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Background: Schwannomatosis is a recently recognized third major type of neurofibromatosis. Our preliminary studies of the *NF2* gene in tumors from schwannomatosis patients reveal a pattern of tumor suppressor gene inactivation not previously reported in any other human disease. **Objective/Hypothesis:** The objective of this project is to clone the locus responsible for familial schwannomatosis. We are exploring two competing hypotheses which address both the non random distribution of LOH observed in schwannomatosis tumors and the high rate of somatic *NF2* mutation seen along the *cis* allele.

Specific Aims:

1. To identify and clinically characterize schwannomatosis patients, and maintain a resource of tumor and blood specimens.
2. To further refine the candidate region on chromosome 22 using linkage and loss of heterozygosity analyses.
3. To determine the molecular mechanism of tumor formation in these patients using complementary molecular and cytogenetic approaches.

Study Design: Schwannomatosis patients and affected relatives will be identified. Blood and tumor specimens will be obtained for linkage, LOH, FISH and mutational analysis of coding and non coding candidate regions. **Relevance:** This study will elucidate the unique pathogenesis of schwannomatosis and provide a means for definitive diagnosis using molecular technology.

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Introduction

Neurofibromatosis (NF) encompasses a diverse group of genetic conditions whose common element is tumors of the nerve sheath. Schwannomatosis is a recently recognized third major type of NF, which results in multiple schwannomas without vestibular tumors diagnostic of NF2. Recent epidemiological studies have shown that schwannomatosis is as common as NF2. Our preliminary studies of the *NF2* gene in tumors from schwannomatosis patients revealed a pattern of tumor suppressor gene inactivation not previously been reported in any other human disease. The objective of this project is to clone the locus responsible for familial schwannomatosis, *sch*. We are exploring two competing hypotheses which address both the non random distribution of LOH observed in schwannomatosis tumors and the high rate of somatic *NF2* mutation seen along the *cis* allele. The first hypothesis is that *sch* is a second tumor suppressor gene which lies near to *NF2* on chromosome 22. In this model, schwannoma formation is dependent on four "hits" (two in the *sch* tumor suppressor, and two in the linked *NF2* tumor suppressor). FISH results have suggested a second hypothesis in which a structural element facilitates loss of the *trans* chromosome by increasing the rate of mitotic recombination. This is an especially attractive hypothesis since rates of mitotic recombination are both highly variable and genetically determined in humans.

Body

This section is organized around the approved statement of work.

Task 1. To develop a resource of study subjects and related biological materials (months 1 through 30):

a. Identify schwannomatosis probands to include two potential familial clusterings and five sporadic patients in each 8 month period (months 1 thorough 24).

Excellent progress has been made regarding this task, with the identification of five kindreds over the period of this progress report (figure 1). Seven new sporadic patients were identified and enrolled.

b. Obtain specimens from confirmed probands and affected relatives (months 6 through 30).

A total of 26 blood specimens were collected from newly identified familial clusterings, newly identified sporadic cases and expansion of previously identified familial clusterings. 11 tumor specimens from clinically indicated surgery were obtained. Two patients died and families consented to autopsy collection of affected and unaffected tissues for study. Paraffin embedded tumor material was obtained from newly identified families QU, Penn 1, and Penn 3. In addition, archived material to reconstruct a deceased, unaffected member of family 9 was collected to refine linkage analysis.

Task 2. To refine the candidate region using LOH and linkage analysis (months 12 through 36):

Although not strictly applicable for the current reporting period, collection of samples from family 9 allowed us to significantly narrow our candidate region by excluding the markers D22S420 and D22S427 (figure 2). Since this area is immediately adjacent to the DiGeorge syndrome (DGS) deletion (Shaikh et al., 2000) and DGS patients do not develop schwannomas even with prolonged life span to the third and fourth decade (clinical features summarized at OMIM: http://www.ncbi.nlm.nih.gov), we are now able to concentrate on a candidate region of approximately 5 megabases.

Task 3. To determine the molecular mechanism leading to schwannomatosis (months 1 through 48):

a. Collection of 20 sporadic and 20 NF2 related non-vestibular tumors for FISH analysis (months 1 to 9).

The identification and retrieval of 20 NF2-associated and 20 sporadic non-vestibular schwannomas with banked frozen tissue and matching blood is underway. Twelve cases have been retrieved from the Siteman Cancer Center tumor bank at Washington University in St. Louis. The remainder of cases are being retrieved from the tumor bank at MGH/Harvard University in Boston.

b. Development and validation of FISH probes on the long arm of chromosome 22 and identification of potential markers and probes on 22p (months 1 to 12)

In preparation for FISH analysis of vestibular schwannomas from different patient populations, we have identified 10 BAC clones relatively evenly spaced along chromosome 22 for mapping of deletions on this chromosome in paraffin embedded tissue specimens (figure 3). The BAC clones have been cultured and the DNA has been isolated and labeled in all cases. Additionally, all 10 clones have been validated for chromosomal localization by FISH on normal human metaphase preparations. Lastly, they have been tested on non-neoplastic paraffin embedded brain tissue samples and all 10 yielded robust hybridization efficiencies with visible green (FITC-labeled) and red (rhodamine-labeled) signals.

c. Screening of candidate regions and/or loci based on results of task 2 (months 30 through 45).

Although not strictly applicable for the current reporting period, the unexpected progress in narrowing the candidate region noted under task 2 has allowed us to begin screening of a refined candidate region. A single transcript lies in the region between the excluded region by linkage and LOH in family 10 ("OE1," accession NT_011520). Exon scanning of the 5 coding exons of this transcript through 10 non founders did not reveal any alterations. We therefore suspected that additional small regions of LOH are present in the family 10 tumor studied, which we subsequently confirmed at marker D22S1164 (figure 2).

MAPK1 at position 20495 within the candidate region is an attractive target for a tumor suppressor gene, being an extracellular signal regulated kinase which acts as an integration point for multiple biochemical signals. However screening of the 8 coding exons through 11 non founders failed to revealed coding alterations. The guanine nucleotide binding protein *GNAZ* and oncogene homolog *CRKL* were also screened by exon scanning, unequal expression assays or both. *GNAZ* was found to have a previously unreported alteration at position within exon 1 which activates a cryptic splice site, but this change was seen in 4 of 11 non schwannomatosis controls and thus could not be considered pathogenic.

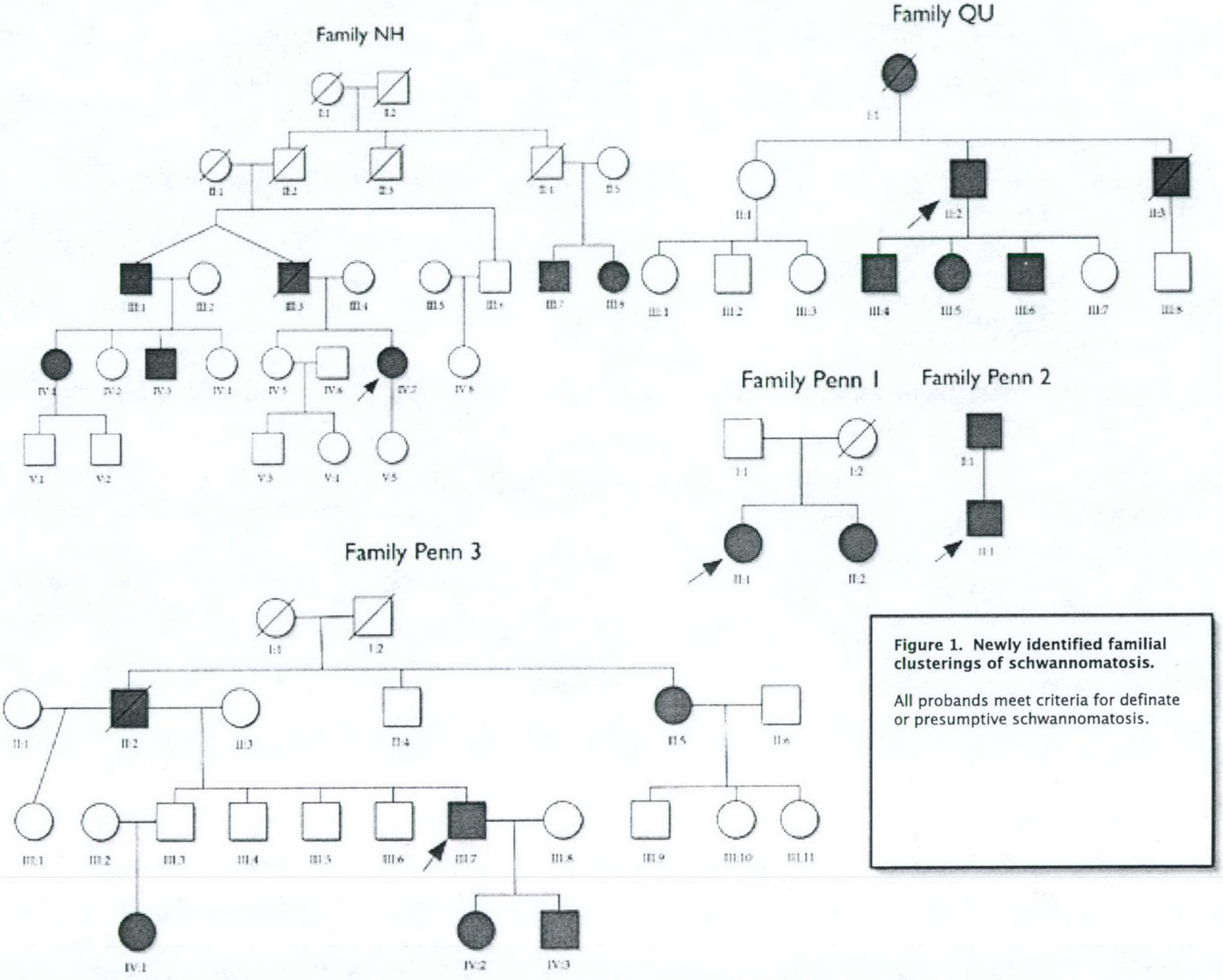


Figure 1. Newly identified familial clusterings of schwannomatosis.

All probands meet criteria for definate or presumptive schwannomatosis.

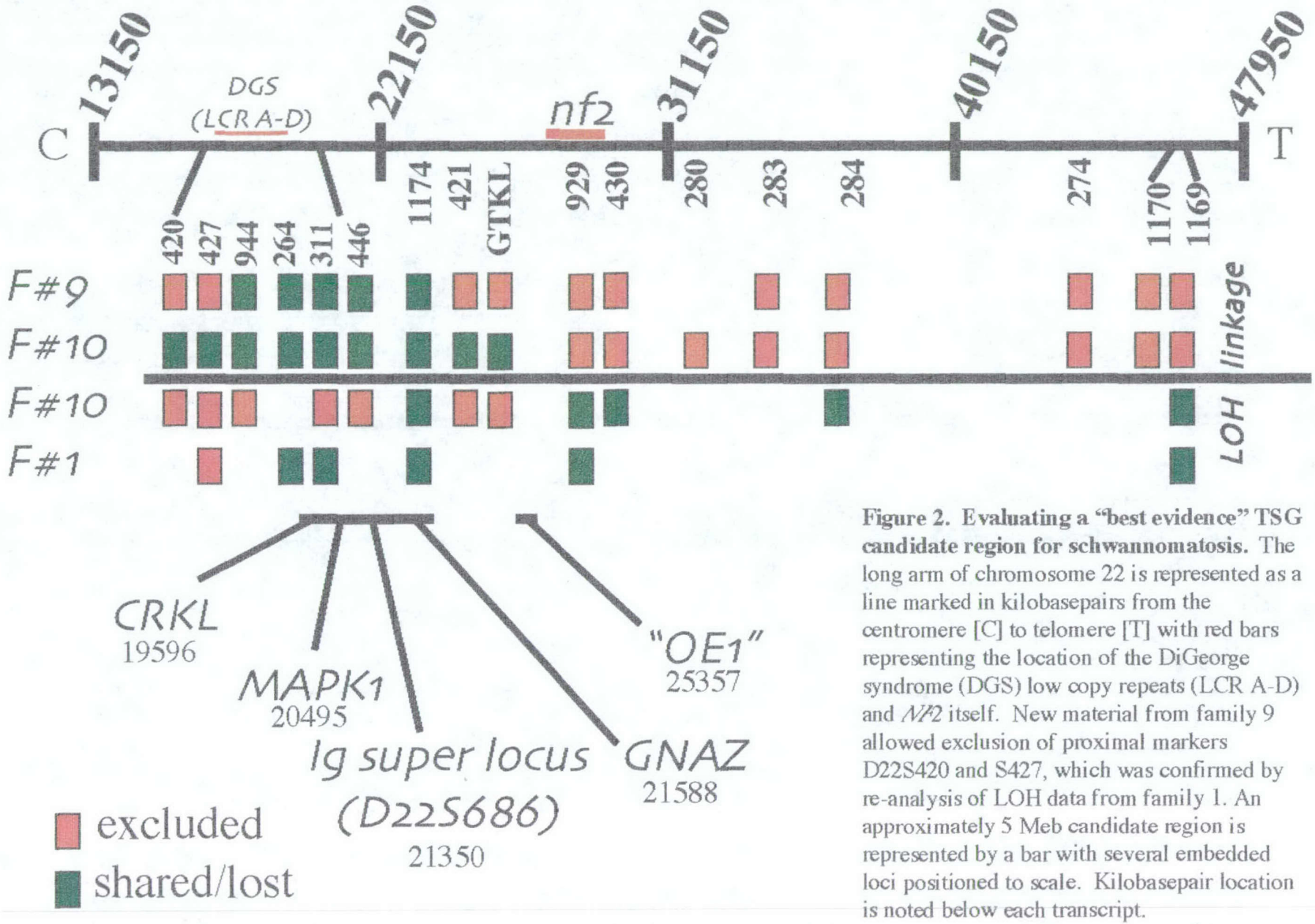
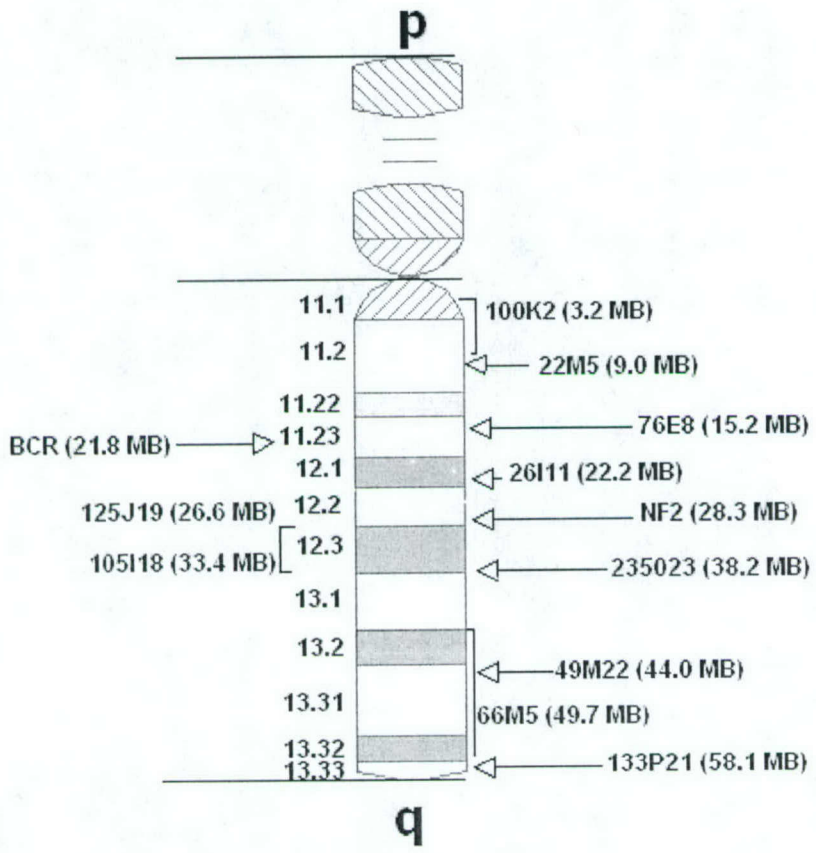


Figure 2. Evaluating a "best evidence" TSG candidate region for schwannomatosis. The long arm of chromosome 22 is represented as a line marked in kilobasepairs from the centromere [C] to telomere [T] with red bars representing the location of the DiGeorge syndrome (DGS) low copy repeats (LCR A-D) and *NF2* itself. New material from family 9 allowed exclusion of proximal markers D22S420 and S427, which was confirmed by re-analysis of LOH data from family 1. An approximately 5 Meb candidate region is represented by a bar with several embedded loci positioned to scale. Kilobasepair location is noted below each transcript.



Chromosome 22

Figure 3. Relative position of BAC clones validated for FISH on normal human metaphase preparations.

Key Research Accomplishments

- Identification of five new schwannomatosis kindreds with significant sample collection
- Refinement of schwannomatosis candidate tumor suppressor gene region to 5 megabases
- Exclusion of 4 candidate tumor suppressor genes
- Successful development of FISH probe panel for chromosome 22

Reportable Outcomes

Presentations:

In conjunction with the National Neurofibromatosis Foundation, a consensus conference on "Diagnosis and Management of Schwannomatosis" with a satellite meeting on cloning of the *sch* locus was held in Boston, Massachusetts on October 27, 2003. A follow-up to this meeting was held in Aspen, Colorado on May 24, 2004. Additional presentations by the PI were made to the Day lab at MGH in January, 2004, the Council of Fellows of the NNFF in March, 2004, and to the Genetics Division of the University of Pennsylvania Medical School in April, 2004.

Funding applied for:

The PI in conjunction with Dr. Hamid Salamipour of the department of radiology submitted a proposal to the NFRP entitled "Clinical and radiographic study of schwannomatosis," proposal log number NF043056 in May, 2004.

Research opportunities:

The PI sponsored Research Science Institute/Center for Excellence in Education student Jason Chu, "Developing a novel mechanism for rapid cloning of tumor suppressor genes via polymorphic analysis," June-August, 2003.

Conclusions

Schwannomatosis is a third major form of NF, which recent epidemiological studies have shown is as common as NF2. However, clinical recognition and molecular characterization have lagged far behind other forms of NF. The clarification of molecular alterations in schwannomatosis will likely have broad implications for other tumor suppressor gene syndromes. We have identified significant resources over the past year to aid in the search for the fundamental alteration in schwannomatosis.

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