Multiplex single-nucleotide primer extension assay for detection of low-penetrance breast cancer susceptibility polymorphisms

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INTRODUCTION

Breast cancer is one of the most common cancers among women in developed countries. Approximately 10% of sufferers have a genetic predisposition and 25% of patients with familial breast cancer carry mutations in *BRCA1* or *BRCA2* high-penetrance genes. At present, it seems to be widely accepted that no further high-penetrance genes accounting for a relevant number of familial cases exist. Generally, two groups of susceptibility factors can be clearly distinguished comparing mean allele frequencies and mean associated risks: rare mutations in genes involved in DNA repair with intermediate penetrance on the one side and low penetrance SNPs on the other (Figure 1).

AIM OF THE STUDY

The aim of this study was to develop an easy, rapid and cost-effective method for genotyping of low-penetrance breast cancer susceptibility polymorphisms within six genes and gene-free genomic regions.

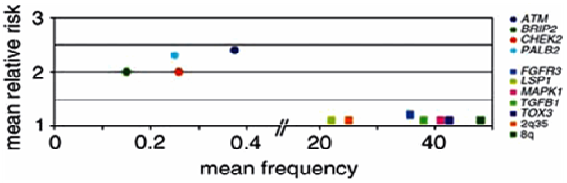

Figure 1. Association of mean frequency and relative risk of breast cancer susceptibility genes and loci.

MATERIALS AND METHODS

We have designed a multiplex single-nucleotide primer extension assay to genotype rs889312 (*MAP3K1)*, rs2981582 (*FGFR2)*, rs3803662 (TNRC9/LOC643714), rs3817198 (*LSP1)*, rs1328615 (8q) and rs1045485 (*CASP8)* in a single reaction. Using this method, we analyzed 92 breast cancer patients, of which 26 with familial breast cancer and 86 controls from the general population.

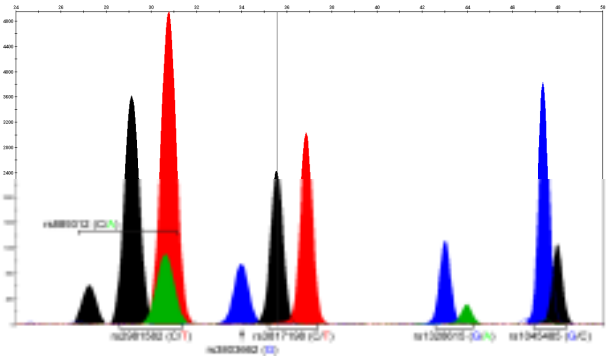

Figure 2. Single-nucleotide primer extension assay for detection of selected SNPs in BRCA genes: the fluorescent peaks formed by specific primer extension products are labeled below the electropherogram; labels correspond to SNPs names.

RESULTS

Our results showed that SNP rs1045485 in *CASP8* is significantly associated with decreased breast cancer risk [p=0.040; OR(95%CI)=0.53 (0.29-0.98)] (Figure 3A), while rs889312 in *MAP3K1* showed a significant association with increased breast cancer risk [p=0.0425; OR(95%CI)=1.91(1.02-3.61)] only among patients with positive family history of breast cancer (Figure 3B).

CONCLUSION

In conclusion, we present an effective genotyping method that can be applied in medium-scale breast cancer association studies.

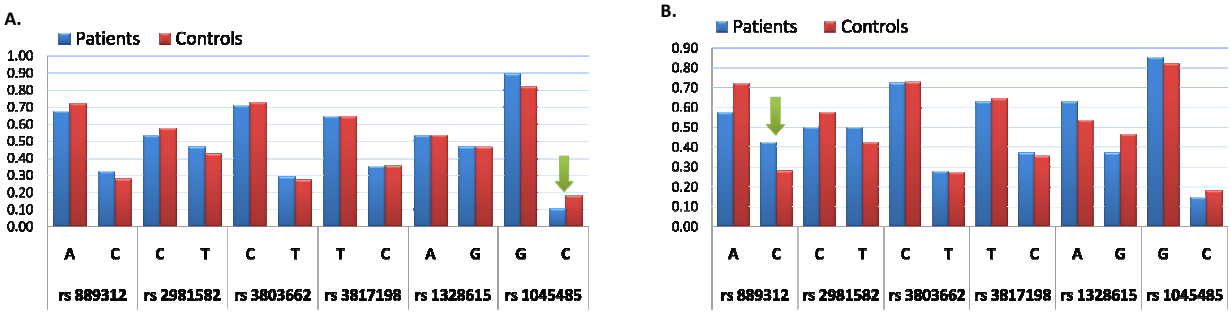

Figure 3. Allelic frequencies of six analyzed SNPs: **A)** all BC patients vs. controls and **B)** BC patients with positive family history vs. controls.

ACKNOWLEDGMENTS

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