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Research Article

Single Nucleotide Polymorphisms in Selected Apoptotic Genes and BPDE-Induced Apoptotic Capacity in Apparently Normal Primary Lymphocytes: A Genotype-Phenotype Correlation Analysis

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Apoptotic capacity (AC) in primary lymphocytes may be a marker for cancer susceptibility, and functional single nucleotide polymorphisms (SNPs) in genes involved in apoptotic pathways may modulate cellular AC in response to DNA damage. To further examine the correlation between apoptotic genotypes and phenotype, we genotyped 14 published SNPs in 11 apoptosis-related genes (i.e., *p53, Bcl-2, BAX, CASP9, DR4, Fas, FasL, CASP8, CASP10, CASP3*, and *CASP7*) and assessed the AC in response to benzo[a]pyrene-7,8-9,10-diol epoxide (BPDE) in cultured primary lymphocytes from 172 cancer-free subjects. We found that among these 14 SNPs, R72P, intron 3 16-bp del/ins, and intron 6 G>A in *p53*, −938C>A in *Bcl-2*, and I522L in *CASP10* were significant predictors of the BPDE-induced lymphocytic AC in single-locus analysis. In the combined analysis of the three *p53* variants, we found that the individuals with the diplotypes carrying 0-1 copy of the common *p53* R-del-G haplotype had higher AC values compared to other genotypes. Although the study size may not have the statistical power to detect the role of other SNPs in AC, our findings suggest that some SNPs in genes involved in the intrinsic apoptotic pathway may modulate lymphocytic AC in response to BPDE exposure in the general population. Larger studies are needed to validate these findings for further studying individual susceptibility to cancer and other apoptosis-related diseases.

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1. INTRODUCTION

Apoptosis, also known as the programmed cell death, is a biological process that regulates physiological cell death and plays an important role in the pathogenesis of a variety of human diseases, including cancer [1]. Resistance to apoptosis or reduced cellular apoptotic capacity (AC) provides a survival advantage of the cells that may develop into cancer cells, commonly seen in almost all types of malignant diseases, and mutations in the genes involved in apoptotic pathways are one of the molecular mechanisms underlying carcinogenesis [2, 3] and cancer therapy [3, 4].

Benzo[a]pyrene (B[a]P) is a classic DNA-damaging carcinogen found in both tobacco smoke and the environment as a result of fuel combustion [5]. Its bioactivated form, benzo[a]pyrene diol epoxide (BPDE), can cause irreversible damage to DNA by forming DNA adducts through covalent binding or oxidation [6, 7]. If these adducts are unrepaired, the cells will undergo apoptosis through activation of p53, caspase-9 (*CASP9*), and caspase-3 (*CASP3*) [8, 9]. As a pivotal regulator of cellular response to DNA damage, the transcription factor encoded by the *p53* tumor suppressor gene has been clearly implicated in B[a]P-induced apoptosis, and the levels of p53 protein expression has been correlated with the levels of B[a]P-DNA adducts [8, 10]. Although details of the signaling pathways that trigger apoptosis in lymphocytes remain not fully understood, possible mechanisms include transcriptional activation of the Bcl-2 family members [11] and transcriptional upregulation of the death receptors (DRs) [12, 13]. These complex proteins participate in the activation of a sequential signaling that modulates two main apoptotic pathways [4]. One is the intrinsic or mitochondrial pathway, in which the stimuli of p53-Bcl-2 pathway lead to the activation of *CASP9* and release

of cytochrome c from the mitochondria [14]. The other, referred to as the extrinsicor cytoplasmic pathway, involves a group of proteins such as the DRs, the membrane-bound Fas ligand, the Fas complexes, the Fas-associated death domain, caspase-8 (*CASP8*), and caspase-10 (*CASP10*) [15, 16]. Activation of these two pathways initiates a common downstream proteolytic cascade that involves CASP3 and caspase-7 (CASP7) [4].

It is likely that the efficiency of these apoptotic pathways is genetically determined. Therefore, we hypothesized that functional polymorphisms in genes involved in these apoptotic pathways may modulate the AC phenotype, thus contributing to individual variation in response to DNA damage. To test this hypothesis, we selected 14 potentially functional polymorphisms in 11 genes, that is, *p53*, *Bcl-2*, *BAX,* and *CASP9* involved in the intrinsic pathway; *DR4*, *Fas*, *FasL*, *CASP8*, and *CASP10* involved in the extrinsic pathway; and *CASP3* and *CASP7,* the effective *CASPs*. We genotyped for these 14 polymorphisms and assessed in vitro AC with a terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labeling (TUNEL) assay using BPDE-treated primary lymphocytes from 172 subjects without cancer to evaluate associations between their apoptotic genotypes and the AC phenotype.

2. MATERIALS AND METHODS

2.1. Study population

Subjects in the current study were the control subjects in a molecular epidemiology study of lung cancer previously described [17]. Briefly, 172 subjects in this study were randomly selected from a pool of cancer-free control subjects recruited from the Kelsey-Seybold Clinics, a large multispecialty physician organization with several clinics throughout the Houston metropolitan area. Each subject was scheduled to be interviewed after a written informed consent was obtained. After the interview, a venous blood sample of about 20 mL was collected from each subject. The research protocol was approved by The University of Texas M. D. Anderson Cancer Center and the Kelsey-Seybold Foundation institutional review boards.

2.2. SNP selection

We used the National Center for Biotechnology Information (NCBI) dbSNP database (http://www.ncbi.nlm.nih.gov), the National Institute of Environmental Health Sciences (NIEHS) Environment Genome Project SNP databases (http://egp.gs.washington.edu/directory.html and http://www.genome.utah.edu/genesnps/) and literature search to identify potentially functional variants in genes involved in both intrinsic and extrinsic apoptotic pathways. Polymorphisms with a minor allele frequency (MAF) of ≥0.05 were included, if they may theoretically result in amino acid changes (nonsynonymous SNP, nsSNP), located at regulating regions such as promoters, or are reportedly associated with known phenotypic effects. Three reported *p53* SNPs were selected, including the well-known codon 72 SNP (R72P, G > C) and two intronic variants (a 16 bp-del/ins in intron 3 and a G-to-A transition in intron 6 because their haplotypes were found to be functional [18]. Two previously reported regulating SNPs in the promoters of the Bcl-2 family members, *Bcl-2* (−938C > A) and *BAX* (−248G > A) [19, 20], were included. For the *CASPs*, we identified one nsSNP from each *CASP8* (D302H, G > C), *CASP10* (I522L, A > T) [21], and *CASP7* (D255E, C > G, http://www.ncbi.nlm.nih.gov) and one of the two *CASP9* nsSNPs in tight linkage disequilibrium (LD) (Q221R, G > A, http://egp.gs.washington.edu/directory.html and [22]. Because no nsSNP was found in the coding region of *CASP3*, we selected one common variant in its promoter region: −1337C > G (http://www.genome.utah.edu/genesnps/). For the death receptor genes, one nsSNP in *DR4* and three promoter SNPs in *Fas* and *FasL* were selected: T209R (C > G) in *DR4*, –1377G > A and –670A > G in *Fas,* and –844T > C in *FasL* [23–26].

2.3. Genotyping

The genotyping methods used to distinguish the 14 selected polymorphisms in 11 apoptosis-related genes are presented in Table 1. Genotyping methods for seven of the polymorphisms were previously described: *p53* R72P [27]), *p53* intron 3 16-bpdel/ins and intron 6 G>A [18], *DR4* T209R [28], *Fas* –1377G>A and –670A>G [29], and *FasL* –844T>C [30]. The remaining seven polymorphisms (i.e., *Bcl-2* −938C>A, *BAX* −248G>A, *CASP9* Q221R, *CASP8* D302H, *CASP10* I522L, *CASP3* –1337C>G, and *CASP7* D255E) were detected by using a primer-introduced restriction analysis (PIRA)—polymerase chain reaction (PCR) assay [31] and summarized in Table 1. Genotyping was performed without knowledge of the subjects' phenotype; more than 10% of the samples were randomly selected for confirmation, and the results were 100% concordant. For the seven self-designed genotyping assays, PCR products containing each target genotype were purified and the sequences were confirmed by direct sequencing.

2.4. Apoptosis assay

The apoptosis phenotype (i.e., apoptotic capacity [AC]) was detected with the TUNEL assay previously described [32]. Briefly, two parallel short-term cultures from each blood sample were incubated at 37°C without CO_2 for 67 hours before BPDE treatment. At the end of the incubation, one of the two parallel cultures was treated with BPDE (98% pure; Midwest Research Institute, Kansas City, Mo, USA) at a final concentration of 4 μM. After an additional 5-hour incubation, all cells were pelleted by centrifugation, resuspended with lysis buffer (Human Erythrocyte Lysing Kit, R&D Systems, Minneapolis, Minn, USA), fixed for 1 hour, rinsed with phosphate-buffered saline, and finally stored in 70% ethanol at –20°C until used for the TUNEL assay.

For the TUNEL assay, we used the APO-BRDU kit (Phoenix Flow Systems, San Diego, Calif, USA) and followed the manufacturer's recommended protocol. The ratio of the

Table 1: Conditions of genotyping assays for the selected polymorphisms of some apoptotic genes.

Gene	Position, base change, and rs#	Genotyping method[a]	Primer	PCR product	Enzyme	Gel band pattern
p53	R72P G>C rs1042522	PCR-RFLP	5′-ATCTACAGTCCCCCTTGCCG-3′ (sense) 5′-GCAACTGACCGTGCAAGTCA-3′ (antisense)	296 bp	*BstU*I	G allele: 169 bp, 127 bp C allele: 296 bp
p53	Intron 3 16-bpins/del rs17878362	PCR	5′-TGGGACTGACTTTCTGCTCTT-3′ (sense) 5′-TCAAATCATCCATTGCTTGG-3′ (antisense)			Del: 180 bp Ins: 196 bp
p53	Intron 6 G>A rs1625895	PCR-RFLP	5′-TGGCCATCTACAAGCAGTCA-3′ (sense) 5′-TTGCACATCTCATGGGGTTA-3′ (antisense)	404 bp	*Msp*I	G allele: 336 bp, 68 bp A allele: 404 bp
Bcl-2	−938C>A rs2279115	PIRA-PCR: mismatch, antisense primer +2 C-to-G	5′-TCCTGCCTTCATTTATCCAGCA-3′ (sense) 5′-CCAGGAGAGAGACAGGGGACA-3′ (antisense)	125 bp	*Nla*III	C allele: 106 bp, 19 bp A allele: 125 bp
BAX	−248G>A rs4645878	PIRA-PCR: mismatch, sense primer –2 G-to-C	5′-CATTAGAGCTGCGATTGGACCG-3′ (sense) 5′-GCTCCCTCGGGAGGTTTGGT-3′ (antisense)	109 bp	*Msp*I	G allele: 89 bp, 20 bp A allele: 109 bp
CASP9	Q221R G>A rs1052576	PIRA-PCR: mismatch, sense primer –2 G-to-C	5′-GGCTTTGCTGGAGCTGGCCC-3′ (sense) 5′-AGTACCCAATGCCTGCCCAGGG-3′ (antisense)	121 bp	*Msp*I	G allele: 102 bp, 19 bp A allele: 121 bp
DR4	T209R C>G rs4871857	PCR-RFLP	5′-ATCCTCTGGGAACTCTGTGG-3′ (sense) 5′-GGGGACAGGCAGATGGAC-3′ (antisense)	300 bp	*Dra*III	C allele: 200 bp, 100 bp G allele: 300 bp
Fas	−1377G>A rs2234767	PIRA-PCR: mismatch, sense primer –2 A-to-G	5′-TGTGTGCACAAGGCTGGCGC-3′ (sense) 5′-TGCATCTGTCACTGCACTTACCACCA-3′ (antisense)	122 bp	*BstU*I	G allele: 104 bp, 18 bp A allele: 122 bp
Fas	–670A>G rs1800682	PCR-RFLP	5′-ATAGCTGGGGCTATGCGATT-3′ (sense) 5′-CATTTGACTGGGCTGTCCAT-3′ (antisense)	193 bp	*ScrF*I	A allele: 193 bp G allele: 136 bp, 57 bp
FasL	−844T>C rs763110	PIRA-PCR: mismatch, sense primer –7 A-to-C	5′-CAATGAAAATGAACACATTG-3′ (sense) 5′-CCCACTTTAGAAATTAGATC-3′ (antisense)	85 bp	*Dra*III	T allele: 66 bp, 19 bp G allele: 85 bp
CASP8	D302H G>C rs1045485	PIRA-PCR: mismatch, sense primer –2 A-to-G	5′-CATTTTGAGATCAAGCCCCGC-3′ (sense) 5′-CCCTTGTCTCCATGGGAGAGGA-3′ (antisense)	132 bp	*BstU*I	G allele: 112 bp, 20 bp C allele: 132 bp
CASP10	I522L A>T rs13006529	PIRA-PCR: mismatch, antisense primer +4 A-to-T	5′-GAGTGGACAAACAGGGAACAAA-3′ (sense) 5′-AGAACCAACAAAAACTCTCTGCAATA-3′ (antisense)	122 bp	*Ssp*I	T allele: 97 bp, 25 bp A allele: 122 bp
CASP3	−1337C>G rs1405937	PIRA-PCR: mismatch, sense primer –2 C-to-A	5′-ATAGGCGCAAGTGTTAGAAACAGGAT-3′ (sense) 5′-CACCAACACATGTGAGCACGAC-3′ (antisense)	112 bp	*Fok*I	C allele: 112 bp G allele: 76 bp, 36 bp
CASP7	D255E C>G rs2227310	PIRA-PCR: mismatch, sense primer –2 G-to-C	5′-CCTGGAGGAGCACGGAAAACA-3′ (sense) 5′-TGGAGACCACACAGGGGATCTG-3′ (antisense)	137 bp	*Pvu*II	G allele: 115 bp, 22 bp C allele: 137 bp

[a]RFLP = restriction fragment length polymorphisme; PIRA = primer-introduced restriction analysis.

difference in the percentages of apoptotic cells in a subject's BPDE-treated and untreated cultures to the percentage of apoptotic cells in the untreated culture was recorded as the AC (AC% = $[AC_{treated}\text{-}AC_{baseline})/AC_{baseline}] \times 100$) [32].

2.5. Statistical analysis

DNA quality or quantity was insufficient for genotyping in 2 subjects; thus, the final analysis included 170 persons.

Differences of the continuous AC measurements between genotypes/diplotypes of apoptotic genes were evaluated by using Student's *t*-test. Trend test was performed by using the general linear regression model with adjustment for age and sex. We dichotomized the continuous phenotype measurements by using the median (150%) as the cutoff value to obtain an almost equal low-AC subgroup (84 subjects) and high-AC subgroup (86 subjects). Logistic regression analyses were used to estimate theodds ratios (ORs) and 95% confidence intervals (CIs) between combined genetic variants and dichotomized AC phenotype with adjustment for age and sex. Alleles/haplotypes associated with the lower AC phenotype in individual polymorphism analysis were termed as "at-risk" alleles hereinafter. We used the PHASE 2.0 program [33] to infer haplotype frequencies based on the observed genotypes for each gene. Diplotype was the most probable haplotype pair for each individual. The potential gene-gene interaction was evaluated by logistic regression analysis and tested by comparing the changes in deviance (–2 log likelihood) between the models of main effects with or without the interaction term. All of the statistical analyses were performed with statistical analysis system software (v.9.1.3; SAS Institute, Inc., Cary, NC, USA).

3. RESULTS

The mean age (±SD, years) of the 170 study subjects (119 males and 51 females) was 57.99 ± 12.10, and we dichotomized age by using the cutoff value of 60 years to facilitate comparisons between age groups. No statistical difference was found in the continuous AC measurements between the subgroups according to age and sex (data not shown). Table 2 shows the continuous AC measurements by genotypes of the selected apoptotic genes. The observed genotype frequencies were all consistent with those expected from the Hardy-Weinberg equilibrium (data not shown). For SNPs in genes involved in the intrinsic apoptotic pathway, variant homozygotes of *p53* intron 3 16-bpins/ins, *p53* intron 6 AA, and *Bcl-2* −938AA all had significantly higher AC than their corresponding wild-type homozygotes (496.07 ± 121.26 versus 204.22 ± 183.21 for *p53* intron 3 16-bpdel/ins, P = .027; 496.07 ± 121.26 versus 199.44 ± 179.10 for *p53* intron 6 G>A, P = .021, and 247.62 ± 225.67 versus 164.06 ± 154.89 for *Bcl-2* −938C>A, P = .046). However, the significant P values for the trend of higher AC with increasing number of the variant alleles were observed only for *p53* R72P (.016) and *Bcl-2* −938C>A (.037) as assessed in the general linear regression model with adjustment for age and sex (Table 2). In contrast, only the variant homozygotes of *CASP10* I522L out of all SNPs in genes involved in the extrinsic apoptotic pathway had significantly lower AC (159.49 ± 171.44) than the II homozygote (239.07 ± 205.18, P = .046) as well as a significant trend of lower AC with increasing number of the variant alleles (P = .046).

Linkage disequilibrium (LD) analysis showed that the three loci in *p53* were in LD (r^2 = 0.14, D' = 0.64 for R72P and intron 3 16-bpdel/ins; r^2 = 0.24, D' = 0.85 for R72P and intron 6 G>A; and r^2 = 0.60, D' = 0.79 for intron 3 16-bpdel/ins and intron 6 G>A). Therefore, we performed haplotype/diplotype inference using the PHASE 2.0 program based on the observed genotypes. Overall, three common hapolotypes were derived (Table 3). The diplotype carrying zero copy of the *p53* R-del-G haplotype and the diplotype carrying two copies of the *p53* P-ins-A haplotype all had significantly higher AC (termed as "protective" hereinafter) and the effect of the R-del-G haplotype was in a dose-response manner (P for trend: .016; Table 3).

4. DISCUSSION

In the genotype-phenotype analysis, we examined the role of potentially functional variants in selected apoptotic genes in the AC phenotype induced by BPDE treatment in primary lymphocytes. We found that R72P, intron 3 16-bpdel/ins, intron 6 G>A in *p53*, –938C>A in *Bcl-2,* and I522L in *CASP10* may be predictors of AC, but the effects of the *p53* variants might also be modulated by its downstream genes involved in the intrinsic pathway. To the best of our knowledge, this is the first multigene genotype-phenotype correlation analysis in relation to the apoptotic pathways in primary lymphocytes at a population level.

Because there is tissue specificity in response to carcinogen exposure, it would be ideal to compare the BPDE-induced AC measurements among different tissues of the same person. However, few reported studies have addressed this tissue specificity, nor did our study have such an opportunity. It was reported that B[a]P-induced apoptosis of murine Hepa1c1c7 cells was through CASP-9 activation related with p53 accumulation and activation [8] and that a decrease in the expression of Bcl-2 to Bax ratio was another hallmark of the process [8, 34]. Although obtained from different cell types, these findings are consistent with our current observations that genetic variants in the genes involved in the intrinsic apoptotic pathway may play an important role in the prediction of AC phenotype. The Bcl-2 family is a group of evolutionarily conserved pro- and anti-apoptotic proteins that play a pivotal role in the regulation of the mitochondrial-mediated (intrinsic) apoptotic pathway [35]. Bcl-2 inhibits apoptosis through heterodimerization with proapoptotic members of the Bcl-2 family, such as Bax and also through formation of channels that stabilize the mitochondrial membrane [36]. Bcl-2 expression has also been implicated in the pathogenesis of cancers [37, 38], and the expression of Bcl-2 to Bax ratio seems to be important in determining both in vitro and in vivo response to chemotherapeutic drugs [39]. Recently, variant allele of *Bcl-2* −938C>A was found to be associated with reduced prostate cancer risk in Caucasians in a small case-control study, possibly due to the elimination of an Sp1 binding site, a downregulation of Bcl-2 mRNA transcript levels, and unregulated programmed cell death [40], which is consistent with what we found in the current study (the variant A allele carriers were associated with high-AC phenotype that may help eliminating possible malignant cells).

Our results on the *p53* polymorphisms were not consistent with published data. For example, the wild-type 72R

Table 2: Comparisons of mean BPDE-induced apoptosis capacity in apparently normal primary lymphocytes by the genotypes of selected apoptotic genes.

Variable	No. (%)	AC (mean ± SD)	*P* value(a)	*P* value(b)
Intrinsic pathway				
p53 R72P				
RR	91 (53.5)	180.47 ± 164.13	Ref.	
RP	65 (38.2)	223.07 ± 196.22	.143	
PP	14 (8.2)	313.40 ± 243.25	.067	**.016**
p53 intron 3				
16-bpdel/del	134 (78.8)	204.22 ± 183.21	Ref.	
16-bpdel/ins	34 (20.0)	204.51 ± 194.62	.993	
16-bpins/ins	2 (1.2)	496.07 ± 121.26	**.027**	.274
p53 intron 6				
GG	135 (79.4)	199.44 ± 179.10	Ref.	
GA	33 (19.4)	224.07 ± 209.17	0.495	
AA	2 (1.2)	496.07 ± 121.26	**.021**	.099
Bcl-2 −938C>A				
CC	53 (31.2)	164.06 ± 154.89	Ref.	
CA	76 (44.7)	216.62 ± 180.38	.087	
AA	41 (24.1)	247.62 ± 225.67	**.046**	**.037**
BAX –248G>A				
GG	144 (84.7)	205.39 ± 189.73	Ref.	
GA	25 (14.7)	225.99 ± 173.85	.613	
AA	1 (0.6)	84.85	—	
CASP9 Q221R				
QQ	53 (31.2)	194.75 ± 169.29	Ref.	
QR	75 (44.1)	213.91 ± 199.95	.571	
RR	42 (24.7)	213.00 ± 187.11	.620	.704
Extrinsic pathway				
DR4 T209R				
TT	52 (30.6)	179.61 ± 179.86	Ref.	
TR	75 (44.1)	229.67 ± 206.45	.160	
RR	43 (25.3)	203.39 ± 155.49	.497	.485
Fas –1377G>A				
GG	126 (74.1)	218.66 ± 195.82	Ref.	
GA	42 (24.7)	179.99 ± 158.13	.248	
AA	2 (1.2)	100.14 ± 92.38	.396	.182
Fas –670A>G				
AA	43 (25.3)	210.82 ± 173.91	Ref.	
GA	86 (50.6)	236.97 ± 203.18	.472	
GG	41 (24.1)	143.07 ± 147.70	.058	.085
FasL –844T>C				
CC	79 (46.5)	225.68 ± 195.41	Ref.	
CT	74 (43.5)	190.11 ± 180.80	.245	
TT	17 (10.0)	200.82 ± 173.81	.629	.464
CASP8 D302H				
DD	127 (74.7)	202.30 ± 184.45	Ref.	
DH	38 (22.4)	210.97 ± 195.10	.802	
HH	5 (2.9)	320.41 ± 183.38	.162	.389
CASP10 I522L				
II	54 (31.8)	239.07 ± 205.18	Ref.	
IL	74 (43.5)	212.19 ± 177.84	.430	
LL	42 (24.7)	159.49 ± 171.44	**.046**	**.046**

Table 2: Continued.

Variable	No. (%)	AC (mean ± SD)	P value[(a)]	P value[(b)]
Effective *CASPs*				
CASP3 –1337C>G				
CC	107 (62.9)	210.98 ± 202.46	Ref.	
CG	51 (30.0)	212.23 ± 166.75	.970	
GG	12 (7.1)	159.34 ± 109.53	.388	.516
CASP7 D255E				
DD	95 (55.9)	214.49 ± 190.29	Ref.	
DE	68 (40.0)	195.79 ± 166.92	.516	
EE	7 (4.1)	231.51 ± 318.48	.829	.801

(a) Two-sided Student *t*-test.
(b) Trend test obtained from general linear regress model with adjustment for age and sex.

Table 3: Comparisons of mean BPDE-induced apoptosis capacity in apparently normal primary lymphocytes by *p53* diplotypes.

p53 diplotypes	No. (%)	AC (mean ± SD)	P value[(a)]	P value[(b)]
R-del-G				
2 copies	81 (47.6)	177.57 ± 159.94	Ref.	
1 copy	74 (43.5)	220.39 ± 197.21	.138	
0 copy	15 (8.8)	307.90 ± 235.37	**.009**	**.016**
R-ins-G				
0 copy	116 (68.2)	190.05 ± 174.57	Ref.	
1 copy	49 (28.8)	238.99 ± 197.24	.116	
2 copies	5 (2.9)	310.86 ± 311.47	.146	.094
P-ins-A				
0 copy	143 (84.1)	202.33 ± 179.92	Ref.	
1 copy	25 (14.7)	215.43 ± 215.45	.745	
2 copies	2 (1.2)	496.07 ± 121.26	**.023**	.154

(a) Two-sided Student *t*-test.
(b) Trend test obtained from general linear regress model with adjustment for age and sex.

allele was found to be associated with an increased ability to induce apoptosis in response to radiation or cytotoxic drugs [18, 41, 42]. However, other data suggested that the 72P allele had a stronger transcription effect in response to DNA damage, leading to enhanced apoptotic phenotype [43]. These previous studies were mainly based on assays with cell lines [18, 41] and suffered with a limited sample size [18, 41, 42]. Most importantly, no previous study took into account other coexisting polymorphisms in the genes involved in the intrinsic pathway, which may play an important role in the B[a]P-induced apoptosis [8]. In the present study with apparently normal primary lymphocytes, we found that all minor variant alleles of the three *p53* polymorphisms were associated with a higher AC phenotype and that the overall effects on the AC phenotype appeared to be affected by the *CASP9* Q221R polymorphism, but the interaction between polymorphisms of *p53* and *CASP9* was only borderline significant due to a limited study power. However, our findings of this intrinsic apoptotic pathway in lymphocytes may be relevant to other types of tissue as well because this kind of induced apoptosis in lymphocytes may be inheritable [44]. Thus these results, although preliminary, need to be substantiated in larger studies.

Previous finding on the *CASP10* I522L polymorphism from a large breast cancer study showed that the variant LL genotype was associated with a borderline significant 1.30-fold increased cancer risk compared with the wild-type II homozygote [21], which is consistent with the notion that the LL homozygote contributes to a diminished AC. However, the role of the extrinsic pathway (or only *CASP10*) in B[a]P-induced apoptosis was not obvious in the present study, which needs further evaluation.

In conclusion, this proof-of-principle study of genotype-phenotype correlation provides evidence that potentially functional polymorphisms in the core genes of the apoptotic pathways may have a role in regulating the apoptotic response to carcinogen exposure, at least in primary lymphocytes, although there is tissue specificity in response to exposure to carcinogens. Such a modification of host carcinogen-induced AC in primary lymphocytes may contribute to variation in individual susceptibility to cancer in the general population. Although this study may be limited due to small sample size, multiple tests, and lack of repeated AC measurements for the same individuals, the findings, if validated in more rigorously designed and larger studies, should facilitate the design of future studies aimed at identifying subpopulations at risk of cancer and other apoptosis-related diseases.

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