Novel Pheochromocytoma Susceptibility Loci Identified by Integrative Genomics


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Abstract

Pheochromocytomas are catecholamine-secreting tumors that result from mutations of at least six different genes as components of distinct autosomal dominant disorders. However, there remain familial occurrences of pheochromocytoma without a known genetic defect. We describe here a familial pheochromocytoma syndrome consistent with digenic inheritance identified through a combination of global genomics strategies. Multipoint parametric linkage analysis revealed identical LOD scores of 2.97 for chromosome 2cen and 16p13 loci. A two-locus parametric linkage analysis produced maximum LOD score of 5.16 under a double recessive multiplicative model, suggesting that both loci are required to develop the disease. Allele-specific loss of heterozygosity (LOH) was detected only at the chromosome 2 locus in all tumors from this family, consistent with a tumor suppressor gene. Four additional pheochromocytomas with a similar genetic pattern were identified through transcription profiling and helped refine the chromosome 2 locus. High-density LOH mapping with single nucleotide polymorphism–based array identified a total of 18 of 62 pheochromocytomas with LOH within the chromosome 2 region, which further narrowed down the locus to <2 cM. This finding provides evidence for two novel susceptibility loci for pheochromocytoma and adds a recessive digenic trait to the increasingly broad genetic heterogeneity of these tumors. Similarly, complex traits may also be involved in other familial cancer syndromes. (Cancer Res 2005; 65(21): 9651-8)

Introduction

Catecholamine-secreting tumors derive from the neural crest and are known as pheochromocytomas if located in the adrenal medulla or as paragangliomas if they arise elsewhere along the distribution of the sympathetic nervous system (1). Although usually sporadic, pheochromocytomas and/or paragangliomas can occur as components of several hereditary syndromes, including multiple endocrine neoplasia type 2A and B (MEN 2A and 2B), von Hippel-Lindau disease (VHL), neurofibromatosis type 1 (NF1), and paraganglioma syndromes types 4 (PGL4), 3 (PGL3), and 1 (PGL1; ref. 2). These disorders are caused by mutations in RET, VHL, NF1, and succinate dehydrogenase (SDH) SDHB, SDHC, and SDHD genes, respectively. However, there are familial clusters of pheochromocytoma for which the primary genetic mutation has not been identified (3). Furthermore, <30% of apparently sporadic pheochromocytomas can be attributed to mutations in the hereditary pheochromocytoma syndromes above (4). It is therefore likely that an additional gene(s) accounts for the primary defect in the remaining cases.

We describe here the identification of two novel pheochromocytoma susceptibility loci by integration of multiple strategies. First, we did conventional and two-locus linkage analysis in a sibship with six cases of pheochromocytoma. A single nucleotide polymorphism (SNP)–based genome-wide scan was then carried out both to increase the density of the linkage analysis and to provide a genome-wide loss of heterozygosity (LOH) map of the linked regions in available tumors. On identification of LOH at one of the target loci in all tumors from this kindred, two parallel approaches were done to narrow down the target regions: (a) expression profiling of these tumors was generated to define a signature that might serve as a filter for similar subtypes of pheochromocytomas and (b) high-density LOH was used to fine map an independent cohort of pheochromocytomas. Our results are consistent with a double recessive digenic inheritance being responsible for the disease phenotype in this family. Furthermore, one of the two loci has features of a tumor suppressor gene. We propose a model to explain the unusual coexistence of recessive inheritance and LOH.

Subjects and Methods

Subjects

A sibship of 13 individuals (FP1 kindred) was studied. Eight adrenal pheochromocytomas, including two bilateral tumors, were detected in six siblings using standard clinical, biochemical, and imaging methods and confirmed by histology and immunohistochemistry (see detailed description in Results).

Samples from additional 96 pheochromocytomas were obtained through an international consortium (the Familial Pheochromocytoma

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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Familial Pheochromocytoma Consortium members and their affiliations are listed in the Appendix.

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Consortium). In total, 49 sporadic and 47 familial tumors were included in this analysis (Supplementary Table S1). Written informed consent was obtained according to institutional ethical committee requirements (Dana-Farber Cancer Institute/Harvard Cancer Center; Hospital das Clínicas, University of São Paulo Medical School, São Paulo, Brazil; and collaborating institutions).

Methods  
DNA and RNA isolation. Blood samples were collected from all 13 consenting FP1 siblings, and genomic DNA was prepared from lymphocyte pellets. DNA was also isolated from snap-frozen or paraffin-embedded material of eight tumors from the six affected cases using the Qiagen kit (Valencia, CA) according to the manufacturer’s instructions. RNA for expression profiling studies was obtained from four of these tumors (individuals 03, 05, 09, and 11, numbered as in Fig. 1 using Trizol; Invitrogen, Carlsbad, CA) following the manufacturer’s recommendations.

The 96 additional pheochromocytomas included in this study were available for expression profiling (n = 34), LOH analysis (n = 38), or both (n = 24). DNA and/or RNA was obtained as described above.

Genome-wide linkage analysis using a 10-cM microsatellite panel. DNA samples from all 13 siblings (6 affected cases and 7 "clinically unaffected" individuals) were used for a genome-wide scan using the 378-marker ABI panel with 10-cM interval spacing. In this study, we adopted a stringent approach by using an affected-only strategy wherein the siblings without a pheochromocytoma diagnosis are considered "unknown" (rather than "unaffected"). The LOD scores thus obtained reflect only the genotype of the affected siblings. Additional markers were subsequently used to provide a 2- to 3-cM spacing coverage in selected regions that yielded results consistent with linkage.

Parametric analysis was initially carried out assuming autosomal dominant (AD) inheritance with risk allele frequency of 0.01%. After observing that all six affected siblings coinherit both maternal and paternal haplotypes at two locations in the genome, parametric analysis was done considering an autosomal recessive (AR) inheritance with the same variables. The analysis was also processed considering other allele frequencies: 1%, 0.5%, and 0.05%. Both parametric and nonparametric linkage analyses were done using GeneHunter versions 1.3 and 2.1 (5), and the nonparametric studies were also confirmed by Multipoint Engine for Rapid Likelihood Inference (MERLIN) software package (6). All genetic distances (based on a sex-averaged map) and marker locations used here correspond to the Marshfield map (http://research.marshfieldclinic.org/genetics/Default.htm).

Genome-wide linkage using a high-density single nucleotide polymorphism panel. The Mapping 10K array (Affymetrix, Santa Clara, CA) was used for LOH analysis (see below) as well as to replicate and refine the genome-wide linkage analysis based on microsatellite markers. Samples were processed according to the manufacturer’s instructions using 250 ng of high molecular weight genomic DNA. The oligonucleotide array used for DNA analysis contains 11,500 SNPs distributed throughout the genome at an average density of 0.2-Mb interval.

10K SNP genotypes were generated from all six affected individuals and two siblings without clinical disease (individuals 13 and 15 in Fig. 1) for linkage analysis. Genotype calls and pedigree file were converted into a format suitable for linkage software MERLIN version 0.10.2 and Gene-Hunter version 2.1 (7). All noninformative markers were removed and genotype inconsistencies were set to "no call." Similar to the conventional linkage analysis, two inheritance models, AD and AR, were considered for this strategy.

Two-locus Locus Analysis
In the single-locus analysis above, the inheritance trait in the FP1 family seemed to be recessive at both chromosomes 2 and 16 loci. To examine the potential interaction between these two loci, we employed a modified version of the GeneHunter Two-Locus software (kindly provided by Dr. J. Dietter, Institute for Medical Biometry, Informatics, and Epidemiology, University of Bonn, Bonn, Germany) as described (8, 9). SNP array genotype data of all affected and two clinically unaffected siblings (individuals 13 and 15 from Fig. 1), as described above, were used for this analysis. In brief, the program was used to test whether the two loci identified by the single-locus analysis interacted. Three models were tested to examine the nature of the interaction between the two loci: a multiplicative or synergistic model, an additive or modulating model, or a heterogeneity model (8, 9). Both parametric (recessive model, allele frequency of 0.01%) and nonparametric (NPL) results were obtained in these three models.

Loss of heterozygosity analysis. LOH analysis was used on all 8 tumors from the FP1 family and an additional 62 pheochromocytomas of various genetic backgrounds. Both chromosomes 2 and 16 microsatellite markers and/or genome-wide SNP-based array (Mapping 10K array) were done as reported previously (10, 11). Twenty-four tumors were analyzed by both methods. Tumor samples from four FP1 individuals, FP03, FP05, FP11, and FP21, were included in the SNP array procedures.

Clustering analysis was done to determine tumors with similar pattern of LOH throughout the genome using the dChipSNP program as described (12). In addition, signal intensity analysis was used to distinguish LOH events caused by hemizygous deletion from those resulting from copy neutral events as reported previously (13).

Expression profiling and pathway enrichment analysis. We have generated gene expression profiles of 76 primary pheochromocytomas representative of all known genetic backgrounds (14). Four tumors from the FP1 family (03, 05, 09, and 11 as labeled in Fig. 1) were included in the transcription analysis. In the present study, we did two strategies. First, we used the transcription clustering distribution as a filter to screen for additional "FP1-like" pheochromocytomas. This approach involved a genome-wide LOH analysis of tumors that clustered with samples from the FP1 family. Next, we extended the supervised analysis by comparing FP1 tumors with well-known genetic defects (i.e., MEN 2, VHL, or samples with SDH mutations) in a pairwise manner. This was done using k-nearest neighbor and weighed voting supervised machine learning algorithms as described (14–16). Models were evaluated using leave-one-out cross-validation (15, 16). Probes to be used in the models were selected by ranking the genes according to the signal-to-noise metric (15).

Gene set enrichment analysis (GSEA) was used to gather insights into the functional classes and mapping position of genes associated with the FP tumors by comparison with the other genetic classes with known mutations (17, 18). GSEA considers predefined gene sets representing pathways or chromosomal areas of interest and determines whether the members of these sets are positively or inversely correlated with a specific phenotype or class distinction.

Results
A familial cluster of pheochromocytoma. A large kindred of Brazilian-Portuguese extract with 13 siblings and their offspring were evaluated and followed clinically (Fig. 1). No consanguineous marriage was reported. The index case, a female patient with typical adrenergic symptoms, was diagnosed with bilateral adrenal pheochromocytoma at age 34 years. Five of 13 screened siblings, ages 36 to 55 years, who were hypertensive, were found to have adrenal pheochromocytoma, including 1 bilateral case. Only minor noradrenaline elevation, and in one case adrenaline elevation, was detected in plasma or urine after multiple samplings of all these individuals. Histologic and immunohistochemical confirmation was obtained in all cases, and areas of hyperplasia were noted adjacent to the limits of pheochromocytoma in each case. No additional pheochromocytomas have been detected in other family members in 8 years since the last diagnosis. Both clinical and genetic screens excluded MEN 2A or B, VHL, NF1, or PGL syndromes as being the primary genetic defect in this family, suggesting that this is a novel hereditary pheochromocytoma variant (19).
Genome-wide scan identifies two loci consistent with linkage. Parental haplotypes were not available and phase was inferred based on the haplotypes of the entire offspring. The results of the genome scan are provided as Supplementary Data. With the AD model, 33% of the genome were excluded with LOD scores lower than 2.0. Another 51% of the genome had scores between 2.0 and 0. Only 7% of the markers produced LOD scores higher than 1 and those regions were reanalyzed at a higher density. Maximum (and identical) LOD scores were found with markers located on chromosomes 2 and 16 (Fig. 2; Supplementary Data).

Haplotype analysis revealed that affected individuals shared both maternal and paternal haplotypes in these two regions. Thus, the analysis was also done under the assumption of an AR model.

On chromosome 2, the peak LOD score of the AR analysis was 2.97 (Fig. 2). Recombination events in individual 09 defined the linked area to 31.5 cM delimited by markers D2S337 and D2S2209. For the AD model, maximum LOD score was 1.50 delimited by recombination events in individuals 09 and 19 between markers D2S337 and D2S368 (Fig. 1). Analysis of chromosome 16 resulted in similar scores: peak LOD of 2.97 was obtained with the AR model and recombination events delimited the region between markers D16S407 and D16S499 (Figs. 1 and 2). Maximum LOD score of 1.50 was found between markers D16S423 and D16S499 using the AD model. Corresponding nonparametric Z-mean in both regions was 7.57 (P < 0.0001).

The linkage results of eight siblings, six affected and two clinically unaffected cases, using SNP genotypes were in complete agreement with the microsatellite-based analysis on both chromosomes 2 and 16 loci and yielded highly similar LOD and Z-mean scores (Supplementary Data). The limits of the linked area defined by recombination events were largely concordant with the conventional linkage data and were delimited in the AR model by a 28.5-cM region on chromosome 2 and a 23.4-cM area on chromosome 16 (Supplementary Data).

Of note, although the frequency of one parental haplotype in the sibship seemed overrepresented at the chromosome 2 region, the boxed area represents the regions shared by all six affected individuals at both parental alleles (one parental allele is dotted and the other is grey). The gray-colored allele was deleted in all tumors from this family. Individuals in the sibship are identified by numbers on top row (same as in Fig. 3).

Figure 1. Digenic inheritance of familial pheochromocytoma. Sibship with six individuals with pheochromocytoma (four unilateral, top left, filled symbols; two bilateral, top right, filled symbols) with haplotypes at chromosomes 2 and 16 loci are displayed. The age at diagnosis and/or screening is indicated for each individual. The boxed area represents the regions shared by all six affected individuals at both parental alleles (one parental allele is dotted and the other is grey). The gray-colored allele was deleted in all tumors from this family. Individuals in the sibship are identified by numbers on top row (same as in Fig. 3).
this difference was not significant when the binomial distribution of the parental haplotypes was estimated ($P = 0.092$ and 0.267 for each parental allele, respectively). Likewise, the frequency of parental haplotype distribution at the chromosome 16 locus was unremarkable ($P = 0.581$ for both parental alleles).

**Two-locus analysis suggests an interactive effect of chromosomes 2 and 16 loci in the familial pheochromocytoma inheritance.** Maximum LOD scores obtained with the two-locus analysis using a multiplicative model were 5.16, higher than those obtained with the heterogeneity and additive models (Table 1). This suggests that the two loci interact in a synergistic manner to determine the pheochromocytoma phenotype. Conversely, the LOD scores obtained with the additive and heterogeneity model were similar to those calculated for each chromosome in the single-locus analysis (Fig. 2). Thus, taken together, the results of the two-locus analysis are consistent with a multiplicative, double recessive model of inheritance whereby chromosomes 2 and 16 genes are equally necessary for tumor development.

**Nonrandom loss of heterozygosity is detected in the novel familial pheochromocytoma tumors at the chromosome 2 locus.** LOH at the chromosome 2 locus was detected in all eight tumors from affected individuals by microsatellite analysis (data not shown) and, in four tumors, by SNP array methods (Fig. 3A). In all cases, the same parental allele was retained, indicating the nonrandom nature of the deletion (Fig. 1). Furthermore, copy number analysis of SNP data based on signal intensity indicates that the observed LOH at 2q is the result of chromosomal loss rather than copy-neutral events, such as mitotic nondisjunction followed by duplication of one parental chromosome (Fig. 3B). The limits of LOH were highly concordant among all tumors from this sibship and the resulting overlap with the linked region by the AR model refined the target locus to an interval delimited by markers D2S2216 and D2S2209. A more conservative view using the larger limits of the linked region predicted by the AD model defines the minimal area of overlap between markers D2S2216 and D2S368.

In contrast to the finding on chromosome 2, despite high-density, detailed analysis, no LOH or copy number changes suggestive of deletion or amplification were detected in the FP tumors at the chromosome 16 locus (data not shown). Only 1 of 36 additional pheochromocytomas tested showed LOH at this locus: this was a metastatic pheochromocytoma with a deletion that spanned all tested markers on 16 as well as on other chromosomes. Due to the high degree of genetic instability of this tumor, the specificity of the 16p finding is unclear. Thus, although the two-locus analysis indicates a requirement for both loci for the disease phenotype, the lack of LOH suggests that the candidate chromosome 16 gene functions through a different mechanism.

**Additional FP-like pheochromocytomas are identified through transcription profiling and loss of heterozygosity screen.** We generated global profiles of four FP tumors to define a transcription signature that might offer insights into the mechanism...
of disruption underlying these tumors as well as to function as a filter for similar subtypes of pheochromocytomas. Pheochromocytomas and paragangliomas of a variety of genetic backgrounds were included in this analysis. FP tumors formed a cluster, which segregated closely with MEN 2- and NF1-derived pheochromocytomas (Fig. 4A), whereas tumors with VHL and SDHB or SDHD (SDH) mutations constituted a separate branch (14). To identify distinguishing features of the FP tumors that might offer clues to the identity of the susceptibility gene, we applied supervised learning methods to the expression data. Independent gene sets were generated from two-class comparisons between FP tumors and either VHL-, SDH-, or MEN 2-related pheochromocytomas (Supplementary Data). These two-class comparisons are efficient methods to highlight genes that underlie the differences between predetermined groups of samples. Cross-validation analysis derived from the comparisons between FP × VHL and FP × SDH tumors indicate that FP is a separable, distinct tumor class based on its gene expression profile (Supplementary Data). A less clear distinction was noted between FP and MEN 2 tumors, indicating that these two tumor classes share similar transcription patterns (Supplementary Data). These analyses were further enhanced by the inclusion of additional FP-like tumors identified by LOH screen (see below; Supplementary Data).

GSEA of the FP tumors identified genes involved in GTPase activity, lysosomal and vacuolar functions, and protein synthesis and translation to be statistically overrepresented in FP tumors in relation to VHL and SDH pheochromocytomas (Table 2). Importantly, chromosome 2q genes present in the array were significantly underexpressed in FP pheochromocytomas (GSEA, \( P < 0.01 \)), in agreement with the genomic loss observed in all tumors from this family.

Because chromosome 2 LOH was the hallmark of FP tumors, we decided to search for new FP-like pheochromocytomas by performing LOH analysis at the target chromosome 2 locus in samples that clustered in the vicinity of the FP samples by expression profiling (Fig. 4A). Thirteen tumors for which DNA was available were used for this screen. Three of these pheochromocytomas were familial cases with no identifiable mutation, one tumor was from a NF1 patient, another from a MEN 2 case, and the remaining eight were apparently sporadic samples. With this approach, we detected four tumors (three unknown familial and one sporadic) with LOH within the target region, one of which displayed a markedly smaller region of deletion that overlapped with the FP locus (Fig. 4B). We next decided to extend the LOH analysis to an independent group of 49 sporadic and familial pheochromocytomas using microsatellite- and/or SNP-based approaches. Fourteen of these tumors revealed LOH of at least two consecutive markers within this area. In total, 18 of 62 (29%) pheochromocytomas had detectable LOH at chromosome 2q (Fig. 4C).

The consensus region of overlap between the LOH area and the linked region is delimited by markers D2S2222 and D2S2209 (Fig. 4C). The length of deletion at the chromosome 2 locus was variable among the 18 tumors with LOH and in some cases did not overlap with the putative linked region. However, six adrenal pheochromocytomas (three bilateral cases) had a pattern virtually identical to the original FP1 tumors (Fig. 4C). Likewise, these bilateral tumors with chromosome 2q LOH were negative for mutations in the known pheochromocytoma susceptibility genes. Although no additional cases were available for linkage analysis, the combination of clinical features (tumor bilaterality), allele-specific LOH, and similar transcription profiling suggest that these three families are likely

<p>| Table 1. Results of two-locus versus single-locus parametric and nonparametric linkage analysis done with SNP-based genotype data of the FP1 kindred |</p>
<table>
<thead>
<tr>
<th>Analysis model</th>
<th>LOD*</th>
<th>NPL</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two locus</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Multiplicative</td>
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<td>7.91</td>
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<td>Heterogeneity</td>
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<td>Single locus</td>
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<tr>
<td>Chromosome 2</td>
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<td>7.5</td>
<td>0.000977</td>
</tr>
<tr>
<td>Chromosome 16</td>
<td>2.97</td>
<td>7.5</td>
<td>0.000977</td>
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*Parametric analysis assumed a recessive trait.
linked to the same locus on chromosome 2. In the absence of a similar marker for the chromosome 16, it is not possible to infer if this second locus contributes to the phenotype of the additional FP1-like families.

### Discussion

Pheochromocytomas represent a unique paradigm of genetic diversity and have therefore served as engines of gene discovery: at least six independent genes have been described in the context of hereditary pheochromocytoma syndromes (2, 20). We report here on a novel familial variant of these tumors. The linkage studies revealed two loci, at chromosomes 2 and 16, with identical scores on both parametric and nonparametric analyses. Although the large number of affected cases in this sibship (6 of 13) approaches the expected frequency for dominantly inherited traits, haplotype analysis revealed that all affected cases share both parental alleles on chromosomes 2 and 16 loci consistent with recessive inheritance from both regions. This differs from other known FP syndromes that are inherited through an AD pattern as well as from other hereditary cancers that only rarely result from recessive traits (21–23). Interestingly, a recessive pheochromocytoma syndrome was reported in rats, and the susceptibility locus was mapped to a region that is syntenic to human chromosome 10 (24, 25).

The hereditary pheochromocytoma syndrome described in our study presents consistent, nonrandom LOH at the chromosome 2 but not chromosome 16 locus. This is in agreement with a classic two-hit model of tumor suppressor genes at the 2q locus (26). To reconcile the unusual combination of a recessive disorder with coexistent LOH at the somatic level, we propose a model whereby one of the two mutant alleles confers a null phenotype, whereas the second allele is hypomorphic at the chromosome 2 locus. This compound “null/hypomorphic” heterozygous state would predispose to cell growth at the somatic tissue and consequently cause a hyperplastic state at the target cells (i.e., chromaffin cells). Deletion of the hypomorphic allele would be required as a second “hit,”

### Table 2. Significantly enriched pathways in FP pheochromocytomas in comparison with other hereditary pheochromocytoma classes

<table>
<thead>
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<tbody>
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<td>Response to stress</td>
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<tr>
<td>Peroxisome</td>
<td>0.004</td>
</tr>
<tr>
<td>DNA repair</td>
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<tr>
<td>Lysosome</td>
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<tr>
<td>Vacuole</td>
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<tr>
<td>Endocytosis</td>
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</tr>
<tr>
<td>Complement activation</td>
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<tr>
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<tr>
<td>Translation factor activity</td>
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<tr>
<td>Calcium channels</td>
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<td>Glutamate metabolism</td>
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<tr>
<td>RNA associated</td>
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*DM_GSEA: significance of enrichment as described in Methods.
which could progressively evolve to pheochromocytoma. Testing of this hypothesis will be possible with characterization of the FP mutation at 2q.

The identification of a second linked locus at chromosome 16p13 in this family is consistent with a digenic inheritance. The two-locus trait analysis supports a multiplicative or synergistic model to explain the relationship between the two loci (9). Of note, the 16p13 region overlaps that of a recently described familial neuroblastoma locus (27). Pheochromocytoma and neuroblastoma share a common sympathoadrenal origin and could conceivably share a genetic link. However, although LOH at the 16p13 region was detected in neuroblastomas (24), we have not identified chromosomal gains and/or losses at the 16p locus in the FP pheochromocytomas. The robust and concordant findings of the two independent linkage analyses are consistent with a role for this locus in the disease phenotype. Hence, it is possible that a distinct mechanism of tumorigenesis may operate at the putative chromosome 16 locus that does not require gross structural defects.

Expression profiling suggests that FP tumors resemble pheochromocytomas derived from MEN 2 and NF1. It is plausible therefore that the FP susceptibility gene(s) may encode functions associated with those of mutated RET and NF1 genes. Most genes in the target region have no ascribed function, but those believed to be involved in the signals highlighted by the GSEA are potential candidates to be further explored. Pairing of expression-based clustering with LOH analysis of pheochromocytomas allowed the identification of additional FP pheochromocytomas. The distinction between the effects of genotype from those of loss of chromosome 2 is not trivial in these tumors, especially because our hypothesis is that these are not independent events. It is possible that low expression of 2q genes in tumors without LOH reflects other mechanisms of down-regulation, such as methylation or microRNA-based repression (28). Our data suggest that integration of global genomic approaches can be successfully used to help in the identification of the primary genetic defect in familial disorders. This concept has been recently validated not only for studies of monogenic diseases (29) but also for quantitative trait loci by deriving genetic transmission patterns of cotranscribed genes (30–32).

In conclusion, we identified two novel susceptibility loci consistent with a recessive form of pheochromocytoma on chromosomes 2 and 16. These findings broaden the spectrum of genetic defects and mode of inheritance of pheochromocytomas and might signal the existence of other familial forms of the tumor that could result from defects in more than one gene. Although the role of these genes in more common forms of disease still remains to be determined, the initial chromosome 2 LOH data from sporadic tumors suggest that the FP locus may be relevant for a broader group of pheochromocytomas.

Appendix

Familial Pheochromocytoma Consortium members and their affiliations: Gail Adler (Brigham and Women’s Hospital, Boston, MA); Seth M. Arum (Boston Medical Center, Boston, MA); Marta Barontini (Center of Endocrinology Investigations, Buenos Aires, Argentina); Diana E. Benn (Royal North Shore Hospital and Kolling Institute of Medical Research, University of Sydney, Sydney, New South Wales, Australia); Ana Valèria B. de Castro (Universidade Estadual Paulista, Botucatu, São Paulo, Brazil); Robert Dluhy (Brigham and Women’s Hospital, Boston, MA); Judy Garber (Department of Population Sciences, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA); John Godine (Massachusetts General Hospital, Diabetes Center, Boston, MA); Ashley Grossman (St. Bartholomew’s Hospital, London, United Kingdom); Shirin Haddady (Division of Endocrinology, University of Massachusetts Memorial Medical Center, Worcester, MA); Shannon Heitritter (Brigham and Women’s Hospital, Boston, MA); Richard Hodin (Department of Surgery, Massachusetts General Hospital, Boston, MA); Marta Korbonits (St. Bartholomew’s Hospital, London, United Kingdom); Francis Moore, Jr. (Brigham and Women’s Hospital, Boston, MA); Célia Nogueira (Universidade Estadual Paulista, Botucatu, São Paulo, Brazil); Vania Nóse (Department of Pathology, Brigham and Women’s Hospital, Boston, MA); I. Tolgay Ocal (Yale University, New Haven, CT); Pascal Pigny (Regional University Hospital, Center Hospitalier Régional Universitaire, Lille, France); James A. Powers (Tufts New England Medical Center, Boston, MA); Bruce G. Robinson (Royal North Shore Hospital and Kolling Institute of Medical Research, University of Sydney, Sydney, New South Wales, Australia); Marie Roses (Division of Endocrinology, University of Massachusetts Memorial Medical Center, Worcester, MA); Gabriela Sanso (Center of Endocrinology Investigations, Buenos Aires, Argentina); Sandro Santagata (Department of Pathology, Brigham and Women’s Hospital, Boston, MA); Kathy Schneider (Department of Population Sciences, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA); Julie Ann Sosa (Yale University, New Haven, CT); and Arthur S. Tischler (Tufts New England Medical Center, Boston, MA).

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