Brief papers

Molecular genetic diagnosis of von Hippel-Lindau disease in familial phaeochromocytoma

Paul A Crossey, Charis Eng, Maria Ginalska-Malinowska, Thomas W J Lennard, Diana C Wheeler, Bruce A J Ponder, Eamonn R Maher

Abstract

Inherited predisposition to phaeochromocytoma is seen in multiple endocrine neoplasia type 2 syndromes, von Hippel-Lindau (VHL) disease, and neurofibromatosis type 1. In addition familial phaeochromocytoma alone has been reported. To investigate the genetic basis for familial phaeochromocytoma alone, we screened three affected kindreds for mutations in the RET proto-oncogene and the VHL tumour suppressor gene. We did not detect MEN 2 associated RET mutations in any family, but missense VHL gene mutations (V155L and R238W) were identified in two kindreds with no clinical evidence of VHL disease. Patients with familial, multiple, or early onset phaeochromocytoma should be investigated for germline VHL and RET gene mutations as the molecular diagnosis of multisystem familial cancer syndromes enables appropriate counselling and screening to be provided.

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Human Molecular Genetics Group, University of Cambridge Department of Pathology, Box 134, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, UK
P A Crossey
E R Maher

CRC Human Cancer Genetics Group, Cambridge University Department of Pathology, UK
C Eng
B A J Ponder

Department of Endocrinology, Child Health Centre, Warsaw, Poland
M Ginalska-Malinowska

Department of Surgery, University of Newcastle, Newcastle upon Tyne, UK
T W J Lennard

Institute of Medical Genetics, University Hospital of Wales, Cardiff, UK
D C Wheeler

Correspondence to:
Dr Maher.

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Phaeochromocytoma is familial in approximately 10% of patients.[1] In some cases, detailed clinical, biochemical, and radiological investigation may lead to a diagnosis of multiple endocrine neoplasia type 2 (MEN 2), von Hippel-Lindau (VHL) disease, or neurofibromatosis type 1 (von Recklinghausen disease).[2] The identification of these dominantly inherited familial cancer syndromes is important in reducing morbidity and mortality from phaeochromocytoma and associated tumours (medullary thyroid carcinoma in MEN 2 and retinal and cerebellar haemangioblastomas and renal cell carcinoma in VHL disease) in at risk relatives.[3 4] Although familial phaeochromocytoma without additional features has been reported, we hypothesised that some of these families might have occult MEN 2 or VHL disease. MEN 2 is caused by germline mutations in the RET proto-oncogene and the VHL gene has also been identified.[5–10] We therefore investigated the potential role of VHL and RET mutations in three kindreds with familial phaeochromocytoma.

Methods

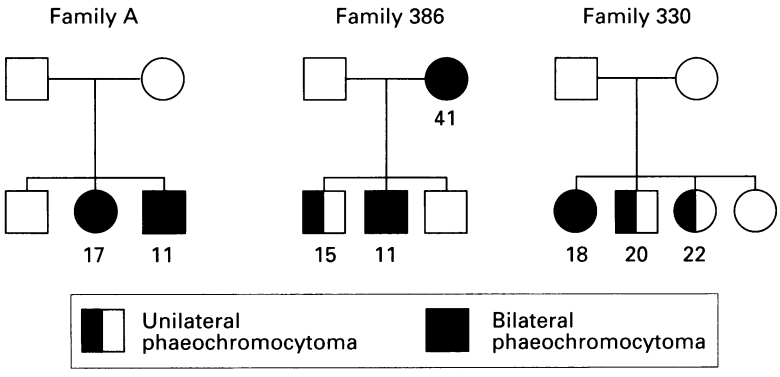
Three families with phaeochromocytoma in three first degree relatives or two relatives one of whom has multiple tumours were studied (see below). No family members had other signs of MEN, VHL, or NF1. Molecular genetic analysis on peripheral blood DNA was performed as described previously.[5 6 8 9 11] Briefly, genomic DNA was isolated from peripheral blood and the coding sequence of the entire VHL gene was amplified by the polymerase chain reaction using six primer pair sets. Each PCR product was analysed by single strand conformational polymorphism (SSCP) and heteroduplex analysis.[11] Samples showing aberrant band patterns were sequenced directly using nested primers. Southern analysis was performed to detect large germline deletions. These approaches can detect germline mutations in approximately 70% of patients with VHL disease. For analysis of the RET gene, PCR amplification of exons 10, 11, 13, and 16 was performed and analysed by direct sequencing or appropriate restriction enzyme digests or both.[5 6 8 9] These methods detect germline RET mutations in approximately 87% of MEN 2A and 93% of MEN 2B patients.[8] Family linkage studies with microsatellite markers linked to RET (D10S141, ZNF22) and VHL (D3S1038) were performed when a mutation could not be detected.[12 13]

Results

Molecular genetic analysis of the VHL and RET genes showed germline VHL mutations in two families (A and 386).

Family A was from Wales and contained two people with bilateral phaeochromocytoma (figure). Ophthalmological and radiological screening for VHL disease was normal in both sibs and their parents. However, SSCP examination of the VHL gene showed a band shift in exon 1 and genomic sequencing indicated a G→T transversion at nucleotide 463, substituting a valine for leucine at codon 155 (V155L). Both affected patients and a non-penetrant parent were heterozygous for this missense mutation. No abnormality was detected on molecular analysis of the RET gene.

Family 386 lived in Poland and contained three patients with phaeochromocytoma (figure). Molecular analysis of the critical exons



Details of three families with familial phaeochromocytoma. The age at diagnosis of phaeochromocytoma is shown underneath each person.

of the RET proto-oncogene showed no abnormalities and haplotype analysis using microsatellite markers (D10S141 and ZNF22) excluded linkage to the RET proto-oncogene. SSCP analysis and genomic sequencing of the VHL gene showed a C→T transition at nucleotide 712 in exon 3 substituting an arginine for tryptophan at codon 238 (R238W) in all three affected relatives.

Family 330 contained three affected sibs (figure). Analysis of the RET and VHL genes showed no abnormality and linkage analysis using microsatellite markers linked to RET (D10S141 and ZNF22) and VHL (D3S1038) could not exclude linkage to either gene.

Discussion

The identification of germline VHL gene mutations in two of the three familial phaeochromocytoma kindreds studied has illustrated the benefits of molecular genetic testing. Although neither family A nor family 386 had clinical evidence of VHL disease, the R238W mutation detected in family 386 has been detected previously in four families with VHL disease and is associated with a high risk of phaeochromocytoma and other VHL related tumours, including renal cell carcinoma. Hence the identification of VHL gene mutations not only allows presymptomatic diagnosis but also changes the management and follow up of a family. The early detection of VHL tumours by presymptomatic screening reduces morbidity and mortality and gene carriers require regular surveillance (including annual ophthalmological examinations and renal imaging). The V155L substitution identified in family A has not been detected previously, but is unlikely to represent a polymorphism as it segregates with the disease, produces a non-conservative amino acid change, and has not been identified on 300 normal VHL alleles (unpublished observations). The failure to detect VHL or RET mutations in family 330 may result from the insensitivity of the mutation detection techniques, or may indicate further loci for familial phaeochromocytoma susceptibility.

Interfamilial differences in the frequency of phaeochromocytoma are well recognised in VHL disease. Missense mutations, in particular those at codon 238, are associated with a much higher frequency of phaeochromocytoma than germline deletions or intragenic mutations which are predicted to cause a truncated protein product.[11] The absence of other features of VHL disease in family A may represent incomplete penetrance or mutation specific variations in phenotype. For example, most VHL gene mutations associated with phaeochromocytoma also predispose to other VHL complications including renal cell carcinoma. However, a single ancestral mutation in families of German origin is associated with a high incidence of phaeochromocytoma but a low incidence of renal carcinoma.[14] This raises the possibility that some VHL mutations might predispose to phaeochromocytoma but be associated with a low risk of other VHL related complications. Further studies of the molecular genetics of familial and early onset phaeochromocytoma patients for RET and VHL gene mutations will provide information on the genetic epidemiology of phaeochromocytoma and provide insights into the genotype-phenotype correlation in VHL disease.

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