Null Results in Brief

No Association Between the *MDM2* −309 T/G Promoter Polymorphism and Breast Cancer in African-Americans or Whites

Robert C. Millikan, Kimberley Heard, Scott Winkel, Edgar J. Hill, Kristin Heard, Beri Massa, Lydia Mayes, Patricia Williams, Rachel Holston, Kathleen Conway, Sharon Edmiston, and Allan René de Cotret

Department of Epidemiology, School of Public Health and Lineberger Comprehensive Cancer Center, School of Medicine, University of North Carolina, Chapel Hill, North Carolina

Introduction

MDM2, a protein that binds and inactivates the tumor suppressor p53, is overexpressed in a variety of human cancers (1). Bond et al. (2) recently identified a single nucleotide polymorphism in the *MDM2* gene, −309 T/G within the *MDM2* promoter (database for single nucleotide polymorphism reference sequence number 2279744; http://snp500cancer.nci.nih.gov). The G allele showed increased affinity for the transcriptional activator Sp1, resulting in elevated *MDM2* transcription, higher MDM2 protein levels, and enhanced p53 inhibition. Among 88 members of Li-Fraumeni syndrome families who carried germ line mutations in *p53*, persons with one or two copies of the *MDM2* −309 G allele showed earlier onset of cancer, including breast cancer, and G/G homozygous individuals showed increased frequency of multiple primary cancers. We examined the association of *MDM2* genotype and breast cancer in the Carolina Breast Cancer Study, a population-based case-control study of African-Americans and Whites in North Carolina.

Materials and Methods

Study Population. Details regarding the Carolina Breast Cancer Study have previously been described (3, 4), including extraction of germ line DNA from peripheral blood lymphocytes (5), analysis of *p53* mutations in tumor blocks (6), p53 immunohistochemistry (7), human epidermal growth factor receptor 2 immunohistochemistry (5), and determination of estrogen receptor and progesterone receptor status (8). *p53* mutational status was determined in the subset of Carolina Breast Cancer Study cases enrolled between 1993 and 1996 (6).

***MDM2* Genotyping.** Genotyping was conducted on germ line DNA using a Minor Groove Binding Eclipse assay developed by Nanogen, Inc. (Bothell, WA). The (G) allele probe was labeled on the 3′ end with the FAM reporter dye (nucleotide sequence, 5′-CCCGCGCCGcAG-3′, variant site in lower case) and the (T) allele probe was labeled on the 3′ end with the TET reporter dye (5′-CCCGCGCCGaA*G-3′). The asterisk denotes a Superbase. Superbases are modified nucleotides that permit highly specific binding to GC-rich DNA templates. The forward PCR primer was 5′-ACCTGCGATCATCCGGACCT-3′ and the reverse primer was 5′-TGCGG*GGCCGCT-3′. Probes and primers were designed for the complementary DNA strand. PCR amplification was done on a GenAmp 9700 thermocycler (Perkin-Elmer, Wellesley, MA) under the following conditions: 1 cycle of 50.0°C for 2 minutes, 1 cycle of 95.0°C for 2 minutes, followed by 50 cycles of 95.0°C for 5 seconds (denature), 28.0°C for 20 seconds (anneal/detection), and 76.0°C for 30 seconds (extension). Post-PCR melt curve analysis was done on the ABI Prism 7700, and data was analyzed using the Minor Groove Binding Eclipse Melt Macro for Microsoft Excel (EclipseMeltMacro_v2.332_050519.xls) provided by Nanogen. Further details are available from the authors upon request.

Genomic DNA samples obtained from the Coriell Cell Repositories (Camden, NJ) were sequenced to determine *MDM2* −309 genotype status and used as positive controls. Positive controls were Coriell sample number NA12749 for G/G (FAM) and NA11587 for T/T (TET). DNA samples that did not amplify or could not be scored were repeated. Samples that did not amplify on the third PCR run were designated as ''missing'' (n = 8). A randomly selected 10% of samples were repeated, and all results matched the initial analysis.

Statistical Analysis. Genotype frequencies were compared using the Cochran Armitage test for trend (9), and allele frequencies were compared using χ^2 tests. Odds ratios (OR) and 95% confidence intervals were calculated using SAS (SAS Institute, Cary, NC) and incorporated offset terms to account for sampling probabilities for cases and controls. Among cases, age at onset of breast cancer was compared across strata defined by *MDM2* genotype using ANOVA for means and the Kruskal-Wallis test for medians.

Results

MDM2 −309 allele and genotype frequencies did not differ between cases and controls, and ORs for breast cancer were close to the null in African-Americans and Whites (Table 1). The frequency of the G allele in Whites was slightly higher than 0.33 [see Bond et al. (2)]. Genotype frequencies did not show significant departures from Hardy-Weinberg equilibrium among African-American (P = 0.22) or White (P = 0.28) cases, but there were more G/G homozygotes than expected among African-American (P = 0.001) and White (P = 0.001)

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Requests for reprints: Robert Millikan, Department of Epidemiology, CB 7435 School of Public Health, University of North Carolina, Chapel Hill, NC 27599-7435. Phone: 215-440-9300; Fax: 215-440-9337. E-mail: millikan@email.unc.edu

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Table 1. Allele frequencies, genotype frequencies, and ORs for *MDM2* −309 genotype and breast cancer in African-Americans and Whites

	Cases (N = 2,037)	Controls (N = 1,813)	OR (95% confidence intervals)
MDM2 −309 alleles	Frequency (95% confidence intervals)	Frequency (95% confidence intervals)	
African-Americans			
T	0.88 (0.86-0.89)	0.89 (0.87-0.90)	
G	0.12 (0.11-0.14)	0.11 (0.10-0.13)	
χ^2 (P = 0.48)			
Whites			
T	0.63 (0.61-0.65)	0.63 (0.61-0.65)	
G	0.37 (0.35-0.39)	0.37 (0.35-0.39)	
χ^2 (P = 0.85)			
MDM2 −309 genotypes	N (%)	N (%)	
African-Americans	N = 767	N = 680	
T/T	594 (77%)	542 (80%)	Referent*
T/G	158 (21%)	121 (18%)	1.2 (0.9-1.6)
G/G	15 (2%)	17 (2%)	0.8 (0.4-1.6)
Cochran Armitage trend test (P = 0.49)			
Whites	N = 1,270	N = 1,133	
T/T	516 (41%)	474 (42%)	Referent*
T/G	573 (45%)	478 (42%)	1.1 (0.9-1.3)
G/G	181 (14%)	181 (16%)	0.9 (0.7-1.1)
Cochran Armitage trend test (P = 0.86)			
Age at diagnosis (cases) or selection (controls), all participants			
<40			
T/T	157	121	Referent†
T/G	119	70	1.2 (0.8-1.9)
G/G	25	23	0.7 (0.4-1.4)
40-50			
T/T	401	395	Referent†
T/G	259	222	1.2 (0.9-1.5)
G/G	82	80	0.9 (0.7-1.4)
>50			
T/T	552	500	Referent†
T/G	353	307	1.1 (0.9-1.4)
G/G	89	95	0.9 (0.6-1.2)

*Adjusted for age and offsets.
†Adjusted for age, race, and offsets.

controls. ORs did not differ according to menopausal status or for subgroups of cases defined by *in situ* versus invasive breast cancer, *p53* mutational status, p53 immunohistochemistry, human epidermal growth factor receptor 2 immunochemistry, estrogen receptor, or progesterone receptor status (data not shown). ORs were unchanged when we adjusted for or stratified on family history of cancer as well as other breast cancer risk factors (data not shown). ORs were close to the null for breast cancer diagnosed before age 40, age 40 to 50, and over age 50 (Table 1).

Table 2. Age at onset of breast cancer among cases according to *MDM2* −309 genotype

MDM2 genotype	Age at onset		
	Mean (SD)	Median	Range
African-American cases	N = 767		
T/T	51.7 (11.6)	51.0	23-74
T/G	51.0 (12.0)	50.0	23-74
G/G	51.9 (11.4)	51.0	33-72
P*	P = 0.76	P = 0.77	
White cases	N = 1,270		
T/T	52.6 (11.4)	50.0	24-74
T/G	51.8 (12.0)	49.0	24-74
G/G	51.6 (11.5)	48.0	29-74
P*	P = 0.46	P = 0.29	
African-American and White cases			
p53 mutation positive	N = 96		
T/T	48.5 (11.6)	47.0	24-74
T/G	48.2 (13.4)	47.0	24-74
G/G	45.3 (8.7)	42.0	37-63
P*	P = 0.81	P = 0.69	
p53 mutation negative	N = 309		
T/T	51.8 (12.1)	49.0	28-73
T/G	50.5 (12.8)	47.5	23-74
G/G	53.2 (11.9)	49.0	36-74
P*	P = 0.47	P = 0.46	

*ANOVA test comparing means, Kruskal-Wallis test comparing medians.

Among cases, *MDM2* genotype frequencies did not differ according to p53 mutational status (P = 0.60), p53 immunohistochemistry (P = 0.70) or human epidermal growth factor receptor 2 immunohistochemistry (P = 0.84). *MDM2* G/G genotype frequencies were slightly higher in estrogen receptor−positive compared with estrogen receptor−negative cases (P = 0.02) and for progesterone receptor−positive versus progesterone receptor−negative cases (P = 0.03). Mean and median age at onset of breast cancer did not differ according to *MDM2* genotype in African-Americans or Whites (Table 2). After stratification on *p53* mutational status, mean and median age at onset were lower among *p53*-positive cases with *MDM2* G/G genotype, but the differences were not statistically significant. Age at onset was not significantly different when we compared G/G carriers to cases with T/T or T/G genotypes (data not shown).

Discussion

Our study had 90% power to detect an OR of ≥1.4 for *MDM2* −309 G/G genotype in Whites, and 90% power to detect an OR of ≥2.6 in African-Americans. We conclude that the *MDM2*

−309 genotype is not associated with breast cancer risk in Whites and does not exhibit a strong association in African-Americans. *MDM2* genotype may be associated with a slightly earlier age at onset of breast tumors containing somatic mutations in *p53*. Additional studies of breast cancer and other cancers are warranted. The search for functional polymorphisms such as *MDM2* −309 that alter transcriptional regulation in biological pathways that influence tumorigenesis is an important research endeavor (10).

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