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The gene for NF1 was identified in 1990 and shortly thereafter, in 1993, the gene causing NF2 was discovered. Typically, when a gene is found, a diagnostic test soon follows. This has not been the case, though, with neurofibromatosis (especially type 1), despite the fact that we have learned a lot in the past decade about the basic mechanisms responsible for the tumors. Recently, however, major challenges have been tackled so that a diagnostic genetic test for NF1 is now available for those patients that need it.

What is *direct* genetic testing?

In general, there are two methods for genetic testing: indirect and direct. *Indirect* testing examines the segregation of markers closely “linked” with the gene in affected individuals in multiple generations. The underlying mutation causing the disorder remains unknown, however. In contrast, *direct* genetic testing analyzes the sequence of a particular gene to search for the presence of a mutation, or change, which is responsible for a specific clinical disorder. The techniques used for direct genetic testing depend on the types of mutations that occur in a specific gene. For some disorders, only one specific type of mutation is found in >99% of affected individuals, making it easy to develop a sensitive direct genetic test. Neurofibromatosis type 1 and 2 are at the other end of the spectrum, with almost every family carrying a different mutation.

Why has it taken so long to develop a genetic test for NF1 or NF2?

Many families – and clinicians – have been frustrated by the fact that it has taken a long time to develop a routine genetic test available for clinical use. Before an efficient and sensitive testing could be offered, it was necessary to precisely define the types of mutations found in patients fulfilling the diagnostic clinical criteria so that accurate information could be reported. In these patients it was necessary to analyze the complete gene with multiple complementary techniques in order to find all NF-causing mutations. Both the *NF1* and *NF2* genes are very large (*NF1* larger than *NF2*) and have a complex structure. Research has shown that the mutations responsible for NF1 and NF2 can reside essentially anywhere within their respective genes, thereby complicating the matter further. Moreover, the *types* of mutations are very diverse, ranging from the total deletion of the *NF1* gene and flanking genes, to a subtle change of only one particular base out of the more than 300,000 bases of the *NF1* gene, even residing somewhere in one of the large non-coding regions. These challenges had to be addressed before a routine *direct* genetic test could be offered for clinical use.

What are the indications for *direct* genetic testing?

Direct genetic testing now allows the establishment of a NF1 diagnosis in those patients that present with only one symptom, such as café-au-lait spots, but do not (yet?) show other symptoms needed to establish the diagnosis on a clinical basis. Café-au-lait spots are often the first signs of NF1 and may already be present at birth, increasing in

number during the first years of life. However, waiting for more symptoms to appear in order to ascertain the diagnosis on a clinical basis can be very stressful for families. Making a definitive diagnosis as early as possible will become even more important as better therapeutic interventions become available.

Direct genetic testing can also help to establish the diagnosis in patients who present with atypical manifestations or unusual combinations of features and will further help to delineate possible subtypes of the neurofibromatoses. Furthermore, *direct* genetic testing and the unequivocal identification of the mutation now provides the patient who is the first affected member of the family with the option to pursue prenatal or preimplantation diagnosis, if desired (see also “Choosing to become a parent in the shadow of Neurofibromatosis”). Finding the pathological mutation remains a major endeavor, with some mutations particularly difficult to identify. These particular mutations will need special focus in the laboratory beyond “routine testing” and will need a longer investigation period to come to a final result. Hence, it is important that patients who want prenatal diagnosis have their mutation identified *before* becoming pregnant.

Can genetic testing predict the severity of the disorder?

Although direct genetic testing for NF1 can predict whether a person has inherited a specific *NF1* mutation, it can not predict the *severity* of the disorder in most cases. It has been widely known that affected members of the same family, although carrying the same mutation, can differ dramatically in the severity of their symptoms.

The only NF1 “genotype-phenotype” correlation identified so far shows that patients carrying the large deletion encompassing the total *NF1* gene as well as a number of flanking genes develop a particularly severe disorder characterized by mild to moderate cognitive impairment and development of a large number of neurofibromas with an earlier age of onset and unusual facial features. Importantly, some sporadically affected patients may carry the total gene deletion in only a proportion of their cells, as the result of a mutation arising *after* fertilization, during fetal development. Although these patients themselves may present with a milder form of NF1, they can pass this deletion to their children, who will develop the more severe phenotype.

Unlike NF1, large deletions of the *NF2* gene have been associated with a milder phenotype. On the other hand, all mutations leading to a premature stop codon (nonsense and frameshift mutations) have been associated with severe disease. In NF2 it is noteworthy that the variability of the disease severity between members of the same family is low, indicating a stronger effect of the type of mutation on the resulting phenotype than is seen in NF1.

Is genetic testing for NF1 or NF2 available today as a clinical test?

Genetic testing has been done in *research* laboratories since the *NF1* and *NF2* genes were identified. The aim was to understand how mutations alter the functions of the genes to cause neurofibromatosis. Patients who submit blood or tissue samples for *research* make an important contribution by allowing scientists to better understand neurofibromatosis, but the patients may not *directly* benefit from the results of such studies. Since research laboratories are not focused on clinical service, they do not necessarily return results.

In contrast, patients who submit blood or tissue for a *clinical* test expect to have their samples analyzed and results reported in a timely fashion for the purpose of diagnosis, treatment or prevention. In the United States, laboratories performing clinical tests must meet quality control and proficiency testing standards and be approved by Clinical Laboratory Improvement Amendment (CLIA) of 1988.

Recently, more laboratories have begun to offer some tests for neurofibromatosis type 1 or 2. Many look for deletions of the entire *NF1* gene using fluorescence *in situ* hybridization (FISH). Only a small minority of NF1 patients (probably less than 5%) can be diagnosed using this method. Some laboratories offer a “linkage-based test” for either NF1 or NF2: the segregation of intra- and/or extragenic markers is followed in affected individuals in multiple generations. This testing only applies to *familial* cases, requires the collaboration of affected relatives, and does not detect the mutation itself. One company in the United States has offered a test for NF1 based on the “protein truncation assay”, an approach that looks for evidence of formation of a shortened protein product of the *NF1* gene. The assay helps to pinpoint a region of interest in about 70% of NF1 patients. The protein truncation assay itself, as it is used in the commercial test, does not further identify the mutation, but only indicates its likely presence. Many scientists and clinicians feel that genetic diagnosis of NF1 should be based on a full identification of the mutation itself, and that the protein truncation assay alone is not sufficient.

There are only a few laboratories worldwide that perform clinical testing for NF1 or NF2 based on study of the *entire* gene using tests that are both highly sensitive and specific and have a fast turn-around time. A good source of information to locate the different laboratories and the tests they are offering is the internet site GeneTests (www.genetests.org). We have developed a multi-step **comprehensive mutation detection protocol** that identifies >95% of pathogenic *NF1* mutations in patients (who are sporadically affected as well as those with a positive family history) fulfilling the NIH diagnostic criteria [[Messiaen et al 2000](#) , [Messiaen et al 2001](#)]. This is the highest detection rate reported. This testing is now available at the UAB Medical Genomics Laboratory as a *clinical* test. The laboratory is both CLIA and CAP certified and is compliant with the new HIPPA rules (detailed information can be accessed at <http://www.genetics.uab.edu/MedicalGenomics>). It is important that the patients who need genetic testing have access to professionals who can explain the indications for testing, what can be expected from the test results, and can provide counseling regarding the use of the test results for medical purposes.

Is genetic testing available for Schwannomatosis and segmental NF?

Schwannomatosis is characterized by the presence of at least 2 pathologically proven schwannomas and no radiographic evidence for vestibular nerve tumor at age above 18 years. Schwannomatosis has been shown to have several different genetic causes, with some patients carrying a mutation in the NF2 gene in all their cells, as found by analyzing the blood lymphocytes. In other patients and families however, although different somatic NF2 mutations have been found in the tumors, it has been shown that the primary *hereditary* gene locus involved lies outside of the NF2 coding region and remains so far unknown. NF2 mutation analysis in blood is useful and clinically

available, but will reveal a mutation that can be transmitted to the offspring only in a fraction of patients presenting with schwannomatosis.

Segmental NF is about 30 times less frequent than NF1. Patients present with one or more NF1-related symptoms, such as neurofibromas and café-au-lait spots, limited to only a certain body region. In some patients with segmental NF, an NF1 mutation has been found in specific cells (such as Schwann cells or fibroblasts) from the affected body region, while in others, this does not appear to explain their clinical manifestations. It is conceivable that in some of these patients, the mutation may be present in either their reproductive cells, and hence the mutation can be transmitted to the offspring. NF1 mutation analysis in blood is useful and clinically available in these patients, but will reveal a mutation only in a fraction of segmental patients. Testing for segmental NF starting from specific cells from the affected region is more powerful in detecting the mutations in these patients and will become clinically available in our laboratory shortly.

Conclusion

It has taken a long time since the NF1 and NF2 genes were identified to develop diagnostic tests that can be used for clinical decision-making. Such tests are now available, and can be used to clarify diagnosis or enable prenatal testing. The tests require careful interpretation, which should be done together with a qualified health provider who is skilled in the use of complex genetic tests.