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Common genetic variation in *TP53* and its flanking genes, *WDR79* and *ATP1B2*, and susceptibility to breast cancer

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Germline mutations in the tumor suppressor gene *TP53* are associated with high incidence of early-onset malignancies, and somatic mutations occur in 20–40% of all breast cancer cases. We investigated the association of common genetic variation in *TP53* and its flanking genes, *WDR79* and *ATP1B2*, with risk for breast cancer. Single nucleotide polymorphisms (SNPs) identified in a re-sequence analysis were genotyped in 2 large case–control studies including 731 cases and 1,124 controls from Norway, and 1,995 cases and 2,296 controls from Poland. Analyses of the pooled data showed no SNPs in *TP53* to be significantly associated with risk for breast cancer. However, we found a significant and consistent association with risk for a SNP in exon 1 (R68G) of the 5′ neighboring gene *WDR79* (rs2287499, OR (95% CI) = 1.08 (0.95–1.23) for CG *vs.* CC and 1.60 (1.04–2.47) for GG *vs.* CC, *p*-trend = 0.01). Stratification by ER and PR status, showed these increases in risk to be limited to ER negative tumors (OR (95% CI) per variant allele: 1.42 (1.18–1.71) *p*-trend = 0.00009). In addition, 2 *TP53* SNPs (rs17887200 3′of STP and rs12951053 in intron 7) showing weak and non-significant overall increases in risk, were also associated with ER negative tumors (1.48 (1.11–1.93) *p*-trend = 0.01 and 1.29 (1.06–1.58) *p*-trend = 0.009, respectively). In conclusion, this comprehensive evaluation of common genetic variation in *TP53* and its flanking genes found no significant overall associations between SNPs in *TP53* and breast cancer risk. However, data suggested that common variation in *TP53* or *WDR79* could be associated with ER negative breast cancers.

Key words: *TP53*; polymorphisms; breast cancer

The tumor suppressor gene *TP53* encodes the p53 protein that participates in multiple cellular functions of critical importance for cell growth and maintenance of genomic stability. Somatic mutations in *TP53*, either inactivating, dominant negative and gain of function, are found in approximately 20–40% of breast tumors.[1] The role of *TP53* in carcinogenesis is evident in the high incidence of early onset malignancies, including breast cancer, in the rare familial Li-Fraumeni Syndrome, which is most commonly characterized by germline mutations in the *TP53* gene.[2] Selected *TP53* mutations and common non-synonymous single nucleotide polymorphisms (SNPs), particularly the P72R change in exon 4, have been shown to differ in their structure as well as their biochemical and biological properties,[3–6] which suggests that *TP53* variation could influence susceptibility to cancer. A recent pooled analysis of the P72R SNP including 8,743 cases and 10,618 controls showed strong evidence against an association between this variant and breast cancer risk.[7] Two other variants in intron 3 (16 bp insertion),[8–13] and intron 6,[9,10,13–15] as well as haplotypes based on 3 polymorphisms,[9,16,17] have also been evaluated in relation to breast cancer risk; however, most studies have been relatively small and have yielded inconclusive results.

To better characterize common genetic variation in *TP53*, we recently conducted an extensive re-sequence analysis of *TP53* and its neighboring region, in 196 unrelated individuals. This effort revealed linkage disequilibrium (LD) between *TP53* and its flanking genes: WD repeat domain 79 (*WDR79*) upstream of *TP53*, and ATPase, Na+/K+ transporting, β 2 polypeptide (*ATP1B2*) downstream of *TP53*.[18] We performed a comprehensive assessment of common genetic variation based on the previous re-sequence analysis, and evaluated the possibility of an association with breast cancer risk in 2 large case–control studies in Norway and Poland. Because it is plausible that genetic variants near *TP53* could affect the regulation of transcription or the function of this key protein, we also included SNPs to capture genetic variation in the 2 flanking genes.

Material and methods

Study populations

Norwegian breast cancer study. Four series of previously described breast cancer patients ($N = 731$) were accrued in accordance with local institutional review board guidelines.

1. Breast cancer patients sequentially enrolled at Ullevål University Hospital from 1990–94, representing the breast cancer population during this period.[19,20] The mean age was 64 years (range, 28–92 years). Blood samples were collected in 1994–96 from 119 patients still alive (~80%), living in the Oslo area, and agreed to give blood (~70% of eligible

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women). Time from diagnosis to blood collection was zero to 6 years.
2. Breast cancer patients admitted to the Norwegian Radium Hospital during 1972–91.[21] The mean age at diagnosis was 57 years (range 27–94). Blood was drawn between 1987 and 1991 ($N = 224$) either at patient control, relapse or at diagnosis.
3. Breast cancer patients diagnosed at the Norwegian Radium Hospital and treated with radiotherapy during 1975–1986. The mean age at diagnosis was 59 (26–75). Blood samples were collected in 1996 from a subset of patients alive (21%) at that time who were part of a treatment evaluation and agreed (82%) to provide a blood sample ($N = 263$).[22]
4. Breast cancer patients with Stage I and II disease enrolled in the Oslo Micrometasteses study between 1995–1998 who had blood samples collected at the time of diagnoses[23,24] ($N = 125$). The mean age at diagnosis was 56 (29–82). Blood sampling was performed just prior to primary surgery for breast cancer from all patients included in the study.

The majority of control subjects ($N = 1,015$) were women with a negative mammogram from the Tromsø Mammography and Breast Cancer Study conducted in 2001 and 2002. About 70% of the women, 55–71 years of age, residing in the municipality of Tromsø, Norway and attending the Norwegian Breast Cancer Screening Program (NBCSP) at the University Hospital of North Norway agreed to participate.[25] The study was approved by the National Data Inspection Board and the Regional Committee for Medical Research Ethics. In addition, we included healthy woman ($N = 109$), 55–72 years of age, participating in the NBCSP in Bergen in 1999, with 2 negative mammogram during a 2-year period.[26] From this latter group, women with oral hormone replacement therapy or history of diabetes or other endocrine disorders were excluded. The mean age (range) for all cases ($N = 731$) and controls ($N = 1,124$), respectively, was 56 (26–93) and 62 (55–72) years.

Polish breast cancer study. A population-based case–control study was conducted in women residing in 2 Polish cities, Warsaw and Lodz.[27] Eligible cases were women aged 20–74 years who were newly diagnosed with either histologically or cytologically confirmed *in situ* or invasive breast cancer in 2000–2003. Cases were identified through a rapid identification system in participating hospitals (about 90% of cases), as well as through cancer registries to ensure complete case ascertainment. Controls with no history of breast cancer were randomly selected from population lists during the case ascertainment period and were frequency matched to cases by city and age in 5-year categories. Institutional Review Board approval was obtained from all participating institutions, and signed informed consent was obtained for all respondents.

A total of 2,386 cases (79% of eligible cases) and 2,502 controls (69% of eligible controls) provided a personal interview on known and suspected risk factors. Blood samples for DNA extraction were obtained from 1,995 cases (84% of participating cases) and 2,296 controls (94% of participating controls). Most cases (94%) were diagnosed with invasive tumors. Cases and controls had a mean (range) age of 56 (27–74) and 56 (24–75) years, respectively.

Genotyping

A re-sequence analysis of over 7,200 bp including 5′, 3′, conserved regions as well as all exons in *TP53* was performed in 94 healthy Norwegian women and 102 individuals in the SNP500Cancer panel.[18] Single nucleotide polymorphisms (SNPs) with a minor allele frequency >0.03 were genotyped in the Norwegian and Polish studies. Genotype analyses using blood DNA were performed at the Core Genotyping Facility (CGF) of the Division of Cancer Epidemiology and Genetics, NCI for 11 SNPs in TP53: rs8079544, IVS1-112G > A; rs1642785, IVS2+38C > G; rs1042522, Ex4 + 119C > G (P72R); rs9895829, IVS4-125T > C; rs2909430, IVS4-91A > G; rs1625895, IVS6 + 62A > G; rs12947788, IVS7 + 72T > C; rs12951053, IVS7 + 92T > G; rs1614984, 21226bp 3′ of STP; rs9894946, 22342bp 3′ of STP; rs17887200, 22369bp 3′ of STP. In addition we genotyped 7 SNPs in the 2 flanking genes, the 5′ neighbor WDR79 (rs2287499, Ex1-230C > G (R68G); rs17885803, IVS1-60C > T; rs2287498, Ex2 + 19C > T (F150F); rs17886268, IVS2-106C > T); and the 3′ neighbor ATP1B2 (rs1641536, −8852T > C; rs1641535 −8703T > C; rs1641512, Ex7 + 414G > A). Description and methods for each genotype assay can be found at http://snp500cancer.nci.nih.gov.[28] Completion was ≥98% for all assays, except for WDR79 rs2287498 (96% in Norway), ATP1B2 rs1641536 (97% in Norway), TP53 rs1614984 (95% in Poland), TP53 rs12947788 (97% in Norway), TP53 rs1042522 (97% in Norway), and *TP53* rs1642785 (97% in Norway and 93% in Poland). Duplicated DNA pairs from 100 subjects in the Polish study showed >99% concordance for all but 1 assay in intron 6 of *TP53* (rs1625895) with 98% concordance, and 1 in intron 2 of *WDR79* (rs17886268) with 97% concordance.

Statistical analyses

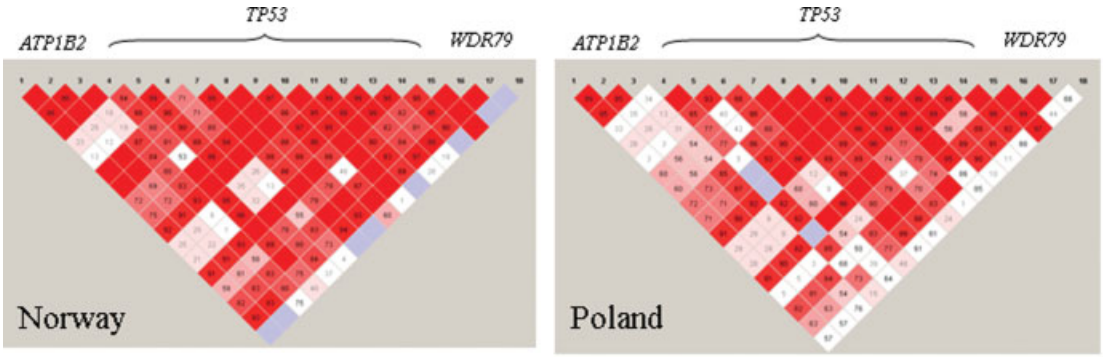
Odds ratios (OR) and their 95% confidence intervals (CI) were derived from unconditional logistic regression models adjusting for age in 5-year categories. The association between genotypes and breast cancer risk was tested using a trend test, except for rare alleles in which heterozygous and homozygous variants were combined. Given that controls from the Norwegian population were all older than 50 years of age, we evaluated interactions between genotypes and age by testing for the association between age and genotypes (both as continuous variables) in the case populations. We also evaluated heterogeneity in the genotype ORs by hormone receptor status in logistic regression models among cases only with receptor status as the outcome variable and genotypes as explanatory variables, adjusting for age and study. Case-control analyses were also performed to estimate associations between genotypes and different tumor types.

Pairwise linkage disequilibrium (LD) was estimated between SNPs based on $D'$and r^2 values using Haploview (http://www.broad.mit.edu/mpg/haploview/index.php). Block structure was determined using genotype data from the control population, and the solid spline of LD option (D' threshold >0.80). Haplotype frequencies within each block, ORs and their 95% CIs were estimated using HaploStats (version 1.2.1; http://mayoresearch.mayo.edu/mayo/research/biostat/schaid.cfm).[29] A global score statistic, adjusted for the matching factors age and study, was used to evaluate the overall difference in haplotype frequencies between cases and controls.

Results

The LD patterns among control populations in Norway and Poland are shown in Figure 1 and, although not identical, showed some similarities. Notably, there was a high degree of LD between SNPs in *ATP1B2* and 3 SNPs in TP53 (rs17887200 3′ of STP and 2 linked SNPs in intron 7, rs12951053 and rs12947788) in the Norwegian but not in the Polish population. The minor allele frequencies in the two control populations were similar, except for frequencies in the *ATP1B2* gene that tended to be higher in the Norwegian than Polish population (Table I). Genotype distributions were in accordance with Hardy-Weinberg equilibrium in the Polish control population, and we observed small but significant departures for 4 SNPs in the Norwegian control population: we observed 46% *vs*. 42% expected heterozygous for rs17887200 3′ of STP in *TP53*; 16% *vs*. 17% and 15% *vs*. 16% heterozygous, respectively, for the correlated SNPs rs1625895 in intron 6 and rs2909430 in intron 4 of *TP53*; and 15% *vs*. 17% heterozygous for rs8079544 in intron 1 of *TP53*.

Variant alleles in 2 linked polymorphisms in the *WDR79* gene: rs2287499 (R68G) in exon 1 and rs2287498 (F150F) in exon 2 ($D' = 1.0/r^2 = 0.78$ and $D' = 0.92/r^2 = 0.63$ for the Norwegian and Polish control populations, respectively) were significantly associated with increased risk of breast cancer, with no evidence for



Color scheme is based on D' and LOD score values: white D' < 1 and LOD < 2, light grey with no numbers D' = 1 and LOD < 2, shades of grey: D' < 1 and LOD ≥ 2, and dark grey D' = 1 and LOD ≥ 2. Numbers in squares are D' values (values of 1.0 are not show). SNPs are in 3' to 5' order: *ATP1B2* (rs1641536, rs1641535, rs1641512), *TP53* (rs17887200, rs9894946, rs1614984, rs12951053, rs12947788, rs1625895, rs2909430, rs9895829, rs1042522, rs1642785, rs8079544), *WDR79* (rs2287499, rs17885803, rs2287498, and rs17886268).

FIGURE 1 – Patterns of linkage disequilibrium across the *TP53* and neighboring genes *ATP1B2* and *WDR79*, in the Norwegian and Polish control populations. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE I – DESCRIPTION OF POLYMORPHISM EVALUATED IN THE NORWEGIAN AND POLISH BREAST CANCER CASE-CONTROL STUDIES

Polymorphism	Location	Norway		Poland	
		MAF	P HWE	MAF	P HWE
ATP1B2					
rs1641536[1]	−8852T>C	0.33	0.95	0.23	0.61
rs1641535[1]	−8703T>C	0.34	0.83	0.23	0.72
rs1641512	Ex7+414G>A	0.46	0.17	0.36	0.19
TP53					
rs17887200	22369bp 3′ of STP A>G	0.11	0.52	0.06	0.87
rs9894946	22342bp 3′ of STP T>C	0.27	0.01	0.28	0.50
rs1614984	21226bp 3′ of STP C>T	0.32	0.96	0.30	0.78
rs12951053[2]	IVS7 + 92T > G	0.06	0.64	0.06	0.48
rs12947788[2]	IVS7 + 72T > C	0.19	0.41	0.17	0.49
rs1625895[3]	IVS6 + 62A > G	0.17	0.05	0.21	0.15
rs2909430[3]	IVS4 − 91A > G	0.16	0.02	0.20	0.12
rs9895829[4]	IVS4 − 125T > C	0.16	0.16	0.11	0.95
rs1042522[5]	Ex4 + 119C > G (P72R)	0.45	0.13	0.40	0.11
rs1642785[5]	IVS2 + 38C > G	0.50	0.14	0.41	0.14
rs8079544[4]	IVS1 − 112G > A	0.16	0.03	0.11	0.97
WDR79					
rs2287499	Ex1-230C > G (R68G)	0.21	0.19	0.23	0.34
rs17885803	IVS1-60C > T	0.18	0.24	0.12	0.59
rs2287498	Ex2+19C > T (F150F)	0.16	0.40	0.15	0.39
rs17886268	IVS2-106C > T	0.03	0.63	0.04	0.08

Minor allele frequencies for 1,124 Norwegian controls and 2,296 Polish controls are shown.

MAF: Minor allele frequency among control populations. HWE: P value for Hardy-Weinberg Equilibrium in the control populations.

Pairs of SNPs with $r^2 > 0.90$ in Norway or Poland:

[1]Pairwise $r^2 > 0.99$ in Norway and Poland.–[2]Pairwise $r^2 > 0.99$ in Norway and Poland.–[3]Pairwise $r^2 = 0.90$ in Norway and $r^2 = 0.93$ in Poland.–[4]Pairwise $r^2 = 0.98$ in Norway and Poland.–[5]Pairwise $r^2 = 0.82$ in Norway and $r^2 = 0.95$ Poland.

study heterogeneity (Table II; see Supplementary Table I for estimates by study). The pooled estimates for heterozygous and homozygous variant genotypes compared to common homozygotes were 1.08 (0.95–1.23) and 1.60 (1.04–2.47) *p*-trend = 0.01 for rs2287499, and 1.15 (1.00–1.32) and 1.22 (0.69–2.19) *p*-trend = 0.04 for rs2287498. There was also a suggestion for a decreased breast cancer risk for a SNP in intron 1 of *WDR79*; however, the evidence came only from the Norwegian study (Supplementary Table I), and the pooled estimates were not statistically significant (Table II).

We observed significant associations with decreased breast cancer risk in the Norwegian study for 2 SNPs in strong LD (rs1641536 and rs1641535, $D' = 1.0$, $r^2 > 0.99$) in the promoter of *ATP1B2*; rs1641512 in exon 7 of *ATP1B2*; rs1625895 in intron 6 and rs9895829 in intron 4 of *TP53*; and rs17885803 in intron 1 of *WDR79*. However, such associations were not observed in the Polish study (Supplementary Table I), and pooled analyses showed no significant overall associations, or significant study heterogeneity (except for rs1641512 that showed an overall reduction in risk (OR (95% CI) 0.91 (0.81–1.02) for GA *vs.* GG and 0.96

TABLE II – BREAST CANCER RISK AND POLYMORPHISMS IN *TP53* AND ITS FLANKING GENES USING POOLED DATA FROM TWO STUDY POPULATIONS IN NORWAY AND POLAND

Gene polymorphism	Genotype	Case	Control	OR[1]	95% CI		*p** study heterogeneity
ATP1B2							
rs1641536	TT	2048	2422				
−8852T > C	TC	597	857	0.88	0.77	0.99	
	CC	47	76	0.80	0.55	1.19	0.0006
	p for trend			0.0005			
rs1641535	TT	2051	2434				
−8703T > C	TC	601	864	0.88	0.77	0.99	
	CC	51	77	0.86	0.59	1.26	
	p for trend			0.001			0.0003
rs1641512	GG	1674	1962				
Ex7 + 414G > A	GA	898	1232	0.91	0.81	1.02	
	AA	124	168	0.96	0.74	1.23	0.17
	p for trend			0.01			
TP53							
rs17887200	AA	2430	3090				
22369bp 3′ of STP A > G	AG	225	256	1.20	0.99	1.45	
	GG	8	7	1.79	0.63	5.04	0.66
	p for trend			0.18			
rs9894946	CC	1908	2393				
22342bp 3′ of STP T > C	CT	727	892	1.02	0.90	1.14	
	TT	72	105	0.87	0.64	1.20	0.21
	p for trend			0.81			
rs1614984	CC	756	903				
21226bp 3′ of STP C > T	CT	1290	1639	0.94	0.83	1.06	
	TT	569	732	0.92	0.79	1.08	0.71
	p for trend			0.30			
rs12951053	TT	2194	2780				
IVS7 + 92T > G	TG	481	575	1.08	0.94	1.24	
	GG	35	35	1.28	0.78	2.08	0.88
	p for trend			0.23			
rs12947788	CC	2188	2760				
IVS7 + 72T > C	CT	486	570	1.09	0.95	1.25	
	TT	31	36	1.09	0.66	1.80	0.76
	p for trend			0.28			
rs1625895	GG	2080	2686				
IVS6 + 62A > G	AG	564	641	1.10	0.96	1.25	
	AA	37	55	0.89	0.58	1.37	0.39
	p for trend			0.18			
rs2909430	AA	2141	2720				
IVS4 − 91A > G	AG	520	599	1.07	0.94	1.23	
	GG	33	50	0.88	0.56	1.40	0.63
	p for trend			0.38			
rs9895829	TT	2395	2949				
IVS4 − 125T > C	CT	302	417	0.96	0.82	1.13	
	CC	17	20	1.12	0.57	2.21	0.09
	p for trend			0.22			
rs1042522	GG	1368	1774				
Ex4 + 119C > G	CG	1021	1249	1.07	0.97	1.21	
P72R	CC	196	228	1.13	0.92	1.40	0.07
	p for trend			0.18			
rs1642785	GG	1302	1688				
IVS2 + 38C > G	CG	1054	1301	1.07	0.96	1.20	
	CC	208	260	1.05	0.86	1.29	0.04
	p for trend			0.45			
rs8079544	GG	2385	2945				
IVS1 − 112G > A	AG	299	410	0.97	0.82	1.14	
	AA	10	22	0.67	0.31	1.44	0.0009
	p for trend			0.08			
WDR79							
rs2287499	CC	2011	2595				
Ex1 − 230C > G	CG	631	732	1.08	0.95	1.23	
R68G	GG	50	40	1.60	1.04	2.47	0.44
	p for trend			0.01			
rs17885803	CC	2356	2902				
IVS1 − 60C > T	CT	322	446	0.96	0.82	1.12	
	TT	16	24	0.95	0.49	1.82	0.48
	p for trend			0.11			
rs2287498	CC	2171	2811				
Ex2+19C > T	CT	460	518	1.15	1.00	1.32	
F150F	TT	24	25	1.22	0.69	2.19	0.19
	p for trend			0.04			
rs17886268	CC	2585	3244				
IVS2 − 106C > T	CT	112	129	0.97	0.74	1.28	
	TT	1	3	0.39	0.04	3.80	0.68
	p for trend			0.66			

*p value for heterogeneity of odds ratios by study population.
[1]Odds ratios adjusted for age and study population.

TABLE III – ASSOCIATION BETWEEN SELECTED POLYMORPHISMS IN *TP53* AND ITS 5′ FLANKING GENE AND BREAST CANCER RISK STRATIFIED BY ER STATUS, IN POOLED DATA FROM TWO STUDY POPULATIONS IN NORWAY AND POLAND

SNP	Genotype	Genotype frequencies			Relative risk per variant allele										*p* heterogeneity*
			ER+	ER−	ER+					ER−					
		Controls	Cases	Cases	OR	95% CI			P trend	OR	95% CI			*p* trend	
TP53															
rs17887200	*AA*	3090	1116	605											
22369bp 3′ of STP A > G	*AG*	256	88	69											
	GG	7	3	3	1.02	0.80	–	1.31	0.78	1.48	1.11	–	1.93	0.01	0.02
rs12951053	*TT*	2780	1008	533											
IVS7 + 92T > G	*TG*	575	209	138											
	GG	35	11	12	1.00	0.85	–	1.09	0.88	1.29	1.06	–	1.58	0.009	0.02
WDR79															
rs2287499	*CC*	2595	937	477											
Ex1 − 230C > G (R68G)	*CG*	732	270	190											
	GG	40	20	13	1.02	0.87	–	1.19	0.45	1.42	1.18	–	1.71	0.00009	0.005
rs2287498	*CC*	2811	993	527											
Ex2 + 19C > T(F150F)	*CT*	518	204	135											
	TT	25	7	8	1.10	0.92	–	1.31	0.38	1.41	1.15	–	1.73	0.001	0.03

*Heterogeneity of OR's by ER status based on case-only analyses.

(0.74–1.23) for AA *vs.* GG) with a significant *p*-trend = 0.01), and no statistical evidence for study heterogeneity).

Evaluation of genotype-disease associations by estrogen receptor (ER) and progesterone receptor (PR) status showed that 2 SNPs in LD in the *WDR79* gene (rs2287499 and rs2287498) were significantly related to increased risk of ER negative breast cancers (pooled OR (95% CI) per variant allele = 1.42 (1.18–1.71) and 1.41 (1.15–1.73), respectively) but not with ER positive breast cancers (1.02 (0.87–1.19) and 1.10 (0.92–1.31), respectively; Table III; Supplementary Table II for analyses by study). Similarly, 2 SNPs in intron 7 (rs12951953) and 3′ of STP region of *TP53* (rs17887200) showing moderate LD ($D' = 0.80/r^2 > 0.36$ and $D' = 0.77/r^2 > 0.20$ in the Norwegian and Polish populations), were also related to increased risk of ER negative breast cancers (1.48 (1.11–1.93) and 1.29 (1.06–1.58), respectively) but not with ER positive breast cancers (1.02 (0.80–1.31) and 1.00 (0.85–1.09) in both populations). The observed heterogeneity of associations by ER status was statistically significant as shown in case-only analyses (Table III; Supplementary Table II for analyses by study).

In case-only analyses, a SNP (rs12947788) in high LD with rs12951953 ($D' > 0.99/r^2 > 0.99$ in both study populations) was also associated with ER tumor status; however, the association was weaker and only borderline statistically significant (Supplementary Table III). Two additional linked SNPs in exon 4 (P72R) and intron 2 of *TP53*, were observed to be associated with ER status in the Norwegian population; however, these associations were not observed in the Polish study population (Supplementary Table III). No significant differences in the genotype distributions studied here were observed by PR status (Supplementary Table IV), and the differences observed when combining the ER and PR status (data not shown) were driven by ER. We found no significant association between the evaluated polymorphism and age among breast cancer cases in the Norwegian or Polish studies, suggesting no evidence for modification of the observed associations by age (data not shown).

Because of substantial evidence for study heterogeneity in individual SNP analyses, and some differences in LD patterns between the 2 study populations, we have not presented haplotype analyses based on pooled data from both studies. Haplotype analyses within each study were generally consistent with individual SNP analyses (Table IV). Of the 4 SNPs associated with the risk of ER negative tumors, rs17887200 3′ of STP region of *TP53* resided in 1 haplotype in block 2 (GCT). This haplotype was associated with an increased risk of ER negative tumors in the Polish but not in the Norwegian study (Supplementary Table V). A haplotype in block 3 (GTGATCCGGCT) contained variants for the 3 other SNPs individually associated with ER negative tumors (rs12951953, rs2287499 and rs2287498). This haplotype was associated with an increased risk of ER negative tumors in both populations, however it was statistically significant only in the Polish population. The codon 72 SNP resided in 3 haplotypes, including GTGATCC GGCT. None of the 3 haplotypes were significantly associated with risk in the Norwegian or Polish populations, with the exception of a borderline statistically significant reduction in risk for the TCGACCCACTC haplotype in the Norwegian study.

Discussion

This comprehensive evaluation of common genetic variation in *TP53* and its flanking genes found no consistent associations between SNPs in *TP53* and overall breast cancer risk. However, data suggested that common variants in regulatory regions of *TP53* and in its 5′ neighbor, *WDR79*, could be related to increased risk for ER negative but not of ER positive breast cancer. An association for ER negative and not for ER positive tumors would consistent with a higher prevalence of *TP53* mutations in ER negative than ER positive tumors,[1,30] and deserves further evaluation in additional study populations.

The SNPs associated with ER negative breast cancer included 2 linked SNPs in *WDR79* (rs2287499 and rs2287498), and 2 SNPs 3′ of STP region (rs17887200) and intron 7 (rs12951053) of *TP53*. The *WDR79* gene is transcribed in an antisense orientation relative to *TP53*, thus juxtaposing probable proximal promoter elements between these 2 genes. Therefore, it could include regulatory elements for the transcription of the p53 messenger RNA.[31] *WDR79* gene codes for a protein that belongs to the large WD-repeat protein family involved in very diverse cellular functions,[32] and thus, it is possible that the protein coded by this gene could affect breast cancer risk. A recently published report from a large case-control study in the US found evidence for a modest increase in overall breast cancer risk for the *TP53* SNP in intron 7 (rs12951053) and no evidence for an increase in risk for the SNP 3′ of STP (rs17887200).[33] Analyses in the US study were not stratified by ER status and thus we cannot assess if associations were stronger or limited to ER negative cancers, as observed in our study. The functional significance of the SNPs in intron 7 and 3′ of STP in *TP53* is unknown, and thus further studies are needed to clarify the potential functional significance of these or other SNPs in LD.

A recent pooled analysis of 8,743 cases and 10,618 controls, including data from the Polish study, showed strong evidence against an association between the putative functional non-synonymous SNP, P72R, in exon 4 of *TP53* and breast cancer risk.[7] This is also consistent with data from the Norwegian study. Similarly, the variant in intron 6 of *TP53* has also been analyzed by

TABLE IV – ASSOCIATION BETWEEN HAPLOTYPES IN *TP53* AND ITS FLANKING GENES AMONG BREAST CANCER CASES IN TWO STUDY POPULATIONS IN NORWAY AND POLAND

Haplotypes	Norwegian study						Polish study					
	Frequency		OR	95% CI		*p*	Frequency		OR	95% CI		*p*
	Cases	Controls					Cases	Controls				
Block 1												
C C A	0.75	0.71	1.00	(reference)			0.79	0.79	1.00	(reference)		
. . G	0.12	0.09	1.18	0.94	1.50	0.16	0.08	0.08	0.94	0.80	1.11	0.48
T T G	0.12	0.19	0.60	0.49	0.74	0.000002	0.13	0.12	1.00	0.85	1.18	0.99
Rare haplotypes			0.78	0.16	3.70	0.75			0.98	0.95	1.01	0.96
Global test						<0.00001						0.92
Block 2												
A C C	0.38	0.35	1.00	(reference)			0.38	0.37	1.00	(reference)		
. . T	0.41	0.43	0.87	0.74	1.03	0.11	0.42	0.43	0.95	0.86	1.05	0.31
. T .	0.15	0.16	0.88	0.71	1.09	0.23	0.15	0.16	0.96	0.84	1.09	0.50
G . T	0.05	0.05	0.88	0.63	1.23	0.45	0.04	0.03	1.24	0.97	1.58	0.09
Rare haplotypes			1.43	0.97	2.10	0.56			1.45	1.19	1.75	0.21
Global test						0.43						0.14
Block 3												
T C G A T G G G C C C	0.65	0.65	1.00	(reference)			0.67	0.68	1.00	(reference)		
. . . . . T .	0.01	0.01	0.99	0.50	1.94	0.97	–	–				
. . . . . G . .	0.02	0.01	1.16	0.61	2.20	0.65	0.03	0.03	0.98	0.77	1.26	0.90
. . . . . C	0.02	0.04	0.40	0.24	0.67	0.0005	–	–				
. . . . C C C A . T .	0.07	0.08	0.74	0.56	0.98	0.04	0.05	0.05	1.04	0.86	1.26	0.67
. . A G . C C	0.10	0.08	1.15	0.90	1.47	0.27	0.10	0.10	1.00	0.87	1.15	1.00
G T	0.02	0.01	2.32	1.18	4.56	0.01	–	–				
G T . . . C C . G . T	0.08	0.08	0.98	0.75	1.27	0.86	0.09	0.07	1.16	0.99	1.36	0.08
Rare haplotypes			0.84	0.53	1.34	0.48			1.02	0.81	1.27	0.88
Global test						0.001						0.63

Haplotype blocks are defined according to the solid spline method on the pooled genotype data from control populations in each study: block 1 (*ATP1B2* rs1641536, rs1641535, rs1641512); block 2 (*TP53* rs17887200, rs9894946, rs1614984); block 3 (*TP53* rs12951053, rs12947788, rs1625895, rs2909430, rs9895829, rs1042522, rs1642785, rs8079544; *WDR79* rs2287499, rs17885803, rs2287498), rs17886268 was not included in haplotype analyses because of very low frequency in the studied populations. See Figure 1 for LD patters in each study population. Reference haploptypes have the common allele for each individual SNP.

several groups,[9,10,13–15] and it is in strong LD with a variant in intron 4 in the Norwegian and Polish populations. Our analyses showed no evidence of a substantial association with breast cancer risk, consistent with most previous studies. However, pooled estimates did not exclude a weak protection associated with the variant allele, which is consistent with estimates, although not significant, from a previous meta-analysis.[34] A 16 bp insertion in intron 3 of *TP53* which has been previously evaluated in relation to breast cancer risk,[8–13] was not measured in our study population; however, it has been found to be strongly correlated with the intron 6 variant evaluated in our study.[35] Haplotype associations were generally consistent with individual SNP analyses; however, the presence of study heterogeneity of results complicated the comparison of findings.

The current study represents one of the most comprehensive evaluation of common variation in *TP53* and its neighboring genes in relation to breast cancer risk to date. The availability of 2 large and independent study populations facilitated the evaluation of the validity of findings, since potential biases that could explain associations are unlikely to be the same in both study populations. The Polish study has one of the highest participation rates attained in population-based studies collecting biological specimens. Although we cannot rule out selection bias, associations with most established risk factors for breast cancer were in the expected direction and magnitude,[27] indicating that selection bias is unlikely to be important in this study. We had less knowledge on participation rates and distribution of breast cancer risk factors among breast cancer cases participating in the Norwegian study. We observed overall associations with breast cancer risk that were not consistent between the 2 study populations, suggesting that these associations might be due to different characteristics between study populations (*e.g.* the marked differences in LD patters between SNPs in the promoter *ATP1B2* and 3′ of STP region in *TP53*). However, inconsistencies could also be due to biases specific to one of the studies or chance. Therefore, findings should be interpreted with caution and replicated in future study populations. Both study populations were of homogeneous ethnic background, thus reducing the possibility of bias due to population stratification.

In conclusion, we found no consistent associations between SNPs in *TP53* and breast cancer risk in 2 large study populations. However, data suggested that common genetic variation in regulatory regions of *TP53* and its 5′ neighbor *WDR79* could be related to increases in the risk of ER negative breast cancer. Confirmation of these findings in other study populations is warranted.

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