Malignant pheochromocytoma in a 16 year old patient with neurofibromatosis type 1

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Abstract

Patients with Neurofibromatosis type I (NF1) feature a high risk of developing benign and malignant tumors, mostly with a neuroectodermal origin, the risk being about four times higher than in the general population. Pheochromocytoma (PHEO) is a sporadic tumor (1:100000) arising from the adrenal medulla. PHEO is rare conditions in Neurofibromatosis type I (NF1) and occurs in about 1% of the patients, rarely in pediatric age. In this study we present a 16-year-old patient with NF1 and malignant PHEO. Loss of heterozygosity (LOH) analysis in PHEOs shows a reduction to homozygosity, observed for both 17p and 17q markers. This case confirms the importance of surveillance for malignant neoplasias in NF1 patients' childhood and adolescence. On the other hand, as 30% of PHEO had germline mutations and more rarely a somatic mutations, patients with PHEO should be investigated for associated genetic syndromes.

Keywords:

Malignant pheochromocytoma; Neurofibromatosis type 1; Loss of heterozygosity; Multicarcinoma syndromes

Introduction

Pheochromocytoma (PHEO) is a rare tumor with an incidence of 1 per 100,000 in the population; the rate of malignancy is about 10% [1]. Genomics of familial PHEOs have shown that even 30% of these tumors arise in individuals with specific mutations or hereditary cancer syndromes [2-4]. Recent studies have shown the involvement of RET, VHL, NF1, SDHAF2, SDHA, SDHB, SDHC, SDHD, TMEM127 and MAX genes in the pathogenesis of these tumors in children [1,5]. Moreover, latest transcriptomic and genomic studies have suggested that the genes involved in hereditary PHEO may be important players also in the sporadic disease [6]. These studies have shown that a large proportion (83%) of sporadic PHEO has an altered copy number in at least one of the known susceptibility genes, often in association with an altered messenger RNA (mRNA) expression; specifically, somatic NF1 mutations were frequent, suggesting that the NF1 gene constitutes the most frequent target of somatic mutations in sporadic PHEO [7-8]. Neurofibromatosis type I (NF1), also known as von Recklinghausen disease (OMIM*162200), is a dominant autosomal disorder characterized by *cafe-au-lait* spots, Lisch nodules in the eye, and skin neurofibromatous tumors. NF1 is caused by mutations in *NF1*, a tumor suppressor gene located on chromosome 17, its 58 exons spanning approximately 300kb. The genetic screening for *NF1* is rarely performed both because of the large size of the gene and because an accurate diagnosis of NF1 is generally possible based on its peculiar phenotype as well as on the family history.

Clinical Report and Methods

The patient is a 16-year-old male with a neurofibromatosis type 1 (NF1), diagnosed at birth based on clinical findings and family history (grandfather, uncle and mother with NF1). The mother died for breast cancer several years earlier. The patient was brought into the emergency department with a weeklong history of visual impairment, headache, nausea and vomiting. Abdominal echography and computed tomography showed an approximately 58 x 52 x 75 mm oval-shaped lesion in the left

adrenal gland. PHEO was considered as a potential cause of the symptoms. The blood pressure was 140-205 mmHg. The endocrinological examination results showed normal homovanillic acid levels (10 ugr/mg cre; reference values 0-38) following 24h urine collection and a markedly increased level of vanillylmandelic acid (49 ugr/mg cre; reference values 0-38). The aldosterone plasma level was high (450 pg/mL; reference values 12-150), the cortisol was increased (26 μg/dL; reference values 4-22). The neoplastic mass removed by surgery, 6 cm in diameter, originated from the left adrenal gland and looked as a capsulated, vessel‑rich tumor. Regional enlarged lymph nodes were also removed.

The surgical specimen was fixed in 4% phosphate buffered formalin, and 5-μm paraffin-embedded sections were stained with hematoxylin and eosin. For immunohistochemistry, antibodies against chromogranin, synaptophysin, and neuron specific enolase, (all antibodies, ready-to-use Dako, DK) were used (positive and negative control tissue were used). Subsequently, streptavidin-biotin-peroxidase staining was performed. Histological and immunohistochemical findings confirmed the diagnosis of PHEO (Fig.1). Due to the presence of neoplastic cells in a lymph node, the definitive diagnosis was malignant PHEO [9] (Fig.1D). The postoperative course was uneventful, the patient's blood pressure was 70-120 mmHg and he completely recovered.

NF1 associated with loss of heterozygosity (LOH) [10-11], along with the inactivation of the remaining NF1 wild type allele in tumor DNA, leads to PHEO. LOH is known to be caused by a number of mechanisms including deletions of genetic material and the loss of a whole chromosome by non-disjunction with or without reduplication. However, mitotic recombination has been demonstrated to be the most common event accounting for LOH in NF1-associated tumors [12]. For this reason genomic DNA was extracted from frozen tumor tissue and from peripheral blood following the QIAMP DNA mini kit protocol (QIAGEN). LOH was determined by typing genomic DNA samples with 21 microsatellite markers (Fig. 2C) scattered along the whole chromosome 17.

These markers were selected from the NCBI-UniSTS website (http://www.ncbi.nlm.nih.gov/unists). Primer sequences were obtained from the Genome Database (www.gdb.org) and from Garcia-Linares et al. [12] PCR was performed in 25-µl mixtures using Kapa2G Fast DNA polymerase (KAPA biosystem) with 1 minute first step denaturation at 95°C, followed by 30 cycles at 95°C (10 sec), 55°C (10 sec), and 72°C (1 sec).

The fluorescently labeled PCR products were analyzed by capillary electrophoresis on an ABI Prism 3130xl (Applied Biosystems). Peak analysis was performed by Genemapper 4.0 software (Applied Biosystems). Q^{LOH} was determined comparing the ratio of fluorescence intensities for each allele in tumor tissue (T) to that in whole blood (N) from the same patient: (T1/T2)/(N1/N2) where T1 is the diminished allele. Q^{LOH} values of 0.8 or less were scored as LOH [13]. LOH analysis displayed that 5 out of 21 microsatellite markers were not informative, the remainign16 detected LOH affecting the whole chromosome 17 in PHEO tissue (Fig. 2).

Discussion

PHEOs are rare (1:100000 in the general population), usually benign tumors that originate in the sympathetic nervous system; only 10% of them are malignant [1]. Generally PHEO is sporadic, but 30% are part of hereditary cancer syndromes. The clinical symptoms are due to increased secretion of catecholamines, especially adrenaline and noradrenaline that cause paroxysmal hypertension and tachycardia, headache, palpitation and weight loss.

Von Recklinghausen disease (NF1 syndrome) occurs in approximately 1:3000 individuals; the gene involved in its etiology [14], *NF1*, encodes for neurofibromin, a protein similar to to RAS/GTPase-activating protein. RAS has a role in controlling cell growth and differentiation through several signaling pathways, including the cAMP one [1]. Affected individuals are at risk of developing benign and malignant tumors, mainly of neuroectodermal origin, the risk being about four times as

high as in the general population [2]. LOH and loss of neurofibromin expression have been shown in mice model [15], in NF1 [10] and non-NF1 patients' tumors [16], including PHEO, neurofibrosarcoma, neuroblastoma, melanoma and malignant peripheral nerve sheath tumors (malignant transformation of plexiform neurofibromas) [11, 17-18].

Recently Bausch et al. studying 177 children with PHEO and paraganglioma, identified 142 (80%) individuals with a germline mutation in one of the susceptibility genes, 4% of which had a mutation in *NF1* gene [5]. In patients with NF1, it has been postulated that the tumor occurs according to Knudson's two-hit model, which requires biallelic inactivation of a tumor suppressor gene [16]. This theory has been confirmed by studies in murine models and humans [10, 17].

The association of PHEO with NF1 is recognized since a long time [19]. Usually, hereditary forms of PHEO are diagnosed earlier compared to sporadic cases [1]. The case herein reported had NF1 and malignant PHEO. The association of NF1 and malignant PHEO in adult is reported in 11.5% [19] and just recently described in childhood [5].

The patient's mother died with breast cancer several years earlier. Therefore it was not possible to study NF1 LOH and BRCA1/2 mutations in her neoplasia. The association between NF1 and breast cancer has been scarcely reported in the literature [20]. However, it is renowned that both NF1 and BRCA1 are located in the long arm of chromosome 17. Women with NF1 mutations have shown to have a higher risk of developing breast cancer, compared with the general population [20].

These multicarcinoma syndromes are usually inherited in an autosomal dominant way, so early diagnosis and treatment is important, especially in cases of malignant, multiple tumors or in a young onset age.

In summary, patients with NF1 are at high risk of developing benign neoplasias; rarely malignant neoplasias may arise. Malignant PHEO could be one of these, also in childhood. Therefore genetic diagnosis and specific management of family members with a neoplastic history could play an

important role in preventive medicine, in the implementation of early and appropriate treatment and in long-term surveillance.

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199

200

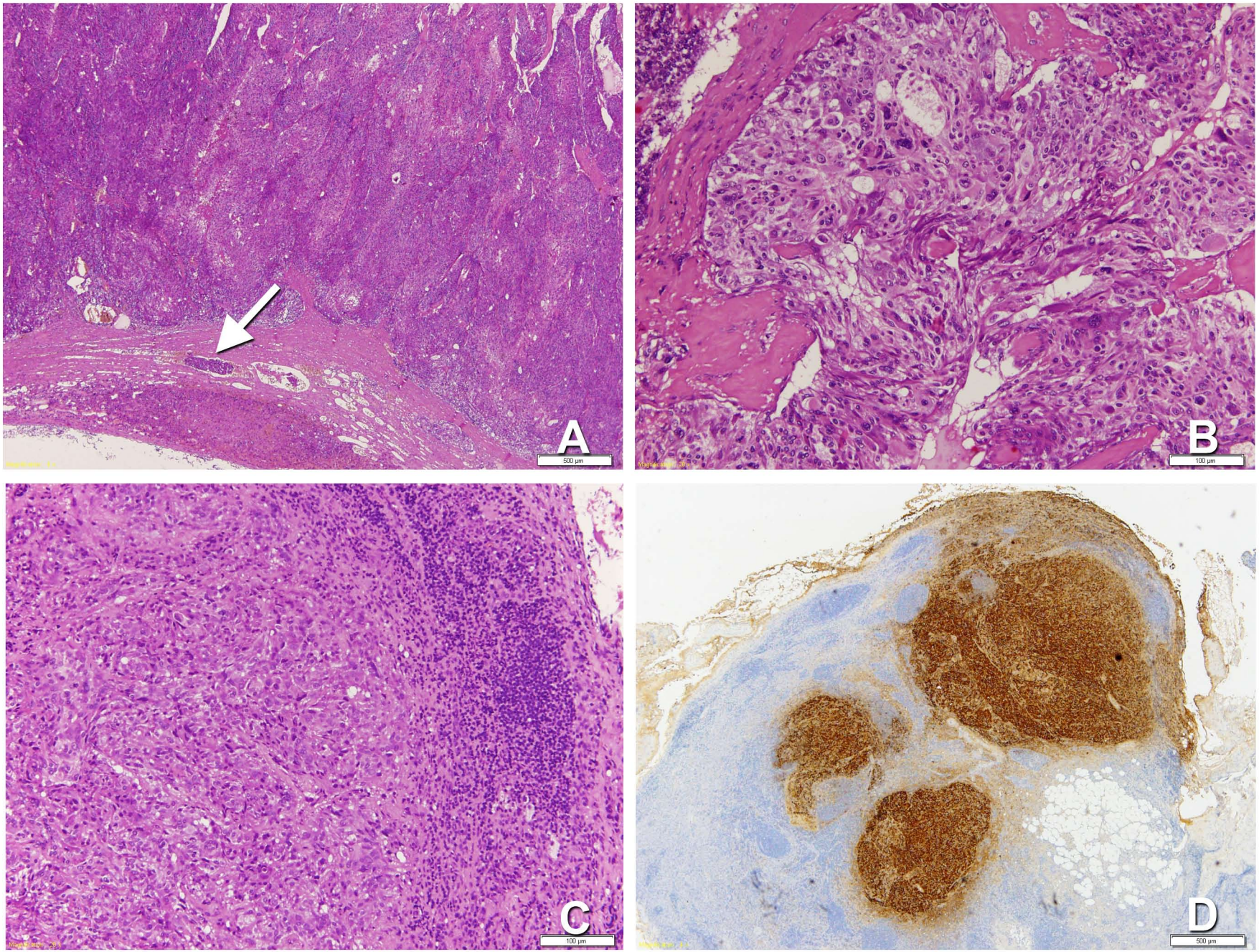
Legend

Fig.1

A, Malignant pheochromocytoma with lymphatic neoplastic emboli (arrow). B, Area with cellular atypia. C, Metastatic lymph node. D, Immunostaining for chromogranin is consistent with PHEO metastasis.

Fig. 2

Electropherograms illustrating LOH at the D17S969 (A) and NF1-53.0 (B) microsatellite markers. The upper panel represents peripheral blood DNA (PB), the lower panel shows tumor DNA. C, Analysis of microsatellite markers covering the chromosome 17. There is clear allelic loss in the tumor DNA markers.


A
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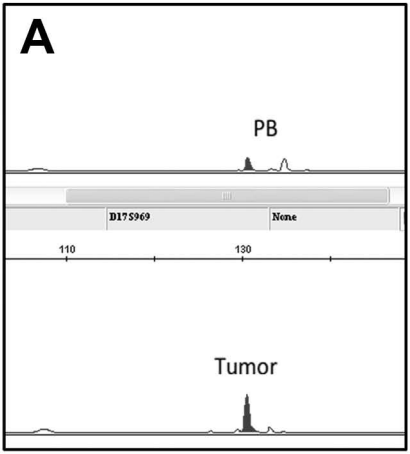

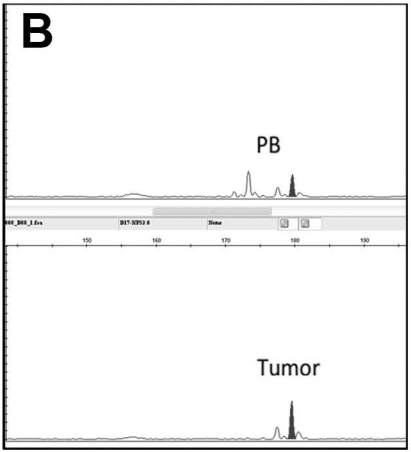

C

PB	Tumor	
284	284	D17S849
116/99	116	D17S1845
148/155	148	D17S1879
135/131	131	D17S969
114/100	100	D17S1856
142/140	140	D17S122
188	188	D17S1843
208/204	204	D17S1307
180/174	180	NF1-53.0
207	207	NF1-28.4
245/240	245	NF1-3
272/266	266	D17S1800
250/247	247	D17S798
191/187	187	D17S933
194/190	194	D17S1299
257	257	D17S1232
140/136	140	D17S1827
171/167	167	D17S1795
178	178	D17S1306
153/149	149	D17S1819
153	153	D17S789