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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*
PTEN is a tumor suppressor gene on 10q23 and encodes a dual specificity phosphatase. One of the major substrates for PTEN is phosphotidylinositol (3,4,5) triphosphate in the PI3 kinase pathway. When PTEN is dysfunctional or absent, P-Akt is high and hence, anti-apoptotic. *PTEN* is a major susceptibility gene for Cowden syndrome (CS), a hereditary disorder with a high risk of breast and thyroid cancer, and appears to be involved in a broad range of tumors. In addition, germline *PTEN*mutations have been found in a developmental disorder, Bannayan-Riley-Ruvalcaba syndrome (BRR) as well. This is an autosomal dominant disorder characterised by macrocephaly, lipomatosis, hemangiomatosis and speckled penis. Previously not thought to be associated with cancer risk, BRR families and cases with germline *PTEN* mutations have recently been shown to be at risk for cancers and especially breast tumors. Between 10-80% (mean 60%) of CS families and 60% of BRR individuals have germline *PTEN* mutations. Families that do not have germline *PTEN* mutations are not inconsistent with linkage to the 10q22-23 region. Thus, genes with related function to *PTEN* in the 10q21-q25 region are good candidates genes for *PTEN* mutation negative CS, BRR and related sporadic tumors, eg, those of the breast and thyroid. *MINPP1* lies no more than 1 Mb upstream of *PTEN* and encodes an inositol polyphosphate phosphatase. In Year 1 of this award, the PI has ascertained 14 unrelated CS probands, 22 BRR probands and 20 CS-like probands known not to harbor germline *PTEN* mutations. To date, they have not been found to carry germline *MINPP1* mutations. We found 4 malignant and 3 benign thyroid tumors to harbor deletion or intragenic mutation of *MINPP1*. More interestingly, IVS3+34T>A was found in about 15% of FA cases and normal controls but not in patients with FTC. These results suggest a role for MINPP1 in the pathogenesis of at least a subset of malignant follicular thyroid tumors, and that *MINPP1* might act as a low penetrance predisposition allele for FTC.

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Year 1 Annual Progress Report

Proposal Title: A novel phosphatase gene on 10q23, *MINPP*, in hereditary and sporadic breast cancer (DAMD17-00-1-0390)

PI: Charis Eng, MD, PhD

INTRODUCTION

PTEN is a tumor suppressor gene on 10q23 and encodes a dual specificity phosphatase. One of the major substrates for PTEN is phosphotidylinositol (3,4,5) triphosphate in the PI3 kinase pathway. Downstream of this pathway lies Akt/PKB, a known cell survival factor. When PTEN is functional and abundant, Akt is hypophosphorylated and hence, pro-apoptotic. Conversely, when PTEN is dysfunctional or absent, P-Akt is high and hence, anti-apoptotic. *PTEN* is a major susceptibility gene for Cowden syndrome (CS), a hereditary disorder with a high risk of breast and thyroid cancer, and appears to be involved in a broad range of tumors. In addition, germline *PTEN*mutations have been found in a developmental disorder, Bannayan-Riley-Ruvalcaba syndrome (BRR) as well. This is an autosomal dominant disorder characterised by macrocephaly, lipomatosis, hemangiomatosis and speckled penis. Previously not thought to be associated with cancer risk, BRR families and cases with germline *PTEN* mutations have recently been shown to be at risk for cancers and especially breast tumors. Between 10-80% (mean 60%) of CS families and 60% of BRR individuals have germline *PTEN* mutations. Families that do not have germline *PTEN* mutations are not inconsistent with linkage to the 10q22-23 region. While breast cancer is a major component of CS and 30-50% of sporadic tumors carry hemizygous deletion in the 10q22-23 region, no or rare sporadic breast carcinomas have somatic intragenic *PTEN* mutations. A gene encoding a novel inositol polyphosphate phosphatase, *MINPP*, with overlapping function with PTEN, has been mapped to 10q23. We hypothesise that *MINPP* will be the susceptibility gene for the remainder of CS and BRR families and might likely be the major tumor suppressor gene on 10q23 which plays a role in the pathogenesis of sporadic breast carcinomas. We hope to explore whether *MINPP* is another CS and BRR susceptibility gene by looking for germline mutations in cases without germline *PTEN* mutations. We will also perform mutation and fine structure deletion analysis of *MINPP* in sporadic breast carcinomas. And finally, to prove that *MINPP* is a tumor suppressor and to begin to explore its relationship with PTEN in breast carcinogenesis, we will perform stable transfection experiments into two breast cancer lines with known genomic *PTEN* status (one PTEN wildtype and one PTEN null) as well as known PTEN protein and P-Akt levels. We will especially determine if MINPP is growth suppressive like PTEN, and determine if growth suppression is mediated by G1 arrest and/or apoptosis. Towards these ends, our specific aims were:

1. To determine if germline mutations of *MINPP* cause *PTEN* mutation negative CS, BRR and CS-like families.
2. To determine if somatic *MINPP* mutations and deletions are associated with sporadic breast carcinomas.
3. To determine if MINPP affects Akt activity and causes G1 arrest and/or cell death in breast cancer cell lines.

BODY

Task 1: Mutation analysis of *MINPP* in germline *PTEN* mutation negative CS, BRR and CS-like Cases

Fourteen unrelated CS probands, 22 unrelated BRR probands and 20 unrelated CS-like probands known not to harbor germline *PTEN* mutations have thus far been ascertained. CS and BRR were diagnosed stringently by the criteria of the International Cowden Consortium (1) and as documented previously (2), respectively. The criteria for the diagnosis of a CS-like individual or family is as previously described (3). Preliminary mutation analysis of all exons, exon-intron junctions and flanking intronic sequences of *MINPP1* have been performed on these subjects. Among a total of 56 subjects, no germline *MINPP1* mutations were found (4) (Eng, unpublished). Please see appended reprint for further details.

We are continuing to accrue germline *PTEN* mutation negative, CS, BRR and CS-like probands for *MINPP* analysis. In addition, we are also examining *PTEN* mutation negative, *MINPP1*mutation negative probands for mutations in genes in the 10q22-24 interval.

Task 2: Mutation and deletion analysis of *MINPP* in sporadic primary human breast carcinomas

To further understand the role of *MINPP* in sporadic counterparts of CS component cancers, we are accruing two series of sporadic tumors, primary adenocarcinomas of the breast and primary follicular thyroid neoplasias. Currently, we have accrued 50 breast cancers and have examined the first 10 for somatic *MINPP* mutations. To date, no obvious pathogenic mutations have been found but N=10 is a small subset of the entire series. Accrual of breast tumors and *MINPP* mutation analysis continues.

We then turned to examining the sporadic counterpart of common CS component neoplasias, namely, sporadic follicular thyroid adenomas (FA) and follicular thyroid carcinomas (FTC). We analyzed DNA from tumor and corresponding normal tissue from 23 patients with FA and 15 patients with FTC for LOH and mutations at the *MINPP1* locus. LOH was identified in 4 malignant and 3 benign tumors. One of these FTC's with LOH was found to harbor a somatic c.122C>T or S41L mutation. We also found two germline sequence variants, c.809A>G (Q270R) and IVS3+34T>A. The c.809A>G variant was only found in one patient with FA but not in patients with FTC or normal controls. More interestingly, IVS3+34T>A was found in about 15% of FA cases and normal controls but not in patients with FTC. These results suggest a role for MINPP1 in the pathogenesis of at least a subset of malignant follicular thyroid tumors, and that *MINPP1* might act as a low penetrance predisposition allele for FTC. See appended reprint for details

Task 3: Functional studies of *MINPP* in *PTEN*+/+ and PTEN null breast cancer cell lines

MINPP1 cDNA constructs are being made in pCR2.1 and in the mammalian expression system pUHD10-3 which contains a tetracycline-suppressible (Tet-off) promoter, as previously described for *PTEN* expression constructs (5).

KEY RESEARCH ACCOMPLISHMENTS

- Germline *MINPP1* mutations likely do not account for a large proportion of germline *PTEN* mutation negative CS, BRR and CS-like probands, although accrual is not sufficient to draw any conclusions at this time.

- Somatic *MINPP1* alterations play some role in the genesis of sporadic follicular thyroid neoplasias.

- Germline *MINPP1* variation may be considered low penetrance alleles for predisposition to FTC.

REPORTABLE OUTCOMES

Dahia PLM, Gimm O, Chi H, Marsh DJ, Reynolds PR, **Eng C**. Absence of germline mutations in *MINPP1*, a phosphatase-encoding gene centromeric of *PTEN*, in patients with Cowden and Bannayan-Riley-Ruvalcaba syndrome without germline *PTEN* mutations. J Med Genet 2000; 37:715-7.

Gimm O, Chi H, Dahia PLM, Perren A, Hinze R, Komminoth P, Dralle H, Reynolds PR, **Eng C**. Somatic mutation and germline variants of *MINPP1*, a phosphatase gene located in proximity to *PTEN* on 10q23.3, in follicular thyroid carcinomas. J Clin Endocrinol Metab 2001; 86:1801-5.

CONCLUSIONS

The first year of work exploring MINPP as an alternative phosphatase in playing a role in the etiology and pathogenesis of CS and sporadic tumors remain inconclusive for reasons of sample size. Our work to date has shown that germline mutations in *MINPP1* do not account for the majority of germline *PTEN* mutation negative CS, BRR and CS-like probands. However, sample size for each category is too small to draw any conclusions at present whether germline *MINPP1* mutations play any role in these syndromes. As originally proposed, accrual of further *PTEN* mutation negative CS, BRR and CS-like individuals continues. Similarly, the somatic analysis of *MINPP1* in sporadic breast carcinomas is still in its infancy with a sample size tested to date of 10. Further samples are being accrued for *MINPP1* analysis. We have shown that somatic *MINPP1* alterations does play a role in the pathogenesis of sporadic FA and FTC. Further, we have some evidence that *MINPP1* might serve as a common low penetrance gene for susceptibility to isolated FTC.

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APPENDIX

Reprints of Articles:

Dahia PLM, Gimm O, Chi H, Marsh DJ, Reynolds PR, **Eng C**. Absence of germline mutations in *MINPP1*, a phosphatase-encoding gene centromeric of *PTEN*, in patients with Cowden and Bannayan-Riley-Ruvalcaba syndrome without germline *PTEN* mutations. J Med Genet 2000; 37:715-7.

Gimm O, Chi H, Dahia PLM, Perren A, Hinze R, Komminoth P, Dralle H, Reynolds PR, **Eng C**. Somatic mutation and germline variants of *MINPP1*, a phosphatase gene located in proximity to *PTEN* on 10q23.3, in follicular thyroid carcinomas. J Clin Endocrinol Metab 2001; 86:1801-5.

NIH-Style Modular Biosketch

of *LDLR* as FH causing, as they appear to have a modest effect on LDL receptor function.

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J Med Genet 2000;37:715–717

Absence of germline mutations in *MINPP1*, a phosphatase encoding gene centromeric of *PTEN*, in patients with Cowden and Bannayan-Riley-Ruvalcaba syndrome without germline *PTEN* mutations

EDITOR—Germline mutations in the dual specificity phosphatase gene *PTEN* (also known as *MMAC1* or *TEP1*) have been associated with susceptibility to two related hamartomatous disorders, Cowden syndrome (CS, MIM 158350) and Bannayan-Riley-Ruvalcaba syndrome (BRR, MIM 153480).[1 2] It has recently been established that PTEN functions as a 3-phosphatase towards phospholipid substrates in the phosphatidylinositol 3-kinase (PI-3 kinase) pathway.[3] Lack of PTEN results in the accumulation of phosphatidylinositol-(3,4,5)-P_3, which is required for activation of protein kinase B (PKB)/Akt, a downstream target of PI3-kinase and a known cell survival factor.[4–8]

While up to 81% of CS and approximately 60% of BRR cases have detectable *PTEN* germline mutations, no mutations in the coding region or exon-intron boundaries of *PTEN* have been found in the remaining affected subjects.[2 9–13] Informative *PTEN* mutation negative families have been shown to be linked to the 10q23 region, where *PTEN* lies,[2 14] although recently there has been a report of two CS families in which linkage to 10q23 has been excluded.[9] This has raised the possibility that either a regulatory region of the *PTEN* gene not included in previous studies, such as the promoter region, or another, closely located gene might be responsible for the CS and BRR cases in which no *PTEN* mutation has been found. The first alternative is unlikely to represent the majority of such cases, as no evidence of *PTEN* transcriptional silencing has been detected in the tissue of affected CS subjects in which no *PTEN* mutation was identified (Dahia and Eng, unpublished observations). Transcription levels of PTEN were found to be similar in affected and unaffected tissues of at least three unrelated CS patients and were equivalent to those of normal subjects. This suggests that methylation of the promoter or mutation within the promoter affecting transcription of *PTEN* does not occur in at least a subset of these *PTEN* mutation negative CS and BRR cases. To investigate the possibility that a closely mapped gene was the target of such mutations, we examined the coding region of a recently identified gene mapping to 10q23, next to D10S579, a marker estimated to lie no more than 1 Mb centromeric of *PTEN*.[15] The multiple inositol polyphosphate phosphatase, known as *MINPP1* or *MIPP*, has been cloned and shown to encode a conserved domain common to histidine phosphatases.[15 16] *MINPP1* codes for an approximately 52 kDa enzyme with the ability to remove the 3-phosphate from inositol phosphate substrates, such as Ins (1,3, 4,5)P_4, as well as other inositol moieties. It has been shown that human *MINPP1* has a wide tissue distribution pattern and its subcellular localisation appears to be targeted to the endoplasmic reticulum (ER).[15 16] While little is known about the human *MINPP1* function, its most well studied homologue, chick *HiPER1*, has a more restricted tissue distribution and appears to be critical to regulate the transition

Table 1 Primer sequences and annealing temperature used in PCRs of the MINPP1 gene

MINPP1 exon	*Forward sequence*	*Reverse sequence*	*Annealing temperature used for PCR*
1-A	MINPP1 5'UTRF CTCCACTGACCGTCCCGA	MINPP1-296R ATCTGTTTGACCGTGGGGTA	54
1-B	MINPP1-145F ACCAAGACTCGCTACGAGGA	MINPP1-556R GTGCTTGGAACTGGTGATGA	54
1-C	MINPP1-535F CTCATCACCAGTTCCAAGCA	MINPP1-I-1R: AGGACCGGGACAGCACAC	61
2	MINPP1-I-2F: CGGCTGTGCGGATTAGTAAG	MINPP1-I-2R: TCCTTATGTTTTCATTTTCACAGTTC	54
3	MINPP1-I-3F: TCCCCAAACTGAAGATGTCC	MINPP1-I-3R: AACCAAATGCAAACAAGCAA	54
4	MINPP1-I-4F: TCAGGGAATCTTGTTATATTTTTGAA	MINPP1-I-4R: TGGGTAGAGTGGAAGGTTCG	54
6*	MINPP1-1093F ATCCTCCAGTTTGGTCATGC or GTCTCAGCCAATTTCTTCTC	MINPP1-1464R TCATAGTTCATCAGATGTACTGTT	54

*In the chick *MINPP1* homologue, *HiPER1*, an extra exon, dubbed exon 5, and not seen in humans, precedes the final exon, named therefore exon 6.

of growth plate chondrocytes from proliferation to hypertrophy.[17] It is presumed that human *MINPP1* plays a role in differentiation and apoptosis, although details on the pathways involved in such signalling are as yet unknown. Thus, owing to its chromosomal location and to the fact that, like *PTEN*, it encodes a phosphatase with activity towards lipid substrates, we sought to investigate whether mutations in *MINPP1* would account for cases of CS and BRR without detectable *PTEN* mutations.

We obtained DNA from 36 subjects who met stringent criteria for the diagnosis of CS (n=14) and BRR (n=22) and in whom no *PTEN* mutation had been detected.[12 13] In at least one of the families, linkage data were compatible with linkage of the CS phenotype with the 10q23 region.[14] The rest of the cases were isolated or belonged to small families where linkage analysis was impossible. Informed consent was obtained from all subjects enrolled in this study, according to institutional Human Subjects Protection Committee protocols. All samples were screened for mutations in the coding region of *MINPP1* and most intron-exon boundaries of the gene by PCR based (primer sequences and PCR conditions in table 1) direct sequence analysis, as previously described.[18] No *MINPP1* mutations were found in germline DNA from any of the subjects examined in the present study. In particular, no mutations were found at the highly conserved histidine phosphatase motif, RHGxRxP, which defines members of the histidine acid phosphatase family. In addition, a second highly conserved site in this group of phosphatases comprising a histidine residue located at position 370 was found to be intact in all samples examined. This represents a proton donor site at the carboxy-terminal region of the protein which appears to be critical for full catalytic activity of this group of enzymes.[15 16] We identified five variations from the reference *MINPP1* sequence from the database in all samples, as well as in three normal controls (GenBank accession number AF046914). All of these sequence variants were identical to the reference *MINPP2* sequence (GenBank accession number AF084943). A sixth variant, c.444A→G, was noted in all our sequences which is in agreement with the *MINPP1* reference sequence, but at odds with that of *MINPP2*. It is likely, therefore, that these variations might represent errors in sequence entry on the database, rather than being associated with any particular phenotype, as they were identical in all samples, including the normal controls.

While described as independent hamartoma syndromes with shared clinical features until recently, it has been generally accepted that only CS bears a higher susceptibility to malignancies.[19 20] A broad analysis of genotype-phenotype data in the largest series of both CS and BRR recently undertaken in our laboratory has suggested that they might in fact represent distinct spectra of the same primary disorder.[13] These findings have clear implications for the follow up of affected subjects, in which systematic cancer surveillance is now recommended for both disorders, and not only for patients with CS.

In several human malignancies, such as breast, prostate, and thyroid cancer with loss of heterozygosity of 10q and in which no *PTEN* mutations have been found, it has been suggested that a region proximal to *PTEN* might be the main target in the tumorigenesis pathway.[18 21-24] It remains to be determined whether somatic abnormalities of *MINPP1* might be related to any of these sporadic tumours.

In conclusion, we have excluded an important candidate gene as the primary genetic abnormality underlying CS and BRR in subjects without identifiable *PTEN* mutation. It is possible that some degree of genetic heterogeneity exists, as suggested by a study that has excluded linkage to 10q23 in two *PTEN* mutation negative CS families.[9] The major genetic defect responsible for CS and BRR in cases without detectable *PTEN* mutation still remains to be established.

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Mosaicism in Alport syndrome and genetic counselling

EDITOR—Alport syndrome is characterised by a progressive glomerulonephritis with typical ultrastructural changes in the glomerular basement membrane. The most frequent, semidominant, X linked type is the result of a variety of mutations (either point mutations or intragenic deletions) of the *COL4A5* gene encoding the α5 chain of type IV collagen.[1]

During SSCP scanning of the *COL4A5* gene, a shift in a segment including exon 44 and flanking intronic sequences was found in a 19 year old proband showing typical ultrastructural changes of the glomerular basement membrane (III.3 in fig 1). Sequence analysis showed a G→C transversion in the 5' splice site of intron 44 (position 4271+1). The mutation introduced an *Alu*I restriction site which divided a 66 bp fragment into two fragments of 39 + 27 bp. All 18 family members were tested using this restriction assay and the mutation was found in the proband's affected brother, his cousin, his mother, and two maternal aunts. Surprisingly, the proband's grandmother was a normal homozygote. The proband's grandfather was dead, but true paternity of all daughters could be (indirectly) ascertained by polymorphic markers.[1]

In this family the mutation is associated with juvenile Alport syndrome in males, suggesting that the splicing defect results in a low level or absence of the protein, in agreement with our previous findings on genotype-phenotype correlations.[1] Interestingly, we noted considerable clinical variability among heterozygous females (n=4), ranging from ESRD at 27 years to absence of microscopic haematuria at 37 years.

Our data strongly suggest mosaicism in the germ cells of either grandparent. Mosaicism in germ cells may be the result of either a mutation in a germ cell that thereafter undergoes mitotic divisions (giving rise to mosaicism confined to germ cells), or an early postzygotic mutation before separation of the somatic/germ cells (giving rise to mosaicism in both the tissues and germline). In the latter case, the phenotype may or may not be expressed in the mosaic subjects, depending on the proportion of mutated cells in the relevant tissues. In order to verify mosaicism in somatic tissues of the living grandmother (I.1 in fig 1), we used Amplification Refractory Mutation System (ARMS-PCR), a tool able to detect known mutations even when present in a low fraction of template molecules.[5] The primer sense for exon 44[1] was used in combination with the following specific antisense primers: normal (5'-GGTATAACTATCTTCAGGAATAAGTCTTAC-3') and mutant (5'- GGTATAACTATCTTCAGGAATAAGTCT TAG-3'). We performed ARMS-PCR on DNA extracted from grandmaternal peripheral blood using progressively lower stringency by lowering the temperature or increasing the PCR cycle number or both, with the aim of reaching a condition where even the very few mutated molecules present in the blood sample would be amplified. This condition was never reached, as the grandmother's DNA always gave the same results as normal homozygous female controls (data not shown).

On analysis of Xq22 DNA polymorphisms, the three carrier females in the second generation were homozygous for one of the maternal haplotypes, which therefore must have been present in the dead grandfather as well, while the single non-carrier female and the unaffected male carried the other maternal haplotype. These data might suggest that the mutation was present in the grandmaternal gonads on

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Somatic Mutation and Germline Variants of *MINPP1*, a Phosphatase Gene Located in Proximity to *PTEN* on 10q23.3, in Follicular Thyroid Carcinomas*

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ABSTRACT

Various genes have been identified to play a role in the pathogenesis of follicular thyroid tumors. Cowden syndrome is the only known familial syndrome with an increased risk of both follicular thyroid adenoma (FA) and carcinoma (FTC). Germline mutations in the tumor suppressor gene *PTEN*, which encodes a dual-specificity phosphatase, have been found in up to 80% of patients with Cowden syndrome suggesting a role of *PTEN* in the pathogenesis of follicular thyroid tumors. Although somatic intragenic mutations in *PTEN*, which maps to 10q23.3, are rarely found in follicular tumors, loss of heterozygosity (LOH) of markers within 10q22–24 occurs in about 25%. Recently, another phosphatase gene, *MINPP1*, has been localized to 10q23.3. MINPP1 has the ability to remove 3-phosphate from inositol phosphate substrates, a function that overlaps that of PTEN. Because of this overlapping function with *PTEN* and the physical location of *MINPP1* to a region with frequent LOH in follicular thyroid tumors, we considered it to be an excellent candidate gene that could contribute to the pathogenesis of follicular thyroid tumors. We analyzed DNA from tumor and corresponding normal tissue from 23 patients with FA and 15 patients with FTC for LOH and mutations at the *MINPP1* locus. LOH was identified in four malignant and three benign tumors. One of these FTCs with LOH was found to harbor a somatic c.122C > T or S41L mutation. We also found two germline sequence variants, c.809A > G (Q270R) and IVS3 + 34T > A. The c.809A > G variant was found in only one patient with FA but not in patients with FTC or normal controls. More interestingly, IVS3 + 34T > A was found in about 15% of FA cases and normal controls but not in patients with FTC. These results suggest a role for *MINPP1* in the pathogenesis of at least a subset of malignant follicular thyroid tumors, and *MINPP1* might act as a low penetrance predisposition allele for FTC. (*J Clin Endocrinol Metab* **86:** 1801–1805, 2001)

FOLLICULAR THYROID TUMORS are a common finding in iodine-deficient areas. By far, the most common tumors are benign follicular thyroid adenomas; only a minority of the tumors are carcinomas. Until today, it is unknown whether an adenoma-carcinoma sequence exists. Data supporting both theories exist (1–3).

The only known familial syndrome with an increased risk of both benign and malignant follicular thyroid tumors is Cowden syndrome (4). Germline mutations of *PTEN*, encoding a dual-specificity phosphatase, are found in up to 80% of patients with Cowden syndrome (5, 6), 60% of patients with Bannayan-Riley-Ruvalcaba syndrome (7), and an unknown proportion of patients with a Proteus-like syndrome (8). Although somatic intragenic *PTEN* mutations are found in only a minority of sporadic follicular thyroid carcinomas (9, 10), loss of heterozygosity (LOH) of markers within 10q23, especially including marker D10S579, has been found in up to 25% of either benign or malignant follicular tumors (9–11). In another study, fine structure deletion analysis of 10q22–24 demonstrated regions of loss that suggest that follicular adenomas and carcinomas develop along distinct parallel neoplastic pathways (11).

A new gene, *MINPP1* (multiple inositol polyphosphate phosphatase), has recently been localized to 10q23.3 in close proximity to marker D10S579 (12). *MINPP1*, also known as *MIPP*, has been shown to encode a conserved domain common to histidine phosphatases (12, 13). This 52-kDa enzyme has the ability to remove 3-phosphate from inositol phosphate substrates, such as Ins(1,3,4,5)P_4, a function that overlaps that of PTEN even though the sequence similarity of *PTEN* and *MINPP1* is only about 16%. MINPP1 is the only

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enzyme known to hydrolyze the abundant metabolites inositol pentakisphosphate and inositol hexakisphosphate. Little is known about human *MINPP1*. It has been shown, however, to be expressed in a wide variety of tissues, including the human thyroid (Gimm, O., and C. Eng, unpublished data). Because of *MINPP1*'s overlapping function with *PTEN* and its physical location within a region of LOH for thyroid tumors, it is an excellent candidate gene that could contribute to thyroid tumorigenesis.

Here, we report the results of mutation analysis of *MINPP1* in benign and malignant follicular thyroid tumors from an iodine-deficient area. Our data might tentatively suggest a role of *MINPP1* in the tumorigenesis of at least a subset of malignant follicular thyroid tumors.

Materials and Methods

Patients and specimens

Paraffin blocks from 38 unselected benign (n = 23) and malignant (n = 15) follicular thyroid tumors were ascertained from Germany and Switzerland. Three malignant tumors were classified as Hürthle cell carcinoma. All samples were obtained with informed consent. In all 38 samples, tumor tissue and corresponding normal tissue (either normal thyroid tissue from a different block or from an area not in proximity to the tumor, or adjacent muscle tissue distant to the tumor site) were available for extraction of paired somatic and "germline" genomic DNA. DNA extraction following microdissection was performed using standard protocols (14).

Mutation analysis

PCR amplification using genomic DNA as template was carried out in 1× PCR buffer (Perkin-Elmer Corp., Norwalk, CT) containing 200 μM dNTP (Life Technologies, Inc., Gaithersburg, MD), 1 μM of each primer (see Table 1), 2.5U *Taq* polymerase (QIAGEN, Valencia, CA), 0.9 mM $MgCl_2$, 1× Q-buffer (QIAGEN), and 50–100 ng of tumor DNA template in a 50 μL volume. PCR conditions were 35 cycles of 1 min at 95 C, 1 min at 58 C, and 1 min at 72 C followed by 10 min at 72 C. All exons were at least divided into two (a and b) because of their large sizes. Exon 1 had to be divided into three fragments (a–c).

Mutation analysis for exon 2 (fragments 2a and 2b), exon 3 (fragments 3a and 3b), and exon 6 (fragments 6a and 6b) was performed with DGGE. Exon 1 and exon 4 of *MINPP1* are very GC rich and therefore less suitable for DGGE. Hence, mutation analysis for these two exons was performed using SSCP (fragments 1b, 1c, 4a, and 4b). No optimal SSCP condition could be found for the 5′ part of exon 1 (fragment 1a) and hence was subjected to direct semiautomated sequence analysis as previously described (5, 15, 16).

Before DGGE, 10 μl of the resulting PCR product were added to 1 μl of Ficoll-based loading buffer. This mixture was loaded onto 10% polyacrylamide gels carrying a 15–65% urea-formamide gradient and a 2–9% glycerol gradient in 0.5 × TAE. The amplicons were electrophoresed at 60 C and 105 V for 16 h. The fragments were visualized with ultraviolet transillumination after staining with ethidium bromide solution (15 μl in 500 ml dH_2O) for 30 min.

Before SSCP, 2 μl of the resulting PCR product were added to 3 μl of formamide buffer and then heated to 95 C for 10 min and subsequently cooled on dry ice. Immediately before SSCP, the samples were quickly thawed and then run through a 10% polyacrylamide/1 × TBE gel. Gels were run either at 100 V for 14 h at room temperature (fragments 1b, 4a, 4b) or at 150 V for 16 h at 4 C (fragment 1c). Subsequent silver staining was performed as previously described (17).

Although DGGE is 100% sensitive and specific in this and other laboratory's hands (18–20), SSCP is acknowledged not to have the same high sensitivity and specificity (21). Routine quality control for both SSCP and DGGE in our laboratory takes the form of subjecting a known positive and known negative to electrophoresis along with the test samples. Further, three random SSCP negative samples are subjected to direct sequence analysis.

If variant DGGE/SSCP banding patterns were observed, the remaining PCR aliquot was subjected to purification and semiautomated sequencing using the above primers and dye terminator technology (see above). If sequencing revealed a variant, the corresponding germline DNA was examined in the same manner to determine whether the sequence variant is somatic or germline.

The frequencies of these sequence variants in patients with follicular thyroid tumors and in a race-matched control group were determined

TABLE 1. *MINPP1* primer sequences and PCR-product sizes

Fragment	Primer name	Primer sequence (5′ to 3′)	PCR-product size
1-a	MINPP1-10F[a]	CCGTCCCGACGATGCTAC	241
	MINPP1-250R[a]	CCGTCCCGACGATGCTAC	
1-b	MINPP1-189F[a]	AACCCCGTGCTATTGTCG	300
	MINPP1-488R[a]	CTGTCGCATATCCTGCCG	
1-c	MINPP1-444F	ATGGACGGGCAGCTAGTAGA	251
	MINPP1-I-1R	AGGACCGGGACAGCACAC	
2-a	MINPP1-I-2F	CGGCTGTGCGGATTAGTAAG	292
	MINPP1-GC-2aR[b]	TCTGGTCCAGTTTTGAAGGC	
2-b	MINPP1-GC-2bF[b]	TTGGACCTCCAACAGTTAATGA	304
	MINPP1-I-2R	TCCTTATGTTTTCATTTTCACAGTTC	
3-a	MINPP1-I-3F	TCCCCAAACTGAAGATGTCC	244
	MINPP1-GC-3aR[b]	TCAAAAACATCACACCAAGGA	
3-b	MINPP1-GC-3bF[b]	CTGTTCATTTGACCTGGCAAT	185
	MINPP1-I-3R	AACCAAATGCAAACAAGCAA	
4-a	MINPP1-I-4F	TCAGGGAATCTTGTTATATTTTTGAA	177
	MINPP1-1071R[a]	CTGCTTTGTCCAAGTGCTGA	
4-b	MINPP1-1027F[a]	GCTGCACCTTGTTTCAGGAT	251
	MINPP1-I-4R	TGGGTAGAGTGGAAGGTTCG	
6-a[c]	MINPP1-E6F	GTCTCAGCCAATTTCTTCTC	294
	MINPP1-GC-6aR[b]	TTTCATTTAATAACATCTGCACTCG	
6-b[c]	MINPP1-GC-6bF[b]	CACTGTGAAAATGCTAAGACTCC	298
	MINPP1-1538R[a]	GCATGTAATCACTCATTGCAGA	

[a] The number refers to the number of the position of the 5′ end of the primer in the sequence available under accession number AF084943; it is not equal to the nucleotide number within the translated coding region.

[b] These primers have in addition a GC-rich clamp (5′-CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCCG-3′) on their 5′ end in order to perform DGGE mutation analysis.

[c] In the chick *MINPP1* homolog, *HiPER1*, an extra exon, dubbed exon 5, and not seen in humans, precedes the final exon, named therefore exon 6.

using peripheral blood leukocyte DNA. This race-matched control group consisted of patients who were admitted to the Department of General Surgery, Halle, Germany, for nonthyroid-related diseases. Informed consent was given in all cases.

LOH analysis

For every germline-tumor pair, PCR reactions were carried out using 0.6 μM each of forward and reverse primer in 1× PCR buffer (QIAGEN), 4.5 mM $MgCl_2$ (QIAGEN), 1× Q-buffer (QIAGEN), 2.5 U HotStarTaq polymerase (QIAGEN), and 200 μM dNTP (Life Technologies, Inc.) in a final volume of 50 μL. Reactions were subjected to 35 cycles of 94 C for 1 min, 55–60 C for 1 min and 72 C for 1 min followed by 10 min at 72 C. LOH analysis for each germline-tumor pair was performed as previously described using markers flanking *MINPP1*, D10S541 (telomeric), D10S2491 (telomeric) (5, 22), and D10S1686 (centromeric) as well as the marker D10S579 that lies in close proximity to *MINPP1* (12). All forward primers were 5′-labeled with either HEX or 6-FAM fluorescent dye (Research Genetics, Inc., Huntsville, AL).

Statistical analysis

Differences in allele frequencies were calculated using the standard Chi-square test. A P value less than 0.05 was considered significant.

Results

Mutation analysis of all 5 exons of *MINPP1* from 38 follicular thyroid tumors revealed variants in 3 exons (fragments 1a, 2b, and 3b). Sequencing revealed one sequence variant each (Table 2). Corresponding germline DNA was examined for the presence of each of these variants.

We detected a sequence variant in one carcinoma, c.122C > T (S41L), in the 5′ end of exon 1 (fragment 1a) (Fig. 1A). This variant was absent in the corresponding germline (DNA from muscle) (Fig. 1B) and most likely represents a somatic missense mutation. Repeat PCR and sequencing confirmed the variant and excluded PCR errors. Thus, somatic S41L was found in 1 out of 15 carcinomas (7%).

The variants in exon 2 (fragment 2b), c.809A > G (Q270R) (Fig. 1, C and D) and fragment 3b, IVS3 + 34T > A (Fig. 1, F and G) were also present in the germline (data not shown). The heterozygous c.809A > G variant was seen in a patient with follicular thyroid adenoma and was never seen in patients with follicular thyroid carcinoma or in a race-matched control group (Fig. 1E and Table 2a).

The germline IVS3 + 34T > A variant was underrepresented in cases with follicular thyroid carcinoma (0%), compared with those with adenoma (15%; $P < 0.03$, Table 2b) or normal controls (14%; $P < 0.04$, Table 2b).

LOH analysis within 10q22–24 was performed for all tumor-germline pairs. We found LOH in seven follicular tumors, four carcinomas (27%), and three adenomas (13%). None of the seven follicular adenomas with IVS3 + 34T > A had LOH. Interestingly, the one carcinoma harboring the somatic mutation S41L showed LOH at D10S579, and the flanking markers D10S2491 and D10S1686 were not informative.

TABLE 2. Allele frequency of *MINPP1* polymorphic sequence variants in patients with follicular thyroid adenoma and follicular thyroid carcinoma and race-matched controls

Codon	Nucleotide (amino acid)	(a) Exon 2 FA	FTC	Controls
270	c.809G (Arg)	1	0	0
270	c.809A (Gln)	45	30	78

FA *vs.* FTC, P = n.s.; FA vs. controls, P = n.s.

Codon	Nucleotide (amino acid)	(b) Exon 3 FA	FTC	Controls
N/A	IVS3+34A (N/A)	7	0	9
N/A	IVS3+34T (N/A)	39	30	57

FTC *vs.* FA, $P < 0.03$; FTC *vs.* controls, $P < 0.04$. n.s., not significant; N/A, not applicable; FA, follicular thyroid adenoma; FTC, follicular thyroid carcinoma.

Discussion

In the present study, we detected a somatic S41L mutation in *MINPP1* together with loss of the corresponding wild-type allele in one follicular thyroid carcinoma. We also found two previously unreported germline sequence variants in *MINPP1*; one, an intronic variant, is underrepresented in cases with follicular thyroid carcinomas, compared with those with follicular thyroid adenomas or normal controls.

The somatic mutation c.122C > T in tumor DNA from one patient with follicular thyroid carcinoma changes serine, a neutral and polar amino acid, at position 41, to leucine, which is also neutral but hydrophobic. This region is highly conserved among several species (human, rat, mouse) (12, 13). Hence, one can speculate that this polar for hydrophobic amino acid substitution changes the structure of *MINPP1*. Postulating that *MINPP1* might act as a tumor suppressor, its functional activity might subsequently be lost or at least decreased. However, functional analysis would be necessary to confirm this premature hypothesis. Nonetheless, loss of the corresponding wild-type allele in this sample lends credence that the somatic S41L mutation is pathogenic and both *MINPP1* alleles inactivated.

The finding of a rare germline sequence variant in one patient with follicular thyroid adenoma is intriguing. This variant was neither observed in 78 control alleles nor found in 36 patients with Cowden syndrome or Bannayan-Riley-Ruvalcaba syndrome (23). One may speculate that this variant, which leads to substitution of a neutral and polar amino acid for a basic amino acid, affects the function of *MINPP1*. Whether this hypothetical change of *MINPP1* function plays a role in the pathogenesis of follicular thyroid adenomas must remain unresolved at this point. Histological appearance did not show any unusual features. Also, there was no family history of follicular thyroid adenomas, but no germline DNA was available from any relative.

The absence of the relatively frequent intronic polymorphic sequence variant IVS3 + 34T > A in follicular thyroid carcinoma patients is intriguing. Even though our numbers are small, at least one or two follicular thyroid carcinomas harboring this sequence variant should have been detected: power calculations reveal that if only 10% of 30 alleles have this variant, our power to detect this in at least one case would exceed 0.92. We also screened 30 patients with breast cancer for variation in *MINPP1* and found about the same frequency (12%) of this polymorphism as found in patients with FA (15%) and controls (14%) (Gimm, O., and C. Eng, unpublished data). Of note, this intronic polymorphism lies on the border of a poly-T/poly-A/poly-T tract. There is some

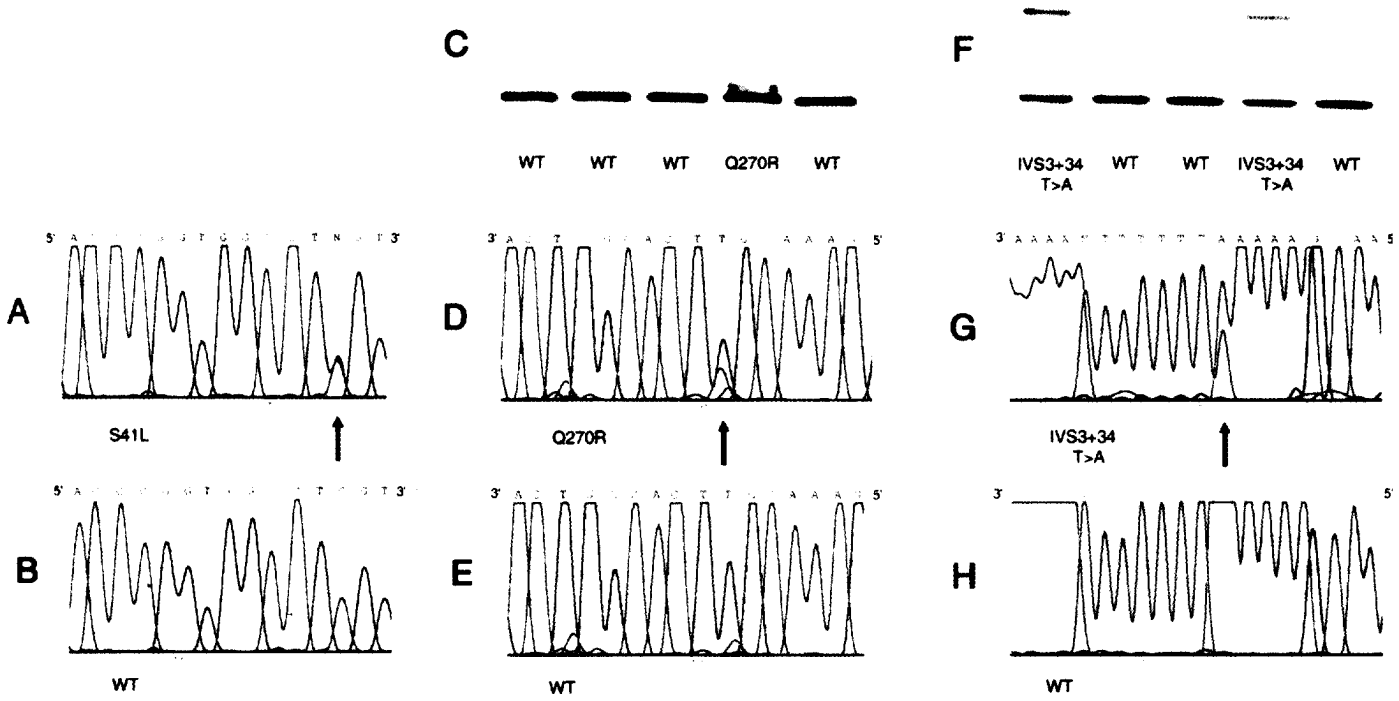

FIG. 1. Sequence variants in *MINPP1* identified by DGGE and/or sequencing. A, Sequence variant c.122C > T (S41L) in one follicular thyroid carcinoma. B, Absence of the sequence variant in the corresponding germline. C, DGGE reveals a variant in exon 2 (fragment 2b) in one follicular adenoma. D, Sequencing identifies the variant c.809A > G (Q270R). E, Example for absence of the variant c.809A > G (Q270R) in blood from one control patient. F, DGGE reveals a variant in exon 3 (fragment 3b) in follicular adenomas. G, Sequencing identifies the variant IVS3 + 34T > A. H, Example for absence of the variant c.809A > G (Q270R) in blood from one control patient. Note: Because the forward primers for the fragments 2b and 3b contain the GC-clamp, the reverse primers have been used for sequencing. Hence, the sequences in D, E, G, and H are reverse.

evidence that poly-N tracts may play important roles in RNA splicing and processing (24, 25). Recently, it has become more evident that development of a cancer can result from an interplay of either a few "high penetrance" mutations in key genes or from several, or many, sequence variants presently of unknown significance. For example, overrepresentation of a rare sequence variant of *RET* has been observed in patients with sporadic medullary thyroid carcinoma (26). Similar observations have been made for polymorphic sequence variants of *RET* in patients with HSCR (27, 28), *Cul2* in sporadic pheochromocytomas (29), and *PPARgamma* in isolated glioblastoma multiforme cases (30). However, the precise mechanism to explain how the intronic sequence variant could "protect" or at least lower the chance of developing follicular thyroid carcinoma is unknown and open to speculation. Possibly, this intronic change affects a splice-donor or splice-acceptor site or enhances a cryptic splice site, which would subsequently lead to a different protein. Unfortunately, complementary DNA was not available to test this hypothesis. The absence of this polymorphism in patients with follicular thyroid carcinoma may also support the hypothesis that follicular thyroid adenomas and carcinomas do not adhere to an adenoma-carcinoma sequence. Fine-structure deletion mapping of 10q22–24 also suggests that sporadic follicular thyroid adenomas and follicular thyroid carcinomas develop along distinct neoplastic pathways (11).

In conclusion, our observations suggest a role for *MINPP1* in the tumorigenesis of malignant follicular thyroid carcinoma. Although it may infrequently contribute to follicular carcinogenesis via the traditional pathway of somatic high penetrance, two-hit (31) mutations, this gene seems to harbor a variant that could act as a common low penetrance susceptibility allele for follicular thyroid carcinoma. Further, the DGGE and SSCP conditions reported here together with the knowledge of the frequency of various sequence variants may help in future mutation analyses of DNA from other cancers with LOH in the 10q23 region in which *PTEN* does not seem to play a major role, such as head and neck carcinomas, lung cancer, and melanomas (32).

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5. **Liaw D, Marsh DJ, Li J, et al.** 1997 Germline mutations of the *PTEN* gene in Cowden disease, an inherited breast and thyroid cancer syndrome. Nat Genet. 16:64–67.
6. **Marsh DJ, Coulon V, Lunetta KL, et al.** 1998 Mutation spectrum and genotype-phenotype analyses in Cowden disease and Bannayan-Zonana syndrome, two hamartoma syndromes with germline *PTEN* mutation. Hum Mol Genet. 7:507–515.
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hemihypertrophy, lower limb asymmetry, arterio-venous malformations and lipomatosis. Hum Mol Genet. 9:765–768.
9. **Dahia PLM, Marsh DJ, Zheng Z, et al.** 1997 Somatic deletions and mutations in the Cowden disease gene, *PTEN*, in sporadic thyroid tumors. Cancer Res. 57:4710–4713.
10. **Halachmi N, Halachmi S, Evron E, Parsons R, Sidransky D.** 1998 Somatic mutations of the *PTEN* tumor suppressor gene in sporadic follicular thyroid tumors. Gene Chrom Cancer. 23:239–243.
11. **Yeh JJ, Marsh DJ, Zedenius J, et al.** 1999 Fine structure deletion analysis of 10q22–24 demonstrates novel regions of loss and suggests that sporadic follicular thyroid adenomas and follicular thyroid carcinomas develop along distinct parallel neoplastic pathways. Gene Chrom Cancer. 26:322–328.
12. **Chi H, Tiller GE, Dasouki MJ, et al.** 1999 Multiple inositol polyphosphate phosphatase: evolution as a distinct group within the histidine phosphatase family and chromosomal localisation of the human and mouse genes to chromosomes 10q23 and 19. Genomics. 56:324–336.
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14. **Eng C, Mulligan LM, Healey CS, et al.** 1996 Heterogeneous mutation of the *RET* proto-oncogene in subpopulations of medullary thyroid carcinoma. Cancer Res. 56:2167–2170.
15. **Gimm O, Marsh DJ, Andrew SD, et al.** 1997 Germline dinucleotide mutation in codon 883 of the *RET* proto-oncogene in multiple endocrine neoplasia type 2B without codon 918 mutation. J Clin Endocrinol Metab. 82:3902–3904.
16. **Mutter GL, Lin M-C, Fitzgerald JT, et al.** 2000 Altered *PTEN* expression as a diagnostic marker for the earliest endometrial precancers. J Natl Cancer Inst. 92:924–931.
17. **Gimm O, Greco A, Hinze R, Dralle H, Pierotti M, Eng C.** 1999 Mutation analysis of *NTRK1* reveals sequence variants in human sporadic medullary thyroid carcinomas. J Clin Endocrinol Metab. 84:2784–2787.
18. **Sheffield VC, Cox DR, Lerman LS, Myers RM.** 1989 Attachment of a 40-base-pair G+C-rich sequence (GC-clamp) to genomic DNA fragments by the polymerase chain reaction results in improved detection of single-base changes. Proc Natl Acad Sci U S A. 86:232–236.
19. **Marsh DJ, Caron S, Dahia PLM, et al.** 1998 Germline *PTEN* mutations in Cowden syndrome-like families. J Med Genet. 35:881–885.
20. **Dahia PLM, Aguiar RCT, Alberta J, et al.** 1999 *PTEN* is inversely correlated with the cell survival factor PKB/Akt and is inactivated by diverse mechanisms in haematologic malignancies. Hum Mol Genet. 8:185–193.
21. **Sheffield VC, Beck JS, Kwitek AE, Sandstrom DW, Stone EM.** 1993 The sensitivity of single-strand conformation polymorphism analysis for the detection of single base substitutions. Genomics. 16:325–332.
22. **Li J, Yen C, Liaw D, et al.** 1997 *PTEN*, a putative protein tyrosine phosphatase gene mutated in human brain, breast and prostate cancer. Science. 275:1943–1947.
23. **Dahia PLM, Gimm O, Chi H, Marsh DJ, Reynolds PR, Eng C.** 2000 Absence of germline mutations in *MINPP1*, a phosphatase-encoding gene centromeric of *PTEN*, in patients with Cowden and Bannayan-Riley-Ruvalcaba syndrome without germline *PTEN* mutations. J Med Genet. 37:715–717.
24. **Leff SE, Evans RM, Rosenfeld MG.** 1987 Splice commitment dictates neuron-specific alternative RNA processing in calcitonin/CGRP gene expression. Cell. 48:517–524.
25. **Niksic M, Romano M, Buratti E, Pagani F, Baralle FE.** 1999 Functional analysis of *cis*-acting elements regulating the alternative splicing of human *CFTR* exon 9. Hum Mol Genet. 8:2339–2349.
26. **Gimm O, Neuberg DS, Marsh DJ, et al.** 1999 Over-representation of a germline *RET* sequence variant in patients with sporadic medullary thyroid carcinoma and somatic *RET* codon 918 mutation. Oncogene 18:1369–1370.
27. **Borrego S, Saez ME, Ruiz A, et al.** 2000 *RET* genotypes comprising specific haplotypes of polymorphic variants predispose to isolated Hirschsprung disease. J Med Genet. 37:572–578.
28. **Borrego S, Saez ME, Ruiz A, et al.** 1999 Specific polymorphisms in the *RET* proto-oncogene are over-represented in individuals with Hirschsprung disease and may represent loci modifying phenotypic expression. J Med Genet. 36:771–774.
29. **Duerr E-M, Gimm O, Kumm JB, et al.** 1999 Differences in allelic distribution of two polymorphisms in the VHL-associated gene *CUL2* in pheochromocytoma patients without somatic *CUL2* mutations. J Clin Endocrinol Metab., in press.
30. **Zhou XP, Smith WM, Gimm O, et al.** 2000 Over-representation of PPARγ sequence variants in sporadic cases of glioblastoma multiforme: preliminary evidence for common low penetrance modifiers for brain tumour risk in the general population. J Med Genet. 37:410–414.
31. **Knudson AG.** 1971 Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci U S A. 68:820–823.
32. **Robertson GP, Herbst RA, Nagane M, Huang HJ, Cavanee WK.** 1999 The chromosome 10 monosomy common in human melanomas results from loss of two separate tumor suppressor loci. Cancer Res. 59:3596–3601.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
ENG, Charis, MD, PhD	Associate Professor of Medicine & Human Genetics

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Chicago, Chicago, IL	B.A.	1978-82	Biological Sci
University of Chicago, Chicago, IL	Ph.D.	1982-86	Development. Bio
University of Chicago, Chicago, IL	M.D.	1982-88	Medicine
University of Cambridge, Cambridge, UK	(Post-Doc)	1992-95	Cancer Genetics

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds three pages, select the most pertinent publications. **DO NOT EXCEED THREE PAGES.**

Professional Positions

1988-91 Residency in Internal Medicine, Beth Israel Hospital, Boston, MA

1991-94 Clinical Fellowship, Medical Oncology, Dana-Farber Cancer Institute, Boston, MA

1992-95 CRC Dana-Farber Fellowship in Human Cancer Genetics, University of Cambridge, UK

1992-95 Senior Registrar in Clinical Cancer Genetics, University of Cambridge Addenbrooke's Hospital, Cambridge, UK and Royal Marsden Hospital, London, UK

1994-95 Instructor in Medicine, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA

1995-98 Assistant Professor of Medicine, Dana-Farber Cancer Institute and Harvard Medical School, Boston

1995-98 Active Staff Physician, Adult Oncology, Dana-Farber Cancer Institute, Boston

1995-98 Associate Physician, Brigham and Women's Hospital, Boston

1998- North American Editor and Cancer Genetics Editor, *Journal of Medical Genetics*

1999- Associate Professor (with tenure) of Medicine , The Ohio State University, Columbus, OH

1999- Director, Clinical Cancer Genetics Program, James Cancer Hospital and Solove Research Institute, Comprehensive Cancer Center, Ohio State University, Columbus, OH

Awards and Other Professional Activities

1982 Phi Beta Kappa

1982 Sigma Xi Associate Membership and Sigma Xi Prize

1987 Sigma Xi Promotion to Full Membership

1988 Alpha Omega Alpha

1995 First Lawrence and Susan Marx Investigator in Human Cancer Genetics, Dana-Farber Cancer Institute

1997-99 National Cancer Institute of Canada Panel J Member

1997-99 MBY3 Study Section, Department of Defence Breast Cancer Research Program Grants

1998 NCCN Genetics/High Risk Screening Panel

1999 American College of Physicians, Promotion to Fellowship

2001 American Society for Clinical Investigation, Elected Membership

Selected Research Projects On-Going or Completed in Last 3 Years

"Genetics of *PTEN* in Cowden syndrome and sporadic breast cancer"
PI: Charis Eng, MD, PhD
Agency: American Cancer Society Research Project Grant (RPG-98-211-01-CCE) 7/1/98-6/30/01
The goal of this project is to determine genotype-phenotype associations in Cowden syndrome and to determine if low penetrance mutations in *PTEN* predispose to apparently isolated breast cancer.

"Genetics of PTEN in Cowden-like syndromes"
PI: Charis Eng, MD, PhD
Agency: Department of Defence Idea Award (DAMD17-98-1-8058) 10/1/98-9/30/01
The goal of this project is to genetically delineate the function of *PTEN* in non-Cowden syndrome hereditary breast cancer and multiple primaries. The focus will be on breast and thyroid as well as breast and uterine cancer cases and families that do not meet the Consortium diagnostic criteria for Cowden syndrome.

"Dissecting out the bifurcation of lipid and protein phosphatase activities in PTEN-mediated growth arrest in a breast cancer model"
PI: Charis Eng, MD, PhD
Agency: Susan G. Komen Breast Cancer Research Foundation Grant 10/1/00-9/30/02
The goal of this project is to determine the pathways downstream of PTEN's lipid and protein phosphatase activities as they related to breast carcinogenesis

"Genetic analysis of the role of the microenvironment in epithelial tumor progression"
PI's: Charis Eng, MD, PhD, Gustavo Leone, PhD and Michael C. Ostrowski, PhD
Agency: V Foundation Jimmy V Golf Classic Research Award 3/1/01-2/28/04
The goal of this award is to provide seed moneys to gather preliminary data and make reagents related to tumor-microenvironmental interactions so that a group grant, eg PPG, may result from such work, as well as novel targets for therapy and prevention

"RET complex polymorphisms in Hirschsprung disease"
PI: Charis Eng, MD, PhD
Agency: National Institutes of Health R01 Research Project Grant 7/1/01-6/30/05
The goal of this project is to identify and characterise common low penetrance alleles within *RET* and the genes which encode its ligands and co-ligands in "sporadic" medullary thyroid carcinoma as well as sporadic Hirschsprung disease

Selected Publications (selected from a total of 167 published and in press peer reviewed original articles)

Nelen MR, Padberg GW, Peeters EAJ, 14 others, Ponder BAJ, Ropers HH, Kremer H, Longy M, **Eng C**. Localization of the gene for Cowden disease to 10q22-23. Nature Genet 1996; 13:114-6.

Eng C, Clayton D, Schuffenecker I, Lenoir G, Cote G, Gagel RF, 22 others, Ponder BAJ, Mulligan LM. The relationship between specific *RET* proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2. International *RET* Mutation Consortium analysis. JAMA 1996; 276:1575-9.

Liaw D, Marsh DJ, Li J, Dahia PLM, Wang SI, Zheng Z, Bose S, Call KM, Tsou HC, Peacocke M, **Eng C***, Parsons R*. Germline mutations of the *PTEN* gene in Cowden disease, an inherited breast and thyroid cancer syndrome. Nature Genet 1997; 16:64-7. (*Joint Senior Authorship noted on the article)

Marsh DJ, Dahia PLM, Coulon V, Zheng Z, Dorion-Bonnet F, Call KM, Little R, Lin AY, Goldstein A, Eeles RA, Hodgson SV, Richardson A-L, Robinson BG, Weber HC, Longy M, **Eng C**. Allelic imbalance, including deletion of *PTEN/MMAC1*, at the Cowden disease locus on 10q22-23 in hamartomas from patients with Cowden disease and germline *PTEN* mutation. Genes Chrom Cancer 1998; 21:61-9.

Marsh DJ, Coulon V, Lunetta KL, Rocca-Serra P, Dahia PLM, Zheng Z, 28 others, Parsons R, Peacocke M, Longy M, **Eng C**. Mutation spectrum and genotype-phenotype analyses in Cowden disease and Bannayan-Zonana syndrome, two hamartoma syndromes with germline *PTEN* mutation. Hum Mol Genet 1998; 7:507-15.

Eng C, Peacocke M. *PTEN* mutation analysis as a molecular diagnostic tool in the inherited hamartoma-cancer syndromes. Nature Genet 1998; 19:223.

Sarraf P, Mueller E, Smith WM, Wright HM, Kum JB, Aaltonen LA, de la Chapelle A, Speigelman BM, **Eng C**. Loss of function mutations in *PPAR*□ associated with human colorectal cancer. Mol Cell 1999; 3:799-804.

Marsh DJ, Kum JB, Lunetta KL, 26 others, Weng LP, Dahia PLM, **Eng C**. *PTEN* mutation spectrum and genotype-phenotype correlations in Bannayan-Riley-Ruvalcaba syndrome suggest a single entity with Cowden syndrome. Hum Mol Genet 1999; 8:1461-72.

Perren A, Weng LP, Boag AH, Ziebold U, Kum JB, Dahia PLM, Komminoth P, Lees JA, Mulligan LM, Mutter GL, **Eng C**. Immunocytochemical evidence of loss of PTEN expression in primary ductal adenocarcinomas of the breast. Am J Pathol 1999; 155:1253-60.

Zhou XP, Marsh DJ, Hampel H, Mulliken JB, Gimm O, **Eng C**. Germline and germline mosaic *PTEN* mutations associated with a Proteus-like syndrome of hemihypertrophy, lower limb asymmetry, arterio-venous malformations and lipomatosis. Hum Mol Genet 2000; 9:765-8

Gimm O, Perren A, Weng LP, Marsh DJ, Yeh JJ, Ziebold U, Gil E, Hinze R, Delbridge L, Lees JA, Robinson BG, Komminoth P, Dralle H, **Eng C**. Differential nuclear and cytoplasmic expression of PTEN in normal thyroid tissue, and benign and malignant epithelial thyroid tumors. Am J Pathol 2000; 156:1693-1700.

Yeh JJ, Marsh DJ, Zedenius J, Dwight T, Delbridge L, Robinson BG, **Eng C**. Fine structure deletion analysis of 10q22-24 demonstrates novel regions of loss and suggests that sporadic follicular thyroid adenomas and follicular thyroid carcinomas develop along distinct parallel neoplastic pathways. Gene Chrom Cancer 1999; 26:322-8.

Weng LP, Smith WM, Dahia PLM, Ziebold U, Gil E, Lees JA, **Eng C**. PTEN suppresses breast cancer cell growth by phosphatase activity-dependent G1 arrest followed by cell death. Cancer Res 1999; 59:5808-14

Mutter GL, Lin M-C, FitzGerald JT, Kum JB, Baak JPA, Lees JA, Weng LP, **Eng C**. Altered *PTEN* expression as a molecular diagnostic marker for the earliest endometrial precancers. J Natl Cancer Inst 2000; 92:924-31.

Gimm O, Attié-Bitach T, Lees JA, Vekemans M, **Eng C**. Expression of the PTEN tumour suppressor protein in human embryonic development. Hum Mol Genet 2000; 9:1633-9.

Kurose K, Zhou XP, Araki T, **Eng C**. Biallelic inactivating mutations and an occult germline mutation of *PTEN* in primary cervical carcinomas. Gene Chrom Cancer 2000; 29:166-72.

Parisi M, Dinulos MB, Leppig KA, Sybert VP, **Eng C**, Hudgins L. The spectrum and evolution of phenotypic findings in *PTEN* mutation-positive cases of Bannayan-Riley-Ruvalcaba syndrome. J Med Genet 2001; 38:52-7.

Perren A, Komminoth P, Saremaslani P, Matter C, Feurer S, Lees JA, Heitz PU, **Eng C**. Mutation and expression analyses reveal differential subcellular compartmentalization of PTEN in endocrine pancreatic tumors compared to normal islet cells. Am J Pathol 2000; 157:1097-1103.

Zhou XP, Gimm O, Hampel H, Niemann T, Walker MJ, **Eng C**. Epigenetic PTEN silencing in malignant melanomas without *PTEN* mutation. Am J Pathol 2000; 157:1123-8.

Weng LP, Brown JL, **Eng C**. PTEN induces apoptosis and cell cycle arrest through phosphoinositol-3-kinase/Akt-dependent and independent pathways. Hum Mol Genet 2001; 10:237-42.

Weng LP, Gimm O, Kum JB, Smith WM, Zhou XP, Wynford-Thomas D, Leone G, **Eng C**. Transient ectopic expression of *PTEN* in thyroid cancer cell lines induces cell cycle arrest and cell type-dependent cell death. Hum Mol Genet 2001; 10:251-8.

Weng LP, Brown JL, **Eng C**. PTEN coordinates G1 arrest by down regulating cyclin D1 via its protein phosphatase activity and up regulating p27 via its lipid phosphatase activity in a breast cancer model. Hum Mol Genet 2001; 10:599-604.

Weng LP, Smith WM, Brown JL, **Eng C**. PTEN inhibits insulin-stimulated MEK/MAPK activation and cell growth by blocking IRS-1 phosphorylation and IRS-1/Grb-2/Sos complex formation in a breast cancer model. Hum Mol Genet 2001; 10:605-16.

Kurose K, Zhou XP, Araki T, Cannistra SA, Maher ER, **Eng C**. Frequent loss of PTEN expression is linked to elevated phosphorylated Akt levels, but not associated with p27 and cyclin D1 expression, in primary epithelial ovarian carcinomas. Am J Pathol 2001; 158:2097-2106

Mutter GL, Ince T, Baak JPA, Kurst GA, Zhou XP, **Eng C**. Molecular identification of latent precancers in histologically normal endometrium. Cancer Res 2001; 61:4311-4.

Zhou XP, Hampel H, Thiele H, Gorlin RJ, Hennekam R, Parisi M, Winter RM, **Eng C**. A subset of Proteus syndrome and Proteus-like syndromes is caused by germline mutation in the *PTEN* tumour suppressor gene. Lancet (in press)

Neumann HPH, Reincke M, **Eng C**. Genetic testing in young patients with apparently isolated pheochromocytoma. N Engl J Med (in press)

Kurose K, Hoshaw-Woodard S, Adeyinke A, Lemeshow S, Watson P, **Eng C**. Genetic model of multi-step breast carcinogenesis involving the epithelium and stroma: clues to tumor-microenvironment interactions. Hum Mol Genet (accepted, revised, resubmitted)