Am. J. Hum. Genet. 62:834–841, 1998

A Clinical Variant of Neurofibromatosis Type 1: Familial Spinal Neurofibromatosis with a Frameshift Mutation in the *NF1* Gene

Elisabet Ars,[1] Helena Kruyer,[1] Antonia Gaona,[1] Pilar Casquero,[2] Jordi Rosell,[3] Víctor Volpini,[1] Eduard Serra,[1] Conxi Lázaro,[1] and Xavier Estivill[1]

[1]Medical and Molecular Genetics Center-IRO, Hospital Duran i Reynals, Barcelona; [2]Unitat de Neurologia, Hospital Verge del Toro, Menorca, Balearic Islands, Spain; and [3]Secció de Genètica, Hospital Son Dureta, Mallorca, Balearic Islands, Spain

Summary

Spinal neurofibromatosis (SNF) has been considered to be an alternative form of neurofibromatosis in which spinal cord tumors are the main clinical characteristic. Familial SNF has been reported, elsewhere, in three families—two linked to markers within the gene for neurofibromatosis type 1 (NF1) and the other not linked to *NF1*—but no molecular alterations have been described in these families. We describe a three-generation family that includes five members affected by SNF. All the affected members presented multiple spinal neurofibromas and café au lait spots, one member had cutaneous neurofibromas, and some members had other signs of NF1. Genetic analysis, performed with markers within and flanking the *NF1* gene, showed segregation with the *NF1* locus. Mutation analysis, performed with the protein-truncation test and SSCP/heteroduplex analysis of the whole coding region of the *NF1* gene, identified a frameshift mutation (8042insA) in exon 46, which should result in a truncated NF1 protein. The 8042insA mutation was detected in all five family members with the SNF/NF1 phenotype. To our knowledge, this is the first time that a mutation in the *NF1* gene has been associated with SNF. The clinical homogeneity in the severity of the disease among the affected members of the family, which is unusual in NF1, suggests that a particular property of the *NF1* mutation described here, a gene closely linked to *NF1*, or posttranscriptional events are involved in this severe neurological phenotype.

Received November 25, 1997; accepted for publication January 30, 1998; electronically published March 18, 1998.

Address for correspondence and reprints: Dr. Xavier Estivill, Medical and Molecular Genetics Center-IRO, Hospital Duran i Reynals, Avia. Castelldefels Km. 2.7, L'Hospitalet, 08907 Barcelona, Catalonia, Spain. E-mail: estivill@iro.es

0002-9297/98/6204-0015$02.00

Introduction

The neurofibromatoses are a group of neurocutaneous syndromes, characterized by growth abnormalities of the nervous system and of various organs, that show extreme clinical heterogeneity (reviewed in Riccardi 1992). Attempts to classify the neurofibromatoses have led to the consideration of two main clinical forms, type 1 (NF1) and type 2 (NF2), as well as several alternate and related forms (reviewed in Riccardi 1992 and in Viskochil and Carey 1994). Of these, only NF1 and NF2 are well-defined clinical entities for which genes have been identified (Cawthon et al. 1990; Viskochil et al. 1990; Wallace et al. 1990; Rouleau et al. 1993; Troffater et al. 1993).

The gene responsible for NF1 maps to chromosome band 17q11.2, spans >350 kb of genomic DNA, and encodes an mRNA, of 11–13 kb, that contains at least 60 exons (Danglot et al. 1995; Li et al. 1995). The gene product of *NF1*, neurofibromin, has a central domain with sequence similarity to the GTPase-activating protein family, which can down-regulate p21-ras (Ballester et al. 1990; Martin et al. 1990; Xu et al. 1990*a*, 1990*b*). Moreover, the identification of somatic mutations in the *NF1* gene, from several NF1- and non–NF1-related tumors (Li et al. 1992; Xu et al. 1992; Andersen et al. 1993; Legius et al. 1993; The et al. 1993; Shannon et al. 1994; Colman et al. 1995; Sawada et al. 1996; Serra et al. 1997), supports the notion that *NF1* is a tumor-suppressor gene. The *NF2* gene, which is also a tumor-suppressor gene, maps to chromosome 22q12 and encodes a 595–amino acid protein, designated "merlin/schwannomin," that has a sequence similar to that of a family of proteins that have been postulated to connect the cytoskeleton to the cell membrane (Luna and Hitt 1992; Rouleau et al. 1993; Troffater et al. 1993).

Spinal neurofibromatosis (SNF) has been considered to be an alternative form of neurofibromatosis. The presence of multiple spinal cord tumors, occurring in several members of the same family and segregating in an autosomal dominant fashion (familial SNF), has been reported, elsewhere, in three families. For two of these

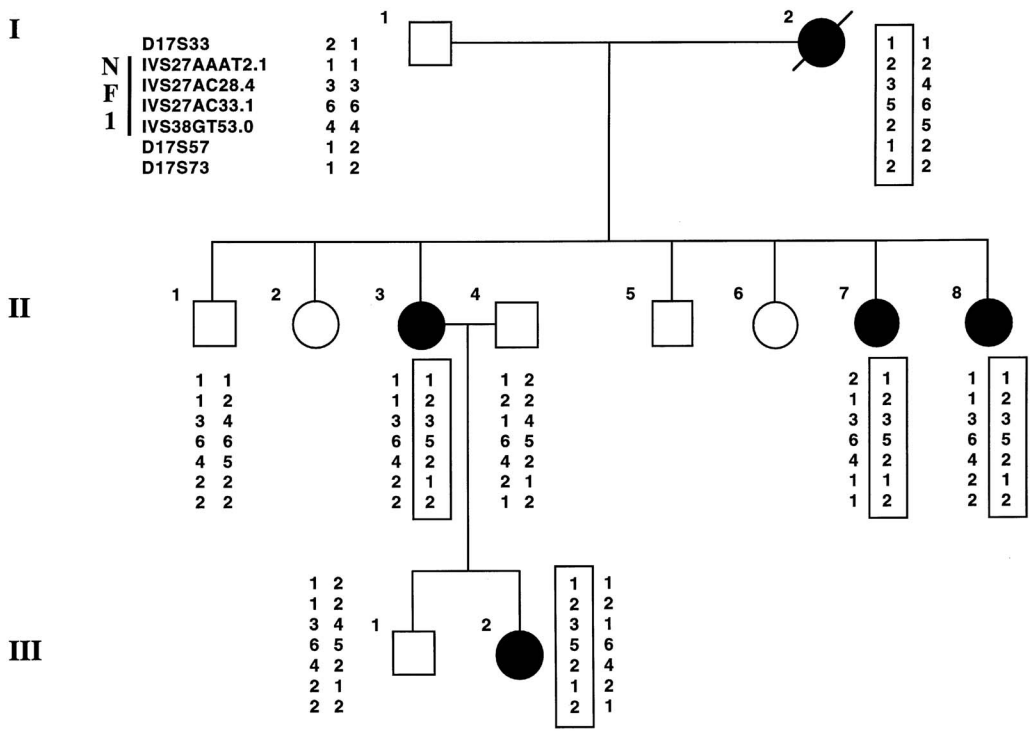

Figure 1 Pedigree of a three-generation family with SNF. Segregation of markers flanking and within the *NF1* gene are shown (*upper left*). Blackened symbols indicate individuals with the NF1/SNF phenotype. The haplotype associated with the disease is boxed.

families, linkage to markers within the *NF1* gene was established; for the remaining family, no linkage to *NF1* was found (Pulst et al. 1991; Poyhonen et al. 1997). No molecular alterations have been detected in these families.

We have analyzed a family that includes five members severely affected by SNF. Mutation analysis, by means of protein-truncation testing (PTT), of the *NF1* cDNA allowed the identification of a frameshift mutation that cosegregates with the disease. This provides further evidence that it is unlikely that SNF/NF1 is genetically distinct from NF1.

Subjects and Methods

Subjects

The family analyzed in this study had five members, in three generations, affected by SNF (fig. 1 and table 1). The five affected members were informed about the study, and consent was obtained from all of them. Patient I-2 was a 58-year-old woman who developed progressive paraparesis of her legs and right arm when she was 45 years old. She had multiple café au lait spots (CLS) but no cutaneous neurofibromas. Magnetic res-

Table 1

Clinical Features of Family Members Affected with SNF

	Patient				
Feature	I-2	II-3	II-7	II-8	III-2
Age (years)	58[a]	34	24	21	12
Spinal neurofibromas	C,D,L	C,D,L	C,D,L	D,L	C,D
Multiple CLS	Present	Present	Present	Present	Present
Cutaneous neurofibromas (*n*)	Absent	Present (3)	Absent	Absent	Absent
Lisch nodules	ND	ND	ND	Present	Present
Paraparesis (age [years])	Present (45)	Present (23)	Absent	Absent	Absent
Other	. . .	Mediastinal neurofibroma	Plexiform neurofibroma	. . .	. . .

NOTE.—C = cervical; D = dorsal; L = lumbar; CLS = café au lait spots; and ND = not determined.

[a] Deceased.

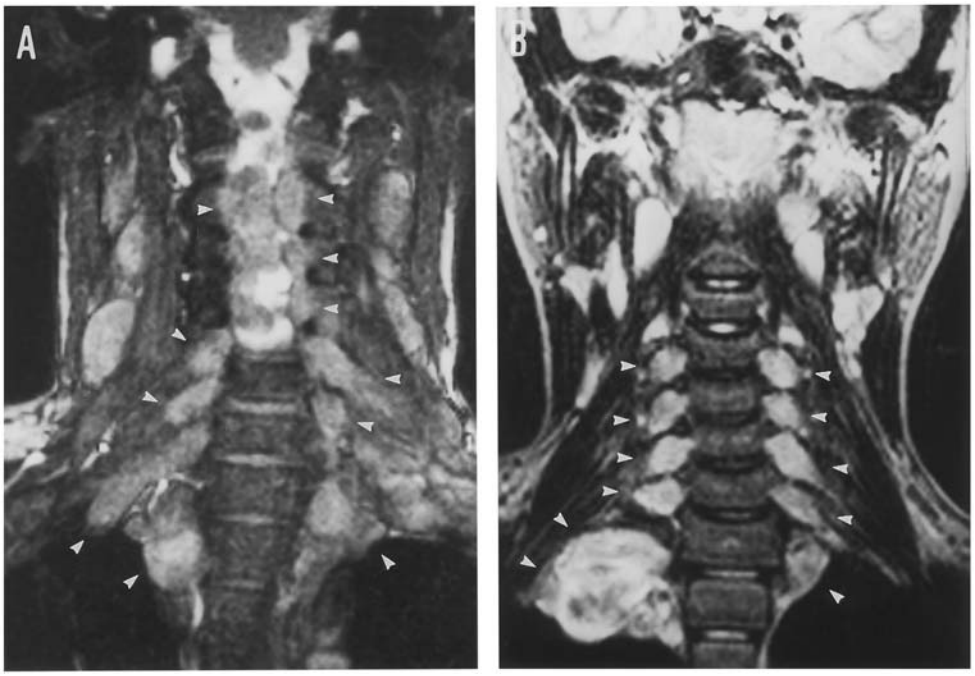

Figure 2 Coronal T2-weighted spinal MRI scan of patients II-3 and III-2. *A,* Patient II-3: multiple bilateral neurofibromas, at cervical and dorsal levels, with the intra- and extraspinal components of a dumbbell neurofibroma growing into both the brachial plexus and the apical region of the lung. *B,* Patient III-2: multiple bilateral, paravertebral tumors, from C2–C3 to D3–D4, and two paravertebral masses, in the right- and left-lung apical regions. The arrowheads point to some of the spinal neurofibromas.

onance imaging (MRI) of the spine detected multiple intradural extramedullary masses, in the cervical, dorsal, and lumbar segments, corresponding to neurofibromas. Some of these masses had both intradural and extradural components, the so-called dumbbell tumors, with a large extraspinal component that produced foraminal enlargement. A surgical intervention to remove intradural neurofibromas from C1–C4 was performed in 1989. The patient had a very torpid clinical evolution that culminated in cardiac insufficiency and subsequent death, from a myocardial infarction, in 1996. Patient II-3 is a 34-year-old woman who had a surgical operation, when she was 16 years old, to remove a mediastinal neurofibroma. She has multiple CLS and three cutaneous neurofibromas. When she was 23 years old, this patient began to have difficulty walking and to show signs of progressive spastic paraparesis. The spinal MRI examination showed multiple tumors at C1–C7 (figs. 2*A* and 3*A*). A laminectomy was performed to remove neurofibromas from levels C1–C5. Shortly after surgery, the patient developed a complete transverse medullar syndrome, at the C2–C3 level, that required mechanical ventilation. Patient II-7 is a 24-year-old woman with multiple CLS and antecedents of surgical resection of a plexiform neurofibroma on the right arm. The spinal MRI examination disclosed multiple intra- and extraspinal neurofibromas in the cervical region, at the D6–D7 level, and in the lumbosacral region. Patient II-8 is a 21-year-old woman with multiple CLS and Lisch nodules. The MRI scans demonstrated bilateral lung apical tumors and a left paravertebral mass, at the L5 level. Patient III-2 is a 12-year-old girl with multiple CLS and Lisch nodules. The spinal MRI showed multiple bilateral tumors, from C2–C3 to D3–D4, and two paravertebral masses in the right- and left-lung apical regions (figs. 2*B* and 3*B*). The physical examination of the other members of the family revealed none of the major clinical features of NF1 (cutaneous neurofibromas, Lisch nodules, or CLS).

RNA/DNA Extraction

Total RNA was extracted, from peripheral blood lymphocytes, by use of the Tripure isolation reagent (Boehringer Mannheim), in accordance with the manufacturer's instructions. DNA was extracted by means of the "salting out" method (Miller et al. 1988).

Reverse Transcription and PTT Analysis

Two to five micrograms of RNA were reverse transcribed with 500 ng random hexamers and 200 U Superscript II reverse transcriptase (RT [Gibco-BRL]) in a

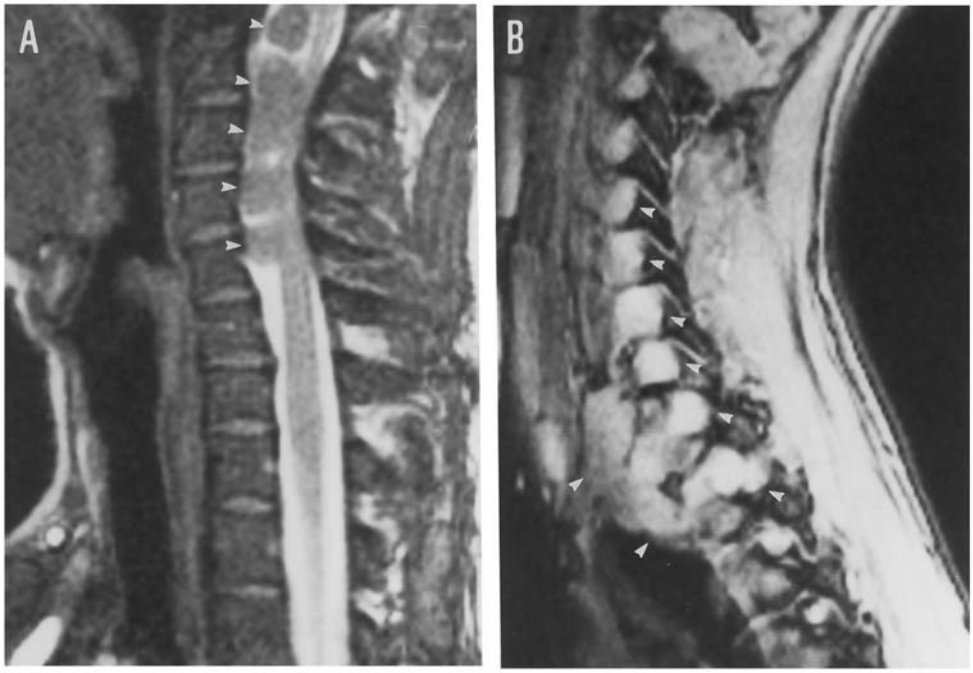

Figure 3 Sagittal T2-weighted spinal MRI scan of patients II-3 and III-2. *A,* Patient II-3: intradural and extramedullary neurofibromas (*arrowheads*), with compression of the cervical cord and enlargement of the cervical spinal canal. *B,* Patient III-3: multiple cervical and dorsal neurofibromas (*arrowheads*), with extension outward from the spinal canal through the intervertebral foramen.

20-μl reaction volume, under conditions recommended by the manufacturer. The entire *NF1* cDNA was then amplified (40 cycles of 1 min at 94°C, 1 min at 54°C, and 1 min at 72°C), with five pairs of overlapping primers that incorporate a T7 promoter and translation initiation site, as described elsewhere (Heim et al. 1995). After in vitro transcription/translation incorporating ^{35}S-methionine, the samples were electrophoresed on a 12% SDS-polyacrylamide gel. The gel was then dried and subjected to autoradiography for 6–48 h. When an abnormal fragment was detected, primers flanking the DNA sequence with the putative mutation were designed, and the RT-PCR product was sequenced by use of an automatic genetic analyzer (ABI PRISM 377 DNA Sequencer).

SSCP/Heteroduplex Analysis

The whole coding region of the *NF1* gene, amplified in 10 overlapping fragments (Hoffmeyer et al. 1994), was also analyzed by SSCP/heteroduplex analysis, as described elsewhere (Sala and Espinosa-Parrilla, in press).

Segregation Analysis

Haplotype analysis for seven *NF1* markers (four intragenic and three extragenic) was performed as described elsewhere (Lázaro et al. 1993). Haplotype analysis for four *NF2* markers (one intragenic CA-repeat and three extragenic markers: *D22S268, D22S273,* and *D22S280*) was performed as described elsewhere (Marineau et al. 1993; Bourn and Strachan 1995). Restriction-enzyme analysis of cDNA, for detection of the 8042insA mutation, was performed as described elsewhere (Sambrook et al. 1989).

Results

Five family members affected with NF1 shared a very severe neurological phenotype that included spinal neurofibromas (fig. 1 and table 1). Two of these five family members had paraparesis; the other three are still young (aged 12–24 years). All five members fulfilled the National Institutes of Health (NIH) diagnostic criteria for NF1 (Stumpf et al. 1988). The SNF/NF1 phenotype segregated with a seven-marker haplotype (four *NF1* intragenic and three extragenic markers) (fig. 1). *NF2* markers (one *NF2* intragenic and three extragenic) did not segregate with the disease (data not shown).

PTT analysis of the fifth segment of the *NF1* cDNA (as indicated by Heim et al. [1995] for PTT analysis) detected a 55.4-kD polypeptide, in addition to the normal 68.5-kD product, for the 3′ end of the *NF1* cDNA (exons 35–49) (fig. 4*A*), which suggests a truncating mutation between nucleotides 8000 and 8100 of the *NF1* coding sequence. Sequencing of the RT-PCR product re-

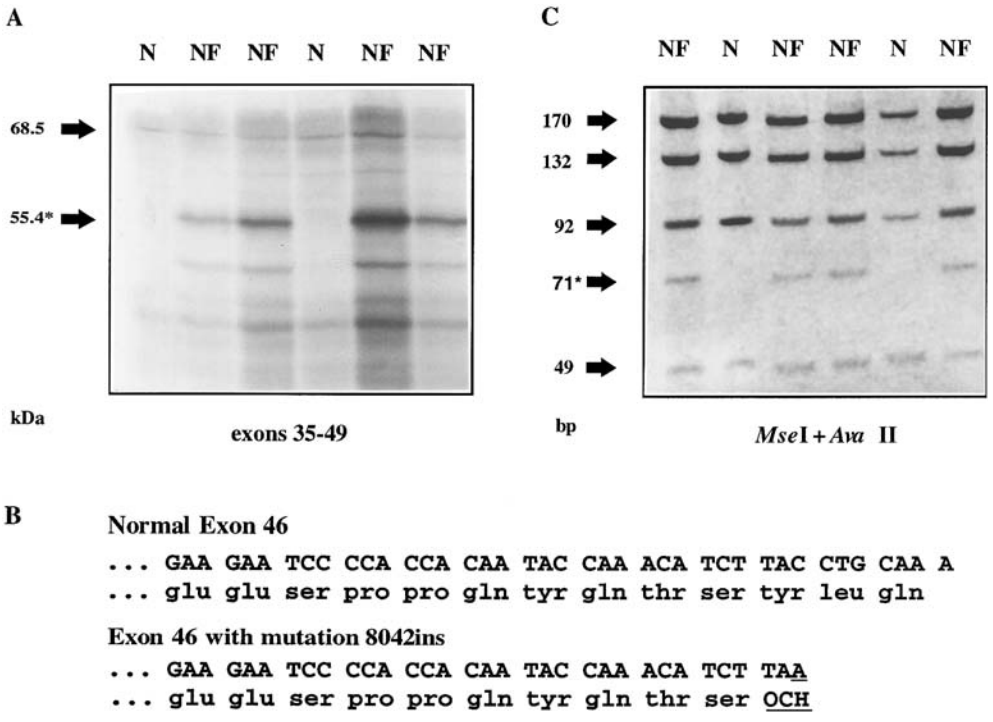

Figure 4 Identification of mutation 8042insA in the SNF family. *A,* PTT analysis, showing a truncated polypeptide, of 55.4 kD(*) , in addition to the normal peptide, of 68.5 kD. The truncated NF1 polypeptide was detected only in the affected patients. *B,* Partial nucleotide and amino acid sequences of exon 46, showing mutation 8042insA, which creates a TAA stop codon at the mutation site. *C,* Detection of mutation 8042insA, which generates an *Mse*I restriction site, by restriction-enzyme analysis. A 488-bp cDNA fragment was amplified, by use of primers flanking the mutation, and was then digested with the restriction enzymes *Mse*I and *Ava*II. This resulted in six fragments, of 170, 132, 92, 49, 23, and 22 bp, in the unaffected members, and in two additional fragments, of 71 bp (asterisk [*]) and 21 bp (not shown), in the affected individuals.

vealed the insertion of an adenine at position 8042, at codon 2681, in exon 46 of the *NF1* gene (fig. 4*B*). Mutation 8042insA creates a TAA stop codon at the mutation site, which leads to a truncated NF1 protein. PTT analysis showed that all affected members of the family produced the truncated polypeptide caused by mutation 8042insA. The mutation generates an *Mse*I restriction site, which allows confirmation of the molecular alteration, at the cDNA level, by digestion (fig. 4*C*). The presence of the mutation in genomic DNA was also confirmed by amplification and digestion with the same enzyme (data not shown).

The PTT analysis of the other regions of the *NF1* gene did not reveal additional alterations. To uncover other potential nucleotide changes that could have escaped the PTT analysis (missense mutations and in-frame insertions and deletions), we studied the complete coding region of the *NF1* gene of the SNF family, by SSCP/heteroduplex analysis of 10 overlapping fragments of the *NF1* cDNA, and we found only the mutation 8042insA.

Discussion

Because of the extreme clinical heterogeneity of the neurofibromatoses, there have been many attempts to classify these disorders into distinct categories (reviewed in Riccardi [1992] and in Viskochil and Carey [1994]). As yet, only NF1 and NF2 have been confirmed as genetically distinct diseases, as a result of the cloning of the respective genes. There are several related or alternate forms of NF1, such as Watson syndrome (Tassabehji et al. 1993; Ahlbom et al. 1995), Noonan syndrome (Tassabehji et al. 1993; Bahuau et al. 1996), autosomal dominant multiple CLS (Brunner et al. 1993; Abeliovich et al. 1995), and LEOPARD syndrome (Wu et al. 1996); however, there exists some controversy about their NF1 molecular basis.

SNF has also been described as an entity that is distinct from NF1 and in which the main clinical characteristic is symptomatic spinal cord tumors, which are present in only 5% of NF1 cases (Huson et al. 1988; Von Deimling et al. 1995) and are actually more common in NF2.

Recently, it has been suggested that spinal neurofibromas associated with NF1 occur more often than initially thought, since they were found, by spinal MRI, in 36% of the NF1 patients studied by Poyhonen et al. (1997) but were associated with clinical symptoms in only 7% of the patients. The infrequent detection, by other investigators, of spinal neurofibromas in NF1 patients could be explained by the lack of routine spinal MRI studies. Although asymptomatic spinal neurofibromas are probably more common than previously thought, the occurrence of multiple spinal neurofibromas in all the affected members of the same family is exceptional.

To date, only three other SNF families have been studied at the genetic level. Pulst et al. (1991) described two families, one of which satisfied NIH NF1 criteria and showed linkage to the *NF1* locus. In the second family, all the affected members had only spinal cord tumors; this family was excluded by linkage from 17q11.2 and is probably linked to *NF2* (Pulst et al. 1991, 1997). Recently, Poyhonen et al. (1997) described a family with multiple CLS, spinal neurofibromas, and some other signs of NF1 (dermal neurofibromas and axillary and inguinal freckles). Linkage to the *NF1* locus was established, and linkage to the *NF2* locus was excluded. No mutations in either *NF1* or *NF2* have been described in any of these families. All the affected members of the three-generation family we describe here have spinal neurofibromas, fulfill NIH NF1 criteria, and have the frameshift mutation 8042insA in the *NF1* gene. This clearly links the *NF1* gene with familial SNF.

A hallmark of NF1 is the high phenotypic variability between affected individuals, even among members of the same family. The only phenotype-genotype correlation described so far in NF1 concerns the mental retardation and/or learning disabilities, mild facial dysmorphology, and large number of early-onset cutaneous neurofibromas associated with large deletions encompassing the whole *NF1* gene (Kayes et al. 1994; Wu et al. 1995; Leppig et al. 1996; Upadhyaya et al. 1996; Wu et al. 1997). One of the striking characteristics of the family reported here and of the SNF families reported elsewhere (Pulst et al. 1991; Poyhonen et al. 1997) is the absence or low number of cutaneous neurofibromas. This contrasts with the high number of early-onset cutaneous neurofibromas seen in patients with deletions of the whole *NF1* gene. The differences between these two phenotypes could be due to the presence, in the patients with SNF/NF1, of a partially functional, truncated NF1 protein that is associated with different consequences at the neurological and dermatological levels.

The NF1 family reported here is unusual because all the affected members present spinal neurofibromas. There are various possible explanations for the severe neurological phenotype in all the affected members of this family. First, it is possible that the predicted 2,680–amino acid truncated neurofibromin of these patients has a negative residual function with a special effect in the development of the neural crest cells that are responsible for the SNF/NF1 phenotype. However, we have found a frameshift mutation, in exon 48, that also gives rise to a stop codon in an NF1 family with a classical NF1 phenotype and without symptomatic spinal neurofibromas (E. Ars, E. Serra, A. Gaona, J. Garcia, H. Kruyer, C. Lázaro, X. Estivill, unpublished data). Second, SNF could be due to a second mutation, either in the normal *NF1* allele or in the same allele that contains mutation 8042insA. However, after an intensive analysis of the whole *NF1* coding region, we were unable to detect a second mutation. Furthermore, the second *NF1* allele of four of the patients reported here has been inherited independently, which excludes the presence of a common variant involved in the SNF phenotype. Third, it could be that a mutation in another gene, closely linked to *NF1,* is the cause of the SNF in this family. In support of this, one member of one of the SNF families described by Pulst et al. (1991) only had multiple CLS, had no spinal neurofibromas, but had inherited the *NF1* haplotype, which suggests that this case could be recombinant for a linked gene involved in the development of SNF. These families and others with concordant or discordant clinical data for SNF and NF1 might be useful in the detection of modifier genes for this SNF/NF1 phenotype. Finally, posttranscriptional events, such as alternative splicing or editing, that modulate the expression of the *NF1* gene (Skuse and Cappione 1997) could contribute to the development of SNF.

Until now, the molecular genetics of families with spinal cord tumors and with clinical features of neurofibromatosis was unsolved. The identification of the mutation 8042insA in the *NF1* gene in the family reported here strongly suggests that other SNF families that satisfy NIH NF1 diagnostic criteria might also have mutations in the *NF1* gene. The genetic characterization of these families should help us to understand the molecular alterations that cause this severe neurological phenotype.

Acknowledgments

The authors wish to thank all the patients and family members who participated in this study. E.A. is a fellow of the Comissió Interdepartamental de Recerca i Innovació Tecnològica (CIRIT) of the Generalitat de Catalunya. This work was supported by Fondo de Investigaciones Sanitarias de la Seguridad Social grants 92-0532, 95-368, and 98-992, by European Union grant BIOMED BMH-4-CT96-1364, and by the Institut Català de la Salut and the Fundació Agust Pi i Sunyer/Marató de TV3. We are indebted to Dr. Michael Lynch, for

advice and help with the manuscript, and to Drs. Narváez and Taboada, for help with the clinical data.

References

Abeliovich D, Gelman-Kohan Z, Silverstein S, Lerer I, Chemke J, Merin S, Zlotogora J (1995) Familial café au lait spots: a variant of neurofibromatosis type 1. J Med Genet 32: 985–986

Ahlbom BE, Dahl N, Zetterqvist P, Anneren G (1995) Noonan syndrome with cafe-au-lait spots and multiple lentigines syndrome are not linked to the neurofibromatosis type 1 locus. Clin Genet 48:85–89

Andersen LB, Fountain JW, Gutmann DH, Tarlé SA, Glover TW, Dracopoli NC, Housman D, et al (1993) Mutations in the neurofibromatosis 1 gene in sporadic malignant melanoma cell lines. Nat Genet 3: 118–121

Bahuau M, Flintoff W, Assouline B, Lyonnet S, Le Merrer M, Prieur M, Guilloud-Bataille M, et al (1996) Exclusion of allelism of Noonan syndrome and neurofibromatosis-type 1 in a large family with Noonan syndrome–neurofibromatosis association. Am J Med Genet 66:347–355

Ballester R, Marchuk D, Boguski M, Saulino A, Letcher R, Wigler M, Collins FS (1990) The NF1 locus encodes a protein functionally related to mammalian GAP and yeast IRA proteins. Cell 63:851–859

Bourn D, Strachan T (1995) Highly polymorphic dinucleotide repeat at the NF2 gene. Hum Genet 95:712

Brunner HG, Hulsebos T, Steijlen PM, der Kinderen DJ, Steen AVD, Hamel BC (1993) Exclusion of the neurofibromatosis 1 locus in a family with inherited café-au-lait spots. Am J Med Genet 46:472–474

Cawthon RM, Weiss R, Xu G, Viskochil D, Culver M, Stevens J, Robertson M, et al (1990) A major segment of the neurofibromatosis type 1 gene: cDNA sequence, genomic structure, and point mutations. Cell 62:193–201

Colman SD, Williams CA, Wallace MR (1995) Benign neurofibromas in type 1 neurofibromatosis (NF1) show somatic deletions of NF1 gene. Nat Genet 11:90–92

Danglot G, Regnier V, Fauvet D, Vassal G, Kujas M, Bernheim A (1995) Neurofibromatosis 1 (NF1) mRNAs expressed in the central nervous system are differentially spliced in the 5′ part of the gene. Hum Mol Genet 4:915–920

Heim RA, Kam-Morgan LNW, Binnie CG, Corns DD, Cayouette MC, Farber RA, Aylsworth AS, et al (1995) Distribution of 13 truncating mutations in the neurofibromatosis 1 gene. Hum Mol Genet 4:975–981

Hoffmeyer S, Assum G, Kaufmann D, Schwenk K, Krone W (1994) A deletion in the 5′-region of the neurofibromatosis type 1 (NF1) gene. Hum Genet 94:97–100

Huson SM, Harper PS, Compston DAS (1988) Von Recklinghausen neurofibromatosis: a clinical and population study in southeast Wales. Brain 111:1355–1381

Kayes LM, Burke W, Riccardi VM, Bennett R, Ehrlich P, Rubenstein A, Stephens K (1994) Deletions spanning the neurofibromatosis 1 gene: identification and phenotype of five patients. Am J Hum Genet 54:424–436

Lázaro C, Gaona A, Ravella A, Volpini V, Casals T, Fuentes JJ, Estivill X (1993) Novel alleles, hemizygosity and deletion at an Alu-repeat within the neurofibromatosis type 1 (NF1) gene. Hum Mol Genet 2:725–730

Legius E, Marchuk DA, Collins FS, Glover TW (1993) Somatic deletion of the neurofibromatosis type 1 gene in a neurofibrosarcoma supports a tumor suppressor gene hypothesis. Nat Genet 3:122–126

Leppig KA, Viskochil D, Neil S, Rubenstein A, Johnson VP, Zhu XL, Brothman AR, et al (1996) The detection of contiguous gene deletions at the neurofibromatosis 1 locus with fluorescence in situ hybridization. Cytogenet Cell Genet 72: 95–98

Li Y, Bollag G, Clark R, Stevens J, Conroy L, Fults D (1992) Somatic mutations in the neurofibromatosis 1 gene in human tumors. Cell 69:275–281

Li Y, O'Connell P, Huntsman-Breidenbach H, Cawthon R, Stevens J, Su G, Neil S, et al (1995) Genomic organization of the neurofibromatosis 1 gene. Genomics 25:9–18

Luna EJ, Hitt AL (1992) Cytoskeleton-plasma membrane interactions. Science 258:955–964

Marineau C, Baron C, Delattre O, Zucman J, Thomas G, Rouleau GA (1993) Dinucleotide repeat polymorphism at the D22S268 locus. Hum Mol Genet 2:336

Martin GA, Viskochil D, Bollag G, McCabe PC, Crosier WJ, Haubruck H, Conroy L, et al (1990) The GAP-related domain of the neurofibromatosis type 1 gene product interacts with ras p21. Cell 63:843–849

Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215

Poyhonen M, Leisti EL, Kytölä S, Leisti J (1997) Hereditary spinal neurofibromatosis: a rare form of NF1? J Med Genet 34:184–187

Pulst SM, Riccardi VM, Fain P, Korenberg JR (1991) Familial spinal neurofibromatosis: clinical and DNA linkage analysis. Neurology 41:1923–1925

Pulst SM, Riccardi V, Mautner V (1997) Spinal schwannomatosis. Neurology 48:787–788

Riccardi VM (1992) Neurofibromatosis: phenotype, natural history and pathogenesis, 2d ed. Johns Hopkins University Press, Baltimore, pp 8–17

Rouleau GA, Merel P, Lutchman M, Sanson M, Zucman J, Marineau C, Hoang-Xuan K, et al (1993) Alteration in a new gene encoding a putative membrane-organizing protein causes neurofibromatosis type 2. Nature 363:515–521

Sala N, Espinosa-Parrilla Y. Direct analysis of the protein S gene in protein S deficiency. In: Passi J, Perry D (eds) Molecular methods in hemostasis. The Humana Press, Totowa, NJ (in press)

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2d ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, sec 5.3, 5.32

Sawada S, Florell S, Purandare SM, Ota M, Stephens K, Viskochil D (1996) Identification of NF1 mutations in both alleles of a dermal neurofibroma. Nat Genet 14:110–112

Serra E, Puig S, Otero D, Gaona A, Kruyer H, Ars E, Estivill X, et al (1997) Confirmation of a double-hit model for the *NF1* gene in benign neurofibromas. Am J Hum Genet 61: 512–519

Shannon KM, O'Connell P, Martin GA, Paderanga D, Olson K, Dinndorf P, McCormick F (1994) Loss of normal NF1

allele from the bone marrow of children with type 1 neurofibromatosis and malignant myeloid disorders. N Engl J Med 330:597–601

Skuse GR, Cappione AJ (1997) RNA processing and clinical variability in neurofibromatosis type 1 (NF1). Hum Mol Genet 6:1707–1712

Stumpf D, Alksne J, Annegers J, Brown S, Conneally P, Housman D, Leppert M, et al (1988) Neurofibromatosis conference statement. Arch Neurol 45:575–578

Tassabehji M, Strachan T, Sharland M, Colley A, Donnai D, Harris R, Thakker N (1993) Tandem duplication within a neurofibromatosis type 1 (NF1) gene exon in a family with features of Watson syndrome and Noonan syndrome. Am J Hum Genet 53:90–95

The I, Murthi AE, Hannigan GE, Jacoby LB, Menon AG, Gusella JF, Bernards A (1993) Neurofibromatosis type 1 gene mutations in neuroblastoma. Nat Genet 3:62–66

Trofatter JA, MacCollin MM, Rutter JL, Murrell JR, Duyao MP, Parry DM, Eldridge R, et al (1993) A novel moesin-like, ezrin-like, radixin-like gene is a candidate for neurofibromatosis-2 tumor suppressor. Cell 72:791–800

Upadhyaya M, Roberts SH, Maynard J, Sorour E, Thompson PW, Vaughan M, Wilkie AOM, et al (1996) A cytogenetic deletion, del(17) (q11.22q21.1), in a patient with sporadic neurofibromatosis type 1 (NF1) associated with dysmorphism and developmental delay. J Med Genet 33:148–152

Viskochil D, Buchberg AM, Xu G, Stevens J, Wolff RK, Culver M, Carey J, et al (1990) Deletions and a translocation interrupt a cloned gene at the neurofibromatosis type 1 locus. Cell 62:187–192

Viskochil D, Carey JC (1994) Alternate and related forms of the neurofibromatoses. In: Huson SM, Hughes RAC (eds) The neurofibromatoses: a pathogenetic and clinical overview. Chapman & Hall, London, pp 445–474

Von Deimling A, Krone W, Menon AG (1995) Neurofibromatosis type 1: pathology, clinical features and molecular genetics. Brain Pathol 5:153–162

Wallace MR, Marchuk A, Andersen LB, Letcher R, Oden HM, Saulino AM, Fountain GW, et al (1990) Type 1 neurofibromatosis gene: identification of a large transcript disrupted in three NF1 patients. Science 249:181–186

Wu BL, Austin MA, Schneider GH, Boles RG, Korf BR (1995) Deletion of the entire NF1 gene detected by FISH: four deletion patients associated with severe manifestations. Am J Med Genet 59:528–535

Wu BL, Schneider GH, Korf BR (1997) Deletion of the entire NF1 gene causing distinct manifestations in a family. Am J Med Genet 69:98–101

Wu R, Legius E, Robberecht W, Dumoulin M, Cassiman JJ, Fryns JP (1996) Neurofibromatosis type 1 gene mutation in a patient with features of LEOPARD syndrome. Hum Mutat 8:51–56

Xu G, Lin B, Tanaka K, Dunn D, Wood D, Gesteland R, White R, et al (1990*a*) The catalytic domain of the neurofibromatosis type 1 gene product stimulates ras GTPase and complements *ira* mutants of *S. cerevisiae*. Cell 63:835–841

Xu G, O'Connell P, Viskochil D, Cawthon R, Robertson M, Culver M, Dunn D, et al (1990*b*) The neurofibromatosis type 1 gene encodes a protein related to GAP. Cell 62: 599–608

Xu W, Mulligan LM, Ponder MA, Liu L, Smith BA, Mathew CGP, Ponder BAJ (1992) Loss of NF1 alleles in phaeochromocytomas from patients with type 1 neurofibromatosis. Genes Chromosom Cancer 4:337–342