Null Results in Brief

Hyaluronan-Mediated Motility Receptor Gene Single Nucleotide Polymorphisms and Risk of Breast Cancer

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Abstract

A recent study used a network modeling strategy to generate a set of genes linked by potential functional associations. The hyaluronan-mediated motility receptor (HMMR) gene was identified as being as functionally associated with BRCA1 and thus a candidate breast cancer gene. SNPs rs10515860, rs299290, and rs7712023 were reported to be significantly associated with breast cancer in a joint analysis of two small case-control studies. We have examined the association of these single nucleotide polymorphisms, together with others tagging the HMMR gene, in a larger, European case-control study and find no association of any of them with risk of breast cancer: rs10515860 [odds ratio (OR; AA/ GG), 0.85; 95% confidence interval (CI), 0.65-1.12; P trend = 0.9], rs299290 [OR (CC/TT), 1.00; 95% CI, 0.87-1.15; P trend = 0.7], rs3756648 (rs7712023) [OR (TT/CC), 0.93; 95% CI, 0.84-1.02; P trend = 0.1], rs299284 [OR (TT/CC), 1.01; 95% CI, 0.76-1.35; P trend = 0.5], and rs13183712 [OR (TT/GG), 1.04; 95% CI, 0.88-1.23; P trend = 0.6]. (Cancer Epidemiol Biomarkers Prev 2008;17(12):3618–20)

Introduction

A recent publication reported significant associations between single nucleotide polymorphism SNPs in the HMMR gene, encoding the hyaluronan-mediated motility receptor, a centrosome unit, and risk of breast cancer (1). The study used a network modeling strategy, combining gene expression profiling with functional genomic and proteomic data from various species, to generate a set of genes linked by potential functional associations. The HMMR gene was identified as being functionally associated with BRCA1 and thus predicted to be a common, low-penetrance breast cancer candidate gene. SNPs rs10515860, rs299290, or rs7712023 were reported to be significantly associated with breast cancer in a joint analysis of two small case-control studies: a population-based case-control study of incident breast cancer in northern Israel and an independent case-control study in Ashkenazi Jewish cohort from New York (Table 1). We have attempted to confirm this finding in the >3-fold larger East Anglian SEARCH breast cancer study.

Materials and Methods

Cases and Controls. The SEARCH breast cancer case-control study has been described previously (2). This study has been approved by the Eastern Region Multicentre Research Ethics Committee, and all patients gave written informed consent. The ethnic background of both cases and controls, as reported on the questionnaires, is similar with >98% being White Europeans. The total number of cases available for analysis was 6,762 and the total number of controls was 6,852.

Selection of Tagging SNPs. TagSNP selection was done using the Tagger feature within the Haploview program (3) on data from the CEU population from HapMap database.5 We aimed to define a set of tagSNPs that tagged all the known common SNPs (with minor allele frequencies of >0.05) in the gene, with a pairwise correlation r² > 0.8.

Genotyping. Genotyping was carried out using Taqman (Applied Biosystems) according to manufacturer’s instructions. Primers and probes were supplied directly by Applied Biosystems as Assays-by-Design. All assays were carried out in 384-well plates. Each plate included negative controls (with no DNA) and positive controls.

5 http://www.hapmap.org

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duplicated on a separate quality control plate. Plates were read on the ABI PRISM 7900 using the Sequence Detection Software (Applied Biosystems). Failed genotypes were not repeated.

**Statistics.** For each SNP, deviations of genotype frequencies in controls from Hardy-Weinberg equilibrium were assessed by a χ² test with one degree of freedom. Genotype frequencies in cases and controls were compared by χ² test for heterogeneity (2 degrees of freedom) and test for trend (1 degree of freedom). Genotype specific risks were estimated as odds ratios with associated confidence intervals using unconditional logistic regression.

**Results and Discussion**

The human *HMMR* gene spans ~31 kb and maps to chromosome region 8q33.2-qter (4). There are 52 SNPs listed on HapMap (HapMap Data Rel 22/phase2 Apr07) within the *HMMR* gene footprint, of which 31 have minor allele frequency of >0.05. We selected 6 tagSNPs to represent all 31 known common SNPs in the gene (rs7712023, rs299284, rs299290, rs13183712, rs299316, and rs10515860) with r² > 0.8. The three SNPs examined in the original paper (rs7712023, rs299290, and rs10515860; ref. 1) were forced into this tagging set. The TaqMan assays for two of the tagSNPs (rs7712023 and rs10515860; ref. 1) were duplicated on a separate quality control plate. Plates were read on the ABI PRISM 7900 using the Sequence Detection Software (Applied Biosystems). Failed genotypes were not repeated. The five assayable SNPs (rs3756648, rs299316, and rs10515860) failed. It was possible to replace SNP rs7712023 by an alternative, perfectly correlated SNP, rs299316 (r² = 1.0), but rs299316 was a singleton (it did not tag any other SNP apart from itself) and, thus, could not be replaced. The five assayable SNPs (rs3756648, rs299284, rs299290, rs13183712, and rs10515860) tagged 30 of 31 known common SNPs in the gene with r² > 0.8. These assays produced reliable genotyping results with allele call rates of >96.7%, and duplicate samples showed >98% concordance in their genotypes.

The 5 *HMMR* tagSNPs were genotyped in 6,762 breast cancer cases and 6,852 controls from East Anglia. We did not identify any significant associations of these SNPs with breast cancer susceptibility (Table 1).

We were unable to confirm the previously reported increased risk of breast cancer associated with SNPs rs10515860, rs299290, or rs7712023 (rs3756648; ref. 1). In the present study, the odds ratios were not different from unity. Furthermore, we found no evidence of breast cancer association with the two other tagSNPs we assayed in this gene. We have done a meta-analysis of our own results together with those of Pujana and colleagues. A joint analysis of the data from the two studies showed no significant association of SNP rs299290 or rs10515860 with breast cancer (Fig. 1A). In the case of rs3756648/rs7712023, there was borderline evidence for the minor allele having a protective effect (Fig. 1B), but this would not have been statistically significant if Pujana’s hypothesis-generating study had been excluded. There is a third SNP rs2069347 perfectly correlated (r² = 1.0) with both rs3756648 and rs7712023 located within another gene, CCNG1, encoding Cyclin G1 15.5 kb upstream of *HMMR*. The reported effect, if any, might be explained by involvement of the cyclin gene rather than originally proposed *HMMR*. Thus, we found no evidence for the existence of common, breast cancer susceptibility alleles in this gene in the East Anglian, British population.

Our study was a size thrice larger than the combined Jewish studies (6,762 cases versus 2,475, and 6,852 controls versus 1,918, as illustrated by the weighting in Fig. 1) and had 90% power to detect a per-allele odds ratio of 1.2 at a *P* value of <0.0001 significance level with a SNP of minor allele frequency of 0.10. Thus, we had

### Table 1. Genotyped tagSNPs and breast cancer association analysis

<table>
<thead>
<tr>
<th>TagSNP genotypes</th>
<th>Our data</th>
<th>Pujana et al., 2007</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls, n (%)</td>
<td>Cases, n (%)</td>
</tr>
<tr>
<td>rs3756648</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>1760 (26.3)</td>
<td>1790 (27.7)</td>
</tr>
<tr>
<td>C/T</td>
<td>3225 (49.8)</td>
<td>3180 (49.1)</td>
</tr>
<tr>
<td>T/T</td>
<td>1598 (23.9)</td>
<td>1504 (23.2)</td>
</tr>
<tr>
<td>rs299284</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>5350 (79.0)</td>
<td>5176 (78.5)</td>
</tr>
<tr>
<td>C/T</td>
<td>1327 (19.6)</td>
<td>1322 (20.1)</td>
</tr>
<tr>
<td>T/T</td>
<td>97 (1.4)</td>
<td>95 (1.4)</td>
</tr>
<tr>
<td>rs299290</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>3861 (56.9)</td>
<td>3708 (56.3)</td>
</tr>
<tr>
<td>T/C</td>
<td>2481 (36.5)</td>
<td>2450 (37.2)</td>
</tr>
<tr>
<td>C/C</td>
<td>445 (6.6)</td>
<td>426 (6.5)</td>
</tr>
<tr>
<td>rs13183712</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>4239 (62.5)</td>
<td>4076 (62.2)</td>
</tr>
<tr>
<td>G/T</td>
<td>2227 (33.0)</td>
<td>2180 (33.2)</td>
</tr>
<tr>
<td>T/T</td>
<td>303 (4.5)</td>
<td>303 (4.6)</td>
</tr>
<tr>
<td>rs10515860</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>5214 (76.6)</td>
<td>5039 (76.3)</td>
</tr>
<tr>
<td>G/A</td>
<td>1479 (21.7)</td>
<td>1472 (22.3)</td>
</tr>
<tr>
<td>A/A</td>
<td>114 (1.7)</td>
<td>94 (1.4)</td>
</tr>
</tbody>
</table>

Abbreviations: OR, odds ratio; CI, confidence interval.

*SNPs rs3756648 and rs7712023 are perfectly correlated, r² = 1.0 in the HapMap CEU population sample.
good power to confirm the effect suggested for SNP rs10515860 (1).

It is possible that the causative variant is a founder mutation, present in the Jewish population, and not found in European populations. Ashkenazi founder mutations exist in the BRCA1 and BRCA2 genes but they do not have high frequencies (minor allele frequencies of <0.02; refs. 5, 6). A rare, founder variant would be very poorly tagged by the common SNPs used here and by Pujana et al. (1). We do not know of any examples of common, founder SNPs in the Ashkenazi population—common SNPs are, by definition, old and probably predate the formation of ethnic groups outside of Africa.

We conclude that the reported association between common SNPs in HMMR and breast cancer risk is likely to be a false positive association. This is not surprising, given that the results did not reach the level of statistical significance generally accepted as providing strong posterior evidence of association. In consequence, although the network modeling approach remains an interesting approach to explore disease biology, its ability to identify cancer susceptibility loci remains, as yet, unproven.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
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References

Figure 1. Meta-analysis of HMMR SNP genotypings in two studies—Pujana et al., 2007 and SEARCH. A. rs3756648/rs7712023, overall P = 0.021. B. rs299290, overall P = 0.335. C. rs10515860, overall P = 0.212; in case of this SNP, the study of Pujana and colleagues contains data from two independent substudies of New York and Israel cohorts.
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