A 39-bp Deletion Polymorphism in *PTEN* in African American Individuals

Implications for Molecular Diagnostic Testing

Xiao-Ping Zhou,* Heather Hampel,*
Jennifer Roggenbuck,[†] Nabil Saba,[†]
Thomas W. Prior,[‡] and Charis Eng*[§]

From the Clinical Cancer Genetics and Human Cancer Genetics Programs, the Comprehensive Cancer Center and the Division of Human Genetics, the Department of Internal Medicine, and the Department of Pathology;[†] The Ohio State University, Columbus, Ohio; the Medical Genetics Program and the Division of Hematology/Oncology;[‡] the Department of Internal Medicine, the Hennepin County Medical Center and the University of Minnesota, Minneapolis, Minnesota; and the Cancer Research Campaign Human Cancer Genetics Research Group;[§] University of Cambridge, Cambridge, United Kingdom*

Germline mutations in the *PTEN/MMAC1/TEP1* tumor suppressor gene cause Cowden syndrome (CS), a hereditary hamartoma-tumor syndrome with an increased risk of breast, thyroid, and endometrial cancers, and seemingly unrelated developmental disorders, such as Bannayan-Riley-Ruvalcaba (BRR) syndrome, *Proteus*, and *Proteus*-like syndromes. Data to date suggest that irrespective of the clinical presentation, the identification of a *PTEN* mutation should trigger medical management which includes cancer surveillance. Clinic-based molecular diagnostic testing for germline *PTEN* mutations has been available for at least 2 years. This study reports on the finding of a previously unobserved heterozygous alteration (IVS7-15→53del39) found in an African American individual who had features of CS. Further investigation revealed that 12 of 42 (28.6%) African American controls, but not individuals of Caucasian or Japanese origin, also carried this heterozygous 39-bp deletion in *PTEN*. Due to its location immediately upstream of the splicing site of exon 8, this polymorphism could be mistaken for a deleterious mutation in the *PTEN*. (J Mol Diagn 2002, 4:114-117)

The *PTEN/MMAC1/TEP1* gene (GenBank accession no. AF067844), located at chromosome sub-band 10q 23.3, encodes a dual-specificity phosphatase with lipid and protein phosphatase activities.¹⁻³ *PTEN* signals down the phosphoinositol-3-kinase (PI3K)/Akt pathway.^{4,5} Via this and PI3K/Akt-independent pathways, proper *PTEN* signaling leads to G1 cell cycle arrest and/or apoptosis.⁵⁻¹²

Germline mutations in *PTEN* cause Cowden syndrome (CS; MIM 158350), an autosomal-dominant multiple hamartoma syndrome with a high risk of breast, thyroid, and endometrial cancers.^{13,14} Germline *PTEN* mutations also cause a subset of seemingly unrelated developmental disorders, such as Bannayan-Riley-Ruvalcaba syndrome (BRR; MIM 153480), *Proteus* syndrome (PS; MIM 176920), and *Proteus*-like syndromes.¹⁵⁻¹⁸ Genotype-phenotype association analyses in CS and BRR have revealed that the presence of germline *PTEN* mutations are associated with the presence of neoplasia irrespective of clinical presentation.^{16,19} Thus, we have proposed that the inherited hamartoma neoplasia syndromes be classified molecularly as this is useful for medical management.^{14,16,18} Clinical molecular diagnostic testing for *PTEN* mutations has been available for at least 2 years.

While scanning for germline mutations in *PTEN* in individuals carrying the clinical diagnosis of CS, BRR, or PS or who have features reminiscent of these syndromes, we noted an IVS7-15→53del39 alteration which has never been observed. Because of its proximity to the intron 7-exon 8 splicing boundary and the number of nucleotides deleted, it was initially considered a deleterious *PTEN* mutation. This heterozygous 39-bp deletion was subsequently found in almost one third of African American control individuals and likely represents a polymorphism in that group, and is worthy of note because it carries implications for molecular diagnostic testing.

Materials and Methods

DNA Extraction from Peripheral Blood

Genomic DNA was isolated from peripheral blood lymphocytes using standard techniques.²⁰ DNA from normal

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Address reprint requests to Charis Eng, Human Cancer Genetics Program, The Ohio State University, 420 W. 12th Avenue, Suite 690 TMRF, Columbus, OH 43210. E-mail: eng-1@medctr.osu.edu.

Figure 1. Characterization of an intronic 39-bp deletion polymorphism in *PTEN*. **a:** PCR products were electrophoresed on 2% agarose gel with 100-bp DNA ladder. **Arrows** denote samples which are heterozygous for the 39-bp deletion. **b:** Fluorescent sizing of the 39-bp deletion. The **upper panel** shows a wild-type homozygote for the full-length 322-bp fluorescence-labeled PCR product. The lower panel shows a heterozygote with one allele containing the 39-bp deletion, which resulted in a short product of 284 bp, and the wild-type allele resulting in a full-length 322-bp product. **c:** Sequencing of the variant (**upper panel**) and the full-length (**lower panel**) PCR products revealed the 39-bp intronic deletion (IVS7-15→-53). The deleted nucleotides are underlined in the **lower panel**.

controls was obtained from 42 unrelated African American individuals, 50 Caucasian American individuals, and 50 Japanese individuals from the Tokyo catchment area. All controls were healthy blood donors.

PTEN Mutation Analysis

PTEN mutation analysis using polymerase chain reaction (PCR)-based denaturing gradient gel electrophoresis (DGGE) was performed using germline genomic DNA as a template, as previously detailed.²¹ Any sample showing DGGE variation was re-amplified with another set of primers specifically for sequence analysis, gel- and column-purified, and subjected to semi-automated sequencing as previously described.²¹ Primers used for sequence analysis in this study were CG3F 5'-CTCA-GATTGCCTTATAATAGTC-3' (intronic) and E8aR 5'-CCTTGTCATTATCTGCACGC-3' (exonic) amplifying a fragment of 322 bp comprising part of exon 8 and intron 7 immediately upstream of the exon-intron boundary.

Screening for the presence of the IVS7-15→-53del39 variant in control individuals of different ethnic backgrounds was performed with a combination of 2% agarose gel electrophoresis, fluorescent sizing, and/or direct sequencing of the PCR products. For fluorescent sizing, the forward "sequencing primer" was fluorescence-labeled at the 5' end, FAM-CG3F. The subsequent fluorescent PCR product was subjected to capillary electro-

phoresis through the PE3700 (ABI, Perkin-Elmer, Norwalk, CT).

Results and Discussion

During routine *PTEN* mutation scanning of an individual with phenotypic features of CS/BRR, we noted a germline heterozygous 39-bp deletion within intron 7, close to the intron 7-exon 8 splice site, IVS7-15→-53del39 (Figure 1). To determine whether this intronic sequence alteration could cause aberrant splicing, RT-PCR was performed using the primers *PTEN* E7F 5'-ACCCACACGACGG-GAA-3' and *PTEN* E9R 5'-TTCATTCTCTGGATCAGAGT-3'. The resulting RT-PCR amplicon, which spans exons 7 to 9, was of the expected size. No other aberrant amplicons were noted (data not shown). When this RT-PCR product was sequenced, only wild-type *PTEN* sequence was obtained. These observations suggest that this intronic sequence variant is not a mutation, but most likely a normal polymorphic variant in *PTEN*. Further investigation revealed that the DNA sample harboring this intronic sequence variation was from an individual of African American origin. This led us to screen lymphocyte-derived DNA from 42 healthy African American blood donors to determine the frequency of the polymorphism. Out of 42 blood donors (84 chromosomes) screened, 12 samples (28.6%; allelic frequency, 14.3%) were found to

have this sequence variation and all were heterozygous. Additional DNA samples from 50 Caucasian Americans and 50 Japanese blood donors were screened and the polymorphism was not found in either of these populations. Thus, both expression analysis and population study confirmed that the observed intronic sequence variation in *PTEN* was indeed a polymorphism, which is common in the African American population.

The cause and biological significance of the presence of this *PTEN* sequence variant in African American individuals, not heretofore found or described in other racial groups, are currently unknown. Different types of polymorphic variation and in certain instances, higher frequencies of genetic polymorphisms in ethnic African populations have been previously described and have been ascribed to the older age of the African population as a whole compared to either European or even Asian populations.²² However, the presence of diverse and often unique polymorphic variation may present challenges in this era of clinical genetic testing for susceptibility to cancer. For example, in the African American population tested for mutations in *BRCA1* and *BRCA2*, there exist multiple missense variants which have neither been previously described as pathogenic nor as polymorphic variations.^{23,24} Such missense variants of unknown significance make it difficult to provide definitive risk assessment information and consequent advice for medical management during genetic cancer consultations.

Truncating mutations including nonsense, frameshift, and splice mutations, are almost always pathogenic. A notable exception is a polymorphic nonsense mutation in the *BRCA2* gene.²⁵ The germline *PTEN* 39-bp deletion in close proximity to a splice site could fall into this category. We initially thought that such a deletion could alter splicing. However, RT-PCR experiments demonstrated no effect on the transcript. Taken together with its frequent occurrence in a normal control population, specifically of ethnic African origin, our observations suggest that the *PTEN* IVS7-15→53del39 is a novel polymorphism possibly unique to African American individuals. Whether this variation might lend low penetrance susceptibility to cancer or other features is as yet unknown. However, given the growing use of clinical *PTEN* mutation analysis, this observation is worthy of note and clinical cancer geneticists, genetic counselors, and laboratory directors should be aware that the IVS7-15→53del39 should be treated as a normal polymorphic variant in the African American population, pending further data. This is a germane observation because finding a pathogenic germline *PTEN* mutation does alter medical and familial management.

The issue of ethnic variation is an important one for all molecular diagnostic testing not limited to germline testing. For example, in somatic testing for translocations, an *Alu* polymorphism in intron 6 of *EWS* present in individuals of African descent can lead to diagnostic errors in Southern blot analysis of this gene.²⁶ Somatic microsatellite stability testing on DNA from tumor blocks using only BAT-25 and/or BAT-26, mononucleotide repeat loci which are monomorphic in the Caucasian population, may lead to false positive results in individuals of African

descent, who show normal polymorphic variation at this locus.²² Thus, caution must be used in applying molecular diagnostic tests in populations other than the ones in which the test was originally established.

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