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Mosaic Neurofibromatosis Type 1 With Multiple Cutaneous Diffuse and Plexiform Neurofibromas of the Lower Leg

REINHARD E. FRIEDRICH[1], CHRISTIAN HAGEL[2], FELIX K. KOHLRUSCH[1], INA SCHANZE[3], ILSE WIELAND[3] and MARTIN ZENKER[3]

[1]*Department of Oral and Craniomaxillofacial Surgery, Eppendorf University Hospital, University of Hamburg, Hamburg, Germany;*
[2]*Institute of Neuropathology, Eppendorf University Hospital, University of Hamburg, Hamburg, Germany;*
[3]*Institute of Human Genetics, Otto von Guericke University Magdeburg, Magdeburg, Germany*

Abstract. *Background: Neurofibromatosis type 1 (NF1) is an autosomal dominant hereditary disease with complete penetrance and a very variable phenotype. Recent research has shown that postzygotic NF1 gene mutations occur to a far greater extent than previously thought. The phenotype of affected individuals reflects the time of somatic mutation and the phenotype is correspondingly diverse. This report describes histological and genetic findings in a case of mosaic NF1, the clinical control of which documents almost stationary skin findings over a period of 9 years. Case Report: The 55-year-old female first presented for advice on a strip of nodular skin tumours of the calf skin. She had no hallmarks of NF1. It was only 9 years later that she had the skin tumours removed, all of which were partially diffuse and partially plexiform neurofibroma. The genetic examination showed an atypical large deletion of the NF1 gene in the skin tumours, but not in overlying skin or blood. Conclusion: Segmental NF1 is a distinct type of mosaic/somatic NF1 mutation. The phenotype of diffuse and plexiform skin neurofibromas can resemble cutaneous neurofibroma. Surgical therapy for segmental neurofibromatosis does not differ from the concepts for treating nerve sheath tumours in NF1 patients with a germline NF1 mutation.*

Neurofibromatosis type 1 (NF1) is an autosomal dominant hereditary disease with complete penetrance and a very variable phenotype (1). The genetic cause of the disease is a mutation on the gene locus 17q11.2 (2). The gene product, called neurofibromin, presumably has several functions and shows properties of a tumour suppressor (3). Neurofibromin is particularly frequently detected in neural cells in the post-embryonic phase of life (4). The development of tumours in NF1 follows Knudson's two-hit hypothesis describing the mutational events in the course of tumorigenesis (5). In addition to constitutive mutation, NF1-associated tumours or lesions exhibit a somatic mutation predominantly in neural-crest derived cells (6). NF1 is characterized by neoplasms that originate from nerve sheath cells or their precursors. The tumours arise particularly frequently and often in large numbers in skin and subcutaneous tissue (7). It has been known for some time that the disease can also arise from postzygotic mutations and that the *NF1* gene mutation status is thus mosaic (8, 9). Previously, this tumour phenotype, limited to individual compartments of the body, was referred to as segmental neurofibromatosis (10). The denomination is derived from the segmental spread of neurogenous tumours in a more or less delimited part of the body (1). Recent study has shown that the term 'segmental' neurofibromatosis is useful for many clinical assessments, for example, surgical treatment (11), but it provides an incomplete description of the genetic predisposition and phenotype of patients affected. In rare cases, mosaic forms are generalized and may remain undetected even in individuals that fulfil the clinical diagnostic criteria of the disease (12). For this reason, the term 'mosaic' NF1 is preferred in patients with postzygotic mutations of the *NF1* gene (12). Mosaic *NF1* is currently calculated to account for about 10% of patients with NF1 (12).

The report is about the genetic analysis of tumours of a patient with multiple cutaneous neurofibromas restricted to a distinct skin area.

Correspondence to: Professor Reinhard E. Friedrich, MD, DMD, Ph.D., FEBOMFS, Department of Oral and Craniomaxillofacial Surgery, Eppendorf University Hospital, University of Hamburg-Eppendorf, 20246 Hamburg, Germany. Tel: +49 40741053259, e-mail: rfriedrich@uke.de

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Case Report

A 55-year-old female visited the outpatient clinic of the Department of Oral and Craniomaxillofacial Surgery for the

first time for assessment of multiple skin tumours that had developed on the dorsal side of the lower leg. She was not able to tell exactly when the tumours had occurred, however, she had noted them several years earlier. The physical examination showed a strip of multiple, slightly raised, rounded, compressible skin tumours in the upper and middle area of the calf (Figure 1). The tumours had developed beneath intact and unremarkable skin. In this skin area and also on a skin tumour, there appeared to be small, healed scars that were interpreted as residues of previous biopsies. The patient recalled local excisions at these sites. However, she was unable to provide any information about the findings besides reporting that these tumours had been diagnosed as neurofibroma. The accumulation of skin tumours of this type was considered an indication of multiple cutaneous neurofibromas. However, visual diagnosis was preliminary and which tissue components really constituted the tumour were unknown. The patient had no other clinical signs or symptoms of NF1 and the family history did not indicate any manifestations of this disease, either for direct ancestors or for her own children. The patient was offered excision of the tumours, which she initially refused.

Nine years later, the patient returned for examination and advice, now with the request for treatment and genetic examination of the tumour material. The extent and number of tumours had remained almost unchanged during that time (Figure 1) but the patient was now worried that she had so far disregarded essential diagnostics for herself and her children. The patient consented in writing to the genetic examination of the tumour material and the publication of the test results in an anonymous form.

The skin tumours were excised and identified as individual tumours. Each tumour was divided and prepared for histological and genetic examination. Wounds were closed with sutures and healing was uneventful. After the findings were reported, the patient refused to take advantage of the additional outpatient care offered.

Histology. All three submitted skin tumour specimens of the lower leg were uniformly diffuse and plexiform neurofibromas growing diffusely in the subepidermal connective tissue and within nerve fascicles (Figure 2). The tumour cells expressed S100. The perineurium of the plexiform tumour parts was stained by antibody to epithelial membrane antigen. Antibody to neurofilament identified small nerve fibres. The proportion of Ki67-positive tumour cells was below 3% in all specimen.

Genetic analysis. Preparation of samples: An EDTA blood sample and three tissue samples from the region affected by neurofibromas were genetically examined. One tissue sample was dissected under macroscopy into overlying skin, one part apparently representing the tumour (neurofibroma 1), and a third fraction was adjacent soft tissue that could not be clearly classified macroscopically. These samples as well as the two separate pieces of tissue representing samples from additional tumours from that region (neurofibromas 2 and 3) were subjected to DNA extraction using QIAamp Fast DNA Tissue Kit (Qiagen, Hilden, Germany). Another part of neurofibroma 3 was used for RNA extraction (Qiagen RNeasy Mini Kit). cDNA was generated from the RNA according to standard procedures.

Analysis. In three samples macroscopically appearing to be independent tumours (neurofibromas 1-3) deletion of the entire *NF1* gene and adjacent genes was found using Multiplex ligation-dependent probe amplification (kits P081-D1, P082-C2; MRC Holland, Amsterdam, the Netherlands) but this was not the case in the tissue fraction of uncertain origin (soft tissue). The deletion was also not detectable in the leukocyte DNA nor in the apparently normal skin overlying one of the tumours. This constellation of genetic findings is to be expected for a mosaic form of NF1.

The deletion was mapped more precisely using a Genome-Wide Human SNP Array 6.0 (Thermo Fisher Scientific, Waltham, MA, USA), which was carried out on DNA from one tumour sample (neurofibroma 2). The tumour harboured a 3.2-Mb deletion in 17q11.2 encompassing *NF1* and at least another 51 genes. This deletion is considerably larger than the recurrent 1.5 Mb *NF1* microdeletions that occur as germline mutations in patients with NF1.

In DNA from the same tissue sample (neurofibroma 2), which clearly showed the *NF1* deletion (estimatedly in 50% of the cells), the *NF1* gene was additionally sequenced by next-generation sequencing with a minimum sequencing depth of 200× using a Nextera Rapid Capture Custom Enrichment Kit (Illumina, San Diego, CA, USA) and an Illumina MiSeq instrument. No *NF1* mutation (for example point mutation) detectable by sequencing was identified.

RNA analysis of tumour tissue (neurofibroma 3) also showed no evidence of a second mutation.

Interpretation of genetic findings. In accordance with the clinical phenotype of segmentally spreading neurofibromas, an atypical *NF1* deletion was detected in the DNA of three apparently separate tumours within the affected segment. The deletion was not detected in the covering skin or in the blood. This clearly confirms the mosaic status for the *NF1* deletion and suggests that it represents the first hit in the affected segment. We were unable to demonstrate a mutation on the second allele in one neurofibroma sample, in which the *NF1* gene was fully sequenced.

Discussion

This report describes the diagnosis and therapy of a mosaic form of NF1, in which the disease led to the development of

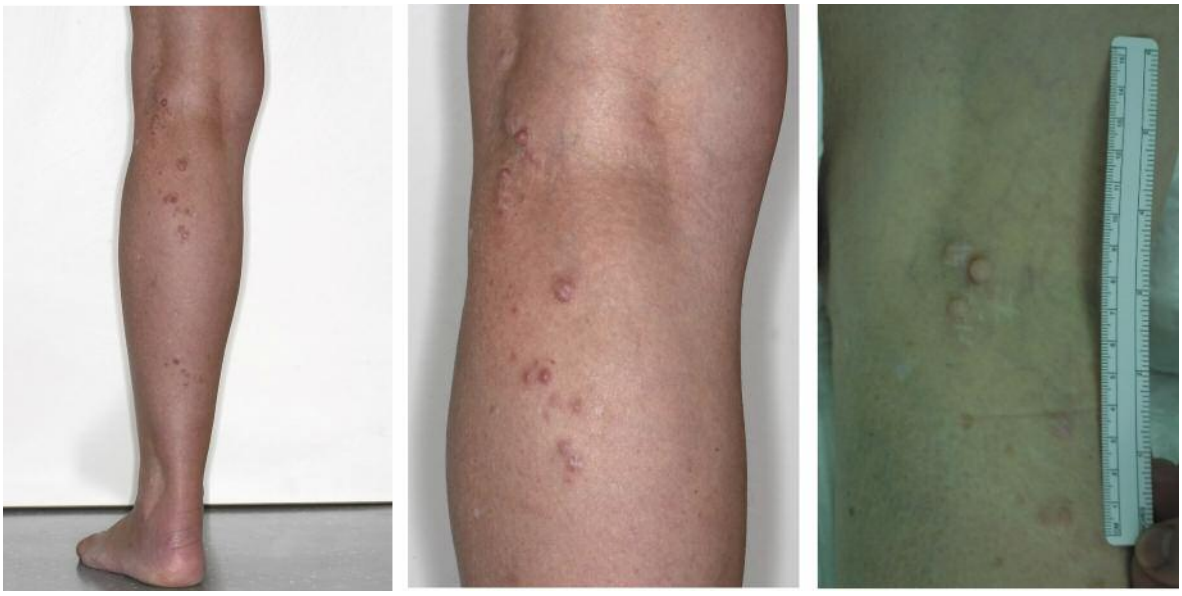

Figure 1. *Left: Dorsal aspect of left calf of the patient at first presentation. Center: Tumour region in close-up. Right: Same region 9 years later. Globular skin tumours of the calf and hollow of the knee had barely increased in size, except for one tumour in the central part of the photograph. Scars testify to previous excisions in this region. New, flat, tumour-like elevations had formed under some scars.*

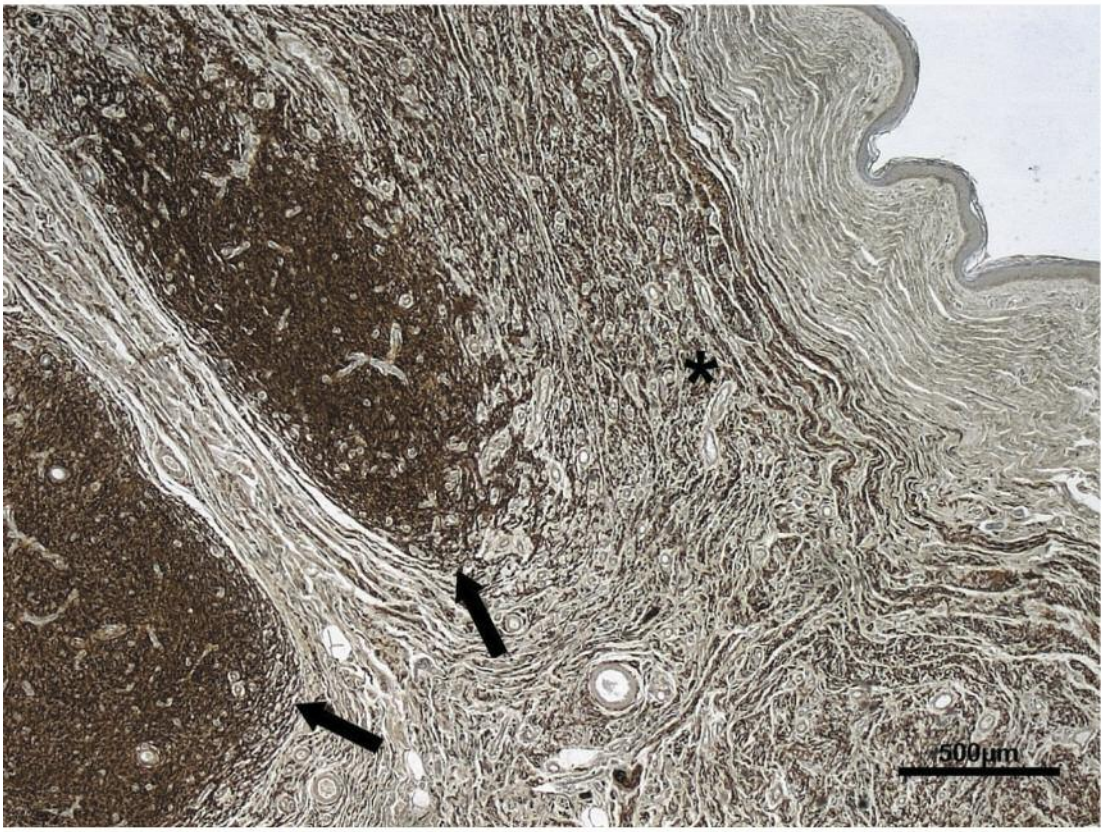

Figure 2. *Intracutaneous diffuse and plexiform neurofibroma. S100 immunohistochemistry (brown staining) depicting diffusely growing tumour cells with wavy contours in the subepidermal connective tissue (*) and sharply demarcated intraneural plexiform tumoural regions (arrows).*

typical tumours in a narrow area of the skin. In principle, the results are also characteristic of the manifestation of sporadic neurofibroma. However, a sporadic neurofibroma is expected to be a solitary tumour finding. In contrast, in the present case, a larger skin segment was found to be affected by tumours of this type, which also had a plexiform differentiation in their histological profile. Plexiform neurofibromas are a rare finding outside of NF1. Surgical therapy consisted of local excision of the tumours. Surgical practice does not differ from the concepts currently preferred for the treatment of NF1-associated skin tumours.

The findings are unusual for several reasons. The histological findings describe diffuse and plexiform neurofibromas. The shape, size and distribution of the skin tumours, based on the assumption of skin neurofibroma, suggested the diagnosis of cutaneous neurofibromas confined to a distinct area. Cutaneous neurofibromas are characterized by restricted growth capacity concerning both total volume and skin area invasion (7). Both the location and size of the lesions were within the expected range. By following-up the patient 9 years after the initial examination, it was demonstrated that the number and volume of the tumours had not noticeably changed during that time. This finding is typical of cutaneous neurofibromas, which usually persist in size after an initial, sudden growth phase associated with the perception of the tumour. Plexiform neurofibroma is rarely identified in mosaic forms of NF1 (13).

The histological diagnosis exemplifies the fact that plexiform neurofibroma does not necessarily refer only to extensive tumours. In clinical observation, the entity typically is associated with severe disfigurement (14). Plexiform neurofibroma is a histologically defined term and the entity is considered to be precancerous, from which malignant peripheral nerve sheath tumours (MPNST) can develop (14-16). Malignancy is a major reason for the lower life expectancy of patients with NF1 compared to the normal population (17). The lifetime risk of a patients with NF1 developing MPNST is much higher compared to that for the normal population (18). In particular, the location of precursor lesions of the trunk and extremities in patients with NF1 should be carefully checked for malignant transformation (19). However, plexiform neurofibromas located in the deeper part of the body are predominantly risk factors for MPNST (19). In the present case, the tumours were localized and arranged in a manner typical for the phenotype of cutaneous neurofibromas. The risk of developing malignant tumours in patients with the mosaic status of *NF1* mutation is unknown. It is also likely to depend on how many cells carry the somatic mutation.

This similarity of plexiform neurofibromas and cutaneous neurofibromas in the present case should also be taken into account when, as in a recent study, the aim is to differentiate the effect of medication on cutaneous neurofibromas according to a proposed classification of cutaneous neurofibromas (20).

The comprehensive molecular genetic analysis showed an unusual deletion that exceeded the size of the recurrent microdeletions typical of germline *NF1* mutations (21). Aside from intragenic *NF1* variants that account for the vast majority of germline mutations associated with NF1, an estimated 5-10% of all patients with NF1 have deletions encompassing the entire *NF1* gene and neighboring genomic regions. Of these, 70-80% are accounted for by a recurrent 1.4-Mb deletion (type 1 deletion), while atypical large deletions, as demonstrated in the present case, are very rarely observed as germline events (22). While the molecular test results confirmed that the clinical phenotype was related to a NF1 defect, it was not possible to completely uncover the genetic basis of tumour development and its chronological sequence, as we only identified the mutation on one allele in a tumour DNA sample. An explanation for this result may be a low level of mutation mosaicism on the second allele making it difficult to detect by cDNA or next-generation sequencing analysis at the applied sequencing depth, or a mutation on the second allele that cannot be detected due to other methodological limitations. The *NF1* deletion was detected in all three tumours, which emerged as distinct entities in a circumscribed skin area of an extremity. This constellation of the clinical and genetic findings makes it probable that the detected deletion was the constitutive *NF1* mutation of the affected nerve sheath cells in the tumour region.

Conclusion

In mosaic type NF1, mutations can occur that lie outside the typical *NF1* mutation spectrum. The histological diagnosis of the resection specimen is indispensable. Differences between clinical findings and histological diagnosis can be considerable. Surgical treatment consists of excising the tumours and covering the skin defects as inconspicuously as possible. The surgical concept does not differ from that for the treatment of cases with germline mutations of the *NF1* gene and local tumours of the skin.

Conflicts of Interest

The Authors state that there are no conflicts of interest regarding the publication of the article.

Authors' Contributions

REF treated the patient and designed the study. REF and FKK researched literature and designed the first article. IS, IW and MK performed the genetic studies. REF and MK carried out the final editing of the article. All Authors gave final approval for submission of the article.

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