

neuro·fibroma·tosis®

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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.



Ludwine Messiaen

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

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Choosing to Become a Parent in the Shadow of Neurofibromatosis Type 1

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The choice of whether to become a parent is a difficult decision for anyone, and even more so for those at risk to pass on a genetic disorder, such as neurofibromatosis, to a child.

A person affected with NF1 has a fifty-percent risk for passing on the disorder, and there is nothing that can be done to either cause or prevent this from happening. In other words, though, there is a fifty percent risk with each pregnancy that the child will *not* inherit NF1. Given the fact that two-thirds of cases are clinically mild, the odds of having a healthy child (either unaffected or mildly affected) are certainly in a couple's favor. For many people, this reassurance may be enough for them to feel comfortable proceeding without further medical assistance.

In other situations, however, a strong desire exists for reassurance that a child will not

inherit NF1. The traditional choices of adoption, sperm or ovum donation, and surrogacy remain open today. Although these techniques prevent biological reproduction for one or both parents, they have led to the formation of many thriving families.

Additionally, with the advent of new methods for detecting mutations, other reproductive options are now available. For people desiring their own biological offspring, preimplantation and prenatal diagnosis are options for determining whether a fetus has inherited NF. Keep in mind, though, that although these tests accurately determine whether an embryo or fetus has inherited a mutation, they do not predict the severity of the condition in that individual.

In preimplantation genetic diagnosis (PGD), the parents' sperm and eggs are combined using in vitro fertilization techniques, allow-

ing several embryos to develop. Each embryo is then individually tested for NF1, and only those that are found to be unaffected are transferred back into the woman's uterus. If a pregnancy has already begun, a fetus can be tested through the methods of chorionic villus sampling (CVS) or amniocentesis. All of these methods carry specific risks and benefits which should be thoroughly discussed with an obstetrician or genetic counselor prior to being performed.

The decision to join the ranks of parenthood can be filled with both joy and fear. Many factors play a role in the decision-making process, only one of which is the basic information discussed here. It is important to carefully consider not only the facts, but your feelings, in relation to what could be the beginning of a beautiful thing. **NF**

With the advent of new methods for detecting mutations, other reproductive options are now available.



WORLD'S NF SCIENTISTS MEET IN ASPEN, CO

The world's leading scientists held their annual meeting as The NNFF International Consortium For The Molecular Biology Of NFI And NF2 in Aspen, CO. This meeting, in 2003 the 18th of its kind, serves scientists to present their latest data, forge collaborations and set new directions for research **NF**

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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)

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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

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DR. KORF NAMED NEW NNFF CHAIRMAN OF MEDICAL AFFAIRS COMMITTEE:

Dr. Bruce Korf, Chair of the University of Alabama's (UAB) new Department of Human Genetics and Wayne H. Finley and Sara Crews Finley Chair of Medical Genetics at UAB, was unanimously elected by the Foundation's Board of Directors to the position of Chairman of the Medical Affairs Committee. In this position Dr. Korf succeeds Dr. Allan Rubenstein who has become the Foundation's Director Emeritus of Medical Affairs. Dr. Korf has long been involved with the Foundation and Neurofibromatosis research. Prior to his recent appointment in Alabama, Dr. Korf served for nearly two decades at the Harvard Medical School, as a teacher, clinical researcher, physician and author. "Dr. Korf's distinguished history and activities in the field of neurofibromatosis will make him a great addition to the Foundation leadership," said NNFF President Peter Bellermann.



ALL GOOD THINGS COME TO AN END Participants in the second session for Returnees, the 2003 NNFF International Summer Camp share a tender moment before departing for their home states. Pictured above at the Salt Lake City airport are (l. to r.) Christine Manolis from Levittown, NY, Erica White from Bellingham, WA, Derrick Barnes from Mora, MN, Kelly Thomas from Kearns, UT, Bill Weiss from Arnold, MO, Brandi Berthold from Colorado Springs, CO, Jenny McElroy from Walnut, CA and Amanda Trima from Attleboro, MA. **NF**

The Sweetest Gift

It is that time of the year again!!

When everyone spends countless hours trying to find the perfect gift. The same gift ideas are flowing in everyone's mind year after year. Let's face the truth, there can only be so many sweaters I can wear, kitchen appliances I need, or gift certificates I will use. As a mother with a child affected by NF, I would like to take this opportunity to suggest a different kind of heart-warming gift; making a contribution to the NNFF.

Your holiday donation will help the Foundation support on-going medical research—research that is making great progress towards rational treatments of NF. It is a great opportunity to make a contribution to the Foundation, which will benefit the thousands of individuals

like my daughter that have been affected by this disorder and deeply touch the heart of those affected by NF.

To acknowledge your donation, the Foundation will send a sweet holiday acknowledgement card to the person whom you have made your generous contribution in honor of. After all, holidays are the time of the year for us to reflect on the things and the people that are most important to us. To me, that means making sure research for NF continues.

Making a generous contribution to the NNFF is the sweetest gift for me this holiday. To make a contribution, please visit <http://www.nf.org> or contact the Foundation at 212-344-6633. **NF**



Above: Paula McDonald and her daughter, Paige Hull (right)

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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. **NF**

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This Year, Raise Funds As Well As Holiday Spirits

Did you know that a percentage of the money that you spend on holiday gifts could go to help NF research? It is true, by shopping on-line at Amazon.com, Vermont Teddy Bear, GreaterGood.com, or Igive.com you can get great gifts, avoid the holiday crush at the mall, and support NF research. All you have to do is follow the link below to these and other great shopping websites, and the company that you purchase your items from will donate a percentage of your purchase price to the NNFF. There is a virtually unlimited selection of gifts on these websites as many of the most popular and well-known brands have signed on to partner with deserving causes like NNFF.

Gone are the days of waiting in long holiday lines and running from store to store trying find the perfect gift! By taking advantage of the foundation's GreaterGood.com, Igive.com or Amazon.com shopping malls, the perfect gift is just a mouse click away. Help Dad get more organized for the New Year by picking out a high-tech pocket organizer at TheSharperImage.com (GreaterGood.com). Or send him to the Golf course in style with his new Taylor Made golf clubs from TheSportsAuthority.com

(GreaterGood.com). Looking for a gift for Mom? No problem! Simply browse through the "Health & Beauty" section of Amazon.com for outstanding choices. Try Amazon's "Toy's & Games" section for children's gifts.

New this year, the NNFF lets you Raise your Paws for a Good Cause—Send a Bear-Gram® gift from the Vermont Teddy Bear Company®! Save 10% off any

Bear-Gram gift & earn an additional 10% for the National Neurofibromatosis Foundation! Order online at www.VermontTeddyBear.com or call 1-800-829-BEAR. Use Coupon Code: NEURO. Show you Care—Send a Bear; The Creative Alternative to Flowers!

What are you waiting for? The possibilities are endless, from gift cards and gift

certificates to clothes and jewelry; so visit the NF web site and try out one of our shopping malls this Christmas! Remember, a percentage of everything you purchase goes towards NF research!

To shop at VermontTeddyBear.com, GreaterGood.com or the Amazon.com NF online mall, simply visit our web site at <http://www.nf.org> and click on the "Shopping For Research" button or look for the special "Holiday Shopping" section. 



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The purpose of the National Neurofibromatosis Foundation, Inc. (NNFF) is to improve the well-being of patients and families affected by NF1 and NF2. The Foundation therefore sponsors scientific research aimed at finding the causes and cures for the neurofibromatoses, promotes the development of clinical activities, works to create public awareness and provides patient support services.

The National Neurofibromatosis Foundation is a founding member of the International Neurofibromatosis Association.

Why do your holiday shopping in a crowded mall when you can shop at the NNFF Online Shopping Mall?