

Atypical neurofibromatosis type 1 with unilateral limb hypertrophy mimicking overgrowth syndrome

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Summary

Neurofibromatosis type 1 (NF1; OMIM 162200), a dominantly inherited multitumor syndrome, results from mutations in the *Neurofibromin 1* (*NF1*) gene. We present the case of a Hungarian woman with the clinical phenotype of NF1 over her whole body and the clinical features of unilateral overgrowth involving her entire left leg. This unusual phenotype suggested either the atypical form of NF1 or the coexistence of NF1 and overgrowth syndrome. Direct sequencing of the genomic DNA isolated from peripheral blood revealed a novel frameshift mutation (c.5727insT, p.V1909fsX1912) in the *NF1* gene. Next-generation sequencing of 50 oncogenes and tumour suppressor genes, performed on the genomic DNAs isolated from tissue samples and peripheral blood, detected only wild-type sequences. Based on these results, we concluded that the patient is affected by an unusual phenotype of NF1, and that the observed unilateral overgrowth of the left leg might be a rare consequence of the identified c.5727insT mutation.

Neurofibromatosis type 1 (NF1; OMIM 162200) is a rare monogenic disease with autosomal dominant inheritance, caused by mutations in the *Neurofibromin 1* (*NF1*) gene.¹ The clinical features of NF1 involve pigmentary changes such as café-au-lait macules and axillary freckling, development of cutaneous fibromatous tumours, and development of hamartomas of the iris known as Lisch nodules.¹ Besides these symptoms, central nervous system and skeletal abnormalities (scoliosis, pseudoarthrosis of the tibia, macrocephaly and short stature) can occur.^{2,3} Frequently reported vascular symptoms include vascular dysplasia with cerebral, gastrointestinal and/or renal involvement, and renovascular hypertension.^{2,3}

NF1 may be caused by many different loss-of-function mutations of the *NF1* gene.¹ In about 5% of

patients with NF1, microdeletions of the *NF1* gene and/or the surrounding genomic region are responsible for the disease.⁴ In patients with *NF1* microdeletion, somatic overgrowth has been frequently observed.⁵ Mosaic overgrowth affecting a well-defined region of the body have only been rarely reported in NF1.

We report the case of a Hungarian woman presenting with the classic clinical features of NF1 and with unusual unilateral overgrowth of her left leg. The symptoms of NF1 were visible over her whole body, while the overgrowth was present only in her left leg. Our aim was to identify the underlying genetic abnormality of this unusual case and determine whether the patient is affected by two independent diseases or whether the symptoms of the left leg are an unusual manifestation of NF1.

Report

A 52-year-old Hungarian woman presented with an unusual phenotype, exhibiting the clinical features of

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NF1. The typical skin symptoms of NF1 were present in the patient, including neurofibromas and cafe-au-lait macules on her body (Fig. 1a,b), and axillary freckling. Ophthalmological examination determined the presence of Lisch nodules. Imaging studies did not find any indication of central nervous system malignancies. Skeletal abnormalities such as scoliosis, tibial pseudoarthrosis, short stature and macrocephaly, were not present. Based on the clinical symptoms, the diagnosis of NF1 was established.

However, in addition to the aforementioned clinical features, the patient was also noted to have hypertrophy of the left leg, resulting in significant differences in the circumference and length of the legs (Fig. 1c,d). This abnormality of the left leg was already present at birth. Imaging studies verified unilateral osteohypertrophy affecting the left leg. The patient was not aware of any relevant chronic diseases or other family members with NF1 or with overgrowth syndromes (Fig. 1e).

Following ethics approved by the internal review board of the University of Szeged (Szeged, Hungary), genetic studies were carried out, conducted according to the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants. Tissue biopsy of the left leg and blood sample were taken from the patient, and genomic DNA was isolated (BioRobot EZ1 DSP Workstation; QIAGEN, Hilden, Germany). Her family members were unwilling to provide material for genetic investigation.

To confirm NF1, the coding regions and flanking introns of the *NF1* gene were amplified and sequenced. Direct sequencing of genomic DNA isolated from the peripheral blood sample revealed a novel frameshift mutation (c.5727insT, p.V1909fsX1912) in exon 39 of the *NF1* gene (Fig. 2). The patient carried the mutation in heterozygous form (Fig. 2a), while unrelated controls ($n=50$) carried the wild-type sequence (Fig. 2b).

To verify overgrowth syndrome, the coding regions and flanking introns of the *PIK3CA* gene were amplified and sequenced. As traditional sequencing of genomic DNA isolated from the peripheral blood and from the tissue sample of the left leg did not identify any putative causative variant of the *PIK3CA* gene, mutational hotspots were screened using an in-house PCR-based restriction fragment assay.⁶ As these also did not identify any overgrowth-causing mutation, next-generation sequencing of approximately 2800 mutations from 50 oncogenes and tumour suppressor genes recorded in the Catalogue of Somatic Mutations in Cancer (COSMIC) was performed (Ion AmpliSeqTMv Cancer Hotspot Panel v2; Thermo Fisher Scientific, Waltham, MA, USA), but also did not identify any putative causative variant that could be responsible for the overgrowth of the left leg.

This patient presented with the clinical features of both NF1 and left leg overgrowth. Such an atypical clinical form of NF1 has not been reported previously. However, the phenomenon of 'vascular neurofibromatosis phenotype' and the relatively frequent

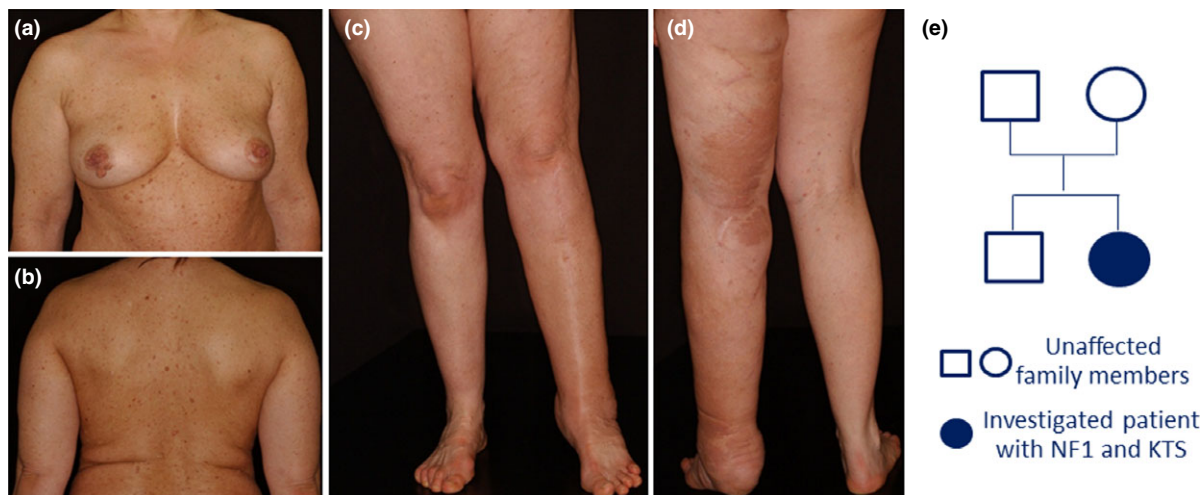


Figure 1 An unusual phenotype of neurofibromatosis (NF1) in a 52-year-old Hungarian woman. The patient presented with the clinical phenotype of NF1 over her whole body, including (a) her chest and (b) back. (c,d) Unilateral osteohypertrophy affected the left leg. (e) The rest of the patient's family were clinically asymptomatic.

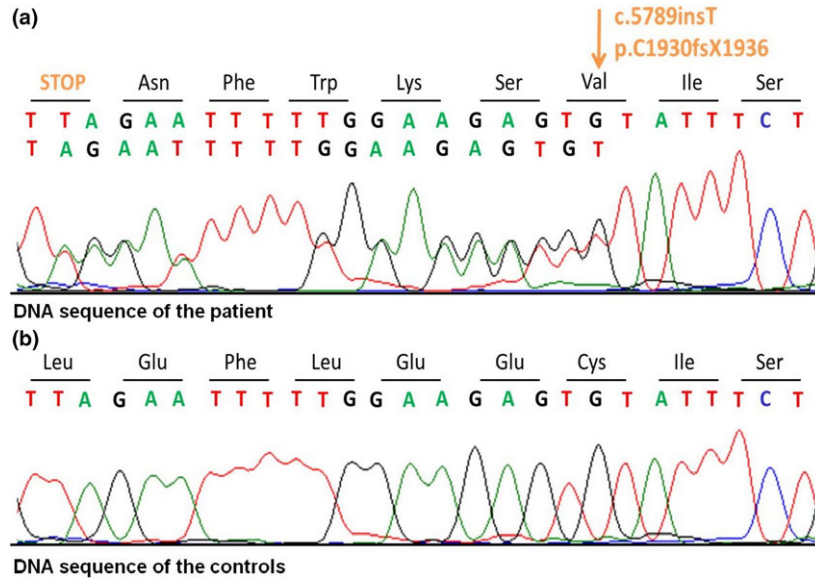


Figure 2 (a,b) Direct sequencing of the *NF1* gene. Direct sequencing revealed a novel single-nucleotide insertion resulting in a frameshift and the formation of premature termination codon (c.5727insT, p.V1909fsX1912). (a) The patient carried the mutation in heterozygous form. (b) Unrelated, healthy controls carried only wild-type sequences.

association of NF1 with vascular dysplasia have been frequently reported in the literature.⁷ NF1 can also be accompanied by skeletal abnormalities, such as sphenoid wing dysplasia, macrocephaly, scoliosis, vertebral disc dysplasia, pseudoarthrosis of tibia and short stature.^{8,9} Therefore, it was important to distinguish whether the patient was affected by two independent rare diseases or the symptoms of the left leg were the results of the atypical vascular and skeletal manifestations of NF1.

Our results demonstrated a novel heterozygous single-nucleotide insertion in the *NF1* gene, leading to a frameshift and the formation of a premature termination codon (c.5727insT, p.V1909fsX1912). Because this mutation was present in the genomic DNA isolated from the peripheral blood of the patient, we suggest that this sequence change is a germline mutation. Considering that there is no other clinically affected member in the patient's family and that NF1 exhibits autosomal dominant inheritance, we hypothesize that this is a *de novo* mutation of the *NF1* gene. The genotype–phenotype correlation is generally poor in NF1, with the exception that patients with large deletions in the *NF1* gene tend to have severe phenotypes.¹⁰ *NF1* mutations usually result in loss of tumour suppressor function by disrupting the NF1 protein's ability to maintain the proto-oncogene *RAS* in an inactive form.¹¹ We suggest that this novel *NF1* mutation is the causative mutation for the

development of NF1, and that it is highly possible that the presence of the unilateral limb hypertrophy is also the consequence of this mutation.

In conclusion, we report a patient with both the clinical features of NF1 and overgrowth of the left leg. To our knowledge, our study is the first to clearly elucidate the genetic background of such a complex case, and further confirms the causative role of the somatic mutations of the *NF1* gene in the development of overgrowth.

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Learning points

- The case reported in this study was presented with the clinical features of both NF1 and left leg overgrowth.

- The unusual clinical phenotype including the left leg overgrowth is the atypical manifestation of NF1.
- The patient carries a disease-causing germline mutation of the *NF1* gene.
- The patient does not carry any somatic mutation of *PIK3CA* or other genes.
- Our study elucidates the genetic background of the investigated case with unusual phenotype, and confirms that the observed left leg overgrowth is the consequence of NF1.

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