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BRIEF ARTICLE

Malignant pheochromocytoma in neurofibromatosis; mutation screening of RET proto-oncogene, *VHL* and *SDH* gene

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Abstract

AIM: To investigate pathogenic mutations related to malignant pheochromocytoma in neurofibromatosis (NF).

METHODS: We present a patient with NF and metastatic pheochromocytoma in whom genetic screening for presence of pathogenic mutations in RET proto-oncogene, von Hippel-Lindau (*VHL*) and succinate dehydrogenase complex subunits B (*SDHB*) genes were investigated. RET proto-oncogene mutation screening for exons 10, 11, 13, 14, 15, 16 were examined by polymerase chain reaction (PCR) and direct DNA sequencing in patient. Mutation screening for exons 1, 2, 3 of *VHL* gene was carried out. Both forward and reverse strands were subjected to direct sequencing after PCR amplification. The entire coding sequence of *SDHB* gene was screened for the presence of pathogenic mutations by PCR-sequencing.

RESULTS: A 45-year-old man presented with abdominal pain and hypertension over the previous year. The patient was a known case of neurofibromatosis type 1 (NF1) who presented at the age of 15 years with hyperpigmented and hypopigmented lesions. After complete evaluation for hypertension, biochemical tests and imagings indicated a malignant pheochromocytoma of 120 mm × 70 mm in size. The patient underwent left adrenalectomy, nephrectomy and splenectomy. After surgery the symptoms improved and blood pressure was controlled. After 5 years he was admitted again for evaluation of hypertensive crisis. Biochemical tests were again consistent with pheochromocytoma and disease relapse. Imaging studies and liver biopsy confirmed metastatic pheochromocytoma to the liver and para-aortic area. 131 Iodine-metaiodobenzylguanidine therapy was carried out. Genetic screening of *VHL* (exons 1, 2, 3), RET proto-oncogene (exons 10, 11, 13, 14, 15, 16) and *SDH* complex subunits revealed no pathogenic mutation.

CONCLUSION: We conclude that mutations in the *NF1* gene are responsible for the patient's clinical findings. However, would be helpful to further examine somatic mutations for a more precise study of genotype-phenotype correlation.

Key words: Neurofibromatosis; Familial pheochromocytoma; Malignant pheochromocytoma; Metastatic pheochromocytoma; RET proto-oncogene; von Hippel-Lindau; Succinate dehydrogenase complex subunits

Core tip: Malignant pheochromocytoma associated with neurofibromatosis (NF) is very rare. We screened for all

possible mutations related to pheochromocytoma in a patient with NF and malignant pheochromocytoma but found no mutations. This negative result shows that the *NF1* gene is responsible for this rare presentation.

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INTRODUCTION

Phaeochromocytomas and paragangliomas (PGLs) are catecholamine-secreting tumors, which arise from chromaffin cells of the adrenal medulla and extra-adrenal sites[1]. Most pheochromocytomas are sporadic tumors. However some patients suffer from the disease as part of a familial disorder (15%-30%). There are several familial disorders associated with pheochromocytoma including von Hippel-Lindau (*VHL*) syndrome, multiple endocrine neoplasia type 2 (*MEN2*), neurofibromatosis type 1 (*NF1*), succinate dehydrogenase (*SDH*) complex subunit mutation-related tumors and occasionally MEN1 syndrome. The approximate frequency of pheochromocytoma in these disorders is 50% in MEN2, 10%-20% in VHL syndrome, and 0.1%-5.7% with NF1[2-5].

Malignant forms of catecholamine-secreting tumors are rare. The malignancy rate is variable from 2.4% to 26%. There are no histological proofs of malignancy for such tumors to date and the only accepted criterion is the presence of metastasis. The distant metastases are usually of hematologic origin, mostly involving bone, liver and lung[2,3]. The prevalence of metastasis is up to 36%-50% for extra-adrenal abdominal pheochromocytoma and 10% and 5% for adrenal and familial forms respectively[6,7]. However, in practice the diagnosis of malignant pheochromocytoma can only be determined by presence of recurrence or metastatic disease at a site where chromaffin cells do not normally exist. NF is the term given to two neurocutaneous genetic conditions. NF1, also known as von Recklinghausen's disease is the most common type of NF, with an incidence of approximately 1 in 2600 to 1 in 3000 individuals[8]. Approximately half of the cases are familial while the remainder are new mutations[9]. The hallmarks of NF1 are the multiple café-au-lait spots and associated cutaneous neurofibromas. Pheochromocytoma has been clinically identified in 0.1%-5.7% of patients with NF. The *NF1* gene has been mapped to chromosome 17q11.2 and cloned[10-12]. Since malignant pheochromocytoma is very rare in NF, we present a case with NF and metastatic pheochromocytoma in which genetic screening in VHL, Ret protooncogene and *SDH* were carried out to investigate the responsible genomic mutation.

MATERIALS AND METHODS

Biochemical testing and Localization Studies

Routine biochemical tests, evaluation of 24 h urine catecholamine metabolites, abdominal computed tomography or magnetic resonance imaging and 131 Iodine-metaiodobenzylguanidine (131I-MIBG) therapy were carried out. The malignant pheochromocytoma was diagnosed according to the presence or absence of metastasis in radiological or pathological reports.

Genetic analysis

RET proto-oncogene mutation screening: Genomic DNA was extracted from peripheral lymphocytes using the salting out technique. RET proto-oncogene mutation screening for exons 10, 11, 13, 14, 15, 16 was performed polymerase chain reaction (PCR) and direct DNA sequencing using the assay described by Alvandi *et al*[13].

***VHL* gene mutation screening:** For *VHL* gene mutation screening, analysis of *VHL* gene was performed using the protocol of Cruz *et al*[2,14]. Exons 1, 2, 3 of *VHL* gene were amplified by PCR with the following primers: 1F - 5' CCATCCTCTACCGAGCGCGCG 3'; 1R - 5' GGGCTTCAGACCGTGCTATCG 2; 3F - 5' TGCCCAGCCACCGGTGTG 2; 3R - 5' GTCTATCCTGTACTTACCACAACA; 3F - 5' CACACTGCCACATACATGCACTC 3'; 3R - 5' ACTCATCAGTACCATCAAAAGCTG 3'. Both forward and reverse strands were subjected to direct sequencing after PCR amplification[2].

***SDH* complex subunits B gene mutation screening:** The entire coding sequence of the succinate dehydrogenase complex subunits B (*SDHB*) gene was screened for the presence of pathogenic mutations by PCR-sequencing based on the assay described previously[15].

RESULTS

Patient

A 45-year-old man presented with abdominal pain and hypertension over the previous year. The patient was a known case of NF1 who presented at the age of 15 years with hyperpigmented and hypopigmented lesions on the trunk, arms, feet and axillary areas. Family history was positive for NF in his mother and brother. Biochemical tests including urine metanephrines and imaging were compatible with malignant pheochromocytoma. In scans there was a large mass in the left adrenal with a size of 120 mm × 70 mm and invasion to left kidney was reported. The patient underwent left adrenalectomy, nephrectomy and splenectomy. After surgery the symptoms improved and blood pressure was controlled. The patient had poor compliance in follow up. After 5 years he was admitted again for evaluation of a hypertensive crisis. Biochemical tests were again consistent with pheochromocytoma and disease relapse. Imaging study and liver biopsy confirmed metastatic pheochromocytoma in the

liver and para-aortic area. 131I-MIBG therapy was carried out.

Genetic analysis

RET proto-oncogene mutation screening: There was no pathogenic mutation in the RET proto-oncogene. Only three single nucleotide polymorphisms were identified in this patient as follows: (1) C1866T in exon 10, causes no AA change (P622P); (2) G2071A in exon 11, causes G691S; and (3) C2712G in exon 15, causes no AA change (S904S).

***VHL* gene and *SDHB* gene mutation screening:** There was no *VHL* gene mutation. In addition, genetic sequencing of 8 exons of *SDHB* gene revealed no pathogenic mutation in this patient.

DISCUSSION

The family described in this study is comprises individuals with NF and one member (a 45-year-old man) with hypertension diagnosed as malignant pheochromocytoma**.** Hypertension is a frequent finding in adults with NF1 and may develop during childhood although pheochromocytoma is a much less common etiology. In these patients, the catecholamine-secreting tumor is usually a solitary benign adrenal pheochromocytoma, occasionally bilateral adrenal pheochromocytoma, and rarely a peri-adrenal abdominal PGL. Although NF1 as an autosomal dominant disorder is the most common familial cancer syndrome predisposing to pheochromocytoma, the risk of pheochromocytoma in this disorder is only about 1%[16,17].

Pheochromocytomas in patients with NF1 occur in the fifth decade. Our patient was a 45-year-old man with an unusual presentation of pheochromocytoma. Currently, except for the presence of the *SDHB* mutation, a large or an extra-adrenal primary tumor, there are no reliable markers for predicting a high likelihood of developing metastatic disease. Pheochromocytoma in NF is usually benign and unilateral. Based on genetic background our expectation before surgery was a benign non-metastatic tumor. As mentioned before, the patient had a metastatic tumor and after 5 years metastasis to liver and para-aortic lymph nodes led to deterioration in the clinical course of disease. It seems to us that a large mass (as detected in this patient initially) as well as local invasion are critical factors for predicting malignancy and may have the highest impact for detecting metastasis in future.

At first we treated the patient with surgical resection of the adrenal mass, but in follow up treatment of distance metastasis the only available modality was MIBG therapy. Given the positive MIBG scan, we predicted that the tumor would up take iodine. To date, beside surgery, 131I-MIBG therapy is the single most valuable option for malignant pheochromocytomas. Results of a phase Ⅱ trial using high dose 131I-MIBG demonstrated 22% partial or complete response and 35% of patients having some degree of response (*i.e.,* biochemical) without demonstrated progressive disease[18]. After 6 mo follow up our patient is alive but needs antihypertentive drugs. In future, our plan could be chemoembolization of the liver if there is persistent disease. Mutation screening were negative in our patient except for three nucleotide changes in the *RET* gene. Among them, nucleotide change C1866T is a new SNP, while changes G2071A and C2712G have been reported in the literature as SNPs. None of these changes are causative mutations for medullary thyroid cancer (MTC) patients although they are more common in patients with MTC than in normal population. Whether there is an interaction between these polymorphisms and other genes related to NF is still a matter of debate.

Mutations in the *NF1* gene result in loss of functional protein, causing the wide spectrum of clinical findings with NF1-associated tumors. No obvious genotype-phenotype correlation between small mutations (< 20 base pairs) of the *NF1* gene and a specific phenotype have been demonstrated, with the exception of the c.2970-2972 delAAT (p.M990del) mutation that is associated with a very mild phenotype in the majority of cases[19]. Genetic testing for NF1 is available but is not routinely performed, as the diagnosis is made based upon clinical phenotype.

In addition, epigenetic factors, particularly DNA methylation, may also play essential roles in regulation of RET, VHL and NF1 pathways. A recent study has identified methylated RET in colorectal cancer[20]. It has been observed that the aberrant methylation of RET correlates with decreased RET expression, whereas the restoration of RET in colorectal cancer cell lines results in apoptosis[21]. Other studies have found epigenetic regulation of *VHL* or *NF1* genes in various carcinomas[22,23]. Therefore, epigenetic regulation of involved genes may also contribute to the pathogenesis of disease in this patient and this requires further investigations.

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We gratefully thank this family for consenting to the publication of this study.

COMMENTS

Background

Neurofibromatosis (NF), is the term given to two neurocutaneous genetic conditions. NF type 1 (*NF1*), also known as von Recklinghausen's is the most common type of NF.

Research frontiers

Hypertension is a frequent finding in adults with NF1 and may develop during childhood but pheochromocytoma is a much less common etiology. In these patients, the catecholamine-secreting tumor is usually a solitary benign adrenal pheochromocytoma, occasionally bilateral adrenal pheochromocytoma, and rarely a peri adrenal abdominal paraganglioma.

Innovations and breakthroughs

At first we treated the patient with surgical resection of the adrenal mass, but in follow up for treatment of distance metastasis the only available modality was metaiodobenzylguanidine therapy.

Applications

Genetic testing for *NF1* is available but is not routinely performed, as the diagnosis is made based upon clinical phenotype.

Peer review

This is a relatively interesting manuscript of a rare case report on Pheochromocytoma in *NF1*. Although mutational studies of potential related genes were performed, the negative findings do not provide any additional molecular data of interest.

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