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Novel neurofibromatosis type 2 mutation presenting with status epilepticus

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ABSTRACT – Neurofibromatosis type 2 (NF2) is a dominantly inherited syndrome caused by mutations of the tumour-suppressor *NF2*, which encodes the merlin protein. Mutations are associated with a predisposition to development of benign tumours in the central nervous system. Even though cerebral cortical lesions are frequently associated with seizures, epilepsy is rarely described in NF2. Here, we describe an adult case of NF2 in which the onset of symptoms was characterised by status epilepticus. In this patient, we identified the novel c.428_430delCTTdel mutation in *NF2*, involving the amino-terminal FERM domain, which is fundamental for the correct tumour suppressor function of the protein. Bioinformatic analyses revealed an important structural perturbation of the FERM domain, with a predicted impairment of the anti-tumour activity.

Key words: neurofibromatosis type 2, epilepsy, status epilepticus, *NF2*, merlin, novel mutation

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Neurofibromatosis type 2 (NF2) is an autosomal dominant inherited disorder caused by various mutations of the tumour suppressor gene *NF2* (which encodes for the merlin protein) on chromosome 22, leading to the development of multiple tumours of the nervous system (Evans, 2009). Patients typically develop schwannomas affecting both vestibular nerves, leading to deafness. Other benign tumours, generally slow growing (typically meningioma, schwannoma, and ependymoma), occur in a significant proportion of patients (50-60% of cases) and involve the brain and spinal cord (Evans *et al*., 2009). Amongst the different manifestations of the disease, epilepsy is described only in a few cases (Evans *et al*., 1992; Parry *et al*., 1994; Mautner *et al*., 1996; Ruggieri *et al*., 2005). Even though brain tumours are

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frequently associated with epilepsy (Michelucci *et al*., 2013), the type and frequency of seizures in NF2 remain to be adequately characterised. Interestingly, a recent retrospective study reported that in NF1, 9.5% of subjects had at least one unprovoked seizure during life and 6.5% had documented epilepsy (Ostendorf *et al*., 2013). Herein, we describe an unusual clinical presentation of NF2 in an adult patient carrying a novel mutation in the candidate gene *NF2*.

Case study

A 23-year-old male patient of South American origin, with unremarkable medical history, presented to our department with a first tonic-clonic generalised seizure, followed by a prolonged loss of consciousness longer than 30 minutes. The EEG during loss of consciousness revealed a generalised continuous epileptiform activity with a frequency of 2-2.5 Hz (*figure 1A*), prevalent on the left hemisphere and consistent with status epilepticus (*SE*). The patient was treated with intravenous 10 mg diazepam, with rapid recovery of consciousness and amelioration of the neurophysiological pattern (*figure 1B*). The remaining neurological examination resulted normal. In the following days, the patient presented with other tonic-clonic generalised seizures and was therefore treated with valproate (1,000 mg daily), without reporting further events. On admission, CSF and blood examination were normal, including bacterioscopic and virological tests; alcohol and drug intake was excluded. Brain and spinal cord MRI revealed the presence of bilateral acoustic neurinomas (*figure 1C*) and multiple tumour lesions bilaterally spread in the cortex (*figure 1D*) and cervical cord (*figure 1E*). Brain CT excluded the presence of cerebral calcifications. Audiological and ophthalmological examinations resulted within the normal range. A dermatological evaluation showed the presence of small *café-au-lait* spots.

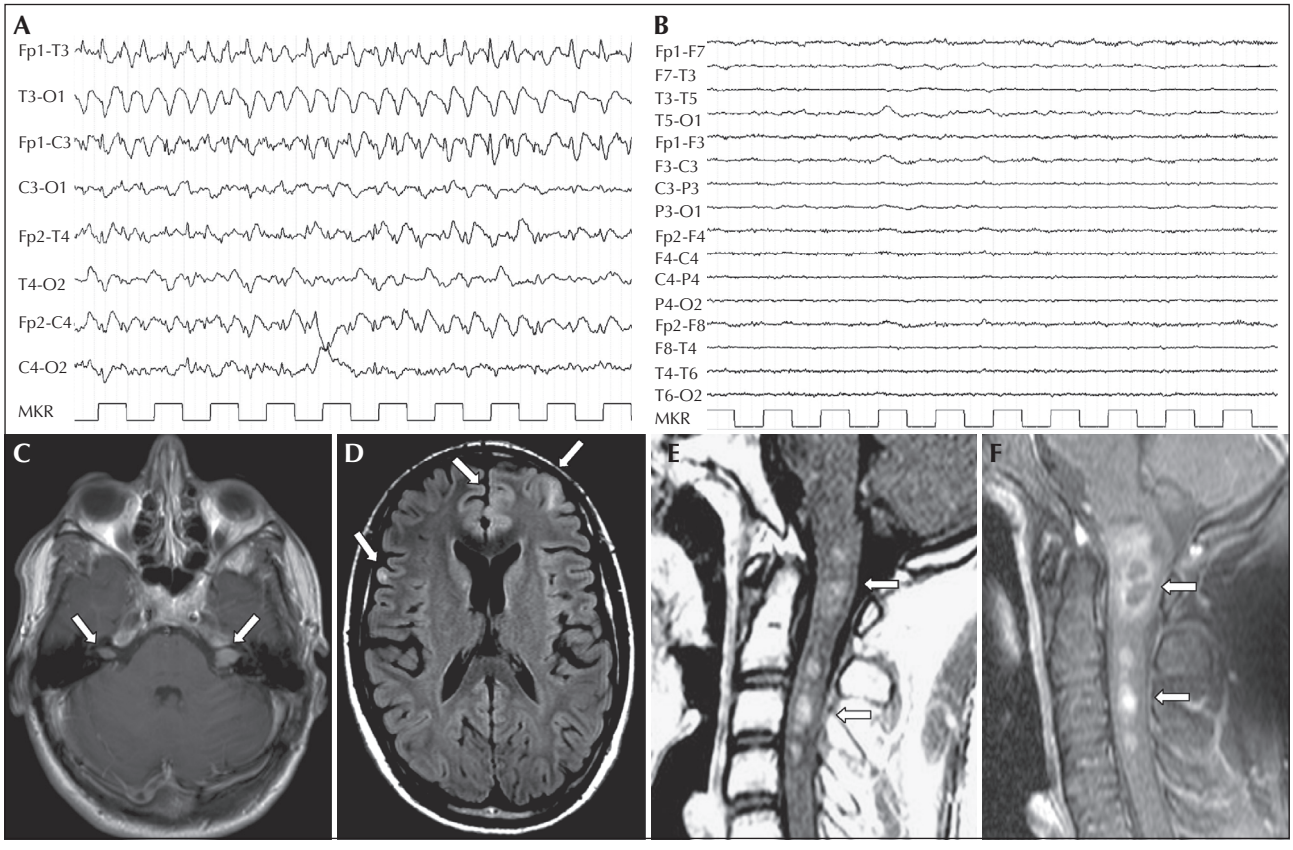

Figure 1. Electroclinical and radiological features of the patient.
(A) First EEG assessed during patient's loss of consciousness showing generalised continuous epileptiform activity prevalent on the left hemisphere, consistent with SE. (B) Treatment with benzodiazepine led to the disappearance of the epileptiform activity. (C) Axial brain MRI showing bilateral acoustic neurinomas (T1 with Gadolinium; arrows) and multiple small tumour lesions, bilaterally spread in the cortex (D; fluid-attenuated inversion recovery-FLAIR; arrows). (E) Sagittal cervical spinal cord MRI showing multiple tumour lesions involving the brainstem and cervical spinal cord, characterised by contrast enhancement (T1 with Gadolinium; arrows). (F) Follow-up MRI, four years later, demonstrating the enlargement of the brainstem lesion, which assumed a cystic shape (T1 with Gadolinium; upper arrow).

According to the Manchester criteria, diagnosis of NF2 was made upon clinical and radiological findings (Evans *et al.*, 1992). In the absence of any other predisposing causes, the epileptic seizures, including SE, were linked to the brain cortical lesions. After four years of clinical stability, without presenting further seizures, the patient presented a slowly progressive left hemiparesis, mainly in the lower limb. In accordance with this evidence, the MRI showed an evident increase of the cervical lesion (*figure 1F*).

Interestingly, the patient's direct relatives along the maternal family line were affected by both bilateral acoustic neurinoma and unclassified "brain tumours", highly suspicious of NF2 (*figure 2A*). It is not clear whether these subjects, all deceased at young age, presented epilepsy during life.

Genetic analysis

Given the clinical features of NF2, the patient underwent genetic screening of *NF2* to support the diagnosis. High molecular weight genomic DNA was extracted from whole blood by phenol/chloroform

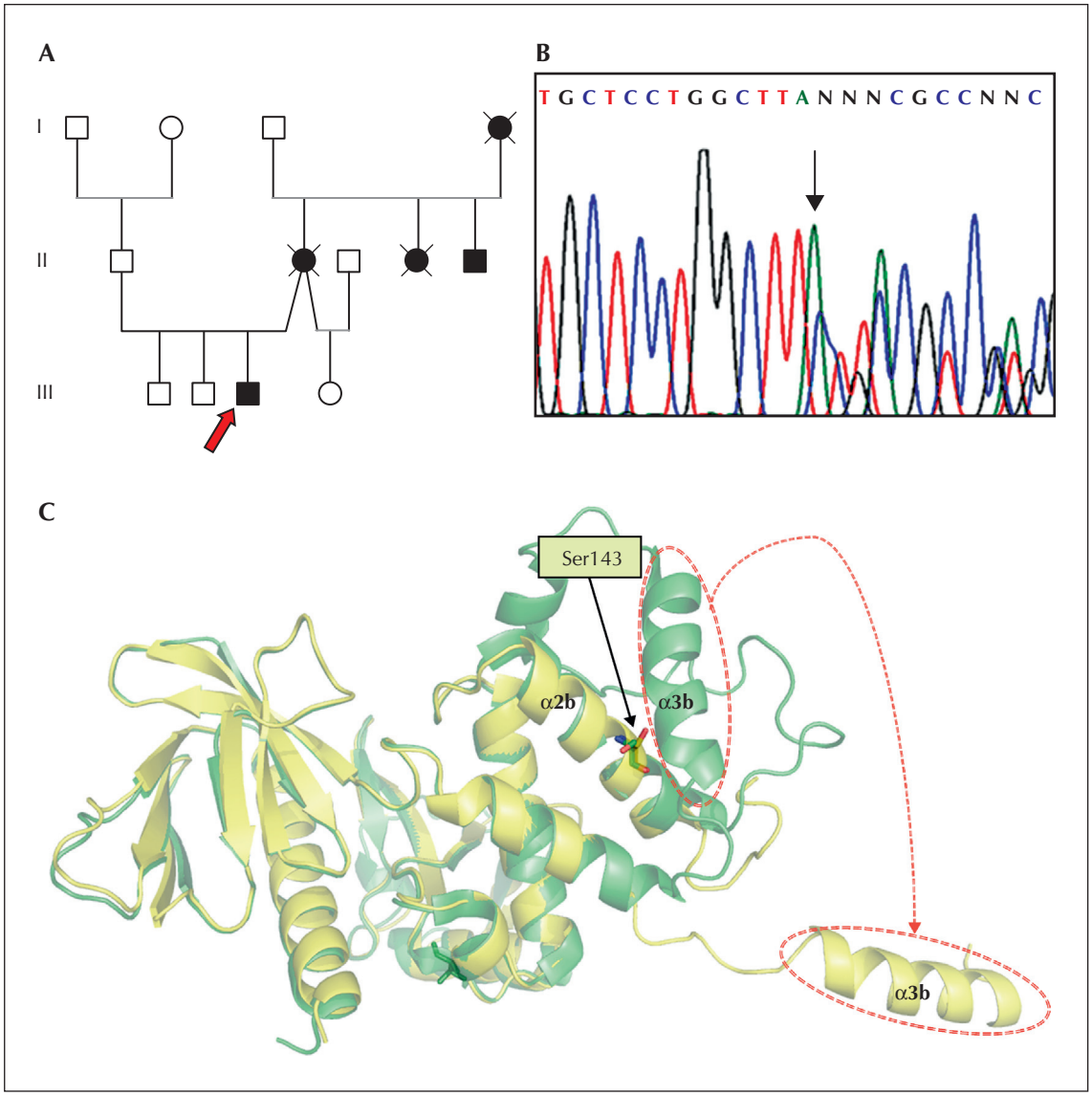

Figure 2. Genetic and bioinformatic aspects of the novel *NF2* p.Ser143del mutation.
(A) The patient's family tree indicating the autosomal dominant transmission of the disease along the maternal line (red arrow indicates the proband carrying the *NF2* novel mutation; black symbols indicate clinically suspected *NF2*). (B) The novel c.428_430delCTT deletion in *NF2* leading to the loss of serine amino acid at position 143. The merlin protein is presented with two alternative conformations, "open" (yellow) and "closed" (green), of the FERM domain. (C) According to *Protein Prediction*, the novel *NF2* p.Ser143 deletion is localised within the mobile portion of the protein, suggesting that this mutation could critically interfere with merlin activity.

extraction and ammonium acetate/ethanol precipitation. The entire coding sequence of *NF2* gene was amplified using primers flanking the intron-exon boundaries (primer sequences available on request). Following purification with QIAquick PCR purification kit (Qiagen, Hilden, Germany), PCR products were sequenced on both strands using the BigDye Terminator v1.1 Cycle Sequencing Kit (Life Technologies Applied Biosystems) and a model 310 automated sequencer (Life Technologies Applied Biosystems). Mutation nomenclature follows the Human Genome Variation Society recommendations (http://www.hgvs.org/mutnomen/). The DNA mutation numbering is based on the *NF2* cDNA sequences (GenBank accession number NM181832.2) with the A of the ATG translation-initiation codon numbered as +1. Amino acid numbering starts with the translation initiator methionine as +1.

Consistent with the clinical diagnosis, the proband's genetic analysis of *NF2* revealed the presence of the novel heterozygous mutation c.428_430delCTT (*figure 2B*), leading to the loss of amino acid serine at position 143 (p.Ser143del). This variant is not present in dbSNP (http://www.ncbi.nlm.nih.gov/snp/) and in the NHLBI Exome Sequencing Project (http://evs.gs.washington.edu/EVS/) databases. This residue, highly conserved within different species, is located in the F2 motif of the FERM domain of merlin, which plays a crucial role in the growth-suppressive function of the protein (Mani *et al*., 2011). Of note, according to *Protein Prediction* analysis, Ser143 deletion induces the loss of the correct three-dimensional conformation of FERM (*figure 2C*), possibly interfering with the tumour-suppressive activity of merlin (for details see the *Supplementary data section at the end of this paper*). This represents a strong indication that this mutation is pathogenetic of the disease. Extending the genetic screening to the patient's healthy siblings (generation III in the family tree; *figure 2A*) did not reveal any mutation in *NF2*, however, we could not retrieve DNA from the relatives with suspected NF2 (generation I and II).

Discussion

Here we describe an adult case of clinically defined NF2, in which the symptoms at onset were characterised by SE. To our knowledge, there are no previous reports of NF2 with evidence of SE, either at onset or during the development of the disease. Of note, seizures do not represent one of the clinical hallmarks for the diagnosis, which is surprising, considering the presence of brain tumours typical of the disease. According to this case and the few descriptions present in the literature (Evans *et al*., 1992; Parry *et al*., 1994; Mautner *et al*., 1996; Ruggieri *et al*., 2005), the frequency of epilepsy may be underestimated in these patients and studies aimed at the evaluation of the impact and characteristics of seizures in NF2 should be assessed. The identification in this patient of the p.Ser143del mutation on the *NF2* gene, strongly corroborates the clinical diagnosis. According to bioinformatic analysis, this novel mutation is predicted to deeply modify the α-helical structure of merlin, leading to important modifications of the physiological tumour suppressive function of the protein. These data extend the spectrum of the possible clinical presentations of NF2 and advance our knowledge of the underlying pathogenetic mechanisms. □

Acknowledgments and disclosures.
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None of the authors have any conflict of interest to disclose.

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Supplementary data *Protein prediction* of the structural changes determined by the novel c.428_430delCTT mutation on the *neurofibromatosis type 2* gene

The neurofibromatosis type 2 tumor suppressor protein (merlin) is an important mediator of contact inhibition. Binding to the amino-terminal FERM domain is required for its conformational activation, proper subcellular localisation and correct tumour suppressor function. The association with the membrane is important for the growth-regulatory function of merlin (Mani *et al*., 2011).

In order to predict the possible conformational changes of the secondary structure of merlin determined by the c.428_430delCTT mutation, both the wild type and the mutated amino acid sequences of the protein were submitted to the *Predict Protein* server (https://www.predictprotein.org). The simulation of the protein's secondary structure did not reveal any change in the helical content of the mutated form, suggesting that Ser143 deletion does not impair the formation of the α2b-helix, comprised between residues Pro135 and Tyr150. The F2 structural motif (comprised between residues 101–158 and 178–215) can be found in the *Protein Data Bank* (PDB, http://www.rcsb.org/pdb) in two different conformations. The first structure (PDB reference code 1H4R) shows that the Pro181-His195 α3b-helix is located in front of α2b-helix (containing Ser143) and adopts a closed conformation (see figure 2C, page 134 of this paper) (Kang *et al*., 2002). In this structure, Ser143 stabilizes a closed conformation establishing an hydrogen link with the side chain of His195, located on α3b-helix. Furthermore, Ser143 strongly interacts with Leu140, Leu141, Tyr144 and Gln147 of the α2b-helix, thus stabilizing the α-helical arrangement in this protein region. The second structure (PDB code 3U8Z) reveals an open conformation, where the two helices in the F2 motif of the FERM domain are located remotely (see figure 2C, page 134 of this paper) (Yogesha *et al*., 2011). This conformation was observed following co-crystallization of the FERM head domain of merlin together with its tail domain. In this case, binding of the tail domain to the head would promote readjustment of the F2 motif, triggering the open conformation, an event that may direct merlin dimerization and/or its interactions with partners required for tumor suppression (Yogesha *et al*., 2011). In conclusion, the F2 motif is considered essential for binding to other partners and/or dimerization of merlin, which is required for the tumor suppressor functions of the protein. Thus, mutations found in the F2 domain may interfere with merlin anti-tumor activity.

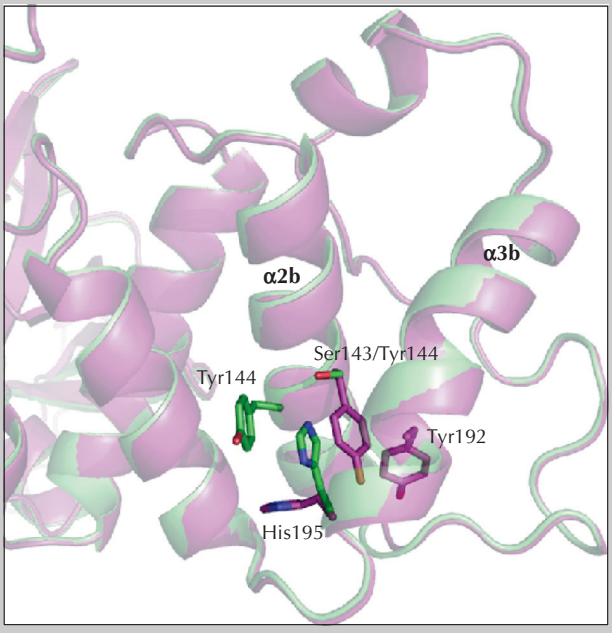

Figure S1. The mutated form of the F2 motif over Ser143 deletion predicted with 3D-JIGSAW (magenta) overlapping the wild-type structure of the FERM domain (green). The mutated form presents important structural rearrangements, involving the residues highlighted. In particular, Ser143 (green) location within the α2b-helix is occupied by Tyr144 (magenta) which, in the mutated form, is rotated by about 45° compared to the position assumed in the wild-type form (green). As a consequence, the His195 side chain on α2b-helix can switch conformation; the new location of Tyr144 appears incompatible with correct α2b/α3b packing, therefore hampering the F2 motif architecture.

The mutated amino acid sequence of merlin was then submitted to the 3D-JIGSAW server (Bates and Sternberg, 1999; Bates *et al*., 2001; Contreras-Moreira and Bates, 2002) (http://bmm.cancerresearchuk.org/~3djigsaw/) to model the structure of the α2b-helix carrying the p.Ser143 deletion (*figure S1*). This mutation induces a shift of all the residues located after the deletion (Tyr144 to Tyr150), resulting in a rotation along the helix axis, and thus altering the amino acid location within the protein.

Even if the α-helix is conserved in this region, Ser143 deletion leads to the loss of important interactions stabilizing the domain, such as Ser143-Tyr144 and Ser143-Gln147. In the prediction of the mutated structure, the location of Ser143 position is occupied by Tyr144 (*figure S1*) and, consequently, His195 side chain loses the interaction with the α2b-helix. Furthermore, an impairment of the packing of α2b-α3b is predicted by the presence of the bulky and polar residue Tyr144 (instead of Ser143) and the close interaction betweenTyr144 and Tyr192 side chains.

In conclusion, all the predicted effects of Ser143 deletion point to an important structural perturbation of the F2 motif that may affect the recognition of merlin's partners, leading to an impairment of the anti-tumour activity.

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