

Short communication

Usefulness of polymorphic markers in exclusion of *BRCA1/BRCA2* mutations in families with aggregation of breast/ovarian cancers

Bohdan GÓRSKI, Tadeusz DĘBNIAK, Anna JAKUBOWSKA, Cezary CYBULSKI, Tomasz HUZARSKI, Tomasz BYRSKI, Elżbieta ZŁOWOCKA, Jan LUBIŃSKI

Department of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland

Abstract. Founder mutations can account for a large proportion of *BRCA1/BRCA2* gene abnormalities in a given population. However there is still a need to study the entire gene in many families, even in countries where founder mutations have been identified. It is possible to decrease the number of cases which are studied by complex and expensive sequencing/Southern blot analyses of *BRCA1/BRCA2* genes by exclusion of common *BRCA1/BRCA2* alleles in a given family by using polymorphic dinucleotide markers. The goal of our study was to assess the effectiveness of this method in exclusion of *BRCA1/BRCA2* constitutional mutations. In each family, blood samples for genetic analyses were taken from two affected relatives from the same generation. Six polymorphic microsatellite markers linked to *BRCA1/BRCA2* genes were analysed. Results obtained with these markers were verified by applying *BRCA1* testing for the most common founder mutations in Poland and using "exon by exon" sequencing of coding fragments of the *BRCA2* gene. Polymorphic markers useful in *BRCA1/BRCA2* analyses included only 3 of 6 examined – D17S855, D13S260 and D13S267. Occurrence of common alleles of *BRCA1* was excluded in 3 families and *BRCA2* in 5 out of 30 families. Results obtained by testing for *BRCA1* Polish founder mutations and *BRCA2* sequencing were in agreement with *BRCA1* findings based on polymorphic markers. The only exception was family 994 with *BRCA1* exon 5 300T/G mutation, in which *BRCA1* mutation carrier was excluded by using D17S855. Among 14 families without *BRCA1* Polish founder mutations in this gene were excluded in 2 families and *BRCA2* mutation was excluded in one family.

Key words: *BRCA1*, *BRCA2*, breast, cancer, inherited predisposition, ovarian.

Received: January 15, 2003. Accepted: May 14, 2003.

Correspondence: B. GÓRSKI, Department of Genetics and Pathology, Pomeranian Medical University, ul. Połabska 4, Szczecin 70-115, Poland, e-mail: gorskib@sci.pam.szczecin.pl

The first major breast cancer and ovarian cancer susceptibility gene, *BRCA1*, was identified in 1994 (MIKI et al. 1994). *BRCA1* (MIM # 113705) is a putative tumor suppresser gene located on chromosome 17q21 that has been implicated in DNA repair recognition (NEWMAN et al. 1988, HALL et al. 1990, CLAUS et al. 1991, NAROD et al. 1991, MIKI et al. 1994, SCULLY et al. 1999). Mutations inactivating *BRCA1* are responsible for tumors in 40-50% of familial site-specific breast cancers and in 80-90% of breast/ovarian cancer syndrome cases (EASTON et al. 1993, FORD et al. 1994).

BRCA1 gene is composed of 22 coding exons distributed over approximately 100 kbp of the genomic DNA with a transcript of 7.8 kb in length, encoding a 1863 amino acid protein (MIKI et al. 1994). In some ethnic or geographically isolated groups, *BRCA1* founder mutations have been described (STRUEWING et al. 1995, TONIN et al. 1995, SZABO, KING 1997, GÓRSKI et al. 2000).

The second breast and/or ovarian susceptibility gene, *BRCA2*, was cloned in late 1995. The *BRCA2* gene is spread over 80 kbp of genomic DNA and consists of 11,385 bp coding sequence distributed over 27 exons. The gene encodes a protein of 3,418 amino acids (WOOSTER et al. 1995). Mutations in the *BRCA2* gene have been found mainly in families with high incidence of female as well as male breast cancers. Mutations are spread over the whole gene, of which the majority consist of small deletions that cause reading frameshifts and premature stop codons (WOOSTER et al. 1995, COUCH et al. 1996).

Founder mutations can account for a large proportion of *BRCA1/2* gene abnormalities in a given population. However, there still remains a need to study the entire gene in many families, even in countries where founder mutations have been identified. It is possible to decrease the number of cases that are studied by complex and expensive sequencing/Southern blot analyses of *BRCA1/BRCA2* genes by exclusion of common *BRCA1/BRCA2* alleles in a given family by using polymorphic dinucleotide markers. The goal of our study was to assess the effectiveness of this method in exclusion of *BRCA1/BRCA2* constitutional mutations.

The study group consisted of 30 Polish families with at least 3 relatives affected by either breast or ovarian cancers, who were drawn from the Hereditary Cancer Center in Szczecin .

In each family, blood samples for genetic analyses were taken from two affected relatives from the same generation. Genomic DNA was prepared from peripheral blood leukocytes by use of the nonenzymatic, rapid method (LAHIRI, SCHNABEL 1993).

DNA samples were investigated for exclusion of the *BRCA1/2* allele by using 6 microsatellite markers for PCR amplification. For the *BRCA1* locus, polymorphic microsatellite markers used were: D17S1322, D17S1323, D17S855. For analysis of the *BRCA2* genetic locus the microsatellite markers D13S170, D13S260, D13S267 were used. Primers included in the Genome Database (gdbwww.gdb.org) were used with a fluorescent tag (6-Fam) attached at the 5' end of the antisense primer.

Results obtained with polymorphic markers were verified by applying *BRCA1* testing for the most common founder mutations in Poland (GÓRSKI et al. 2000) and using “exon by exon” sequencing of coding fragments of the *BRCA2* gene.

Occurrence of common alleles of the *BRCA1* gene was excluded in 3 families – 994, 3865 and 4628. The only useful marker was D17S855. No case was excluded by two other markers: D17S1322 and D17S1323.

Occurrence of common alleles of the *BRCA2* gene was excluded in 5 families – 126, 569, 1279, 1759 and 2710. *BRCA2* was excluded by D13S260 in families 126 and 569 and by D13S267 in families 1279, 1759 and 2710. No case was excluded by using D13S171.

It was possible to exclude neither *BRCA1* nor *BRCA2* in 22 families.

Results obtained by testing for Polish *BRCA1* founder mutations and *BRCA2* sequencing were in agreement with findings based on polymorphic markers. The only exception was family 994 in which the *BRCA1* mutation carrier status was excluded by using D17S855 but *BRCA1* testing made it possible to detect exon 5 300T/G mutation.

Among 14 families without *BRCA1*, Polish founder mutations in this gene were excluded in 2 families – 3865 and 4628, and the *BRCA2* mutation was excluded in one family – 569.

A few years ago the search for *BRCA1/BRCA2* mutations in families with aggregation of breast/ovarian cancers was time-consuming, laborious and thus expensive, because sequencing and Southern blot analyses had to be performed and both of above genes are large. Recently, in several countries founder mutations have been identified. This simplified *BRCA1/BRCA2* testing. In our series of 30 families selected for studies with samples available from 2 affected women from one generation, it was possible to detect *BRCA1* founder mutations characteristic for Poland in 16 (54%) cases. In studies performed on 200 Polish families with at least 3 relatives affected by breast/ovarian cancer we found common Polish *BRCA1* founder mutations in around 60% of cases (data not shown). It seems that analysis of 3 mutations (300T/G, 4153 del A and 5382 ins C) enables detection of *BRCA1* abnormalities in Poland with sensitivity achieving 80-90 %. Up to now no Polish *BRCA2* founder mutation has been identified. Therefore, in around 30-40 % of families we have to perform complete *BRCA1/BRCA2* sequencing.

Herein, we showed that it is justified to precede such sequencing by analyses of at least two relatives with the use of polymorphic markers. In 3 of 14 families without *BRCA1* founder effect, simple analysis of polymorphism allowed to exclude one gene from further analysis. Judging by our experience, situations when two affected sisters or cousins or proband plus affected aunt are alive and additionally peripheral blood from both of them can be taken, are rare. However, it is usually possible to achieve paraffin blocks from which DNA useful for analyses of polymorphisms can be easily extracted.

Cost reduction achieved by application of polymorphic markers is probably at the level of 10-20 %. This amount is not significantly high.

In our opinion, due to the limited amount of resources that can be spent on routine medical diagnostic procedures, it is justified to overcome some inconveniences related to search for paraffin blocks from affected relatives, because of lowering the number of cases which we to be studied by sequencing and Southern RFLP analyses.

Acknowledgements. This work was supported by a grant (4P05A 132 18) from the State Committee for Scientific Research, Warsaw, Poland.

REFERENCES

- CLAUS E.B., RISCH N., THOMPSON W.D. (1991). Genetic analysis of breast cancer in the Cancer and Steroid Hormone Study. *Am. J. Hum. Genet.* 48: 232-242.
- COUCH F.J., FARID L.M., DESHANO M.L., TAVTIGIAN S.V., CALZONE K., CAMPEAU L., PENG Y. et al. (1996). *BRCA2* germline mutations in male breast cancer cases and breast cancer families. *Nat Genet.* 13: 123-125.
- EASTON D.F., BISHOP D.T., FORD D., CROCKFORD G.P. (1993). Genetic linkage analysis in familial breast and ovarian cancer: Results from 214 families. *Am. J. Hum. Genet.* 52: 678-701.
- FORD D., EASTON D.F., BISHOP D.T., NAROD S.A., GOLDFAR D.E. (1994). Risks of cancer in *BRCA1*-mutation carriers. *Breast Cancer Linkage Consortium. Lancet.* 343: 692-695.
- GÓRSKI B., BYRSKI T., HUZARSKI T., JAKUBOWSKA A., MENKISZAK J., GRONWALD J., PLUZANSKA A., BEBENEK M., FISCHER-MALISZEWSKA L., GRZYBOWSKA E., NAROD S.A., LUBIŃSKI J. (2000). Founder mutations in the *BRCA1* gene in Polish families with breast-ovarian cancer. *Am. J. Hum. Genet.* 66(6): 1963-1968.
- HALL J.M., LEE M.K., NEWMAN B., MORROW J., ANDERSON A., HUEY B., KING M-C. (1990). Linkage analysis of early onset familial breast cancer to chromosome 17q21. *Science.* 250: 1684-1689.
- LAHIRI D.K., SCHNABEL B. (1993). DNA isolation by rapid method from human blood samples: effects of $MgCl_2$, EDTA, storage time, and temperature on DNA yield and quality. *Biochem. Genet.* 31: 321-328.
- MIKI Y., SWENSEN J., SHATTUCK-EIDENS D., FUTREAL P.A., HARSHMAN K., TAVTIGIAN S., LIU Q.Y. et al. (1994). A strong candidate for the breast and ovarian cancer susceptibility gene *BRCA1*. *Science.* 266: 66-71.
- NAROD S.A., FEUNTEUN M., LYNCH H.T., WATSON P., CONOWAY T., LYNCH J., LENOIR G.M. (1991). Familial breast-ovarian cancer locus on chromosome 17q12-23. *Lancet.* 338: 82-83.
- NEWMAN B., AUSTIN M.A., LEE M., KING M-C. (1988). Inheritance of human breast cancer: evidence for autosomal dominant transmission in high risk families. *Proc. Natl. Acad. Sci. USA.* 85: 3044-3048.
- SCULLY R., GANESAN K., VLASAKOVA K., CHEN J., SOCOLOVSKY M., LIVINGSTON D.M. (1999). Genetic analysis of *BRCA1* function in a defined tumour cell line. *Mol. Cell.* 4: 1093-1099.

- STRUEWING J.P., ABELIOVICH D., PERETZ T., AVISHAI N., KABACK M.M., COLLINS F.S., BRODY L.C. (1995). The carrier of the *BRCA1* 185delAG mutation is approximately 1 percent in Ashkenazi Jewish individuals. *Nat. Genet.* 11: 198-200.
- SZABO C., KING M.C. (1997). Population genetics of *BRCA1* and *BRCA2*. *Am. J. Hum. Genet.* 60: 1013-1020.
- TONIN P., SEROVA O., LENOIR G., LYNCH H., DUROCHER F., SIMARD J., MORGAN K. et al. (1995). *BRCA1* mutations in Ashkenazi Jewish women. *Am. J. Hum. Genet.* 57: 189.
- WOOSTER R., BIGNELL G., LANCASTER J., SWIFT S., SEAL S., MANGION J., COLLINS N. et al. (1995). Identification of the breast cancer gene *BRCA2*. *Nature.* 378: 789-792.