

## The parental origin of new mutations in neurofibromatosis 2

Lan Kluwe · Victor Mautner · Dilys M. Parry · Lee B. Jacoby · Michael Baser · James Gusella  
Kevin Davis · Dimitros Stavrou · Mia MacCollin

Received: December 21, 1999 / Accepted: February 28, 2000 / Published online: May 9, 2000  
© Springer-Verlag 2000

### ABSTRACT

Neurofibromatosis 2 (NF2) is an autosomal dominant disorder characterized by schwannomas and meningiomas that develop after inactivation of both copies of the *NF2* gene. Approximately half of all patients with NF2 have unaffected parents and the disease results from new mutations at the *NF2* locus. Loss of heterozygosity (LOH) in tumor specimens due to deletions covering the normal *NF2* allele can be used to infer the haplotypes surrounding underlying mutations and determine the allelic origin of new mutations. We studied 71 sporadic NF2 patients using both LOH and pedigree analysis and compared the parental origin of the new mutation with the underlying molecular change. In the

45 informative individuals, 31 mutations (69%) were of paternal and 14 (31%) were of maternal origin ( $P=0.016$ ). Comparison with corresponding constitutional mutations revealed no correlation between parental origin and the type or location of the mutations. However, in 4 of 6 patients with somatic mosaicism the *NF2* mutation was of maternal origin. A slight parent of origin effect on severity of disease was found. Further clinical and molecular studies are needed to determine the basis of these unexpected observations.

**Key words** Parental origin · Neurofibromatosis 2 · Allele loss · Mosaicism

### INTRODUCTION

Neurofibromatosis 2 (NF2) is an autosomal dominant disorder characterized by bilateral vestibular schwannomas (BVS) and intracranial meningiomas, as well as a variety of spinal tumors [1–3]. NF2 is caused by mutations in the *NF2* gene located on the long arm of chromosome 22 [4, 5] and has an incidence at birth of 1:40,000. Half of all NF2 patients have no family history of the disease [2, 6] and the disease is due to new mutations. Somatic mosaicism of the *NF2* gene, resulting in typical and atypical phenotypes, has been widely reported [7–10].

The *NF2* gene product functions as a tumor suppressor, with inactivation of both copies of the gene leading to development of disease-associated tumors. Since the cloning of this gene, a large number of mutations has been found in over 50% of NF2 patients studied by exon scanning of genomic DNA [11–16]. Nonsense and frameshift mutations, which are expected to lead to truncated *NF2* gene products, account for approximately 65% of all constitutional mutations. Changes in the conserved splicing sites have been found in approximately 25% of all cases [17]. Missense mutations and in-frame deletions and insertions have been found only in about 10% of cases.

L. Kluwe (✉)

Laboratory for Brain Tumor Biology, Department of Neurosurgery, University Hospital Eppendorf, Martinistrasse 52, 20246 Hamburg, Germany  
e-mail: kluwe@uke.uni-hamburg.de  
Tel.: +49-40-428032767  
Fax: +49-40-428035982

V. Mautner

Department of Neurology, General Hospital Ochsensoll, Hamburg, Germany

D.M. Parry

Genetic Epidemiology Branch, National Cancer Institute Bethesda, Maryland, USA

L.B. Jacoby · J. Gusella

Molecular Neurogenetics Unit, Massachusetts General Hospital, Charlestown, Massachusetts, USA

M. Baser

11746 Bellagio Road, no. 308, Los Angeles, California, USA

K. Davis, M. MacCollin

Department of Neurology, Massachusetts General Hospital, Boston, Massachusetts, USA

D. Stavrou

Department of Neuropathology, University Hospital Eppendorf, Hamburg, Germany

A similar spectrum of mutations has been found in NF2-related tumors, primarily schwannomas [18, 19] and meningiomas [20, 21]. Allelic loss of the *NF2* locus has been found in up to 60% of these tumors, as evidenced by loss of heterozygosity (LOH) of flanking and/or intragenic markers. These observations are consistent with the first constitutional genetic event in NF2-associated tumors often being small alterations, such as point mutations or short insertions or deletions, while the second somatic event is often the loss of large genomic fragments that cover the entire *NF2* region.

Although the parental origins of new mutations have been determined for a number of genetic diseases using family studies, this approach is usually limited by the need for three-generation pedigrees. In tumor suppressor gene syndromes, use of LOH analysis simplifies the study of the origin of new mutations by requiring only two generations. Because the "two-hit" model predicts that the allele lost in tumor formation is the normal allele, the allele retained may then be inferred to be that carrying the constitutional mutation. Only one previous study has addressed the question of parental origin of new mutations in NF2 patients [22]. Utilizing restriction fragment length polymorphism (RFLP) markers in NF2-related tumors, the authors found paternal origin of new mutations in six of eight sporadic NF2 patients. Due to the small number of patients studied, this observation did not reach statistical significance. In the present study, using highly polymorphic microsatellite markers flanking or within the *NF2* gene, we studied 71 sporadically affected patients with NF2 and related phenotypes, including 9 patients with somatic mosaicism and two kindreds with germline mosaicism. In 45 patients in whom the parental origin could be determined, we further examined the relationship between mutation type and mosaicism, respectively, and the parental origin of these mutations.

## SUBJECTS AND METHODS

### Patients and samples

Sixty-five sporadically affected patients (37 males and 28 females) were identified in whom one or more NF2-related tumors were available for study. Of these 65 patients, 59 had BVS with or without other NF2-related findings, meeting the NIH clinical criteria for NF2 [23]. One patient had an affected sibling due to germline mosaicism in a parent [14]. Four were clinically suspected mosaics with unilateral vestibular tumors and multiple other NF2-related tumors meeting the National Neurofibromatosis Foundation's category of "presumed NF2" [24]. Two patients had schwannomatosis previously demonstrated to be due to somatic mosaicism for *NF2* mutation [8]. There were 110 tumor samples available from these 65 patients.

An additional 6 patients (4 males and 2 females) were identified as second-generation members of three-generation pedigrees, in which the first generation was living and unaffected and one or more members of the third generation were affected. All 6 patients had BVS and all affected third-generation members met NIH criteria for NF2 or were shown to carry the causative mutation. Of the 6 patients, 1 had an affected sibling with an identical mutation, suggesting germline mosaicism in a parent.

Clinical characteristics of the patients, including age of onset of symptoms, severity of disease, and pathological classification of the tumors, were determined by review of medical records.

Genomic DNA was extracted from peripheral blood leukocytes or from Epstein-Barr virus-transformed lymphoblastoid cell lines, as previously reported [8, 13]. Tumor tissue not needed by pathology departments for diagnosis was retrieved from clinically indicated procedures and frozen in liquid nitrogen or dry ice. Genomic DNA was extracted from pulverized frozen tissue as previously reported [8, 10]. When prospectively collected frozen tissue was not available, paraffin-embedded blocks of tumor were obtained from pathology department archives and DNA was extracted as previously reported [8, 10]. This study was approved by the Institutional Review Boards of the participating institutions and informed consent was obtained from all patients and participating family members.

### Microsatellite marker analysis

The six microsatellite markers used in this study are listed in Table 1. *CRYB2*, the most-proximal marker, is approximately 4.3 Mb centromeric to *NF2* [25]. D22S430, the most-distal marker, is approximately 430 kb distal to *NF2* [25]. Products were labeled with either <sup>32</sup>P or fluorescent dyes and separated on 6% polyacrylamide gels or on an automated Genetic Analyzer ABI310.

### Mutational analysis

Mutational analysis of the *NF2* gene was performed on a single specimen of blood or tumor from each patient studied. The 17 known exons of the *NF2* gene were amplified from genomic DNA and scanned using single-strand conformation polymorphism analysis or temperature gradient gel electrophoresis, as previously described [8, 13]. Aberrant mobility of single- or double-stranded products was identified by comparison with known positive and negative controls. When aberrations were detected by these methods, the sequence basis was determined by direct sequencing of re-amplified products. In the case of complex insertions or deletions, or of alterations near amplification primers, the exact sequence of the change was determined by cloning the polymerase chain reaction (PCR) products using a vector with T overhang (pGEM, Promega Life Sciences). When a mutation was detected in a tumor specimen, side by side analysis with the paired blood specimen and other available tumor specimens was performed to determine if the alteration was constitutional or somatic.

**Table 1** Microsatellite markers used for genotyping (*NF2* neurofibromatosis 2)

Marker	Location to <i>NF2</i>	Heterozygosity <sup>a</sup>	Reference
CRYB2	Centromeric	60%	[26]
D22S193	Centromeric	Not reported	Genome Database, version 5.6
D22S275	Centromeric	83%	Genome Database, version 5.6
NF2CA3 (D22S929)	Intragenic	83%	[27]
D22S268	Telomeric	60%	[28]
D22S430	Telomeric	68%	[29]

<sup>a</sup> Heterozygosity as reported in reference given. The URL for the Genome Database is <http://www.gdb.org/>

## RESULTS

### LOH analysis

All 65 affected patients for whom tumor specimens were available were heterozygous in the blood samples for one or more of the microsatellite markers studied. Comparison of the 110 tumor specimens with corresponding blood samples revealed LOH of informative markers in 65 tumors (59%) from 48 patients (Table 2). In all cases in which more than one marker was informative, all markers were either lost or retained, indicating that no tumor carried a breakpoint within this small region. Of the 65 patients, 24 had multiple tumors analyzed. In 4 patients with multiple tumors (3 patients with three tumors and 1 patient with two tumors) all tumors had LOH. In 4 patients with multiple tumors (range two to seven tumors), no tumor had LOH; 16 individuals had both tumors with and without LOH. Overall, 11 patients had more than one tumor with LOH, and in all 11 the lost alleles were the same in each tumor of a patient, as predicted by the two-hit model.

Parental blood samples were available for 39 of 48 patients in whom LOH was seen in one or more tumors. In 34 patients, both parental blood samples were available, and in 5 patients blood samples were only available from a single parent with the transmitted haplotype of the other parent inferred. In 29 of 39 patients (74%), the lost allele was of maternal origin, implying a paternal origin of the new constitutional mutation (Fig. 1). In all cases in which more than one informative marker was available, the lost alleles were from the same parent, as expected.

### Pedigree analysis

For each index patient from the six three-generation families, at least two markers were informative. In four of six three-generation families, the parental origin of the new mutation in the second generation was maternal, as evidenced by its transmission of the maternal genotype to an affected member of the third generation (Fig. 2).

### Mutational analysis

Of the 45 sporadic NF2 patients for whom the parental origin of the new mutation could be determined, 41 underwent mutational analysis of a blood or tumor specimen. Causative alterations in blood specimens were found in 29 of 41 patients (Table 3). Six additional patients were found to harbor somatic mosaicism, as determined by multiple tumors with identical mutations not detectable in the blood specimen or by single tumors with mutations not detectable in the blood specimen and LOH. Mutations included nonsense ( $n=13$ ),

**Table 2** Loss of heterozygosity (LOH) of chromosome 22 alleles in different tumor types

Tumor type	<i>n</i>	% LOH
Vestibular schwannoma	28	64
Non-vestibular schwannoma <sup>a</sup>	51	55
Meningioma	19	74
Neurofibroma	9	33
Ependymoma	2	100
Not classified	1	100
Total	110	59

<sup>a</sup> Predominantly spinal and cutaneous schwannomas

frameshift ( $n=10$ ), splice site alterations ( $n=10$ ), and two in-frame deletions. No correlation was seen between the type of mutation and the parental chromosome on which it arose, although all six mutations not detected by exon scanning were paternal in origin. In 4 of the 6 patients with mosaicism, the mutation arose on the maternal allele. In both cases of suspected gonadal mosaicism, the affected siblings shared identical mutations not found in the maternal sample.

### Patient characteristics

The 45 patients for whom parental origin could be determined included 26 males and 19 females. The percentage of male probands with a paternal origin of their mutation (17 of 26, 65%) was similar to the percentage of female probands with paternal origin (14 of 19, 74%). All non-mosaic patients for whom parental origin was determined had the severe subtype of NF2 (more than two non-vestibular intracranial tumors or more than four spinal tumors, or age of onset younger than 20 years). The mean age of onset of symptoms in these 45 patients was 13.5 years (range 2–32 years, Table 3). The mean age of onset of symptoms for probands with paternal origin was 11.3 years, compared with 18.2 years amongst probands with maternal origin ( $P=0.015$ ). When the 6 patients with mosaicism were excluded, the mean age of onset was 10.3 years for those with paternal origin and 16.4 years for those with maternal origin ( $P=0.056$ ).

## DISCUSSION

In the present study, we determined the parental origin of mutations in 45 sporadically affected patients with NF2 and related conditions. Because three-generation NF2 pedigrees are relatively rare, we primarily utilized LOH analysis in blood-tumor pairs to infer parent of origin when only two generations were available. The new mutation was of paternal origin in 31 (69%) of these 45 patients and of maternal origin in 14 (31%) patients ( $P=0.016$ ). Four (29%) patients with maternal origin were somatic mosaics and 14% of mutations of

**Table 3** Characteristics of sporadic NF2 patients in whom parental origin of mutation was determined (*NA* not available, *SCH* schwannomatosis, *BVS* bilateral vestibular schwannoma, *UVS* unilateral vestibular schwannoma, *NS* nonsense, *FS* frameshift, *SP* splice site alteration, *NT* non-truncating deletion)

Patients with paternal origin of mutation						
Patient	Phenotype <sup>b</sup>	Sex	Age of onset (years)	Mutation <sup>c</sup>	Predicted effect	Reference
KM107	BVS	M	8	Not found <sup>d</sup>		
KM148.1	UVS	M	19	*1396C → T	NS	
KM154	BVS	F	4	Not found <sup>d</sup>		
KM161	BVS	F	2	675+5G → C	SP	[17]
KM17	BVS	M	25	Not found <sup>d</sup>		
KM191.3	BVS	F	5	Not found <sup>d</sup>		
KM205	BVS	M	2	600-3C → G	SP	
KM21	BVS	F	25	586C → T	NS	
KM216	BVS	M	9	1069G → T	NS	
KM217	BVS	M	6	1580delA	NS	
KM26	BVS	F	28	1574+1G → C	SP	[17]
KM3	BVS	M	11	717delG	FS	[13]
KM37	BVS	F	6	448-1G → A	SP	[17]
KM54	BVS	M	7	532-534del	NT	
KM71	BVS	F	9	Not found <sup>d</sup>		
KM91	BVS	F	10	Not found <sup>d</sup>		
MB010	BVS	M	25	Not done		
MB073	BVS	M	11	430insA	FS	
GUS14817	BVS	M	3	1396C → T	NS	[11]
GUS16206	BVS	F	3	1198C → T	NS	[14]
GUS16212	BVS	M	18	448-2A → T	SP	[14]
GUS16215	BVS	M	10	997delC	FS	[14]
GUS18773	BVS	F	12	586C → T	NS	[30]
GUS19030	SCH	F	30	*577-580del	FS	[8]
GUS21772	BVS	M	8	447+1G → C	SP	[31]
GUS22804	BVS	M	12	408-422del	NT	
GUS22987	BVS	F	NA	545delA	FS	
GUS23040	BVS	F	12	Not done		
GUS23428	BVS	M	3	805A → T	NS	
GUS2613	BVS	M	5	269delA	FS	[5]
GUS26677	BVS	F	10	Not done		
Patients with maternal origin of mutation						
KM10	BVS	F	20	1562-1564insA	FS	
KM101	BVS	F	8	1282C → T	NS	[13]
KM13	BVS	F	25	*586C → T	NS	[10]
KM20	BVS	M	22	448-1G → T	SP	[13]
KM47	BVS	M	15	169C → T	NS	
KM72	BVS	M	21	*169C → T	NS	
MB012	BVS	M	24	Not done		
FF5863	BVS	M	8	331C → T	NS	[14]
GUS16039 <sup>a</sup>	BVS	M	29	999+1G → A	SP	[11]
GUS16209	BVS	F	6	448-2A → G	SP	[14]
GUS17692 <sup>a</sup>	BVS	M	4	169C → T	NS	[14]
GUS18813	UVS	F	32	*932delG	FS	[19]
GUS19131	SCH	M	13	*233, complex rearrangement	FS	[8]
GUS19640	BVS	M	28	447G → A	SP	

<sup>a</sup> GUS16039 and GUS17692 had an identically affected sibling indicating germline mosaicism in a parent

<sup>b</sup> BVS with or without other NF2-related findings; UVS and other NF2-related tumors

<sup>c</sup> Numbering of bases showing alteration is given relative to the cDNA sequence, with the initiator ATG beginning at base 1. Start point of insertions and deletions is first possible base pair affected. \*Mutations found to be somatic mosaic

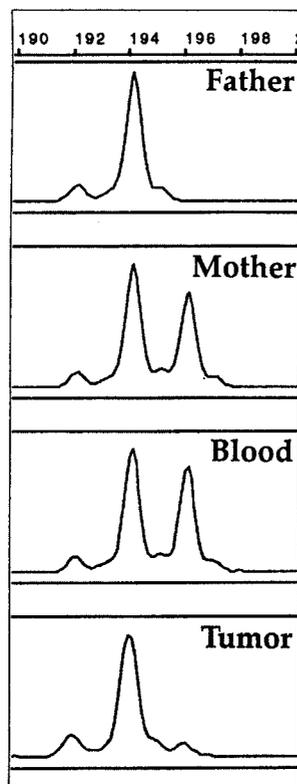
<sup>d</sup> No mutation found in blood sample; DNA quality in tumor sample precluded complete screening

maternal origin were associated with gonadal mosaicism in the mother. Perhaps reflecting the relative severity of the disease in the affected population that we studied, mutations were detected in 85% of patients who underwent exon scanning. When patients with mosaicism were excluded, no relationship was found be-

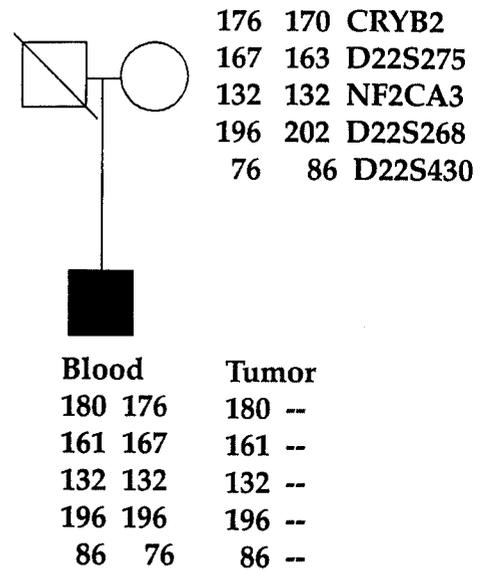
tween the type or location of mutation or the severity of disease and the parent of origin.

Heterozygosity analysis as an indicator of allelic loss in tumor tissues has been widely reported. In the *NF2* gene, frequency of allelic loss has primarily been studied in sporadic tumors. Reports of LOH of chromo-

**Fig. 1** Loss of heterozygosity analysis in the determination of parental origin of neurofibromatosis 2 (*NF2*) mutation. **A** Automated genotyping for marker D22S268 in blood and tumor specimens from sporadic *NF2* patient KM148.1 and the unaffected parents. The tumor has lost the maternal allele, inferring a paternal origin of mutation. **B** Genotyping across the *NF2* region in sporadic *NF2* patient KM3. No paternal blood sample was available; however, the mother's sample does not have the allele retained in the tumors from the patient, indicating that this allele was paternally derived and thus the lost allele was maternal



A



B

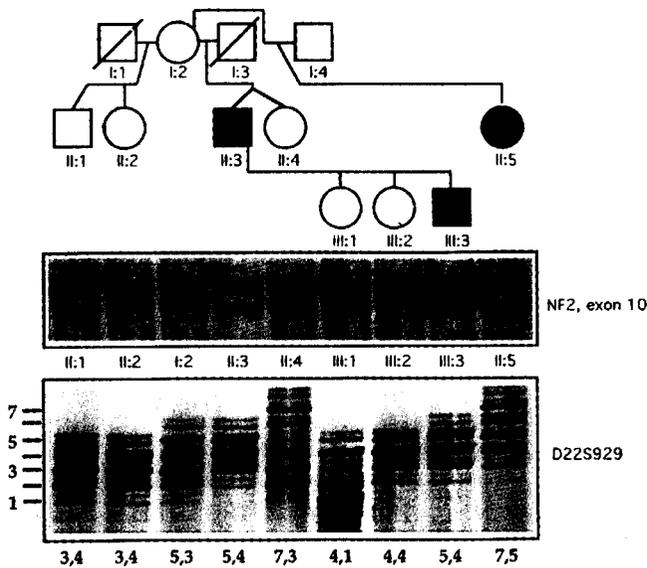
some 22 markers in sporadic vestibular schwannomas have ranged from 31% (8 of 26 tumors [18]) to 50% (24 of 48 tumors [19]). In the same two studies, 6 of 12 sporadic non-vestibular schwannomas showed LOH, although no dermal tumors were reported. Interestingly, although these studies showed a relatively low rate of LOH in schwannomas from *NF2* patients (4 of 19 tumors examined in both studies, 21%), we found a significantly higher rate of allelic loss amongst the schwannomas of our *NF2* patients (Table 2). Since identical methods were used, the reason for this discrepancy is unclear. In sporadic meningioma, several studies have found that LOH for chromosome 22 markers is seen in 60% of all tumors, and that LOH is tightly associated with the finding of primary *NF2* mutation [12, 20, 21, 32]. This has led to the speculation that meningiomas without LOH of chromosome 22 markers may result from involvement of a second locus, not linked to the *NF2* gene. These studies included few meningiomas from *NF2* patients. In the current study, *NF2* mutation was identified in 4 of 5 meningiomas that retained both chromosome 22 alleles (data not shown).

The occurrence of nerve sheath tumors with a pathological appearance of neurofibroma is rare in *NF2* [33, 34], which is reflected in current *NF2* diagnostic criteria [24]. Our study was limited to review of pathological reports, leaving open the possibility that some of these tumors may have been schwannomas on expert review. Loss of chromosome 22 alleles has not been previously reported in *NF2*-associated neurofibromas and LOH was seen at a lower rate in neurofibromas than in any

other tumor type we examined. This may reflect a different pathological mechanism for the generation of neurofibroma in *NF2* patients, or neurofibromas as a mixed cell population, with only one component having a *NF2* alteration and thus LOH.

The parent of origin of new mutations has now been examined for a number of human disorders. In autosomal dominant disorders such as MEN2b, achondroplasia, and Apert syndrome, in which a small number of mutations arise independently and repeatedly in sporadic patients, the parental origin appears to be exclusively paternal [35–37]. In autosomal dominant disorders without common recurrent mutations, parental origin is over 90% paternal [38–42]. The bias towards paternal origin has been ascribed to the greater number of mitotic events in the paternal germ line, and in some cases has been associated with increased paternal age at conception [36, 37]. Although paternal age at conception was not available for our patients, increased paternal age within the sporadic *NF2* population has not been seen in other studies [6]. Parental origins of mutations other than small genetic alterations are more heterogeneous. For example, in contrast to small genetic changes, large deletions in the *NF1* gene are primarily maternal in origin [43, 44].

To our knowledge, this is the first study to examine parent of origin of mutations in multiple patients with somatic mosaicism at the same locus. Interestingly, we found a tendency towards maternal origin of mutation in patients with somatic mosaicism, despite an overall predominance of paternal origin. Since the number of



**Fig. 2** Determination of parental origin of *NF2* mutation in a three-generation family. Single-strand conformation polymorphism (SSCP) analysis of exon 10 in patient GUS16039 (II.3) revealed an alteration also present in his affected half-sister (II.5) and 1 of 3 at-risk children (III.3). Patient III.3 was subsequently found to have small asymptomatic bilateral vestibular schwannoma on magnetic resonance imaging. Sequence analysis revealed that the SSCP alteration was due to a splice site alteration, the effects of which were confirmed by cDNA analysis. Microsatellite analysis revealed that the affected child inherited his father's mother's allele, indicating a maternal origin of mutation in the founding generation. The mother of patient GUS16039 (I.2), who was clinically unaffected, does not carry the SSCP alteration, confirming the clinical impression of germline mosaicism

mosaics in our study was small, studies are now underway to examine the parental origin of the mutation in a larger number of somatic mosaics. Our study included two kindreds in which presumed gonadal mosaicism of an asymptomatic parent produced identical disease in two offspring, and in both cases the originating parent was the mother. A similar *NF1* kindred in which gonadal mosaicism was proven in a non-expressing father has been reported [45]. Although gonadal mosaicism raises difficult genetic counselling issues, the overall incidence in the NF population appears to be sufficiently low as to make routine screening of siblings unnecessary. Our results show, however, that when gonadal mosaicism is present the origin can not be presumed to be paternal.

Two previous studies of *NF2* reported a parent of origin effect on severity, with a mean age of onset in paternally inherited cases of 24 years and in maternally inherited cases 18 years [6, 46]. These clinical studies examined only the age of onset in non-founding generations. Subsequent work did not confirm this disparity [2], which may instead reflect reduced reproductive fitness of severely affected men versus women, combined with the intrafamilial homogeneity of the disorder. Surprisingly, we found a statistically significant opposite effect in this group of sporadic cases with a younger age

of onset amongst the patients with paternal origin, even when corrected for the mitigating effects of known mosaicism. Although the reasons for this discrepancy remain unclear, one possibility is that of unsuspected mosaicism amongst other maternally derived cases. Further molecular studies will help to clarify this point.

A number of potential sampling biases exist in this work. Theoretically, reliance on LOH analysis may effectively exclude individuals with one parent of origin from study if imprinting or other factors prevent loss of one of the parental alleles in specific tumor types [47]. In *NF2*, this appears unlikely since we found loss of both maternal and paternal alleles, and the majority of patients with multiple tumors had tumors both with and without loss. This effect should also be apparent if sporadically occurring tumors are examined. Previous studies have found loss of both maternal and paternal chromosome 22 in sporadic schwannomas [22] and meningiomas [48]. These studies of sporadic tumors did show an unexplained tendency towards maternal allele loss (21 of 32 tumors examined, 66%) that was only slightly less than our findings in *NF2*-associated tumors.

Finally, a number of parameters show that our patient population predominantly included severely affected individuals. This may reflect the increased likelihood of severely affected patients requiring surgery, combined with the greater probability of severely affected patients with young ages of onset having living parents. We were able to ascertain only six three-generation pedigrees that might mitigate this effect, since mildly affected patients may be more likely to have children. Because a severe phenotype in *NF2* is overwhelmingly associated with truncating mutations, our findings of predominantly paternal origin may not apply to those patients with other types of mutations. Alternative mechanisms of determination of parental origin will need to be developed to address this issue.

**Acknowledgements** Supported by grants from the US Army MRC and the Child Neurology Society to M.M., by Deutsche Krebshilfe 70-1947-Ma I and Wilhelm-Sander-Stiftung 93.052.2 to L.K., and by US PHS grant CA51410 to L.B.J. We would like to thank the many patients and families whose participation made this work possible. Proband 16039 and affected and unaffected family members were ascertained by Ms. Lois Forehand. The Los Angeles *NF2* Study Group (Stefan-M Pulst, Vincent Riccardi, Niccola Ragge, and Sushi Baat) contributed to patient identification and sample collection. Presented in part at the 25th National Meeting of the Child Neurology Society, Minneapolis, Minnesota, USA, 27 September, 1996.

## REFERENCES

- Evans DGR, Huson SM, Donnai D, Neary W, Blair V, Newton V, Harris R (1992) A clinical study of type 2 neurofibromatosis. *Q J Med* 84:603-618
- Parry DM, Eldridge R, Kaiser-Kupfer MI, Bouzas EA, Pikus A, Patronas N (1994) Neurofibromatosis 2 (*NF2*): clinical characteristics of 63 affected individuals and clinical evidence for heterogeneity. *Am J Med Genet* 52:450-461

3. Mautner VF, Lindenau M, Baser ME, Hazim W, Tatagiba M, Haase W, Samii M, et al (1996) The neuroimaging and clinical spectrum of neurofibromatosis 2. *Neurosurgery* 38:880-885
4. 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
5. Trofatter JA, MacCollin MM, Rutter JL, Murrell JR, Duyao MP, Parry DM, Eldridge R, et al (1993) A novel moesin-, ezrin-, radixin-like gene is a candidate for the neurofibromatosis 2 tumor suppressor. *Cell* 72:791-800
6. Evans DGR, Huson SM, Donnai D, Neary W, Blair V, Teare D, Newton V, et al (1992) A genetic study of type 2 neurofibromatosis in the northwest of England and the UK. I. Prevalence, mutation rate, fitness and confirmation of maternal transmission effect on severity. *J Med Genet* 29:841-846
7. MacCollin M, Jacoby LB, Jones D, Ojemann R, Feit H, Gusella J (1997) Somatic mosaicism of the neurofibromatosis 2 tumor suppressor gene. *Neurology* 48:A429
8. Jacoby LB, Jones D, Davis K, Kronn D, Short MP, Gusella J, MacCollin M (1997) Molecular analysis of the *NF2* tumor-suppressor gene in schwannomatosis. *Am J Hum Genet* 61:1293-1302
9. Evans DGR, Wallace A, Wu C, Trueman L, Ramsden R, Strachan T (1998) Somatic mosaicism: a common cause of classic disease in tumor-prone syndromes? Lessons from type 2 neurofibromatosis. *Am J Hum Genet* 63:727-736
10. Kluwe L, Mautner VF (1998) Mosaicism in sporadic neurofibromatosis 2 patients. *Hum Mol Genet* 7:2051-2055
11. MacCollin M, Ramesh V, Jacoby LB, Louis DN, Rubio MP, Pulaski K, Trofatter JA, et al (1994) Mutational analysis of patients with neurofibromatosis 2. *Am J Hum Genet* 55:314-320
12. Merel P, Hoang-Xuan K, Sanson M, Moreau-Aibry A, Bijlsma E, Lazaro C, et al (1995) Predominant occurrence of somatic mutations of the *NF2* gene in meningiomas and schwannomas. *Genes Chromosom Cancer* 13:211-216
13. Kluwe L, Bayer S, Baser ME, Hazim W, Haase W, Funsterer C, Mautner VF (1996) Identification of *NF2* germ-line mutations and comparison with neurofibromatosis 2 phenotypes. *Hum Genet* 98:534-538
14. Parry DM, MacCollin M, Kaiser-Kupfer MI, Pulaski K, Nicholson HS, Bolesta M, Eldridge R, et al (1996) Germ-line mutations in the neurofibromatosis 2 gene: correlations with disease severity and retinal abnormalities. *Am J Hum Genet* 59:529-539
15. Ruttledge MH, Andermann A, Phelan CM, Claudio JO, Han F, Chretien N, Rangaratnam S, et al (1996) Type of mutation in the neurofibromatosis type 2 gene (*NF2*) frequently determines severity of disease. *Am J Hum Genet* 59:331-342
16. Evans DGR, Trueman L, Wallace A, Collins S, Strachan T (1998) Genotype/phenotype correlations in type 2 neurofibromatosis (*NF2*): evidence for more severe disease associated with truncating mutations. *J Med Genet* 35:450-455
17. Kluwe L, MacCollin M, Tatagiba M, Thomas S, Hazim W, Haase W, Mautner VF (1998) Phenotypic variability associated with 14 splice-site mutations in the *NF2* gene. *Am J Med Genet* 77:228-233
18. Bijlsma EK, Merel P, Bosch DA, Westerveld A, Delattre O, Thomas G, Hulsebos TJ (1994) Analysis of mutations in the *SCH* gene in schwannomas. *Genes Chromosom Cancer* 11:7-14
19. Jacoby LB, MacCollin M, Barone R, Ramesh V, Gusella JF (1996) Frequency and distribution of *NF2* mutations in schwannomas. *Genes Chromosom Cancer* 17:45-55
20. Ruttledge MH, Sarrazin J, Rangaratnam S, Phelan CM, Twist E, Merel P, Delattre O, et al (1994) Evidence for the complete inactivation of the *NF2* gene in the majority of sporadic meningiomas. *Nat Genet* 6:180-184
21. Wellenreuther R, Kraus JA, Lenartz D, Menon AG, Schramm J, Louis DN, Ramesh V, et al (1995) Analysis of the neurofibromatosis 2 gene reveals molecular variants of meningioma. *Am J Pathol* 146:827-832
22. Fontaine B, Sanson M, Delattre O, Menon A, Rouleau G, Seizinger B, Jewell A, et al (1991) Parental origin of chromosome 22 loss in sporadic and *NF2* neuromas. *Genomics* 10:280-283
23. Mulvihill J, Parry D, Sherman JL, Pikus A, Kaiser-Kupfer MI, Eldridge R (1990) Neurofibromatosis 1 (Recklinghausen disease) and neurofibromatosis 2 (bilateral acoustic neurofibromatosis): an update. *Ann Intern Med* 113:30-54
24. Gutmann D, Aylsworth A, Carey J, Korf B, Marks J, Pyeritz R, Rubenstein A, Viskochil D (1997) The diagnostic evaluation and multidisciplinary management of neurofibromatosis 1 and neurofibromatosis 2. *JAMA* 278:51-57
25. Durham I, Shimizu N, Roe BA, Chisoe S (1999) The DNA sequence of human chromosome 22. *Nature* 402:489-495
26. Marineau C, Rouleau GA (1991) Dinucleotide repeat polymorphism at the human *CRYB2* gene locus (22q11.2). *Nucleic Acids Res* 20:1430
27. Bourn D, Strachan T (1995) Highly polymorphic dinucleotide repeat at the *NF2* gene. *Hum Genet* 95:712
28. Marineau C, Baron C, Delattre O, Zucman O, Thomas G, Rouleau G (1993) Dinucleotide repeat polymorphism at the D22S268 locus. *Hum Mol Genet* 2:336
29. Sainz J, Nechiporuk A, Kim UJ, Simon MI, Pulst SM (1993) CA-repeat polymorphism at the D22S430 locus adjacent to *NF2*. *Hum Mol Genet* 2:2203
30. MacCollin M, Braverman N, Viskochil D, Ruttledge M, Davis K, Ojemann R, Gusella J, Parry DM (1996) A point mutation associated with a severe phenotype of neurofibromatosis 2. *Ann Neurol* 40:440-445
31. Jacoby LB, MacCollin M, Parry DM, Kluwe L, Lynch J, Jones D, Gusella J (1999) Allelic expression of the *NF2* gene in neurofibromatosis 2 and schwannomatosis. *Neurogenetics* 2:101-108
32. Papi L, De Vitis L, Vitelli F, Ammannati F, Mennonna P, Montali E, Bigozzi U (1995) Somatic mutations in the neurofibromatosis type 2 gene in sporadic meningiomas. *Hum Genet* 95:347-351
33. Halliday A, Sobel RA, Martuza RL (1991) Benign spinal nerve sheath tumors: their occurrence sporadically and in neurofibromatosis types 1 and 2. *J Neurosurg* 74:248-253
34. Mautner VF, Lindenau M, Baser M, Kluwe L, Gottschalk J (1997) Skin abnormalities in neurofibromatosis 2. *Arch Dermatol* 133:1539-1543
35. Carlson K, Bracamontes J, Jackson C, Clark R, Lacroix A, Wells S, Goodfellow P (1994) Parent-of-origin effects in multiple endocrine neoplasia type 2B. *Am J Hum Genet* 55:1076-1082
36. Moloney D, Slaney S, Oldridge M, Wall S, Sahlin P, Stenman G, Wilkie A (1996) Exclusive paternal origin of new mutations in Apert syndrome. *Nat Genet* 13:48-53
37. Wilkin D, Szabo J, Cameron R, Henderson S, Bellus G, Mack M, Kaitila I, et al (1998) Mutations in fibroblast growth-factor receptor 3 in sporadic cases of achondroplasia occur exclusively on the paternally derived chromosome. *Am J Hum Genet* 63:711-716
38. Zhu X, Dunn J, Phillips R, Goddard A, Paton K, Becker A, Gallie B (1989) Preferential germline mutation of the paternal allele in retinoblastoma. *Nature* 340:312-313
39. Dryja T, Mukai S, Petersen R, Rapaport J, Walton D, Yandell D (1989) Parental origin of mutations of the retinoblastoma gene. *Nature* 339:556-558
40. Jadayel D, Fain P, Upadhyaya M, Ponder M, Huson S, Carey J, Fryer A, et al (1990) Paternal origin of new mutations in Von Recklinghausen neurofibromatosis. *Nature* 343:558-559

41. Stephens K, Kayes L, Riccardi V, Rising M, Sybert V, Pagon R (1992) Preferential mutation of the neurofibromatosis type 1 gene in paternally derived chromosomes. *Hum Genet* 88:279-282
42. Kato M, Ishizaki K, Shimizu T, Ejima Y, Tanooka H, Takayama J, Kaneko A, et al (1994) Parental origin of germ-line and somatic mutations in the retinoblastoma gene. *Hum Genet* 94:31-38
43. Ainsworth P, Chakraborty P, Weksberg R (1997) Example of somatic mosaicism in a series of de novo neurofibromatosis type 1 cases due to a maternally derived deletion. *Hum Mutat* 9:452-457
44. Lazaro C, Gaona A, Ainsworth P, Tenconi R, Vidaud D, Kruyer H, Ars E, et al (1996) Sex differences in mutational rate and mutational mechanism in the *NF1* gene in neurofibromatosis type 1 patients. *Hum Genet* 98:696-699
45. Lazaro C, Ravella A, Gaona A, Volpini V, Estivill X (1994) Neurofibromatosis type 1 due to germ line mosaicism in a clinically normal father. *N Engl J Med* 331:1403-1407
46. Kanter WR, Eldridge R, Fabricant R, Allen JC, Koerber T (1980) Central neurofibromatosis with bilateral acoustic neuroma: genetic, clinical and biochemical distinctions from peripheral neurofibromatosis. *Neurology* 30:851-859
47. Toguchida J, Ishizaki K, Sasaki M, Nakamura Y, Ikenaga M, Kato M, et al (1989) Preferential mutation of paternally derived *RB* gene as the initial event in sporadic osteosarcoma. *Nature* 338:156-158
48. Sanson M, Delattre O, Couturier J, Philippon J, Cophignon J, Derome P, Rouleau G, Thomas G (1990) Parental origin of chromosome 22 alleles lost in meningioma. *Am J Hum Genet* 47:877-880