MedicalXpress

A new, clinically validated diagnostic test for detecting BRCA1 and BRCA2 mutations

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The recognition of a causal link between mutations in BRCA1 and BRCA2 genes and increased risk of developing breast and ovarian cancer has intensified the demand for genetic testing. Identifying mutations in these large genes by conventional methods can be time consuming and costly. A report in the November issue of the *Journal of Molecular Diagnostics* describes a new technique using second-generation sequencing technology that is as sensitive as the standard methodology but has the potential to improve the efficiency and productivity of genetic testing laboratories.

"In our laboratory, approximately 25% of high risk patients who undergo BRCA1 or BRCA2 testing will generate a result with a real or ambiguous relationship to hereditary cancer risk, and so testing for these mutations is an important tool to identify individuals who would benefit from preventative surgery or increased breast cancer surveillance," says lead investigator Aly Karsan, MD, of the Genome Sciences Centre and Department of Pathology of the BC Cancer Agency.

Dr. Karsan, who says his institution currently receives over 500 requests annually for such genetic testing, expects demand to rise and wait times to increase as public awareness broadens, especially following such high-profile patients as Angelina Jolie. Fueling the demand will be identification of additional suspect genes and discovery of genetic factors predictive of response to new therapies. As a result, there is a need for faster and low-cost testing with additional analytic capabilities.

Increased efficiency of the methodology developed offers additional benefits to patients. The investigators envision that more women will be able to be tested, including those without family history of breast or ovarian cancer. Another potential advantage will be that more genomic regions can be analyzed by a single test, allowing simultaneous analysis of other genes that also may be contributing to breast or ovarian cancer susceptibility.

The investigators warn that as more women undergo genetic testing, there is increased likelihood of finding variants of unknown significance or incidental discoveries. They caution that interpretation of these variants can be difficult and time consuming, and procedures should be developed for reporting these results to physicians and patients.

Technical details of the study

Next-generation sequencing (NGS) refers to technologies that share the ability to parallel sequence millions of DNA templates. The terms second-generation (and third-generation) sequencing are used to describe the evolution of sequencing technology from the first-generation, dideoxy 'Sanger' sequencing. The new DNA sequencing technologies are expected to have a significant impact on the detection, management, and treatment of genetic diseases such as ovarian and breast cancer.

The second-generation sequencing assay described in the current report uses automated small amplicon PCR followed by sample pooling and sequencing with a second-generation instrument. The target region selected was thought to encompass the majority of pathogenic sequence changes in BRCA1 and BRCA2.

The investigators tested the assay using a set of 91 patient genomic DNA samples, 48 selected retrospectively and 43 prospectively. Comparing their results to those obtained by the standard dideoxy sequencing

methodology, the researchers found 100% concordance between the two methods, with no false-positive or false-negative predictions. The method generated high-quality sequence coverage across all targeted regions with median coverage greater than 4,000-fold for each pooled sample. After some technical adjustments (such as setting the maximum depth parameter to an arbitrarily high value of 500,000 using SAMtools software and selecting 100,000 as the on-target alignments threshold), the method proved sensitive and specific for detecting variants in genetic sequences.

More information: "A clinically validated diagnostic second-generation sequencing assay for detection of hereditary BRCA1 and BRCA2 mutations," by Ian E. Bosdet, T. Roderick Docking, Yaron S. Butterfield, Andrew J. Mungall, Thomas Zeng, Robin J. Coope, Erika Yorida, Katie Chow, Miruna Bala, Sean S. Young, Martin Hirst, Inanc Birol, Richard A. Moore, Steven J. Jones, Marco A. Marra, Rob Holt and Aly Karsan, DOI: dx.doi.org/10.1016/j.jmoldx.2013.07.004. *Journal of Molecular Diagnostics*, Volume 15, Issue 6 (November 2013)

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