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Missense Variants in *ATM* in 26,101 Breast Cancer Cases and 29,842 Controls

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Abstract

Background—Truncating mutations in *ATM* have been shown to increase the risk of breast cancer but the effect of missense variants remains contentious.

Methods—We have genotyped five polymorphic (MAF 0.9% to 2.6%) missense single nucleotide polymorphisms (SNPs) in *ATM* (S49C, S707P, F858L, P1054R, L1420F) in 26,101 breast cancer cases and 29,842 controls from 23 studies in the Breast Cancer Association Consortium (BCAC).

Results—Combining data from all five SNPs, the OR was 1.05 for being a heterozygote for any of the SNPs and 1.51 for being a rare homozygote for any of the SNPs with an overall trend OR=1.06 (P_{trend}=0.04). The trend OR among bilateral and familial cases was 1.12 (95% CI 1.02-1.23; P_{trend}=0.02).

Conclusions—In this large combined analysis, these 5 missense *ATM* SNPs were associated with a small increased risk of breast cancer, explaining an estimated 0.03% of the excess familial risk of breast cancer.

Impact—Testing the combined effects of rare missense variants in known breast cancer genes in large collaborative studies should clarify their overall contribution to breast cancer susceptibility.

INTRODUCTION

Ataxia-telangiectasia (A-T) is an autosomal recessive disorder characterized by cerebellar ataxia, telangiectases, immune defects, radiosensitivity and a predisposition to malignancy (MIM #208900). The gene that is mutated in A-T, *ATM* (MIM #607585), encodes a protein kinase that plays a key role in cellular responses to DNA damage. The large majority of A-T cases are known to harbour mutations in *ATM* leading to a truncated or absent protein. Epidemiological studies of families of A-T patients have shown a two to fivefold increased risk of breast cancer for female relatives who are obligate heterozygous carriers of an A-T mutation (1, 2).

The increased risk of breast cancer in *ATM* mutation carriers has been confirmed by direct analysis of *ATM* mutations in breast cancer cases compared to controls. In a study of British familial breast cancer cases and controls, Renwick and colleagues identified nine mutations that result in premature termination or exon-skipping among 443 strongly familial cases (2.0%) compared to two in 551 controls (0.4%, P = 0.028) (3). They also found three cases and no controls who carried one of two missense variants for which there is strong a priori evidence of a pathogenic phenotype in individuals with A-T (V2424G or SV2855_2856RI). Bernstein and colleagues identified seven heterozygotes for the V2424G missense variant among 3,743 population-based breast cancer cases (0.2%) unselected for family history and none among 1,268 controls (P = 0.1) (4). Based on the breast cancer history of first- and second-degree relatives of carrier cases, the breast cancer risk to age 70 years for heterozygotes was estimated to be 52% (95% CI: 28 - 80%; P <0.0001).

An association between other *ATM* variants, particularly amino acid substitutions that are not expected to be associated with A-T, and breast cancer has also been hypothesised (5),

but to date there has been little evidence to support this (6, 7). In a previous study we genotyped nine missense variants in *ATM* in 473 bilateral breast cancer cases and 2,463 controls as part of a high-throughput screen of 1,037 non-synonymous single nucleotide polymorphisms (nsSNPs) within candidate "cancer genes" (8). None of these variants was common, with minor allele frequencies (MAFs) in controls ranging from <0.1% (0/4924 chromosomes) to 2.4% (116/4926 chromosomes). Although no single *ATM* missense variant was significantly associated with breast cancer risk there was a significant trend in risk with increasing numbers of variant *ATM* SNPs (odds ratio (OR) = 1.27, 95%CI: 1.04 - 1.56; P_{trend}= 0.02). We selected the 4 variants with MAF>1% (S707P (rs4986761), F858L (rs1800056), P1054R (rs1800057) and L1420F (rs1800058)) for further analysis in 26,101 invasive breast cancer cases and 29,842 controls in 23 studies within the Breast Cancer Association Consortium (BCAC). We also included a fifth variant (S49C (rs1800054)) with MAF 1.2% which was not genotyped in our previous analysis (8) but for which there had been some prior evidence of an association with breast cancer risk (OR = 1.13, 95% CI 0.99 - 1.30 P = 0.08) in an earlier BCAC analysis (9) that included a subset of the current studies.

MATERIALS AND METHODS

Study populations and genotyping

Table 1s (supplementary online) summarises study details and genotyping platform for all studies that contributed data. Genotyping was performed by 5′ nuclease assay (Taqman®), Sequenom iPLEX or Illumina Golden Gate technology. Taqman genotyping reagents were designed by Applied Biosystems as Assays-by-Design^{SM} and distributed by the University of Cambridge group to each of the centres that used this technology. Genotyping was performed using the ABI PRISM 7900HT or 7500 Sequence Detection Systems according to manufacturer's instructions. For five studies, SNPs were genotyped using matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) for the determination of allele-specific primer extension products using Sequenom's MassARRAY system and iPLEX technology (Sequenom, San Diego, CA, USA). The design of oligonucleotides was carried out according to the guidelines of Sequenom and performed using MassARRAY Assay Design software (version 1.0). In one study SNPs were genotyped using customised Illumina Sentrix Bead Arrays according to manufacturers instructions.

Quality control criteria

We applied BCAC standard quality control (QC) guidelines (http://www.srl.cam.ac.uk/consortia/bcac/). In addition, we imposed a threshold of 99% for the call rate (compared with the standard threshold of 95%) and we excluded SNPs from studies where cluster plots, scored from 1 (poor) to 4 (good), scored by a single reader blinded to identifiers scored 2 or less. These more stringent thresholds were imposed because the minor alleles of these SNPs are rare, and therefore more susceptible to differential calling between cases and controls. S49C was not genotyped by 3 studies and data were excluded from analyses for QC criteria for 3 studies. S707P was not genotyped by 2 studies and data were excluded from analyses for QC criteria for 8 studies. F858L was genotyped by all studies; data were excluded from analyses for QC criteria for 1 study. P1054R was genotyped by all studies and data were excluded from analyses for QC criteria for 3 studies. L1420F was not genotyped by 2 studies and data were excluded from analyses for QC criteria for 8 studies. Full details of studies that contributed data for each SNP, numbers of cases and controls genotyped by each study and genotypes of cases and controls for each SNP are given in supplementary tables 1s and 2s online.

Statistical methods

The OR for each SNP and for being a carrier or rare homozygote for any SNP was tested using logistic regression with "study" as a stratifying covariate. To maximise the amount of data included in the analysis, SNPs that were not genotyped by a study or were excluded for QC criteria were coded as 0 for all subjects for the analysis of being a carrier or rare homozygote for any SNP. The effect of this will be to bias our OR estimate, marginally, towards the null.

LD metrics between SNPs (r^2 and D′, supplementary table 3s) were computed separately for each study using the Tagzilla module as implemented in GLU version 1.0a6. rs1800056 (F858L) and rs1800057 (P1054R) are correlated (r^2=0.38 – 0.71; supplementary table 3s), otherwise these rare SNPs are independent of each other (r^2<0.001). Maximum likelihood estimates of haplotype frequencies for the four alleles defined by F858L and P1054R (namely F858+P1054, F858+1054R, 858L+1054R, and 858L+P1054) were estimated in cases and controls separately and in each of the studies separately using HaploStats (http://mayoresearch.mayo.edu/mayo/research/schaid_lab/software.cfm); supplementary table 3s). ORs for F858+1054R, 858L+1054R and 858L+P1054 versus the common allele F858+P1054 were estimated using unconditional logistic regression weighted for the phase assignment probability and with study as a stratifying covariate.

Statistical analyses were performed using STATA version 10 (State College, Texas, US). All P values reported are two-sided. Meta analyses (Figure 1) were carried out using the Metan routine within STATA, using inverse variance weighting of the study specific estimates. Cochran's Q statistic and the I^2 statistic (10) to quantify the proportion of the total variation due to heterogeneity between studies were calculated.

Results

The distribution of genotypes in cases and controls in each study for each *ATM* SNP is shown in table 2s (supplementary online). Subjects reporting ethnicities other than Caucasian were excluded (table 2s, footnote). The MAFs for each of the five SNPs genotyped in this analysis differed significantly (P<0.007, footnote table 1s) between the 22 studies of Caucasian subjects; medians (and ranges) were: S49C 1.2% (0.2%-1.7%), S707P 0.9% (0.6%-1.6%), F858L 1.5% (0.2%-2.4%), P1054R 2.6% (0.6%-3.7%) and L1420F 1.6% (0.2%-2.7%). In the one study in which the majority of subjects were of Asian ethnicity (SEBCS) three SNPs were monomorphic (S49C, S707P and F858L) and for the other two SNPs (P1054R and L1420F) there was only one carrier among 872 control subjects.

In the combined analysis across studies, the point estimates for each of the heterozygote ORs were above 1.0 and the estimates of the homozygote ORs were higher (table 1). The only significantly elevated OR was for L1420F homozygotes (OR=5.31, 95%CI 1.35-20.87). Two SNPs F858L (rs1800056) and P1054R (rs1800057) are correlated (r^2 0.38 to 0.71 across studies, supplementary table 3s). The G allele of rs1800057 (1054R) is more common than the C allele of rs1800056 (858L; table 1s) thus the rare C allele of rs1800056 (858L) is almost completely contained on the rare G allele of rs1800057 (1054R) such that there are 3 main haplotypes for these two allelic variants (F858_P1054, F858_1054R and 858L_1054R) and one extremely rare haplotype (858L_P1054, supplementary table 3s). The trend OR estimates for each of the two haplotypes that carried the rare (C) allele of rs1800056 (858L_1054R and 858L_P1054) compared to the most common haplotype (F858_P1054) were 1.05 (95% CI; 0.95-1.16, P=0.47) and 1.12 (95% CI; 0.61 – 2.05, P=0.71) respectively. The OR estimate for the haplotype that carried the rare (G) allele of

rs1800057 with the common T allele of rs1800056 (F858_1054R) was 0.97 (95% CI; 0.86-1.10, P=0.65).

Combining data from all five SNPs, the OR was 1.05 for being a heterozygote for any of the SNPs and 1.51 for being a rare homozygote for any of the SNPs (P_{trend}=0.04), with an overall OR_{trend} of 1.06 (table 1) and no evidence of heterogeneity between studies (figure 1a, Cochrane Q=21.5 21df, P=0.43, I^2=2.4%). Restricting the analysis to bilateral cases and those with a family history of breast cancer the overall OR_{trend} was stronger (OR_{trend}=1.12, P_{trend}=0.02, table 1) with no evidence of heterogeneity between studies (figure 1b, Cochrane Q=15.5 18df, P=0.62, I^2=0%)

Discussion

Based on Swift's demonstration that carrier status for recessively inherited A-T is associated with a three-fold increase in risk of female breast cancer (1) and the more recent molecular validation of this observation (3) it is arguable that there is a high prior likelihood that a subset of polymorphic (MAF>1%) missense ATM variants will be associated with a modest increase in breast cancer risk. In our previous analysis of the combined effects of nine missense ATM variants (MAF <0.1% -2.4%) we demonstrated that on average, each missense ATM SNP was associated with an OR of 1.27 (95%CI: 1.04-1.56) in bilateral breast cancer cases, implying an OR of 1.13 (95% CI: 1.02–1.25) for cases with a single primary breast cancer (11, 12).

We selected five SNPs for further investigation. Despite restricting our follow-up analysis to SNPs with MAFs estimated to be ≥1% we did not have power to estimate individual effects for these SNPs or the effects of individual haplotypes. The aim of this present analysis was, therefore, to test the composite hypothesis that rare polymorphic ATM variants are, on average, associated with an increased risk of breast cancer. The five SNPs we genotyped in this analysis had a combined carrier frequency of ~12.5%; by genotyping 20,000 cases and 20,000 controls we had 90% power at 1% significance to detect an OR of 1.10.

Our OR estimate of 1.06 (95% CI 1.00-1.12) provides independent evidence that polymorphic missense variants in ATM are associated with a very modest increase in breast cancer risk, albeit at a nominal level of statistical significance (P=0.04). The stronger OR estimate for bilateral cases and cases with a family history of breast cancer (OR=1.12, 95% CI; 1.02-1.23, P=0.02) provides additional support.

We identified four previous studies (13-16) in which at least 100 Caucasian breast cancer cases and 100 Caucasian controls were genotyped and for which individual effect sizes for S49C (rs1800054), S707P (rs4986761), F858L (rs1800056), P1054R (rs1800057) or L1420F (rs1800058) were reported (table 2); we also obtained data for all five variants from the Wellcome Trust Case Control Consortium analysis (Table 2, (17)). For three of these (13, 14, 16), the case control series overlap with the current analysis; the other two (15, 17) do not support an association but are entirely consistent with a per SNP OR of 1.06. A recent analysis of rare (MAF<1%), evolutionarily unlikely missense substitutions in ATM (18) reported a per SNP OR estimate of 1.14 (0.90-1.44, P=0.39) for the combined effects of 121 variants in 1,948 cases and 1,852 controls. We also identified two studies that compared the frequency of ATM variants in bilateral breast cancer cases versus unilateral breast cancer cases. One (19) reported no difference in the frequency of missense variants between bilateral cases and unilateral cases overall but a longer median time to developing a second cancer in carriers of a missense variant who also received radiotherapy. In the other (20), a study of gene-environment interactions (WECARE study) in which bilateral cases were counter-matched to unilateral “controls” on the basis of exposure to radiotherapy, rare

(MAF<1%) A-T associated variants and those that were classified as deleterious according to the prediction algorithm SIFT (21) were associated with a non-significantly increased risk of a second breast cancer while those that were classified as tolerated and several of the more common missense variants were associated with a protective effect. For the linked variants F858L and P1054R, this was statistically significant (OR=0.5, 95% CI 0.3-1.0 and OR=0.5, 95% CI: 0.3-0.9 for F858L and P1054R respectively) raising the possibility of an interaction between radiotherapy and a subset of ATM variants.

It is not yet clear whether polymorphic (MAF>1%) missense variants in ATM and other validated breast cancer genes could make a contribution to explaining the excess familial risk of breast cancer. With a combined carrier frequency of 12.6% in Caucasian controls and an estimated average OR of 1.06, these five *ATM* variants explain 0.03% of excess familial risk of breast cancer, compared to between 0.07% and 1.7% explained by each of the common variants identified in recent GWA studies (7, 22-27). Rare SNPs (MAF≤5%), however, account for a relatively large proportion of genetic variation (28); there are 83 rare missense SNPs in *ATM* listed in dbSNP (including the five genotyped in this study) and large numbers in other breast cancer genes. (29-32).

Testing the combined effects of rare missense variants in known breast cancer genes in large collaborative studies should, eventually, clarify their overall contribution to breast cancer susceptibility. Gutierrez-Enriquez et al (33) compared radiosensitivity of lymphoblastoid cell lines (LCLs) from breast cancer cases who were carriers of one or more rare allele(s) of S707P, F858L, P1054R and L1420F to LCLs from healthy controls. They demonstrated increased radiosensitivity in the LCLs from the breast cancer cases compared to controls generally, and specifically for the six LCLs from patients with at least one copy of the 858L + 1054R haplotype. Incorporating information from such functional assays and from next-generation *in silico* prediction algorithms may help to identify a subset that are most likely to be predictive of risk (34-36).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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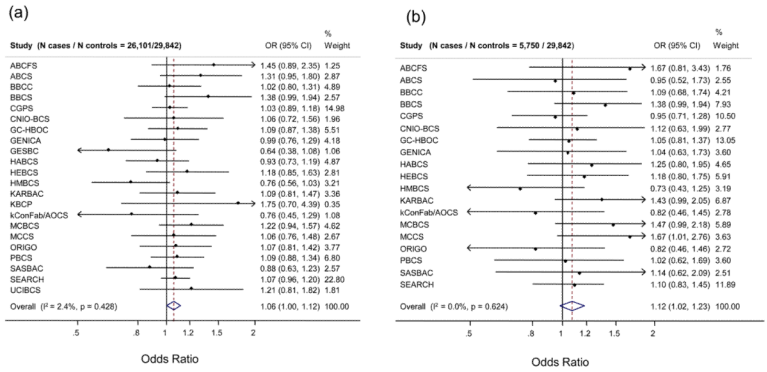

Figure 1. Trend OR estimates for S49C, S707P, F858L, P1054R and L1420F combined by study in (a) all cases and all controls and (b) bilateral cases and cases with a family history of breast cancer and all controls

ORs and P_{trends} were calculated coding individuals who were common homozygotes for all genotyped SNPs as 0, individuals who were heterozygous for any rare variant as 1 and individuals who were rare homozygotes as 2 (statistical methods). Horizontal lines represent 95% CIs. The diamond represents the combined, fixed-effects estimate of the OR and 95% CI. The vertical line indicates the null effect (OR = 1.0).

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Table 1

Summary heterozygote, homozygote and trend odds ratios for S49C, S707P, F858L, P1054R and L1420F

SNP	MAF [1] (range)	N_{cases} $N_{controls}$	Heterozygote OR (95% CI)	Homozygote OR (95% CI)	Trend OR (95% CI)
S49C	1.2 (0.2, 1.7)	22,011 25,865	1.08 (0.95 – 1.22)	1.44 (0.39 – 5.32)	1.08 (0.96 – 1.22)
S707P	0.9 (0.6, 1.6)	17,068 22,330	1.1 (0.96 – 1.26)	5.56 (0.58 – 53.02)	1.12 (0.97 – 1.28)
F858L	1.5 (0.2, 2.4)	26,455 29,785	1.03 (0.93 – 1.14)	1.58 (0.62 – 4.05)	1.04 (0.94 – 1.15)
P1054R	2.6 (0.6, 3.7)	24,191 27,048	1.01 (0.93 – 1.10)	1.04 (0.57 – 1.89)	1.01 (0.94 – 1.10)
L1420F	1.6 (0.2, 2.7)	18,607 22,565	1.05 (0.95 – 1.17)	5.31 (1.35 – 20.87)	1.07 (0.97 – 1.20)
F858L P1054R haplotype [2]					
858L+1054R	1.5 (0.2, 2.4)	24,191 27,048	1.04 (0.94 - 1.16)	1.67 (0.59 - 4.73)	1.05 (0.95 - 1.16)
F858+1054R	1.1 (0.4, 1.9)	24,191 27,048	0.98 (0.87 - 1.10)	0.72 (0.21 - 2.46)	0.97 (0.86 - 1.10)
858L+P1054	0.1 (0.04, 0.2)	24,191 27,048	1.06 (0.53 - 2.12)	1.93 (0.22 - 16.67)	1.12 (0.61 - 2.05)
Any SNP					
All cases	6.3 [3]	26,101 29,842	1.05 [4] (0.99 – 1.11)	1.51 [5] (0.95 – 2.41)	1.06 (1.00 – 1.12)
					P_{trend} =0.04
Bilateral & familial cases		5,750 29,842	1.12 (1.02 – 1.23)	1.22 (0.55 – 2.72)	1.12 (1.02 – 1.23)
					P_{trend} =0.02

CI; confidence interval, MAF; minor allele frequency in controls expressed as a percentage, OR; odds ratio, N/A; Not available

[1] Median and range

[2] the OR for being a compound heterozygote was 1.04 (0.94 - 1.15). Due to the correlation between F858L and P1054R, however, 1587/1690 (93.9%) of compound heterozygotes were carriers of the 858L 1054R haplotype.

[3] To calculate the combined MAF we assumed all carriers of the rare allele of F858L also carried the rare allele of P1054R and independence between the other SNPs

[4] Heterozygote for any of the five SNPs

[5] Rare homozygote for any of the five SNPs

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Table 2

Summary of previously published and publicly accessible data on S49C, S707P, F858L, P1054R, L1420F

Study (reference)	Dork (13)	Spurdle (14)	Bretsky (15)	Stredrick (USRT) (16)	Stredrick (Poland) (16)	WTCC (17)
Nos cases/controls	**1000/500**	**1453/793**	**110/110**	**856/1042**	**1978/2286**	**1045/1476**
S49C	—	—	—	1.60 (0.88 – 2.90)	1.87 (1.14 – 3.11)	1.26 (0.81 – 1.96)
S707P	2.4 (1.0 – 5.6)	1.08 (0.59 – 1.97)	0.66 (0.05 – 5.90)	0.47 (0.23 – 0.93)	1.25 (0.80 – 1.94)	0.90 (0.55 – 1.46)
F858L	1.4 (0.7 – 2.7)	—	2.02 (0.10 – 120.15)	2.03 (1.05 – 3.90)	1.12 (0.67 – 1.86)	0.66 (0.40 – 1.10)
P1054R	1.4 (0.8 – 2.2)	1.35 (0.85 – 1.98)	0.83 (0.19 – 3.36)	—	—	0.84 (0.58 – 1.22)
L1420F	1.5 (0.9 – 2.7)	—	0.66 (0.05 – 5.90)	—	—	0.93 (0.63 – 1.35)
Combined	1.56 (1.11 – 2.20)	1.25 (0.89 – 1.77)	0.75 (0.25 – 2.25)	1.22 (0.84 – 1.77)	1.37 (1.04 – 1.81)	0.96 (0.78 – 1.18)