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Review of: A DGGE system for comprehensive mutation screening of *BRCA1* and *BRCA2*: application in a Dutch cancer clinic setting

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Abstract of the original article:
Rapid and reliable identification of deleterious changes in the breast cancer genes *BRCA1* and *BRCA2* has become one of the major issues in most DNA services laboratories. To rapidly detect all possible changes within the coding and splice site determining sequences of the breast cancer genes, we established a semiautomated denaturing gradient gel electrophoresis (DGGE) mutation scanning system. All exons of both genes are covered by the DGGE scan, comprising 120 amplicons. We use a semiautomated approach, amplifying all individual amplicons with the same PCR program, after which the amplicons are pooled. DGGE is performed using three slightly different gel conditions. Validation was performed using DNA samples with known sequence variants in 107 of the 120 amplicons; all variants were detected. This DGGE mutation scanning, in combination with a PCR test for two Dutch founder deletions in *BRCA1* was then applied in 431 families in which 52 deleterious changes and 70 unclassified variants were found. Fifteen unclassified variants were not reported before. The system was easily adopted by five other laboratories, where in another 3593 families both exons 11 were analysed by the protein truncation test (PTT) and the remaining exons by DGGE. In total, a deleterious change (nonsense, frameshift, splice-site mutation or large deletion) was found in 661 families (16.4%), 462 in *BRCA1* (11.5%), 197 in *BRCA2* (4.9%), and in two index cases a deleterious change in both *BRCA1* and *BRCA2* was identified. Eleven deleterious changes in *BRCA1* and 36 in *BRCA2* had not been reported before. In conclusion, this DGGE mutation screening method for *BRCA1* and *BRCA2* is proven to be highly sensitive and is easy to adopt, which makes screening of large numbers of patients feasible. The results of screening of *BRCA1* and *BRCA2* in more than 4000 families present a valuable overview of mutations in the Dutch population.

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Review

The breast cancer susceptibility genes, *BRCA1* (MIN# 113705, located at band q21 on chromosome 17) and *BRCA2* (MIN# 600185, located at band q12.3 chromosome 13), are commonly mutated in the germline of 5–10% of all breast cancer cases and 10–15% of all cases of ovarian cancer. Therefore, familial clustering of breast and/or ovarian cancer increases ones risk of having inherited a deleterious *BRCA1* or *BRCA2* mutation [1]. Germline *BRCA1* and *BRCA2* mutations in men have also been associated with an increased risk of prostate and breast cancer, while *BRCA2* mutations are associated with increased risk of pancreatic and stomach cancer and melanoma [2]. Detecting *BRCA1* or *BRCA2* mutations is therefore important in the clinical management of a number of cancers, particularly breast cancer in women.

Genetic testing to identify deleterious *BRCA1* or *BRCA2* mutations in high-risk breast cancer families are routine practice in most genetic diagnostic laboratories. Mutation screening for *BRCA1* and *BRCA2* is however challenging, not only due to the size of these genes, 7.8 kb mRNA and 24 exons (22 coding for 1863 amino acids) [3], and 10 kb mRNA and 27 exons (26 coding for 3418 amino acids) [4], respectively, but also due to the occurrence of over 1500 different and individually rare disease-associated mutations currently identified across the *BRCA1* and *BRCA2* coding/regulatory regions (http://research.nhgri.nih.gov/bic/). With the exception of a few founder mutations identified in isolated populations (including in the Netherlands), diagnostic laboratories are required to perform comprehensive analysis of both genes.

DNA sequencing of the coding exons and flanking regulatory regions is currently considered the gold standard for mutation detection in the *BRCA1* and *BRCA2* genes, with companies like Myriad Genetics Laboratories in the United States monopolising the commercial service. This method is not only costly, but requires comprehensive, time-consuming data interpretation. For this reason, a number of diagnostic and research laboratories employ pre-screening (pre-sequencing) methods for detection of unknown mutations. These methods can broadly be divided into gel-based or non-gel-based (capillary) methods. The gel-based methods traditionally used for *BRCA1* and *BRCA2* mutation detection are single-stranded conformation polymorphism (SSCP) analysis and heteroduplex analysis (HA). The reported sensitivity of these methods range from 80% to 95%, often requiring several electrophoretic conditions [5]. Adaptations of these methods to capillary-based systems for *BRCA1* and/or *BRCA2* mutation screening include the SSCP/duplex system combining both SSCP and HA [6], capillary restriction endonuclease fingerprinting SSCP (REF-SCCP) [7], conformation-sensitive gel electrophoresis (CSGE) based on HA [8] and HA by capillary array electrophoresis (HA-CAE) method [9,10]. Another commonly used method for *BRCA1* and *BRCA2* mutation detection is the PTT, which although appropriate for the detection of larger genomic rearrangements is unable to detect missense mutations and cDNA-based PTT is particularly labour intensive [11]. The common capillary-based systems, namely denaturing high-performance liquid chromatography (dHPLC) and the more recent high-resolution technique, although highly sensitive, both require specialised equipment that is more costly than the gel-based systems and are less sensitive for detection of unknown homozygotes unless spiked with a known control. Although dHPLC has been used for *BRCA1* and *BRCA2* screening [12,13], the high-resolution technique has not as yet been comprehensively validated for the detection of unknown *BRCA1* and *BRCA2* mutations. In this study DGGE, arguably the most sensitive of the gel-based methods, was chosen as the method of choice for comprehensive mutation detection of *BRCA1* and *BRCA2*.

DGGE is based upon the different melting properties of double stranded DNA in an increasing gradient of denaturant, urea and formamide, at a fixed elevated temperature. The addition of a CG-rich fragment (known as a GC-clamp) during amplification, as well as post-PCR heteroduplexing (the formation of mismatched heteroduplexes during the denaturation and re-annealing of target DNA containing two different alleles) facilitates sensitivity of mutation detection [14]. If appropriate computer programs are utilised to determine the melting behaviour of the DNA sequence for optimal primer selection [15], and optimal gel and electrophoretic conditions are adhered to [16], then 100% mutation detection rate as suggested by the authors is certainly feasible.

In this study, the Dutch group has utilised this seemingly 'old-fashioned' gel-based technique for its clear advantages of a 'logic-basis' and easy interpretation to design a comprehensive mutation detection screening assay for the entire coding regions (including splice junctions) of the *BRCA1* and *BRCA2* genes. To facilitate throughput, the 120 DGGE amplicons (45 *BRCA1* and 75 *BRCA2*) were amplified at the same annealing temperature and PCR products pooled (up to 5 amplicons per pool) for layered analysis using a single DGGE gel gradient per gene. The assay was tested on 175 known sequence variants across 107 amplicons (43 *BRCA1* and 64 *BRCA2*), with 100% mutation detection rate. Implementation of the assay in the local diagnostic setting resulted in the identification of 52 deleterious mutations and 70 unclassified sequences variants in 431 breast cancer families. Further adoption of the DGGE assay across

the Netherlands (identifying 16.5% of 4024 families with deleterious mutations), clearly demonstrates the reproducibility and effectiveness of this assay as an appropriate, cost-effective diagnostic laboratory test.

Although conventional sequencing and pre-sequencing techniques, such as described in this paper, are ideal for the detection of point mutations and small deletions/insertions, these techniques would miss mutations caused by genomic rearrangements (e.g. large deletions or duplications). It is estimated that approximately 12% of *BRCA1* and *BRCA2* mutations will be missed in high-risk breast cancer families using these methods [17]. Additional techniques such as multiplex ligation-dependent probe amplification (MLPA) are therefore required to assess for these types of variants. Another disadvantage of the described method is lack of automation, with the use of gels carrying the potential of human error. The effective use of gradient pouring stations should largely reduce or even exclude any major concern in this area.

In conclusion, comprehensive mutation screening of the *BRCA1* and *BRCA2* genes is now a necessity for all genetic-based laboratories offering DNA-based testing, with the detection of all possible deleterious mutations being critical to the management and prevention of breast cancer within high-risk families. The assay described in this article allows for a cost-effective, easily interpretable approach to comprehensive mutation screening of these large genes in a relatively efficient and reasonable time frame as desirable for any diagnostic setting. The assay has not only been appropriately validated, but also widely implemented across the entire country, servicing a 16.5 million strong population.

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