More genes, more problems? Benefits and risks of multiplex genetic testing

Barbara M. Norquist and Elizabeth M. Swisher

Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, University of Washington, Seattle, WA, USA

Corresponding author: Barbara Norquist, MD
Division of Gynecologic Oncology
Department of Obstetrics and Gynecology
University of Washington
Seattle, WA, USA
Phone: 206-543-3669
Fax: 206-543-3915
E-mail: bnorquis@uw.edu

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In 1990, Dr. Mary-Claire King announced that she and her team had localized a gene for hereditary breast cancer to chromosome 17q21,¹ which she later named *BRCA1*. This discovery led to an intense, international 4-year race to clone and sequence the gene, and the advent of commercially available genetic testing. That race was ultimately won in 1994 by a team lead by Mark Skolnick,² who went on to found a company called Myriad genetics. Myriad acquired patents for the genomic sequence of *BRCA1* itself (among other patents), followed by similar patents on *BRCA2* after it was sequenced the following year. Shortly after that, commercial genetic testing became available, and for the next 17 years, (1996 – 2013), a single company provided all commercial testing for *BRCA1* and *BRCA2* in the United States, using an approach based on PCR amplification and Sanger sequencing of those individual genes. *BRCA1* and *BRCA2* are the most common causes of hereditary ovarian cancer. Further research identified

other genes implicated in hereditary ovarian cancer risk, such as the DNA mismatch repair genes *MSH6*, *PMS2*, *MLH1*, and *MSH2*, followed more recently by genes in the BRCA-Fanconi anemia pathway such as *BRIP1*, *RAD51D*, and *RAD51C*.³⁻⁶ For women with ovarian cancer, these mutations are individually rare,⁷ but when present confer a significantly increased risk of ovarian cancer. Testing for all genes associated with hereditary ovarian cancer risk in a “gene by gene” fashion is expensive and inefficient. Next generation sequencing technology paved the way for multiplex testing, allowing highly accurate sequencing of many genes simultaneously at no additional cost,⁸ however, due to Myriad’s gene patents, the initial cancer gene panels did not include *BRCA1* and *BRCA2*. Therefore, for families with breast and/or ovarian cancer, multiplex testing was initially offered only to the highest risk individuals after negative *BRCA1* and *BRCA2* testing. This all changed in June of 2013, when the Supreme Court reached the unanimous decision to overturn Myriad’s gene patents based on the premise that a part of nature (e.g., a genetic sequence) cannot be patented.⁹ That very day, two vendors immediately offered comprehensive genetic tests that included *BRCA1* and *BRCA2* and all other known breast and ovarian cancer genes.

Over the next year, many other companies, some new to cancer genetic testing, started offering cancer gene panels. The number and variety of vendors and their cancer gene panels continues to grow, and multiplex testing is rapidly becoming standard practice for cancer genetic risk assessment. Dr. Frey and colleagues have sought to answer an important clinical question – what is the yield of multi-gene panel testing after negative “single” gene testing? This study provides some interesting data that highlights a number of the benefits and risks of multiplex genetic testing.

The main benefit of multiplex testing is the ability to detect mutations in many genes affecting cancer risk with a single test, thus increasing efficiency and decreasing cost. Re-screening of patients with negative single gene testing by Frey et al identified mutations in some genes with available estimated risks of cancer, including *BRIP1*, *RAD51D*, *ATM*, and even *BRCA2* (with a mutation that was missed on previous *BRCA2* sequencing). The knowledge of

cancer risks associated with rare mutations in newer cancer susceptibility genes is rapidly expanding, allowing providers to better counsel patients on their risks and ways to modify them. Identification of a mutation in a clearly cancer-associated gene also allows for a more accurate assessment of risks to other family members; If a mutation that clearly explains risk is found, then risk reduction can be targeted to those relatives with the mutation and excess interventions avoided in those without.

The yield of repeat multiplex testing following an initial negative genetic test will vary by patient population. Similar to the initial testing, the pre-test probability of reflexive testing is driven by the risk factors of the individual; a woman with negative BRCA testing and a personal history of both breast and ovarian cancer will have a higher likelihood of an informative multiplex test result than an unaffected patient with a single relative with breast cancer. The study by Frey and colleagues is relatively small and includes a heterogeneous patient population, which does not help us parse out which patients we should select for retesting. Studies of retesting on a large cohort of women with breast cancer yielded an informative mutation rate of approximately 3-5%.¹⁰ Though similarly large retesting series of ovarian cancer patients has not been done, we would predict a similar mutation rate, given the known 3-5% rate of non-BRCA mutations in unselected women with ovarian cancer.

Nearly half of the pathogenic mutations identified by Frey et al occurred in genes not associated with the patient phenotype. The identification of *MUTYH* and *APC* mutations in the absence of a personal or family history of colon cancer or polyps are likely incidental findings and should not be counted as a positive result for patients undergoing testing for other cancer phenotypes. Heterozygous mutations in *MUTYH* are relatively common, with a 1-2% carrier rate in some populations, and are not associated with a high cancer risk or any specific screening recommendations. In addition to “incidental” mutations, multiplex testing can result in the reporting of deleterious mutations in genes of uncertain significance. Just because a gene is included in a multiplex cancer risk test does not necessarily mean it is associated with a proven

cancer risk. Genes of questionable significance that are present on multiple panels include *RAD50* and *MRE11A*.

Finally, the elephant in the living room of multiplex cancer gene testing is the high frequency of variants of uncertain significance (VUS). Frey et al found VUS in 46% of patients undergoing testing, which is on the high end of published rates of VUS from commercial cancer gene panels. Despite the fact that the vast majority of VUS will not prove to be pathogenic, the reporting of a VUS can cause patient distress and clinical uncertainty. In many cases, VUS are incorrectly interpreted by patients and providers as associated with cancer risk, causing patients to undergo unindicated interventions. Strategies do exist to reduce the rate of VUS. In a manuscript currently under review, Shirts et al describe the use of consensus-based review of variants utilizing multiple experts, available literature, and multiple prediction tools, allowing them to get the VUS rate down to <10%, in a single-institution academic setting. When VUS are identified, patient recommendations should be based on that individual's personal and family history, not the VUS.

In summary, when assessing “positive” results of panel testing, the clinician should always ask the following questions: Does this mutation clearly damage the protein product of the gene (i.e., a real mutation)? Is this gene convincingly associated with cancer risk (i.e., a real gene)? Both must be true in order to base treatment recommendations on the genetic results. Also, “negative” results are only as negative as the test allows and should be interpreted in the context of the personal and family history. Clinicians should consult with genetic counselors and other genetics experts to aid in interpretation as necessary. Fortunately, as more people are tested the quality of this information will improve as researchers, clinicians, and genetic testing companies share data. VUS rates will go down, and risk characterization of uncertain genes will become more accurate. So, while the number of potential problems does increase with the number of genes assessed, with careful attention to interpretation of results and improved data over time, the benefits can be real.

References

1. Hall JM, Lee MK, Newman B, et al. Linkage of early-onset familial breast cancer to chromosome 17q21. *Science*. Dec 21 1990;250(4988):1684-1689.
2. Goldgar DE, Fields P, Lewis CM, et al. A large kindred with 17q-linked breast and ovarian cancer: genetic, phenotypic, and genealogical analysis. *Journal of the National Cancer Institute*. Feb 2 1994;86(3):200-209.
3. Loveday C, Turnbull C, Ramsay E, et al. Germline mutations in RAD51D confer susceptibility to ovarian cancer. *Nat Genet*. Sep 2011;43(9):879-882.
4. Loveday C, Turnbull C, Ruark E, et al. Germline RAD51C mutations confer susceptibility to ovarian cancer. *Nat Genet*. May 2012;44(5):475-476; author reply 476.
5. Meindl A, Hellebrand H, Wiek C, et al. Germline mutations in breast and ovarian cancer pedigrees establish RAD51C as a human cancer susceptibility gene. *Nat Genet*. May 2010;42(5):410-414.
6. Rafnar T, Gudbjartsson DF, Sulem P, et al. Mutations in BRIP1 confer high risk of ovarian cancer. *Nat Genet*. 2011;43(11):1104-1107.
7. Walsh T, Casadei S, Lee MK, et al. Mutations in 12 genes for inherited ovarian, fallopian tube, and peritoneal carcinoma identified by massively parallel sequencing. *Proc Natl Acad Sci U S A*. Nov 1 2011;108(44):18032-18037.
8. Walsh T, Lee MK, Casadei S, et al. Detection of inherited mutations for breast and ovarian cancer using genomic capture and massively parallel sequencing. *Proc Natl Acad Sci U S A*. Jul 13 2010;107(28):12629-12633.
9. Association for Molecular Pathology v. Myriad Genetics. June 13, 2013;WL 2631062.
10. Desmond A, Kurian AW, Gabree M, et al. Clinical Actionability of Multigene Panel Testing for Hereditary Breast and Ovarian Cancer Risk Assessment. *JAMA Oncol*. Oct 1 2015;1(7):943-951.