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The *ATM* missense mutation Ser49Cys and risk of breast cancer

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Abstract

Homozygous mutation in the *ATM* gene causes ataxia telangiectasia and heterozygous mutation carriers may be at increased risk of breast cancer. We studied a total of 22 *ATM* variants in two large population-based studies of 2856 breast cancer cases and 3344 controls from the U.S. and Poland. The missense mutation Ser49Cys (S49C), carried by approximately 2% of subjects, was more common in cases than controls in both study populations, combined odds ratio (OR) 1.69, 95% CI 1.19 – 2.40, $P = 0.004$. Another missense mutation at approximately 2% frequency, F858L, was associated with a significant increased risk in the U.S. study but not in Poland, combined OR of 1.44,

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Electronic Database Information

dbSNP, (<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Snip>).

SNP500, NCI Core Genotyping Facility (CGF), (http://snp500cancer.nci.nih.gov/assay_list.cfm *ATM* mutation database, http://www.vmrsearch.org/investigators/concannon_patrick/atmut-t.htm

95% CI 0.98 – 2.11, $P = 0.06$. These analyses provide the most convincing evidence thus far that some missense mutations in *ATM*, particularly S49C, may be breast cancer susceptibility alleles. Because of their low frequency, even larger sample sizes are required to more firmly establish these associations.

Introduction

Ataxia telangiectasia (AT) is a rare autosomal recessive disease (frequency of 1/40,000 or less, heterozygote carrier frequency $< \sim 1\%$) characterized predominantly by severe progressive cerebellar degeneration and increased rates of leukemia and lymphoma (McKinnon 2004). For nearly 20 years it has been suggested that obligate heterozygote mutation carriers, who are phenotypically normal, are at increased risk of breast cancer (Swift et al. 1987). Although somewhat controversial (Khanna 2000), epidemiologic studies among relatives of AT patients have generally confirmed a modest breast cancer risk among obligate heterozygotes (Swift et al. 1991; Geoffroy-Perez et al. 2001; Olsen et al. 2001; Thompson et al. 2005b).

The *ATM* (ataxia telangiectasia mutated) gene, cloned in 1995, is very large and mutations occur throughout its amino acid coding portions (Savitsky et al. 1995). Although somewhat biased, owing to the mutation screening methods applied to such a large gene, like the protein truncation test, most of the mutations identified thus far in AT patients are protein-truncating. The initial cancer observations in obligate carriers from AT families imply that AT-causing mutations underlie the increased breast cancer risk. Complete mutation screening of the *ATM* gene in breast cancer patients, however, has generally not identified a significant number of AT-related mutations in cases compared to controls, although the diversity and extremely low frequency of detected mutations has resulted in studies of insufficient power (FitzGerald et al. 1997). Additionally, haplotypes defined by common SNPs across the gene have not shown any association with breast cancer (Tamimi et al. 2004; Lee et al. 2005).

To explain the inability to identify protein-truncating *ATM* mutations in breast cancer patients, a model has been proposed suggesting that missense and truncating mutations could be associated with distinct phenotypes, namely, cancer and neurological diseases (Gatti et al. 1999). This reasoning is not entirely satisfactory, however, because it does not explain how the initial breast cancer risk association was discovered – namely that relatives of AT patients, who necessarily have mutations that are associated with severe neurological symptoms and are usually truncating, are at increased risk of breast cancer. None-the-less, recent evidence suggests that indeed some missense mutations are functionally relevant and predispose heterozygous carriers to breast cancer. For example, the rare missense mutation V2424G has been identified in two British AT patients who have a less severe level of cerebellar degeneration (Stankovic et al. 1998). It appears to confer an increased risk of breast cancer in these families, and shows “dominant-negative” biochemical effects (Stankovic et al. 1998; Chenevix-Trench et al. 2002; Scott et al. 2002; Thompson et al. 2005a). Missense mutations V2761A, R2849P, and G2876R, identified in AT patients, also showed dominant-negative effects, whereas of the missense mutations S2592C, A2274T, G2287A, C2464R, and G2772R, initially identified in breast cancer patients, only S2592 showed dominant-negative effects when studied biochemically (Scott et al. 2002). Although past studies have been underpowered for analysis of low frequency variants, several studies of breast cancer subjects have identified missense mutations more commonly in breast cancer patients, including the S49C mutation (Izatt et al. 1999; Atencio et al. 2001; Dork et al. 2001; Teraoka et al. 2001; Maillet et al. 2002; Rodriguez et al. 2002; Sommer et al. 2002; Bretsky et al. 2003; Buchholz et al. 2004).

Since complete sequence analysis of the entire *ATM* gene is still prohibitively expensive for an adequately powered breast cancer case-control study, we analyzed 22 individual *ATM*

variants, including AT-associated mutations, missense mutations, and non-coding variants, in two independent study populations in the U.S. and Poland.

Methods

Study Populations

United States Radiologic Technologist Study—The study of *ATM* was initiated in a nested breast cancer case-control study within the US Radiologic Technologist (USRT) cohort study, a group with occupational exposure to medical sources of ionizing radiation (Sigurdson et al. 2003; Sigurdson et al. 2004; Yoshinaga et al. 2004). Subjects included 861 prevalent breast cancer cases, both *in situ* and invasive, identified through questionnaires mailed during 1983–1988 and 1994–1998, and 1048 control subjects without breast cancer as of 1999, frequency matched to the cases on year of birth. Blood samples were collected between 1999 and 2004, with participation rates of 58% among cases and 48% among controls. Calendar year of breast cancer diagnoses ranged from 1955 to 2000.

Breast cancer case-control study in Poland—The second study is a case-control investigation of breast cancer conducted in Poland from 2000 – 2003 (Garcia-Closas, Submitted). Eligible cases were women 20–74 years of age, residents of Warsaw and Łódź, who were newly diagnosed with either histologically or cytologically confirmed *in situ* or invasive breast cancer. Population registries were used to randomly select controls, stratified by city and age in 5 year categories. We identified 3,037 eligible cases and 3,639 eligible controls through the study period. Of these, 2,386 (79%) cases and 2,503 (69%) controls agreed to participate in a personal interview on known and suspected breast cancer risk factors. The present study is limited to women with blood DNA samples: 1,995 cases (6% *in situ*) and 2,296 controls, which represent 84% and 92%, respectively, of those interviewed.

Both study protocols were reviewed and approved by local and NCI Institutional Review Boards. All participants provided written informed consent. Demographic characteristics of the two study populations are shown in Table S1.

Laboratory Methods & Variant Selection—We developed Taqman 5'-nuclease allele discrimination, denaturing high-performance liquid chromatography (dHPLC), or PCR-RFLP assays for the following types of nucleotide changes: AT-associated mutations (n=6) identified in five or more families of diverse ethnicity (as of 2002) or in British AT patients (http://www.vmrsearch.org/investigators/concannon_patrick/atmut-t.htm); variants with functional significance by biochemical assay (n=4) (Scott et al. 2002); and non-conservative missense mutations with a minor allele frequency < 5% (n=5). A single silent mutation, P1526P, was also studied because its frequency differed between breast cancer cases and controls in a prior study (Teraoka et al. 2001).

Interim analyses of association with breast cancer in the USRT study (Struewing et al. 2004) identified four variants (S49C, S707P, F858L, and P1526P) with $P \leq 0.1$, prompting us to evaluate these mutations in the Polish breast cancer study. In the Polish study, six additional non-coding SNPs, chosen from among those available at the National Cancer Institute's Core Genotyping Facility (<http://snp500cancer.nci.nih.gov>), were also assayed (Bonnen et al. 2002; Packer et al. 2004; Tamimi et al. 2004; Lee et al. 2005). Additional details for all assays are available in Table S2.

Genotyping quality control (QC) was assessed with duplicated DNA samples intermixed with study samples (87 DNA aliquots from 8 non-study subjects in the USRT study, and 100 DNA pairs in the Polish study). Laboratory personnel were blinded as to the QC samples, which showed $\geq 99\%$ concordance for all but one *ATM* assay (rs600329 in IVS31, 97% concordance).

We observed no significant departures from Hardy-Weinberg equilibrium in the control populations for any of the assays.

Statistical Methods—We compared genotype frequencies between cases and controls using contingency table analyses and unconditional logistic regression modeling, adjusting for age and study site. Adjustment for additional variables, such as age at menarche, parity, and age at first birth within the individual study groups did not appreciably alter the estimated genotype odds ratios. For age-adjusted analyses, age was considered the age at diagnosis for cases; for controls from Poland, age was considered the age at interview, and in the USRT study, the age as of 1999. For variants where all three genotype possibilities were observed, we tested for overall differences in genotype frequency between cases and controls using a likelihood ratio chi-square test with 2 degrees of freedom (does not assume dominant or recessive inheritance model). We tested for homogeneity of odds ratios between study groups using the Breslow-Day test as implemented in SAS 9.1 (SAS Institute, Inc., Cary, NC).

We calculated the probability of a false association, or false positive report probability (FPRP) (Wacholder et al. 2004), to assess whether our findings were “noteworthy” and reflect true associations. The FPRP was calculated for prior probabilities ranging from 0.1 to 0.0001, based on the statistical power to detect a true OR of 1.5. This range of probabilities was chosen to reflect our priors for the SNPs under study, i.e. 0.1 for the AT-related and missense mutations and 0.0001 for the non-coding mutations. We considered associations with an FPRP below 0.20 (i.e. less than 20% probability of being false positive findings) to be noteworthy.

Genotype frequencies were compared for cases with various survival times in the USRT study and there were no statistically significant differences. For example, the prevalence of S49C heterozygotes was 3.6% in subjects recruited within 10 years of their breast cancer diagnosis and 4.1% among those diagnosed more than 10 years prior to recruitment and blood collection ($P = 0.8$). We also noted no statistically significant differences in the genotype frequencies for any variant between invasive and *in situ* cases for either study group (data not shown). Therefore, estimates of relative risk are presented for both types of tumors combined.

Results

We observed a significantly increased risk of breast cancer among women who were heterozygous carriers of the rare missense mutation S49C (Table 1). Among USRT subjects, 3.9% of cases and 2.6% of controls were heterozygous for S49C, while in Polish subjects 2.3% of cases and 1.2% of controls carried this mutation. Estimated ORs were not significantly different between the two study populations ($P = 0.5$), and the adjusted OR for the combined data was 1.69 (95% CI 1.19 – 2.40, $P = 0.004$). S49C heterozygotes were found more commonly among cases under age 50 than among older cases, whereas the frequency was similar among controls (Table 2), resulting in a stronger association with breast cancer risk in younger (OR = 2.21, 95% CI 1.09 – 4.46) than older (OR 1.53, 95% CI 0.99 – 2.38) women ($P = 0.1$ for heterogeneity of ORs). The F858L missense mutation was associated with significantly increased breast cancer risk in the USRT study, being carried by 3.5% of cases and 1.8% of controls ($P = 0.03$) (Table 1). Its frequency did not vary significantly between cases and controls in the Polish study. The study-specific ORs for F858L were not significantly different from each other ($P = 0.2$), however, and the combined OR was 1.44 (95% CI 0.98 – 2.11, $P = 0.06$).

Table 2 shows S49C and F858L heterozygote frequencies for various subgroups of subjects. The two SNPs were slightly more common in the US subjects, but generally showed consistency in their association with breast cancer. Heterozygote frequencies for F858L were slightly higher in subjects with a first degree relative with breast cancer compared to those with a negative family history, but S49C did not vary by family history.

The FPRP, or probability that the observed association with S49C is a false positive finding, was 11% for a prior probability of 0.1 and a true effect size of 1.5. Lower prior probabilities, down to 0.05, yielded FPRP values of 20% or below, while a prior probability of 0.01 results in a FPRP of 57%. The FPRP for the borderline significant association for F858L was above 20%, even for high priors (FPRPs 49% to >99% for priors of 0.1 to 0.0001, respectively, and true OR of 1.5), indicating that this finding is likely to be a false positive.

The S49C and F858L mutations were two of four coding mutations genotyped in both the USRT and Poland study groups because they showed some evidence of association ($P \leq 0.1$) in an interim analysis of the USRT data (Struewing et al. 2004). (Tables S3 & S4) The P1526P silent mutation, observed commonly in a previous series of breast cancer cases (Teraoka et al. 2001), was somewhat less common among cases in USRT but was at the same frequency among the Polish cases and controls. The S707P missense mutation was significantly less common among USRT cases compared to controls (OR = 0.47, 95% CI 0.23 – 0.93) but showed the opposite effect in the Polish study group (OR = 1.25, 95% CI 0.80 – 1.94). Overall, there was no significant association between S707P with breast cancer risk, but there was evidence for significant heterogeneity in the OR between study groups ($P=0.02$).

Of the 10 AT-related mutations studied in the USRT population, only four were heterozygous in at least one subject: one case carried the 1563delG and one carried the 7636del9 mutation, while three cases and four controls were heterozygous for IVS10-6T>G and four cases and three controls carried S1691R. In total, nine cases and seven controls carried one of these mutations specified *a priori* as likely to be causally related to the AT phenotype, an insignificant difference. The frequency of all variants studied in the USRT study group are shown in Table S3. In the Poland study group, there were no differences in genotype frequency between cases and controls for the non-coding variants, as shown in Table S4.

Discussion

We have analyzed a number of rare (heterozygote frequencies < 10%) coding variants in the *ATM* gene in two large breast cancer studies of 2856 breast cancer cases and 3344 controls from the U.S. and Poland. We provide evidence that at least one of them, S49C, may be a breast cancer susceptibility allele. The S49C missense mutation, carried by less than 3% of subjects, was associated with a significantly elevated odds ratio of 1.69 (95% CI 1.19 – 2.40, $P = 0.004$). False positive report probability calculations indicated that this association is unlikely to be a false positive finding. The variant was slightly more common in US compared to Polish subjects, but the association with breast cancer risk was consistent between the two study populations. The variant was more common among early-onset breast cancer cases (diagnosed before age 50), but there was little difference for subjects with and without a first degree relative with breast cancer.

F858L, another missense mutation at about the same frequency as S49C, was also more common among cases but the association did not reach even nominal statistical significance. The association was mostly driven by the US study group, although the test for heterogeneity of the ORs between studies was not statistically significant. The S707P mutation was significantly less common in cases than controls in the USRT study, but is was more common in cases than controls in the Polish study. The P1526P variant was significantly lower in cases from the USRT study in an interim analysis, but upon analysis of the complete data set and when combined with the Polish data, was not associated with breast cancer risk.

Because complete sequence analysis of the *ATM* gene is prohibitively expensive in large epidemiologic studies, we developed assays for a set of individual AT-causing mutations. While individually rare, we hoped that combining less frequent AT-related mutations would

permit us to determine whether they are associated with breast cancer. Most of the mutations we assayed were not detected in any of the USRT subjects and the summed frequency of AT-related mutations was not different between cases and controls. Two of the mutations included in our *a priori* list, S1691R and the intron 10 splicing mutation, were detected in more than one subject, but it may be that neither of them are true mutations: a study of the intron 10 splicing mutation published since we initiated our analyses suggests it may not increase the risk of breast cancer (Szabo et al. 2004), and the S1691R was studied because it was identified in a single British AT patient, which may, in fact, not be the AT-causing mutation in this subject. Excluding these two variants, we detected two clear AT-causing mutations in cases and none in controls. We thus were not able to contribute much information on whether truncating, AT-causing mutations are more common in breast cancer patients compared to controls.

Our most interesting association was S49C and breast cancer. This mutation, initially identified in a small series of 38 sporadic breast cancer cases (Vorechovsky et al. 1996), has also been detected in 1.6% of 192 hospital-based breast cancer cases (Dork et al. 2001) and in a study of 92 North American AT patients (Castellvi-Bel et al. 1999). It has been evaluated in at least two previous epidemiologic studies of breast cancer. Its frequency did not differ between breast cancer cases and controls in the Multiethnic Cohort study (Bretsky et al. 2003), although there were two or fewer carriers in each of the four ethnic groups. S49C was significantly more common among 75 Caucasian breast cancer patients, enriched for second cancers and positive breast cancer family history, studied at M. D. Anderson Cancer Center compared to 940 blood bank donor controls (6.7% vs 1.3%) (Buchholz et al. 2004).

The Ser → Cys mutation is a non-conservative amino acid substitution at residue 49, but there have been no biochemical studies of its effect on ATM function. This amino acid maps to the recently described chromatin association domain (Young et al. 2005), but does not map to the well-known functional domains of this protein. *In silico* analysis of the potential functional significance of this and other missense mutations were inconclusive: the S49C has a potentially intolerant “Sorting Intolerant from Tolerant” (SIFT) score, but is not predicted to be pathological with PolyPhen, while the F858L was possibly damaging in PolyPhen analyses but tolerated in SIFT analyses (Ng and Henikoff 2001; Ramensky et al. 2002).

The other coding SNPs that we evaluated have been studied previously in epidemiologic settings, but owing largely to their small sample size, the results have been inconsistent. For example, F858L has sometimes been observed more frequently in breast cancer cases than controls (3.6% vs 2.6%, 1500 total subjects) (Dork et al. 2001) while in another setting it was less frequent (2.1% vs 7.4%, 223 total subjects) (Teraoka et al. 2001). In the Multiethnic Cohort study, its frequency was similar between cases and controls (1.3% vs 1.1%, 854 total subjects) but there were only nine heterozygote carriers (Bretsky et al. 2003). The S707P mutation has usually been observed more frequently in breast cancer cases than controls (Dork et al. 2001; Teraoka et al. 2001; Bretsky et al. 2003), but it was significantly less frequent in cases from our USRT study. Whether the observed differences in our two study populations might reflect true differences in the effect of S707P on breast cancer risk due to different environmental or genetic backgrounds is unknown. We did not find any evidence for associations with two of the more common missense variants, P1054R and L1420F, which have shown inconsistent results in previous studies, nor with the very common non-coding polymorphisms studied previously (Dork et al. 2001; Teraoka et al. 2001; Bretsky et al. 2003; Tamimi et al. 2004; Lee et al. 2005).

In summary, we find that the *ATM* missense mutation S49C is likely to be a breast cancer susceptibility allele. The mutation is rare, and even in our combined study population of 6200 subjects, we observed it in only 133. It will be important to confirm this association in even

larger study populations that can be attained through data pooling efforts, such as the Breast Cancer Association Consortium.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Association between four *ATM* SNPs and breast cancer.

SNP Name (rs num ^a)	Genotype ^b	USRT			Poland			Combined				<i>p</i> ^e			
		Cases N	Controls N	<i>P</i>	Cases N	Controls N	OR (95% CI) ^c	<i>P</i>	Cases		Controls N		Controls %	OR (95% CI) _d	
									N	%					
S49C (1800054)	Hz WT	821	1013		1933	2258	1.0		2754	97.2	3271	98.4	1.0	0.5	
	Het	33	27	0.1	45	28	1.60 (0.88)	0.009	78	2.8	55	1.7	1.69 (1.19 – 2.40)		0.004
							–								
S707P (4986761)	Hz WT	837	999		1923	2231	1.0		2760	98.0	3230	97.8	1.0	0.02	
	Het	14	34	0.03	42	39	2.90) 1.0 0.47 (0.23)	0.3	56	2.0	73	2.2	0.92 (0.65 – 1.32)		0.7
							–								
F858L (1800056)	Hz WT	826	1023		1924	2230	1.0		2750	97.9	3253	98.5	1.0	0.2	
	Het	30	19	0.03	30	31	1.94) 1.0 2.03 (1.05)	0.7	60	2.1	50	1.5	1.44 (0.98 – 2.11)		0.06
							–								
P1526P (1800889)	Hz WT	791	951		1702	1962	1.0		2493	88.3	2913	87.7	1.0	0.2	
	Het	47	87		273	308	3.90) 1.0 0.75 (0.49)		320	11.3	395	11.9	0.93 (0.79 – 1.09)		
							–								
	Hz Var	2	0		7	14	1.13) Inf.		9	0.3	14	0.4	0.75 (0.32 – 1.74)		
							–								
							1.43)								
								0.5 (df=2) <i>f</i>						0.5 (df=2) <i>f</i>	

^aReference SNP number (<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Snp>).

^bHz WT subjects are homozygous for the reference/wild-type allele; Het subjects are heterozygous for the variation; Hz Var subjects are homozygous for the non-reference/variant allele.

^cOdds ratio, adjusted for age (below 50, 50–59, 60–69, 70+).

^dAdjusted for study group (USRT or Poland) and age (below 50, 50–59, 60–69, 70+).

^eP value for Breslow-Day test for homogeneity of the ORs across study groups (USRT vs Poland).

^fP value for unadjusted likelihood ratio chi square test with 2 degrees of freedom for overall difference in genotype frequencies between cases and controls.

Table 2

Heterozygote frequencies for the two *ATM* missense mutations S49C & F858L among subgroups of study subjects.

Breast Cancer Case/ Control	Subgroup	<i>ATM</i> SNP					
		S49C			F858L		
		Total N	No. Het	% Het	Total N	No. Het	% Het
Cases	All	2832	78	2.8	2810	60	2.1
Controls	All	3326	55	1.7	3303	50	1.5
Cases	USRT ^a	854	33	3.9	856	30	3.5
	Warsaw ^b	1255	31	2.5	1242	18	1.4
	Łódź ^b	723	14	1.9	712	12	1.7
Controls	USRT	1040	27	2.6	1042	19	1.8
	Warsaw	1467	20	1.4	1450	17	1.2
	Łódź	819	8	1.0	811	14	1.7
Cases	Age <50	1095	37	3.4	1089	27	2.5
	Age 50+	1736	41	2.4	1720	33	1.9
Controls	Age <50	807	11	1.4	803	11	1.4
	Age 50+	2519	44	1.7	2500	39	1.6
Cases	FH + ^c	398	10	2.5	395	10	2.5
	FH –	2432	68	2.8	2413	50	2.1
Controls	FH +	296	5	1.7	294	5	1.7
	FH –	3028	50	1.7	3007	45	1.5
Cases	Invasive- USRT	729	28	3.8	731	28	3.8
	<i>In situ</i> - USRT	125	5	4.0	125	2	1.6
	Invasive- Poland	1862	41	2.2	1839	30	1.6
	<i>In situ</i> - Poland	116	4	3.4	115	0	0.0

^aU.S. Radiologic Technologist Study subjects.

^bWarsaw and Łódź are the two study sites for the Polish Breast Cancer Case-control Study.

^cFH + subjects report at least one first degree relative with breast cancer. FH – subjects have no first degree relatives with breast cancer.