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Short Communication

Analysis of the Neurofibromatosis 2 Gene Reveals Molecular Variants of Meningioma

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There is evidence from cytogenetic and loss of heterozygosity studies for the involvement of a tumor suppressor gene on chromosome 22 in the formation of meningiomas. Recently, the NF2 gene, which causes neurofibromatosis type 2 and which is located in the affected region on chromosome 22, has been identified. A previous study on 8 of the 17 exons of the NF2 gene described mutations in 16% of meningiomas. We have analyzed the entire coding region of the NF2 gene in 70 sporadic meningiomas and identified 43 mutations in 41 patients. These resulted predominantly in immediate truncation, splicing abnormalities, or an altered reading frame of the predicted protein product. Although there was no evidence for distinct hotspots, all mutations occurred in the first 13 exons, the region of homology with the filopodial proteins moesin, ezrin, and radixin. The association of loss of heterozygosity on chromosome 22 with mutations in the NF2 gene was significant. These data suggest that NF2 represents the meningioma locus on chromosome 22. NF2 mutations occurred significantly more frequently in fibroblastic meningioma (70%) and transitional meningioma (83%) than in meningiothelial meningioma (25%), thus indicating a differential molecular pathogenesis of these meningioma variants. (Am J Pathol 1995, 146:827–832)

Involvement of chromosome 22 in the formation of meningioma was initially suggested by cytogenetic studies.[1] Subsequently, loss of heterozygosity (LOH) studies demonstrated frequent allelic deletions on chromosome 22q (LOH22).[2–4] This region overlapped with the neurofibromatosis 2 (NF2) gene, which has been mapped to chromosome 22q12.[5] The recently cloned NF2 gene[6,7] is a candidate for the meningioma-associated gene on chromosome 22 because NF2 patients frequently develop meningiomas. Two studies revealed mutations in the NF2 gene in sporadic meningiomas.[4,8] In these studies it was estimated that up to 60% of sporadic meningiomas may carry a NF2 gene mutation.

To examine the role of the NF2 gene in sporadic meningiomas we analyzed a series of 70 tumors of different histopathological grades and subtypes by single strand conformation polymorphism (SSCP) analysis and direct sequencing for mutations in the entire coding region of the NF2 gene and for LOH22. The question of whether subtypes of meningiomas differ with respect to their frequency of NF2 gene mutations was addressed by correlating molecular genetic data with histopathological data.

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Materials and Methods

Tumor and blood samples were obtained from 70 patients treated at the University Hospital, Bonn, Germany, the Hospital Cologne-Merheim, Cologne, Germany, and the University Hospital, Zurich, Switzerland. All tumors were classified by the same neuropathologist (AvD) and graded according to the guidelines of the World Health Organization (WHO).[9] All patients have given consent to the analysis of their tumors. The group of 70 meningiomas consisted of 24 meningiothelial meningiomas, WHO grade I; 18 transitional meningiomas, WHO grade I; 10 fibroblastic meningiomas, WHO grade I; 10 atypical meningiomas, WHO grade II; and 8 anaplastic meningiomas, WHO grade III. All tumors were examined by frozen sections for contaminating tissue before DNA extraction. DNA was obtained by phenolic extraction as described.[10]

The primers and conditions for polymerase chain reaction (PCR)-mediated amplification of the exons of the NF2 gene have been published elsewhere.[11] PCR was performed in a final volume of 10 µl containing 10 ng of DNA, 50 mmol/L KCl, 10 mmol/L Tris-HCl, pH 8.3, 200 mmol/L of each dNTP, 0.1% gelatin, and 20 pmol of each primer. Either Taq-polymerase or Pfu-polymerase was used. The $MgCl_2$ concentrations ranged from 1.0 to 2.0 mmol/L, depending on the primer pair. Initial denaturation at 95 C for 3 minutes was followed by 30 cycles on an automated thermocycler (Hybaid, Omnigene). These included denaturation at 95 C for 30 seconds, annealing at temperatures from 52 to 59 C for 40 seconds, and extension at 72 C for 30 seconds. A final extension step of 10 minutes at 72 C was used.

Eight microliters PCR product were mixed with an equal amount of loading dye (formamide, xylenecyanol, bromphenol blue) and denatured for 5 minutes at 95 C. Electrophoresis was carried out on long acrylamide gels (8 to 12% acrylamide, 1X TBE) on a sequencing apparatus (Pokerface II, Hoefer Scientific) at room temperature or at 4 C with 3 to 30 watts.

For the staining of large, thin acrylamide gels, we developed a staining frame that allows multiple incubation and washing steps without wrinkling or damaging the gel. Technical details of this frame are described elsewhere.[12] For the detection of the amplification product, an established silver stain protocol was employed.[13,14]

Variant SSCP bands were excised from the silver-stained gel, and the single stranded DNAs in these bands were extracted.[10] Upon reamplification with the same set of primers, the PCR products were analyzed by direct sequencing. All amplicons were sequenced bidirectionally. Sequence analysis was carried out with a semiautomated sequencer (Applied Biosystems model 373) and the corresponding Taq cycle sequencing kit.

The following primer pairs on chromosome 22 were used to detect LOH by microsatellite analysis: D22S258 at 22q,[15] PDGFB at 22q12.3-q13.1,[15] and D22S445 and microsatellite GATA4F03 (no locus designation yet), both from Research Genetics (Huntsville, AL). Alleles and allelic losses were scored as previously described.[16]

Results

Aberrant migration patterns of PCR-amplified exons of the NF2 gene were seen in 43 exons from 41 individuals in a series of 70 meningioma patients. Two meningiomas (cases 632 and 2684) had two exons with altered PCR fragments. Representative data are shown in Figure 1. We also examined the constitutional DNA of patients with aberrantly migrating DNA fragments. With one exception (case 632), all patients with an abnormal signal in tumor DNA showed the wild-type pattern in their constitutional DNA. Case 632 exhibited SSCP shifts in exons 3 and 11; the latter was also present in constitutional DNA.

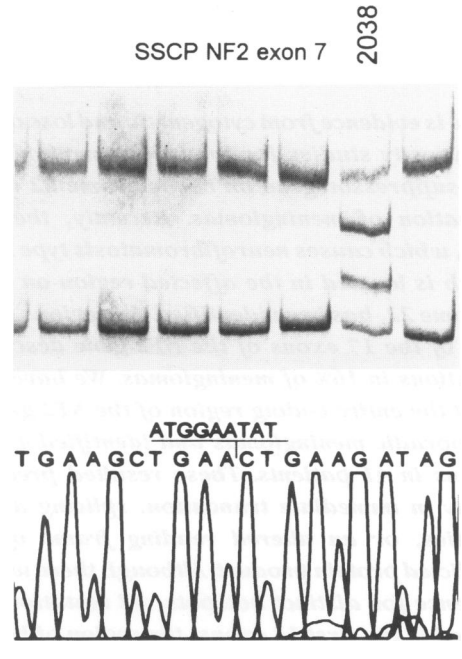

Figure 1. *Representative example of SSCP and DNA sequence analysis of the NF2 gene exon 7 in meningiomas. Case 2038 shows an altered migration pattern of SSCP fragments* (upper panel) *resulting from a 9-bp deletion* (lower panel).

DNA sequence analysis revealed an altered nucleotide sequence in all tumors with aberrantly migrating SSCP bands. We detected 43 mutations in the NF2 gene in 41 of 70 patients with sporadic meningiomas. There were no obvious hotspots for mutational activity. Only two mutations occurred twice in our series (cases 582/864 and 992/2818). All mutations were localized in the first 13 exons of the NF2 gene. The positions and the types of mutations are summarized in Table 1. In 16 tumors, we detected a deletion in the coding region; 13 of which resulted in a frameshift, and 2 of these also affected a splice site. In 4 tumors, there was an insertion in the coding region, all of which caused frameshifts. A nonsense mutation that generated a stop codon at the mutated site was seen in 10 tumors. Only 1 tumor (case 2376) had a missense mutation, which led to the replacement of a glycine by a leucine residue. In 9 tumors a splice site was affected, and 3 tumors (cases 632, 2064, and 2206) had intronic mutations in the vicinity of a splice site. In cases 2432 and 2684, we found intronic mutations. Cases 632 and 2684 were affected by two mutations, each in different exons. Cases 582 and 864 had the same nonsense mutation in exon 6, and cases 992 and 2818 had the same nonsense mutation in exon 11. All other mutations were unique.

For statistical analysis, each patient with a NF2 gene alteration was scored only once, ignoring that two patients (cases 632 and 2684) had two mutations each. Therefore, in this series 41 of 70 (59%) patients had NF2 mutations. These mutations occurred in 6 of 8 (75%) anaplastic meningiomas, WHO grade III; 7 of 10 (70%) atypical meningiomas, WHO grade II; 7 of 10 (70%) fibroblastic meningiomas, WHO grade I; 15 of 18 (83%) transitional meningiomas, WHO grade I; and 6 of 24 (25%) meningiothelial meningiomas, WHO grade I. The frequency of NF2 mutations in meningiothelial meningioma was significantly lower than in all other subgroups ($P < 0.001$, χ^2 test).

These tumors were also examined for LOH22 by microsatellite analysis. Our study included 65 meningiomas that were informative in at least one locus on chromosome 22. LOH22 was detected in 39 of 65 (60%) meningiomas. The figures for the histopathological variants were 6 of 10 (60%) atypical meningiomas, WHO grade II; 7 of 7 (100%) anaplastic meningiomas, WHO grade III; 8 of 10 (80%) fibroblastic meningiomas, WHO grade I; 11 of 15 (73%) transitional meningiomas, WHO grade I; and 7 of 24 (29%) meningiothelial meningiomas, WHO grade I. The frequency of LOH22 in meningiothelial meningioma was significantly lower than in all other subgroups ($P < 0.01$, χ^2 test).

Among all meningiomas, NF2 mutations were closely linked to LOH22 ($P < 0.002$, Yates χ^2 test). In this series, 34 of 39 (87%) patients with LOH22 had NF2 mutations, but only 4 of 26 (15%) patients without LOH22 had NF2 mutations.

Discussion

This study on 70 sporadic meningiomas provides additional evidence for involvement of the NF2 gene in the formation of meningiomas. We detected NF2 mutations in 59% of these tumors. In addition, our data show that histopathological variants of meningioma differ significantly in their frequency of NF2 gene mutations. This indicates genetic subtypes of meningiomas with different pathogenetic mechanisms of tumor formation.

Eight exons of the NF2 gene have been previously examined for mutations in 151 meningiomas, and 24 mutations (16%) have been detected.[4] A 32% incidence of NF2 mutations has been reported in a cDNA analysis of 44 sporadic meningiomas.[8] Compared with our analysis, these two studies showed a significantly lower overall rate of NF2 mutations in meningiomas. The analysis of only one-half of the NF2 exons[4] and the less sensitive screening of large PCR fragments[8] probably accounts for this difference. It also cannot be excluded that meningiothelial meningioma with a low incidence of NF2 mutations was overrepresented in the latter report. A striking feature of both studies was the high incidence of nonsense mutations, frameshift mutations, mutations in the splicing sites, and the virtual absence of missense mutations. The present study, which detected 43 NF2 gene mutations, and two reports on NF2 mutations in schwannomas show a similar distribution of mutation types.[11,17] Most frequently, we found deletions or insertions in coding regions that in 17 of 20 tumors, resulted in an altered reading frame. Ten tumors had nonsense mutations leading to a stop codon, and in nine tumors there were splice site alterations. Only a single tumor had a missense mutation. As previously suggested, this implies that a severe alteration in the NF2 gene product, merlin, is required for meningioma formation and that amino acid exchanges may generally not suffice.[4] The missense mutation in exon 10 of our case 2376 may be localized in a critical functional region of the NF2 gene. Surprisingly, all 43 mutations were seen in the first 13 exons of the NF2 gene (see Figure 2). This region of merlin shows a high homology to the filopodial proteins ezrin, radixin, and moesin and contains an α-helix analogous to protein band 4.1.[6] It has been proposed that the ezrin, ra-

Table 1. *Clinical Data and NF2 Point Mutations in Sporadic Meningiomas*

ID	Sex	Age	Diagnosis	22q	Exon	DNA sequence alteration*	Codon change†	Consequence
864	M	65	Mal WHO III	LOH	E6	586 C → T	Arg196X	Nonsense
878	F	51	Mal WHO III	LOH	E2	162/5 del 1 bp (*G*)	Gly55fs → 128X	Frameshift
2064	F	49	Mal WHO III	–	E11	1000 – 7 c → g		Splice acceptor?
2226	F	70	Mal WHO III	LOH	E10	934/6 del 1 bp (*A*)	Lys312fs → 322X	Frameshift
2608	M	70	Mal WHO III	LOH	E3	241 – 3 to –15 del 13 bp		Splice acceptor
						241 to 242 del 2 bp (*GT*)	Val81fs → 85X	Frameshift
2686	F	71	Mal WHO III	LOH	E8	785/6 ins 1 bp (*G*)	Arg262fs → 269X	Frameshift
596	F	49	Mal WHO III	LOH				
2070	M	50	Mal WHO III	LOH				
582	M	21	Atyp WHO II	LOH	E6	586 C → T	Arg 196X	Nonsense
812	F	70	Atyp WHO II	LOH	E1	114 + 1g → t		Splice donor
2038	F	37	Atyp WHO II	LOH	E7	613 to 621 del 9 bp		
2060	F	37	Atyp WHO II	LOH	E1	70/1 ins 1 bp (*T*)	Val24fs → 49X	Frameshift
2066	F	65	Atyp WHO II	12	E1	70 del 1 bp (*G*)	Val24fs → 40X	Frameshift
2220	M	49	Atyp WHO II	LOH	E2	115 – 1 g → c		Splice acceptor
2794	F	67	Atyp WHO II	LOH	E11	1009 C → T	Gin337X	Nonsense
610	F	64	Atyp WHO II	12				
2068	F	57	Atyp WHO II	12				
2482	F	70	Atyp WHO II	12				
916	F	59	Fib WHO I	12	E12	1340 + 1 g → a		Splice donor
988	F	52	Fib WHO I	LOH	E13	1389 to 1408 del 20 bp	Glu463fs → 489X	Frameshift
2082	F	73	Fib WHO I	LOH	E5	484/8 ins 1 bp (*T*)	Leu163fs → 203X	Frameshift
2312	F	59	Fib WHO I	LOH	E6	592 to 593 del 2 bp (*CG*)		Frameshift
						595 to 599 + 3 del 8 bp		Splice donor
2376	F	44	Fib WHO I	LOH	E10	971 A → T	Gln324 → Leu	Missense
2432	F	82	Fib WHO I	LOH	E2	240 + 22 del 1 bp (*a*)		Splice donor?
2694	F	68	Fib WHO I	LOH	E12	1228 C → T	Gln410X	Nonsense
914	F	57	Fib WHO I	12				
2020	F	53	Fib WHO I	LOH				
2034	F	76	Fib WHO I	LOH				
632	F	40	Trans WHO I	LOH	E6	599 + 1 g → t		Splice donor
					E11	1000 – 4 g → a		Splice acceptor?
810	F	44	Trans WHO I	LOH	E1	95 to 96 del 2 bp (*AG*)	Glu32fs → 47X	Frameshift
992	F	67	Trans WHO I	LOH	E11	1021 C → T	Arg321X	Nonsense
998	F	66	Trans WHO I	LOH	E2	174 to 197 del 24 bp		
2014	F	48	Trans WHO I	LOH	E3	298/301 del 1 bp (*T*)	Tyr101fs → 123X	Frameshift
2016	F	77	Trans WHO I	12	E4	439 C → T	Arg196X	Nonsense
2094	M	66	Trans WHO I	LOH	E6	531 T → G	Tyr177X	Nonsense
2188	F	59	Trans WHO I	LOH	E4	430/1 ins 1 bp (*A*)	Tyr144fs → 153X	Frameshift
2206	F	55	Trans WHO I	LOH	E8	676 – 9 to –22 del 14 bp		Splice acceptor?
2426	F	74	Trans WHO I	NI	E3	346 to 347 del 2 bp (*CA*)	His116fs → 129X	Frameshift
2428	F	42	Trans WHO I	12	E7	610 del 1 bp (*G*)	Glu204fs → 209X	Frameshift
2576	F	87	Trans WHO I	LOH	E5	453 to 464 del 13 bp		Frameshift
2684	F	38	Trans WHO I	LOH	E3	354/7 to 356/9 del 3 bp (*CTT*)		
					E8	676 – 10 to –11 del 2 bp (*tg*)		Splice acceptor?
2818	F	19	Trans WHO I	LOH	E11	1021 C → T	Arg341X	Nonsense
3300	F	80	Trans WHO I	–	E7	675 + 1 to +3 del 3 bp (*gtg*)		Splice donor
784	F	53	Trans WHO I	NI				
2080	F	72	Trans WHO I	12				
2260	F	43	Trans WHO I	12				
634	F	71	Men WHO I	LOH	E3	241 – 2 to –9 del 8 bp		Splice acceptor
678	F	59	Men WHO I	LOH	E8	784 C → T	Arg262X	Nonsense
942	F	75	Men WHO I	LOH	E7	600 – 3 c → g		Splice acceptor
2122	M	53	Men WHO I	LOH	E6	551 G → A	Trp184X	Nonsense
2172	F	39	Men WHO I	LOH	E11	1082 to 1106 del 25 bp	Leu361fs → 367X	Frameshift
2418	F	63	Men WHO I	LOH	E3	346 del 1 bp (*C*)	His116fs → 123X	Frameshift
618	M	63	Men WHO I	12				
654	F	41	Men WHO I	12				
660	F	70	Men WHO I	12				
774	F	59	Men WHO I	12				
834	F	63	Men WHO I	12				
886	F	53	Men WHO I	12				
930	F	53	Men WHO I	12				
982	M	53	Men WHO I	12				
2026	F	59	Men WHO I	12				
2042	F	66	Men WHO I	12				
2046	F	32	Men WHO I	12				
2072	F	57	Men WHO I	12				
2266	F	34	Men WHO I	12				
2310	F	69	Men WHO I	12				
2332	M	62	Men WHO I	NI				
2422	F	63	Men WHO I	12				
2688	F	43	Men WHO I	12				
2698	F	66	Men WHO I	LOH				

Mal, = anaplastic meningioma; atyp, atypical meningioma; fib, fibroblastic meningioma; trans, transitional meningioma; men, meningiothelial meningioma; 12, heterozygous; NI, noninformative; –, no data available.

*Numbering of bases showing alteration is given relative to the cDNA sequence with the initiator ATG beginning as base 1. All coding sequences are given in upper case. When the alteration affects intronic sequence, it is presented in lower case and numbered as – (5′ intron) or + (3′ intron) the requisite number of bases from the first or last base of the exon, respectively. For deletions, the span of deleted bases (numbered as above) is given, followed by the deletion size (del). For deletions of less than 5 bp, the deleted bases are also named. When the start position of the deletion is uncertain, the alternative ranges of bases deleted are shown. Insertion is indicated by ins followed by the number of bases inserted and their identity.

†Original amino acid and position of the residues in the protein (numbered from the initiator Met as 1) is followed by the new amino acid for a missense mutation, X for a nonsense mutation, or fs for a frameshift, followed by the position of the next in-frame stop codon.

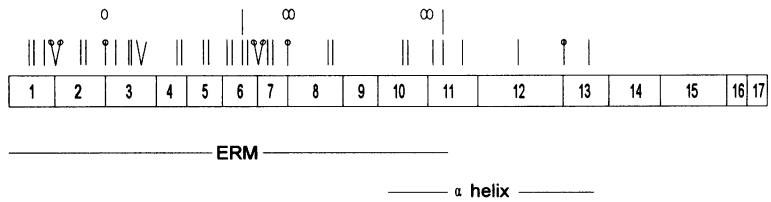

Figure 2. *Distribution of NF2 gene mutations. All mutations were located in the first 13 exons of the NF2 gene, ie, the region homologous with the protein 4.1 family. ERM, ezrin-moesin-radixin homologous region; vertical line, exonic mutation; vertical line with circle on top, mutation in splice site; 0, intronic mutation; two vertical lines on top of each other indicate identical mutations.*

dixin, moesin homologous domain of merlin associates with the cell membrane whereas the protein 4.1 homologous α-helical region interact with structures of the cytosol. Mutations in either of these regions would impair the function of merlin. Mutations in the carboxyl-terminal region of merlin may not be sufficient for inducing meningiomas. An analysis of the NF2 gene in schwannomas, however, has shown mutations in the carboxyl-terminal region of merlin.[11]

The combined analysis of molecular genetic and histopathological findings yielded a striking association. NF2 mutation rates differed significantly between histological subtypes of meningiomas. Fibroblastic meningiomas, which are characterized by fibrous appearance and abundant reticulin, had NF2 mutations in 7 of 10 (70%) cases. On the other hand, meningiothelial meningioma, characterized by syncytial growth and paucity of reticulin, had NF2 mutations in only 6 of 24 (25%) cases. Transitional meningiomas appearing as intermediate forms with features of both fibroblastic and meningiothelial tumors had NF2 mutations in 15 of 18 (83%) cases. The atypical and anaplastic meningiomas showed mutations in 7 of 10 (70%) and 6 of 6 (75%) cases, respectively. This indicates that meningiothelial meningiomas have a significantly lower rate of mutation in the NF2 gene than other histopathological subtypes ($P < 0.001$). With a 25% incidence of mutations, the NF2 gene appears to be involved in at least a fraction of meningiothelial meningiomas as well. However, it remains to be examined whether the meningiothelial meningiomas with NF2 mutations represent purely meningiothelial tumors or transitional meningiomas with a prominent meningiothelial component. At present, genes affected in the majority of meningiothelial meningiomas are unknown. Genes for products that functionally interact with merlin may be involved in this subgroup. Alternatively, NF2-related genes could be altered in meningiothelial meningiomas. The high sequence homology of the first 11 exons of the NF2 gene with the moesin, ezrin, and radixin genes and the predominant occurrence of NF2 gene mutations in this homologous region may point to a potential role of this gene family.

The nearly identical rate of NF2 mutation in fibroblastic (70%) and transitional (83%) meningiomas suggests that these tumors have a common pathogenetic origin. In our study, atypical meningioma, WHO grade II, and anaplastic meningioma, WHO grade III, ie, variants with an increased rate of recurrence, have incidences of NF2 mutations of 70 and 75%, respectively, which equals the incidences in fibroblastic and transitional meningiomas. One potential conclusion from these data is that atypical and anaplastic meningioma arise through progression from fibroblastic or transitional meningiomas. In this model, NF2 mutations would not participate in the development of malignancy. Therefore, other genes must play a role in malignant progression of meningiomas. A potential candidate locus has recently been described on the short arm of chromosome 1.[18]

In our study, 34 of 39 (87%) of the meningiomas with LOH22 but only 4 of 26 (15%) of the meningiomas without LOH22 had NF2 mutations. This shows a tight association of LOH22 with NF2 mutations ($P < 0.002$). This correlation was established for all meningioma subtypes including meningiothelial meningiomas that showed considerably lower LOH22 and NF2 mutations. These data strongly support the hypothesis that NF2 represents the meningioma locus on chromosome 22. Additional meningioma-associated genes on chromosome 22 have been proposed.[19] However, the present study has not provided evidence for such additional loci. Of 39 meningiomas with LOH22 in the present series, only 5 (13%) were without NF2 mutations. This most likely reflects the expected 85% detection rate of our SSCP assay.[20]

In conclusion, our study shows that the NF2 gene is mutated in the majority of sporadic meningiomas, indicating that NF2 plays a critical role for the pathogenesis of these tumors. The significant difference in the incidence of NF2 mutations between distinct histological variants of meningiomas strongly suggests that meningiomas arise through different molecular genetic pathways. A potential future implication of these findings would be the molecular classification of meningiomas into subtypes with NF2 mutations and with mutations in yet unknown genes.

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