High frequency of novel germline mutations in the VHL gene in the heterogeneous population of Brazil

J C C Rocha, R L A Silva, B B Mendonça, S Marui, A J G Simpson, A A Camargo

Von Hippel-Lindau disease (VHL) (MIM 193300) is a heritable autosomal dominant disease characterised by predisposition to the development of a combination of benign and malignant tumours affecting multiple organs. The main features of the disease include retinal angiomas (RA), haemangioblastomas of the central nervous system (HB), clear cell renal carcinomas (RC), pheochromocytomas (PH), multiple renal and pancreatic cysts, adenomas and carcinomas of the pancreas, neuroendocrine tumours, endolymphatic sac tumours, and papillary cystadenomas of the epididymis and broad ligaments.1–12 Reported birth incidence ranges from 1:36 000 to 1:45 000.13 Penetration is essentially complete at 65 years of age and subjects at risk may develop a combination of clinical manifestations during their lifetime.

Usually the disease presents with a family history, but de novo mutations have been reported in as many as 23% of VHL patients.1 In general, the incidence of HB, AR, RC, and PH are approximately 60.2%, 41%, 25.3%, and 14.5%, respectively. The diagnostic criteria for VHL are based on the presence of RA, HB, visceral lesions (RC, PH, or multiple pancreatic cysts) and a family history of similar lesions. If a family history of VHL disease is present, only one RA, HB, or visceral lesion is required to make the diagnosis of VHL. However, the presence of two or more RA/RC or one RA/RC and a visceral lesion is required in cases without a family history.

The VHL gene was mapped to 3p25-2614 and cloned in 1993 by an international cooperative study.15 The gene was shown to be mutated in the germline of VHL patients and to be inactivated in most sporadic tumours associated with VHL.15–21 The coding sequence of the VHL gene spans three exons and has two transcriptional start codons that result in protein products (pVHL) of 213 and 160 amino acid residues, respectively.

pVHL is multifunctional and is associated with the inhibition of angiogenesis, cell cycle exit, extracellular fibronectin matrix assembly, and proteolysis.22–23 It forms a multimeric complex that contains, at minimum, elongin B, elongin C, Cul2 and Rbx1 (VCB), and which controls the stability and degradation of the α and β subunits of hypoxia inducible transcription factor 1 (HIF-1) in response to available oxygen levels.24 Wild type pVHL polyubiquitinites HIF-1 subunits when oxygen is available, targeting them for degradation by the 26S proteasome. Mutation in the VHL gene results in constitutive expression of many HIF-1 inducible genes, including vascular endothelial growth factor (VEGF), erythropoietin (EPO), and platelet derived growth factor B chain (PDGF-B).25–27 In addition, it has recently been shown that pVHL plays a crucial role in cytoskeletal organisation, focal adhesion formation, and motility.28

Several groups in North America, Europe, and Japan have collectively reported more than 224 distinct intragenic germ-line mutations (HGMD online), although the frequency of mutation detection has varied considerably among studies.23 24 25 26 However, the recent improvement in mutation detection methodologies has raised this rate to virtually 100% in confirmed VHL patients.13

Phenotypes vary among families and reflect genotypic differences.13 24 25 26 Currently, VHL is subclassified into type 1 and type 2 according to the low or high risk of developing PH, respectively. Type 2 VHL is subclassified into type 2A (low risk for RC and high risk for HB), type 2B (high risk for RC and HB), and type 2C (with familial PH).1–5 As long as VHL type 2 is associated with missense mutations in more than 90%, most type 1 VHL families present large deletions or protein truncating mutations.13 27–31

Most deleterious VHL germ-line mutations damage the pVHL-elongin C binding α domain or affect the HIF-1 binding site at the pVHL β domain.27 Mutations associated with RC cause complete HIF-1 deregulation and loss of p220 (fibronectin) binding, whereas the products of such type 2 VHL alleles retain the ability to downregulate HIF-1 but are defective for promotion of fibronectin matrix assembly, suggesting that loss of other pVHL functions are necessary for PH susceptibility.27–30
Here, we report the nature of VHL gene mutations in 20 consecutive VHL patients belonging to distinct families, mainly from Brazil. Mutations were detected in all VHL patients using a combination of direct sequencing and quantitative Southern blotting. To our knowledge, this is the first study of molecular diagnosis of VHL in Latin America. The study contributes eight novel germline VHL mutations and discusses whether increased risk of RC observed within these families may be attributable to mutations affecting the HIF-1 binding domain of the pVHL and/or the VCB complex.

SUBJECTS AND METHODS

Patients and clinical evaluation

Seventeen affected probands with a family history of VHL (15 from Brazil, one from Portugal, and one from Ecuador) and three Brazilian VHL patients without a family history (defined by clinical criteria) were included in this study. Fifteen of these were clinically evaluated at the Department of Oncogenetics of the Hospital do Câncer - São Paulo, Brazil, and the others were referred by other centers that were following the families. All families received appropriate pre- and post-test genetic counselling.

Blood samples from the affected probands were collected following receipt of signed informed consent. Clinical status was determined by physical examinations (including ophthalmoscopy), radiological evaluations, and laboratory testing according to methods described elsewhere. Previous medical records of affected members and follow up were reviewed and registered in a database.

Amplification and sequencing of the VHL gene

Genomic DNA was extracted from 10 ml of peripheral blood using phenol-chloroform by standard procedures after separation of mononuclear cells and stored at 4°C. The three exons of the VHL gene were amplified by PCR in a total volume of 25 µl containing 50 ng of template DNA, 1× PCR buffer (20 mmol/l Tris-HCl, 50 mmol/l KCl), 200 µmol/l each dNTP (Promega, USA), 1.5 mmol/l MgCl₂, 0.25 units of Taq polymerase (Gibco-BRL Life Technologies, USA), and 0.5 µmol/l each primer set. Primers used for the amplification of the coding region of the VHL gene were: 1F 5′ CCATCTCTTACCGAGCGCGCC 3′ and 1R 5′ GGCCCTCACGGCGTCATGTCG 3′ for exon 1 yielding a fragment of 522 bp; 2F 5′ TGGCCGACCGCTGGTGG 3′ and 2R 5′ CTGATCGTCTACCTACAAACA 3′ for exon 2, 232 bp; and 3F 5′ CACACTGGCCAATCATGCATTCTGCTC 3′ and 3R 5′ ACTCGATGATCCTTACAAACGTG 3′ for exon 3, 383 bp. Reactions were incubated at 95°C for five minutes and amplified for 35 cycles of 95°C for one minute, 58°C for one minute (exons 2 and 3), and 68°C (exon 1) for one minute, and 72°C for one minute, with a final extension cycle of 72°C for five minutes. PCR products were sequenced in both strands with Big Dye terminators (Applied Biosystems) and sequences were collected and analyzed on an ABI Prism 377 (Applied Biosystems). The same PCR primers were used in the sequencing reactions, with the exception of exon 3, which was sequenced in the sense direction with an internal primer 3′ CGTTCCTTGATCGAGCCCT 3′.

Southern blot analysis

Germline rearrangements involving part or the entire VHL gene were detected by quantitative Southern blotting using g7 cDNA and a β globin probe, as control, as previously described. In brief, 10 µg of genomic DNA were digested with the endonucleases EcoRI and AseI and DNA fragments were separated by electrophoresis. The DNA was transferred to a nylon membrane and hybridised to random primer labelled probe specific for the VHL gene (g7) and for the β globin gene. Filters were exposed to x-ray film (Kodak X-AR, USA) in a film cassette with two intensifying screens at −70°C for one to three days. Quantification was possible by comparing the intensity between the two bands. DNA from normal subjects and from patients with known rearrangements in the VHL gene (kindly provided by Dr. Catherine Stolle, University of Pennsylvania, USA) was used as negative and positive controls, respectively. In normal subjects, a single 9.7 kb EcoRI and AseI fragment is detected by hybridisation with the g7 probe, whereas in subjects with partial deletions of the VHL gene, a slower or faster migrating fragment was detected in addition to the normal sized fragment. Complete deletion of the VHL gene produced a single 9.7 kb band with 50% relative intensity after normalisation, which was confirmed by densitometry of the bands.

RESULTS

Twenty VHL probands and their families were evaluated in this study, of whom 17 presented with a family history (15 from Brazil, one from Portugal, and one from Ecuador). Fifteen familial VHL and two VHL patients were classified as having type 1 VHL (without PH) and two familial VHL and one VHL patient were classified as having type 2 VHL (with PH). Ten families were large enough (four or more affected subjects from at least two generations) for full phenotype analysis (table 1).

We were able to define germline VHL gene mutations in all probands who fulfilled the clinical criteria for VHL. In 16 cases (80%), these were point mutations involving either the coding region or exon boundaries of the VHL gene (nine missense, three frameshift, one in frame deletion, two splice defect, one nonsense), while four (20%) were large deletions of which three were partial deletions and one a complete deletion of the VHL gene (table 1). Eight of the 16 point mutations (50%) have not been previously described and thus represent novel mutations. The point mutations were not distributed uniformly along the VHL gene and most were concentrated within exons 1 and 2 (87.5%). The 13 point mutations associated with type 1 VHL occurred in this region in contrast to the findings of others. We were able to analyse the germline mutation linkage to disease in nine families (families 2, 8, 9, 12, 14, 15, 16, 18, and 20). Four of these families carried known point mutations described before by others, two presented large deletions, and three had novel point mutations. The novel mutations 238A>C, 344delA, and IVS1+7G>A were detected in five, three, and two affected family members, respectively. The pathogenic nature of the IVS1+7G>A remains to be confirmed at the mRNA level; however, the fact that this alteration was not detected in one unaffected first degree relative (after clinical and radiological screening) suggests that it may be associated with the disease. Additionally, none of the novel mutations were represented in 120 alleles from normal subjects in the Brazilian population suggesting that these base changes are not common polymorphisms.

Linkage analyses were not possible in eight of the VHL families owing to the absence of live family members (families 1, 3, 4, 5, and 7) or relatives alive but not assessable (families 11, 17, and 19). Only in family 1 was genetic testing performed in a non-symptomatic boy, since no relatives were alive for testing. Although many members of this family had already presented with HB, the diagnosis of VHL was confirmed after necropsy of the proband’s father, who died at 37 years from complications following emergency surgery for cerebellar HB. The necropsy showed many occult lesions related to VHL, including RC, multiple pancreatic and renal cysts, and cystadenum of the epididymis.

From the eight families in which linkage to disease could not be established, four carried mutations previously described as having a deleterious effect (table 1), two had partial deletions of the VHL gene, and two had novel missense mutations. Although these two missense mutations have not been previously described, they affect amino acid residues for which
other different mutations have already been described. Additionally, these mutations (Trp88Arg and Val130Phe) affect the HIF-1α binding site within the β domain of pVHL and the hydrophobic core of pVHL responsible for the structural integrity of the β sheets, respectively. Thus, in both cases these novel mutations are predicted to result in significant alteration of pVHL function.

Three novel germline mutations were detected in all VHL patients with no family history: frameshift mutation 305delC, frameshift mutation 436delC, and missense mutation Thr124Ile. Both novel frameshift mutations introduce a premature stop codon leading to a truncated protein and the missense mutation was shown to be at least 1% in normal DNA samples from the Brazilian population (a minimum of 120 alleles evaluated) (table 1). In contrast, none of the novel point mutations described in the present study were detected in normal subjects.

Genotype-phenotype correlations were consistent with those reported in other studies. Most of the mutations found in the 17 families classified as type 1 VHL resulted in a truncated protein (66%), whereas all three mutations found in families whose phenotype included PH (type 2 VHL) predicted a full length protein. There was no correlation between the type of germline mutation and the occurrence of HB or RA, since these manifestations were present in almost all families analysed. Two families harboured distinct mutations in codon 167, a VHL hotspot associated with high risk of RC, HB, and PH (type 2B). However, RC was not observed in these families, and we considered that a larger number of affected members and a longer period of follow up will be necessary for full phenotypic characterisation in these families. Eleven out of 17 type 1VHL families had a RC phenotype (table 1). Almost all mutations detected in these families affect residues in the type 1 VHL families had a RC phenotype (table 1).

### Table 1 Clinical manifestation of 20 consecutive families with von Hippel-Lindau disease (VHL). Details of germline mutations found in VHL families without (type 1) and with (type 2) phaeochromocytoma

<table>
<thead>
<tr>
<th>VHL Family</th>
<th>VHL type</th>
<th>Family history</th>
<th>No</th>
<th>HB</th>
<th>RA</th>
<th>RC</th>
<th>PC</th>
<th>PH</th>
<th>Mutation at nt level of ORF</th>
<th>Exon</th>
<th>Consequence to pVHL</th>
<th>Type of mutation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Yes</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>226-228 del TCT</td>
<td>1</td>
<td>delPhe76</td>
<td>In frame del</td>
<td>41</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>Yes</td>
<td>10</td>
<td>7</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>238A&gt;C</td>
<td>1</td>
<td>Ser80Arg</td>
<td>Missense Novel</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>Yes</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>262T&gt;C</td>
<td>1</td>
<td>Trp88Arg</td>
<td>Missense Novel</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>Yes</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>280G&gt;T</td>
<td>1</td>
<td>Gln94Stop</td>
<td>Nonsense</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>Yes</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>302T&gt;G</td>
<td>1</td>
<td>Leu101Arg</td>
<td>Missense Novel</td>
<td>31</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>No</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>305 del C</td>
<td>1</td>
<td>Framework at aa 102</td>
<td>Frameshift Novel</td>
<td>11</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>Yes</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>320G&gt;C</td>
<td>1</td>
<td>Arg137Pro</td>
<td>Missense</td>
<td>33</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>Yes</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>IVS1+7G&gt;A</td>
<td>Intron 1</td>
<td>Splicing defect</td>
<td>Novel</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>Yes</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>344 del A</td>
<td>2</td>
<td>Frameshift at aa 115</td>
<td>Frameshift Novel</td>
<td>14</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>No</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>371C&gt;T</td>
<td>1</td>
<td>Thr124Ile</td>
<td>Missense Novel</td>
<td>12</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>Yes</td>
<td>14</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>388G&gt;T</td>
<td>2</td>
<td>Val130Phe</td>
<td>Missense Novel</td>
<td>13</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>Yes</td>
<td>8</td>
<td>7</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>407T&gt;C</td>
<td>2</td>
<td>Phe136Ser</td>
<td>Missense</td>
<td>12</td>
</tr>
<tr>
<td>13</td>
<td>1</td>
<td>No</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>436 del C</td>
<td>2</td>
<td>Frameshift at aa 146</td>
<td>Frameshift Novel</td>
<td>14</td>
</tr>
<tr>
<td>14</td>
<td>1</td>
<td>Yes</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>IVS2+1G&gt;A</td>
<td>Intron 2</td>
<td>Splicing defect</td>
<td>Novel</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>1</td>
<td>Yes</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>499C&gt;T</td>
<td>3</td>
<td>Arg167Pro</td>
<td>Missense Novel</td>
<td>12</td>
</tr>
<tr>
<td>16</td>
<td>1</td>
<td>Yes</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>500G&gt;A</td>
<td>3</td>
<td>Arg167Gln</td>
<td>Missense</td>
<td>12</td>
</tr>
<tr>
<td>17</td>
<td>1</td>
<td>Yes</td>
<td>10</td>
<td>10</td>
<td>1</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>Partial deletion</td>
<td>–</td>
<td>Rearrangement</td>
<td>–</td>
<td>14</td>
</tr>
<tr>
<td>18</td>
<td>1</td>
<td>Yes</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>Partial deletion</td>
<td>–</td>
<td>Rearrangement</td>
<td>–</td>
<td>14</td>
</tr>
<tr>
<td>19</td>
<td>1</td>
<td>Yes</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>Partial deletion</td>
<td>–</td>
<td>Rearrangement</td>
<td>–</td>
<td>14</td>
</tr>
<tr>
<td>20</td>
<td>1</td>
<td>Yes</td>
<td>6</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>Complete deletion</td>
<td>–</td>
<td>Rearrangement</td>
<td>–</td>
<td>14</td>
</tr>
<tr>
<td>9, 17</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>183C&gt;G</td>
<td>1</td>
<td>Synonym</td>
<td>Polymorphism 1.16%</td>
<td>1</td>
</tr>
<tr>
<td>16</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>IVS2-95T&gt;A</td>
<td>Intron 2</td>
<td>Apparently no effect</td>
<td>Polymorphism 1.69%</td>
<td>1</td>
</tr>
</tbody>
</table>

*Further investigation showed that the mother of the patient was an asymptomatic carrier of the mutation. One patient per family with renal tumour <3 cm under follow up detected by imaging. Although we did not observe RC, the germline mutations are described as associated with the VHL type 2B phenotype. Pancreatic cystadenomas. The proband presented with one tumour which biopsy showed to be a non-functional paraganglioma. Nomenclature according to Antonarakis et al (1998). Proteins stops at residue 142. Protein stops at residue 157. New at nucleotide level, but already described at aa level. Allele frequency in normal Brazilian population.

No = number of affected members in each VHL family; HB = haemangioblastoma of CNS; RA = retinal angioma; RC = renal carcinoma; PC = pancreatic carcinoma; PH = phaeochromocytoma; nt = nucleotide; ORF = open reading frame; aa = amino acid; new = novel germline mutation.
The spectrum of known mutations in the VHL gene is enormous and includes missense and nonsense mutations, deletions or insertions of one or more exons, mutations, and rearrangements consisting of partial or complete deletion of the gene. Here, we found that by complete sequencing of the coding regions of the VHL gene, followed by quantitative Southern blotting when a sequence mutation could not be detected, we were able to reproduce the results obtained by Stolle et al. in that we detected germline mutations in all 20 distinct VHL families analysed. Because of the relatively short length of the VHL gene, no screening method based on single stranded DNA conformation, such as SSCP or CDGE, was used before sequencing. Since not all initial screening achieved the same results, we consider that both methodology and patient selection are important for achieving a high efficiency of mutation detection in VHL. We believe that the high rate of mutation detection achieved in this work can also be explained by the stringent clinical criteria used for family selection. All patients selected for the study had the diagnosis of VHL confirmed by clinical, pathological, or radiological evidence.

We found that molecular screening was particularly invaluable in situations where the diagnosis of VHL could not be achieved by clinical criteria alone, such as where family data were not available, or where patients had a negative family history. In addition, the genetic test was important to determine the status of the parents in the apparently de novo VHL patients. In one case, it was possible to detect the same mutation in the mother of the patient, with implications for the risk assessment and screening of other family members. Indeed, we emphasise here that the study of the patient's parents is essential for the characterisation of de novo mutations, and for risk assessment and counselling of other relatives. Recent studies have shown that about 25% of probands have parents and sibs with no history of the disease at the time of the diagnosis.

We have contributed eight novel germline mutations, including four missense mutations, three frameshift mutations, and one splice site mutation (table 1). Five novel mutations occurred with a familial pattern. However, two of these could not be analysed further in other family members: the missense mutation Trp88Arg, because the mother of the proband had died and was adopted, and the missense mutation Val30Phe, because there were no available relatives at the time of the study. Although the mutation IVS1+7G>A does not affect the invariant $3'$ AG splicing donor site, segregation and population analysis presented in this work suggest it can be a pathogenic mutation. Splicing mutations outside the invariant receiver or donor site have been reported in many cancer susceptibility genes, including VHL, TP53, BRCA1, and hMLH1, and they represent as many as 27% of the splice sites mutations reported in these genes (HGMD online). None of the novel point mutations described in this study were represented in the normal population, excluding the possibility that they might be common polymorphisms.

Germline mutations in the VHL gene are distributed throughout the coding region of the gene. Although other groups have found that the distribution of the mutations within the VHL gene tended to concentrate in exon 1 and exon 3, we observed in our series that most point mutations (87.5%) occurred in exon 1 (43.7%), exon 2 (31.3%), and the boundaries of these exons (12.5%). We found large deletions of the VHL gene in four of our families (20%), including three partial deletions and one complete deletion. Previous studies showed a frequency of partial VHL gene deletion of 25% and complete VHL gene deletion in 9% of the VHL families. In general, genotype-phenotype correlations observed in this study were consistent with those reported by others. It is known that distinct phenotypes can result from almost identical mutations. Here we found that the mutation Ser80Gln detected in a large family conferred a high risk for RC, HB, and RA, but no apparent risk for PH. A similar missense mutation in this same codon, Ser80Gln, has been reported to be associated with type 2C VHL (only PH). It should be emphasised that many mutation hotspots are conserved in the VHL gene, suggesting that mutations occurring at these sites may be influenced by the genetic background of the population. Thus, it should be emphasised that distinct phenotypes can result from almost identical mutations. Here we found that the mutation Ser80Gln detected in a large family conferred a high risk for RC, HB, and RA, but no apparent risk for PH. A similar missense mutation in the same codon, Ser80Gln, has been reported to be associated with type 2C VHL (only PH). It should be emphasised that many mutation hotspots are conserved in the VHL gene, suggesting that mutations occurring at these sites may be influenced by the genetic background of the population.
REFERENCES


High frequency of novel germline mutations in the \textit{VHL} gene in the heterogeneous population of Brazil

J C C Rocha, R L A Silva, B B Mendonça, S Marui, A J G Simpson and A A Camargo

\textit{J Med Genet} 2003 40: e31
doi: 10.1136/jmg.40.3.e31

Updated information and services can be found at:
http://jmg.bmj.com/content/40/3/e31

These include:

References
This article cites 51 articles, 22 of which you can access for free at:
http://jmg.bmj.com/content/40/3/e31#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections

- Pancreas and biliary tract (110)
- Screening (oncology) (234)
- Urological cancer (70)
- Epidemiology (630)
- Molecular genetics (1254)
- Reproductive medicine (519)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/