A Multi-Centre Study of *CASP8* polymorphisms in Breast Cancer

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ABSTRACT

Work is underway to investigate the genetic components involved in the aetiology of breast cancer (OMIM 1144880). The current work takes a candidate gene approach to investigate the role of polymorphisms in the *CASP8* gene which is involved in the apoptosis and programmed cell-death (PCD) pathways. Previous work has demonstrated that a SNP within this gene (rs1045485) reduced the risk of breast-cancer **?**, and the current work seeks to test additional polymorphisms for association.

Methods

The Sheffield Cohort consisted of 1233 cases recruited from surgical outpatients at the Royal Hallamshire Hospital, Sheffield and 1232 controls recruited from the Sheffield Breast Screening Service. A subset of 135 samples were genotyped for 33 Single Nucelotide Polymorphisms (SNPs) spanning a 50kb region across the *CASP8* gene and Principal Components Analysis was used to select tagging SNPs for genotyping in the remaining cohort **?**. Genotyping of cases and controls was performed using the ABI SNPlex genotyping platform.

Statistical Analysis

All loci were tested for departure from Hardy-Weinberg equilibrium in controls. Single-locus test for association were performed using the trend-test **?** and per-allele Odds-Ratios' were calculated under an additive model. The distribution of linkage disequilibrium across the region was assessed via the metrics D' and r^2 and haplotype associations of sliding windows of haplotypes were tested using the software haplo.stats() **?**.

Replication

SNPs that demonstrated significant association were genotyped in two additional cohorts. The Utah cohort comprised 754 unrelated BRCA1/2 negative cases and 440 unrelated sex and age matched controls from the Utah Breast Cancer Study. The German cohort consisted of 1228 BRCA1/2 negative cases collected from the German Consortium for Hereditary Breast and Ovarian Cancer and 1667 unrelated controls recruited from the Red Cross Blood Service.
Hardy-Weinberg equilibrium tests were repeated in the controls and the single-point trend-test and per-allele Odds-Ratios' were calculated in each cohorts. In additio, logistic regression with dummy variables was performed to determine genotype relative risks (GRR). A pooled meta-analysis of the raw data was carried and Monte-Carlo simulations (N = 5000) performed to derive empirical confidence intervals to account for the relatedness of Utah samples using the software PedGenie **? ?**.

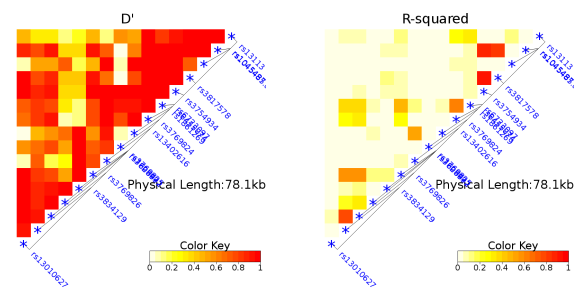
Results : Sheffield Cohort

One SNP (rs3820792) was found to deviate from Hardy-Weinberg equilibrium in the Sheffield controls ($p_{exact} = 0.049$). Three SNPs demonstrated significant ($p_{trend} \leq 0.05$) under the trend test rs3834129 ($p_{trend} = 0.027$); rs6435074 ($p_{trend} = 0.042$) and rs6723097 ($p_{trend} = 0.024$), the previously associated rs1045485 showed border-line significance ($p_{trend} = 0.070$). Two of these SNPs also demonstrated significant association under an additive disease model (rs3834129 ($p_{allele} = 0.027$); rs6723097 ($p_{allele} = 0.022$)); rs1045485. The locus rs6435074 showed border line significance under an additive model ($p_{allele} = 0.060$). Full results are summarised in table 1.

Table 1 Association at SNPs in the *CASP8* gene in the Sheffield Cohort.

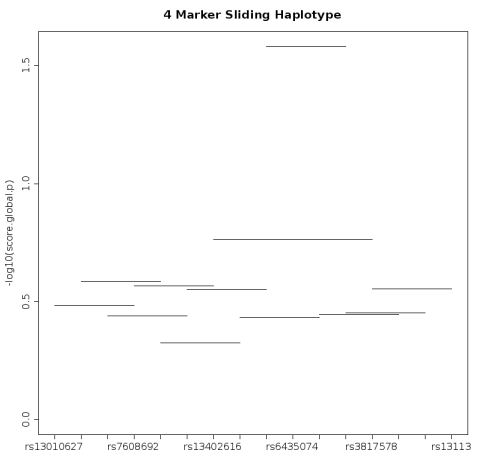
dbSNP	p_{HW}	MAF	p_{trend}	Per-Allele OR (95% CI)
rs13010627	1.000	0.059	0.818	1.028 (0.804-1.315)
rs3834129	0.178	0.468	0.027*	0.876 (0.779-0.985)*
rs3769826	0.952	0.450	0.102	1.104 (0.982-1.241)
rs7608692	0.096	0.215	0.502	0.961 (0.831-1.110)
rs3820972	0.049*	0.085	0.486	1.063 (0.847-1.334)
rs3769825	0.713	0.419	0.083	1.110 (0.988-1.249)
rs13402616	0.612	0.061	0.341	1.128 (0.893-1.425)
rs1861269	0.397	0.036	0.457	0.897 (0.650-1.239)
rs6435074	0.338	0.245	0.042*	1.136 (0.995-1.297)
rs6723097	0.845	0.350	0.024*	1.152 (1.020-1.301)*
rs3754934	0.508	0.048	0.555	0.937 (0.700-1.255)
rs3817578	0.509	0.049	0.822	1.033 (0.777-1.374)
rs1045485	0.742	0.156	0.070	0.848 (0.721-0.998)*
rs1045487	0.718	0.043	0.961	1.013 (0.761-1.349)
rs13113	0.759	0.451	0.373	0.947 (0.841-1.066)

Linkage Disequilibrium



Haplotype Association

The strongest association was seen with a 4 markers haplotype consisting of rs1861269, rs6435074, rs6723097, rs3754934.



Results : Replication Cohorts

Four SNPs with the strongest evidence for association in this study and previous work were genotyped in the Utah and German cohorts (rs3834129, rs6435074, rs6723097, rs1045485).
With the exception of the Utah cohort (rs1045485 ; $p_{HWexact} = 0.025$) all loci were in Hardy-Weinberg equilibrium for the four SNPs in the German and Utah cohorts.
The direction of single-point associations in the German cohort was similar to that observed in the Sheffield cohort, however, the Utah cohort did not show any evidence of association across the loci (see tables 2 and 3).

Results : Meta-Analysis

Pooled analysis of the four SNPs shows that three loci have a significant effect on breast cancer risk. The rs3834129 locus reduces risk in an additive manner, whilst rs6435074 and rs6723097 both increase risk in an additive manner. Despite previous reports of a reduction in risk by rs1045485 this was not observed in the pooled analysis of these cohorts (see tables 2 and 3)

Table 2 Trend Test of Association at SNPs in the *CASP8* gene in all Cohorts.

dbSNP	rs3834129	rs6435074	rs6723097	rs1045485
Sheffield	0.027	0.042	0.024	0.070
Utah	0.797	0.193	0.120	0.782
Germany	0.046	0.298	0.022	0.200
Pooled	0.009	0.018	0.0005	0.032

Table 3 Genotype Relative Risks at SNPs in the *CASP8* gene in all Cohorts.

dbSNP	Genotype	rs3834129	rs6435074
Sheffield	Het.	0.90(0.74-1.11)	1.20(1.02-1.43)
	Hom. Mut.	0.77(0.61-0.96)	1.16(0.83-1.63)
Utah	Het.	1.01(0.74-1.35)	1.20(0.92-1.56)
	Hom. Mut.	1.05(0.73-1.50)	1.14(0.68-1.89)
Germany	Het.	0.96(0.78-1.18)	1.01(0.85-1.21)
	Hom. Mut.	0.77(0.61-0.98)	1.32(0.96-1.82)
Pooled	Het.	0.95(0.83-1.08)	1.12(1.00-1.26)
	Hom. Mut.	0.82(0.70-0.95)	1.22(0.97-1.55)

dbSNP	Genotype	rs6723097	rs1045485
Sheffield	Het.	1.14(0.95-1.37)	0.88(0.73-1.06)
	Hom. Mut.	1.34(1.02-1.75)	0.59(0.34-1.03)
Utah	Het.	1.21(0.91-1.60)	1.07(0.76-1.50)
	Hom. Mut.	1.29(0.84-1.98)	0.91(0.27-3.08)
Germany	Het.	1.11(0.93-1.34)	0.87(0.64-1.17)
	Hom. Mut.	1.36(1.05-1.77)	0.52(0.16-1.66)
Pooled	Het.	1.14(1.00-1.30)	0.91(0.79-1.05)
	Hom. Mut.	1.34(1.12-1.61)	0.65(0.41-1.04)

Conclusion

This clearly demonstrates the role of *CASP8* in breast cancer susceptibility, however to date no clear functional or regulatory polymorphism has been identified that may explain the observed associations. Sequencing of the region to identify additional polymorphisms is underway, and testing of epistatic interactions with polymorphisms in genes in the same apoptosis and PCD pathways is planned.

Acknowledgements

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References