ELECTRONIC LETTER

Rapid, inexpensive scanning for all possible *BRCA1* and *BRCA2* gene sequence variants in a single assay: implications for genetic testing

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Recent advances in functional genomics have fostered the vision of a future health care that will be tailored to the specific needs of a person by using detailed genetic information about disease predisposition, disease progression, and the response to preventive or therapeutic medication.[1 2] To confirm that this concept of personalised medicine is viable, proof of principle studies are required, entailing extensive efforts to identify and functionally characterise individual gene variants in human populations and their association with disease or therapy related phenotypes. For this purpose, as well as for the ultimate widespread genetic testing that may result from these studies, practical, cost efficient methods of analysing thousands of genes in large human populations for all possible sequence variants are critically important. Such methods are currently not available, the main stumbling block being the high costs associated with comprehensive genotyping of large numbers of DNA samples. Costs are determined to some extent by intellectual property rights on the genes and gene variants (in fee for service testing[3]), but mostly by the type of assay that is used. When a genetic test can be based on one or few mutations, the costs are generally low, owing to the recent increase in assays capable of confirming the presence or absence of a previously known mutation or single nucleotide polymorphism (SNP).[4] Unfortunately, even relatively simple monogenic diseases, such as cystic fibrosis, can be caused by many different mutations in the same gene and it will never be possible to test only for a limited number of variants.[5]

The situation is different for the genetic component of complex diseases. The question here is if such diseases involve combinations of a few common SNP haplotypes or many rare alleles.[6] Recent results indicate that most gene variants occur at low frequency,[7] which suggests that association or family studies on the basis of a limited number of common SNPs in candidate genes would not be the optimal strategy to find all the relevant variants that could serve to guide future management of such diseases. Instead, it might be necessary exhaustively to interrogate the entire coding and regulatory regions of many candidate genes in large populations to ensure discovery of all relevant variants, including the ones that may be found associated with a disease or therapeutic phenotype only in some but not all populations. When all clinically relevant variants of a gene are identified the number may be so large that, like in the discovery phase, resequencing might still be the most practical option, also in the clinical setting. Hence, there is a need for comprehensive screening methods that are not limited to a given number of common gene sequence variants and that can be applied cost effectively to many different genes.

At present, nucleotide sequencing is the gold standard in genetic testing, as exemplified by the *BRCA1/BRCA2* genetic test for breast and ovarian cancer offered by Myriad Genetics for $2800. While this price tag is generally considered high, it is doubtful that even on a non-for profit basis a significant reduction is feasible, for example, by 10-100-fold. To address this problem, several alternative methods for comprehensive genotyping of subjects have been developed.[8] Most of these methods suffer from a lack of either accuracy or applicability in clinical routine. Virtually all of them are based, like sequencing, on the PCR amplification of individual target sequences (that is, exons and their surrounding intronic regions) followed by their serial analysis for sequence variation. An exception is the use of microarrays, pioneered by Affymetrix, to interrogate each nucleotide position in a gene, using PCR amplified coding regions as probe.[9] At present, this method is not yet fully developed and still too costly.

A mutation detection principle with both high accuracy and the potential to be developed into a high throughput screening system at low cost is denaturing gradient gel electrophoresis (DGGE). It has been convincingly shown that DGGE has the same or higher sensitivity as nucleotide sequencing.[10 11] DGGE has been converted into a system capable of assessing multiple fragments in parallel using a combination of multiplex

Key points

- Great efforts are currently devoted to the characterisation and functionalisation of gene sequence variation in relation to human disease, with the ultimate aim of directing the practice of medicine to the individual person rather than the average patient.
- This path to personalised medicine is greatly hampered by the lack of efficient and cost effective methods for the comprehensive analysis of the possibly thousands of clinically relevant human genes for all possible DNA sequence variants in different populations.
- We have previously reported an inexpensive, simple test protocol for such gene variant scanning of the breast and ovarian cancer susceptibility gene *BRCA1*, involving extensive multiplex PCR followed by two dimensional electrophoretic separation on the basis of size and base pair sequence (van Orsouw *et al*, *J Med Genet* 1999;**36**:*747*).
- Here we present a similar, but significantly improved test for *BRCA1* and *BRCA2* together, using a recently developed, high speed, automated 2D electrophoresis system and three colour fluorescent detection of PCR fragments.
- The further increase in speed and cost efficiency resulting from these improvements would enable the large scale population studies necessary to identify the relationships between specific genotypes and known disease states or pharmacological responses, as well as the widespread genetic testing that may result from such studies.

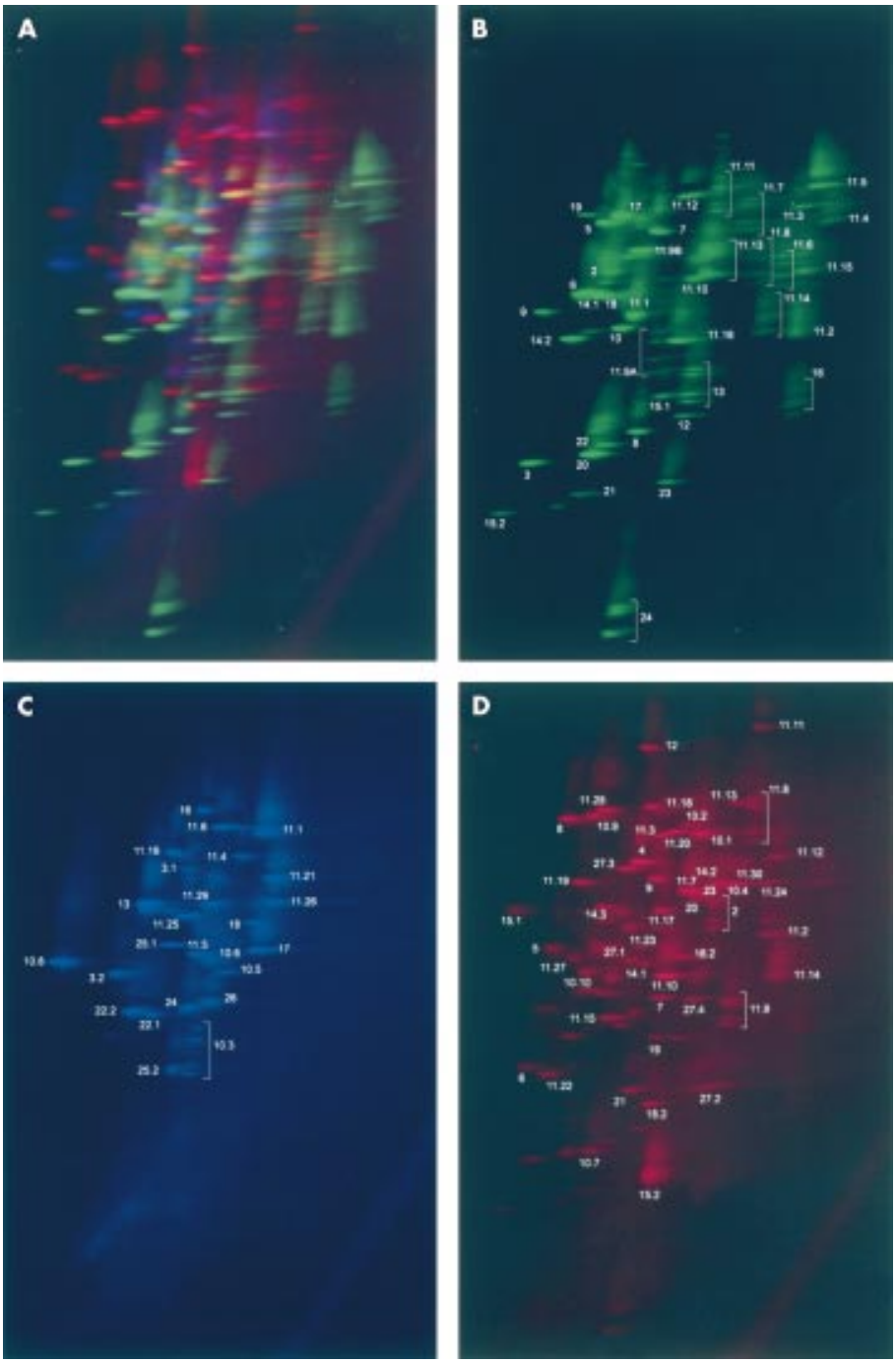

Figure 1 Two dimensional gene variant scanning of *BRCA1* and *BRCA2* in a single assay. (A) Complete separation of 115 fragments corresponding to the entire coding regions of *BRCA1* and *BRCA2*. Primers for each TDGS fragment were designed according to optimal melting behaviour, with a GC clamp attached to one primer of each pair. Primers were fluorescently labelled with HEX (green spots, representing 40 *BRCA1* fragments), FAM (blue spots, representing 25 *BRCA2* fragments), and Texas Red (red spots, representing 50 *BRCA2* fragments). To increase specificity, PCR amplification was performed in two steps. First, three long distance multiplex PCRs were performed to amplify 14 large fragments encompassing the coding regions of the *BRCA1* and *BRCA2* genes. These products served as template for 12 multiplex short PCRs to amplify all 115 target fragments. One µl of each multiplex short PCR was combined (12 µl total) and loaded onto a 0.25 mm polyacrylamide denaturing gradient gel (0-75% urea/formamide). Separation according to size and base pair sequence (melting temperature) was for three hours and 27 minutes. The entire gel cassette was then scanned using an FMBIO II Fluorescent Image Scanning Unit. (B) Separate TDGS pattern for 40 HEX labelled *BRCA1* fragments. Sequence variants were detected in exons 11.6, 11.7, 11.8, 11.9A, 11.11, 11.13, 11.14, 13, 16, and 24. (C) Separate visualisation of the TDGS pattern for 25 FAM labelled *BRCA2* fragments. A sequence variant was detected in exon 10.3. (D) Separate TDGS pattern for 50 Texas Red labelled *BRCA2* fragments.

PCR and two dimensional electrophoresis.[12] Termed two dimensional gene scanning (TDGS), this method has now been shown to be useful in screening a large variety of genes for all possible sequence variation, including some major disease genes such as *BRCA1*,[13] *TP53*,[14] and *MLH1*.[15] In a recent comparison with three other assays for detecting unknown mutations in *BRCA1*, that is, single strand conformational polymorphism analysis (SSCP), conformation sensitive gel electrophoresis (CSGE), and denaturing high performance liquid chromatography (DHPLC), TDGS and DHPLC performed best with a 91 and 100% detection rate, respectively.[16]

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DHPLC is based on the same denaturing gradient principle as TDGS, but has a much lower throughput since it operates on a fragment by fragment basis, making interpretation of the results easier. So far, TDGS has only been used in the research setting, but with the development of an instrument for automated 2D electrophoresis and the use of multicolour fluorescent labels high throughput applications in the routine setting have become feasible.[17] Here we show the utility of a single, low cost TDGS test as an objective screen for all possible mutations and SNPs in the *BRCA1* and *BRCA2* coding regions.

METHODS AND RESULTS

Using PCR amplification in 12 multiplex groups with three different fluorophores, all 115 gene coding fragments were obtained and subsequently subjected to automatic 2D denaturing gradient gel electrophoresis (procedures are briefly summarised in the legend to fig 1 and provided in detail on http://fgoa.stcbmlab.uthscsa.edu/supplemental/AGC-BRCA1+2/). After fluorescent scanning of the gel, the complete three colour pattern was first visualised on the screen (fig 1A). The pattern of each colour group was then interpreted separately by eye directly from the screen and sequence variants (in brackets) were scored electronically (fig 1B-D). As described previously,[13] it is possible to differentiate between common SNP variants or recurrent mutations and new or rare sequence variants on the basis of the unique spot configuration of each heterozygous variant. Variant fragments for which the spot configuration indicated a new mutation or SNP are subjected to confirmatory testing using nucleotide sequencing.

DISCUSSION

In view of its low cost and simplicity, TDGS has the potential of becoming a method of choice for large scale genetic epidemiological research until more advanced sequencing methods currently under development become available. Based on the empirically determined cost of about $50 for screening the 115 fragments of the large *BRCA1* and *BRCA2* genes on one gel (see http://fgoa.stcbmlab.uthscsa.edu/supplemental/AGC-BRCA1+2/ for details), the costs of analysing a gene with an average coding region of about 2000 bp (corresponding to 20 or less TDGS fragments) would be only about $10. This would permit population studies of significant sample size and number of candidate genes. For example, a study of 100 genes and a sample size of 10 000 people would cost less than $10 million.

Similarly, for future application in clinical practice we have calculated the theoretical cost of screening a person during his/her lifetime for a total of 5000 genes of average size. In this respect the assumption was made that only a fraction of all human genes will prove to be diagnostically relevant and that 5000 genes would be sufficient to account for such phenotypes as predisposition, drug efficacy, and toxicity associated with the most common diseases. Based on the same 20 TDGS fragments per 2000 bp average gene coding region, the maximum cost of a lifetime of diagnostic genotyping would be about $40 000. Against an average lifetime health care cost of over $350 000 in the USA (based on a life expectancy of about 80 years and the figures for the year 2000 from the US Health Care Financing Administration; www.hcfa.gov), this seems a modest amount, provided that it would lead to an increased quality of life and extensive cost savings owing to increased opportunities to prevent costly interventions and/or a more effective use of drugs. Although current knowledge on the benefits of genetic testing on this scale is still insufficient actually to warrant its routine inclusion by the health care industry, our results show that technological constraints to this end have been effectively removed.

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REFERENCES

1 **Meyer JM**, Ginsburg GS. The path to personalized medicine. *Curr Opin Chem Biol* 2002;**6**:434-8.
2 **Johnson JA**, Evans WE. Molecular diagnostics as a predictive tool: genetics of drug efficacy and toxicity. *Trends Mol Med* 2002;**8**:300-5.
3 **Merz JF**, Kriss AG, Leonard DG, Cho MK. Diagnostic testing fails the test. *Nature* 2002;**415**:577-9.
4 **Syvanen AC**. Accessing genetic variation: genotyping single nucleotide polymorphisms. *Nat Rev Genet* 2001;**2**:930-42.
5 **Bobadilla JL**, Macek M Jr, Fine JP, Farrell PM. Cystic fibrosis: a worldwide analysis of CFTR mutations–correlation with incidence data and application to screening. *Hum Mutat* 2002;**19**:575-606.
6 **Kruglyak L**, Nickerson DA. Variation is the spice of life. *Nat Genet* 2001;**27**:234-6.
7 **Stephens JC**, Schneider JA, Tanguay DA, Choi J, Acharya T, Stanley SE, Jiang R, Messer CJ, Chew A, Han JH, Duan J, Carr JL, Lee MS, Koshy B, Kumar AM, Zhang G, Newell WR, Windemuth A, Xu C, Kalbfleisch TS, Shaner SL, Arnold K, Schulz V, Drysdale CM, Nandabalan K, Judson RS, Ruano G, Vovis GF. Haplotype variation and linkage disequilibrium in 313 human genes. *Science* 2001;**293**:489-93.
8 **Vijg J**, Suh, Y. Screening for mutations in cancer predisposition genes. In: Eeles RA, Easton DF, Eng C, Ponder B, ed. *Genetic predisposition to cancer*. 2nd ed. London: Edward Arnold, 2003.
9 **Hacia JG**, Sun B, Hunt N, Edgemon K, Mosbrook D, Robbins C, Fodor SP, Tagle DA, Collins FS. Strategies for mutational analysis of the large multiexon ATM gene using high-density oligonucleotide arrays. *Genome Res* 1998;**8**:1245-58.
10 **Gejman PV**, Cao Q, Guedj F, Sommer S. The sensitivity of denaturing gradient gel electrophoresis: a blinded analysis. *Mutat Res* 1998;**382**:109-14.
11 **Trulzsch B**, Krohn K, Wonerow P, Paschke R. DGGE is more sensitive for the detection of somatic point mutations than direct sequencing. *Biotechniques* 1999;**27**:266-8.
12 **Vijg J**, van Orsouw NJ. Two-dimensional gene scanning: exploring human genetic variability. *Electrophoresis* 1999;**20**:1239-49.
13 **van Orsouw NJ**, Dhanda RK, Elhaji Y, Narod SA, Li FP, Eng C, Vijg J. A highly accurate, low cost test for BRCA1 mutations. *J Med Genet* 1999;**36**:747-53.
14 **Rines RD**, van Orsouw NJ, Sigalas I, Li FP, Eng C, Vijg J. Comprehensive mutational scanning of the p53 coding region by two-dimensional gene scanning. *Carcinogenesis* 1998;**19**:979-84.
15 **Smith WM**, Van Orsouw NJ, Fox EA, Kolodner RD, Vijg J, Eng C. Accurate, high-throughput "snapshot" detection of hMLH1 mutations by two-dimensional DNA electrophoresis. *Genet Test* 1998;**2**:43-53.
16 **Eng C**, Brody LC, Wagner TM, Devilee P, Vijg J, Szabo C, Tavtigian SV, Nathanson KL, Ostrander E, Frank TS, on behalf of the Steering Committee of the Breast Cancer Information Core (BIC) Consortium. Interpreting epidemiological research: blinded comparison of methods used to estimate the prevalence of inherited mutations in *BRCA1*. *J Med Genet* 2001;**38**:824-33.
17 **McGrath SB**, Bounpheng M, Torres L, Calavetta M, Scott CB, Suh Y, Rines D, van Orsouw N, Vijg J. High-speed, multicolor fluorescent two-dimensional gene scanning. *Genomics* 2001;**78**:83-90.

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