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REVIEW ARTICLE

Utilisation of the Malaysian BRCA variants database to construct a target panel for biosensor-based genetic breast cancer screening

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Abstract

Breast cancer remains a significant health concern, particularly in Malaysia, where it stands as the most prevalent cancer among women. The BRCA, implicated in inherited breast cancer syndromes, has garnered considerable attention due to its role in tumorigenesis. Utilising next-generation sequencing and bioinformatic tools, researchers have compiled a comprehensive database of BRCA variants specific to the Malaysian population. This article reviews the distribution of these variants across different ethnic groups in Malaysia and explores their implications for biosensor development. By leveraging this database, researchers aim to construct biorecognition elements for electrochemical biosensors, enabling affordable and accessible genetic screening for breast cancer mutations. The article underscores the importance of adhering to technical standards and considering ethnic diversity in selecting biorecognition elements. Ultimately, the integration of Malaysian BRCA variants into biosensor technology holds promise for enhancing early detection and improving clinical management of breast cancer in the Malaysian population.

Keywords: breast cancer, BRCA1 protein, BRCA2 protein, germline mutation, electrochemical techniques, biosensing techniques.

INTRODUCTION

Breast cancer stands as the predominant cancer impacting women in Malaysia. Over the period from 2016 to 2020, 29,434 new cases of breast cancer emerged, signifying that, based on the Malaysian National Cancer Registry, the estimated lifetime risk of breast cancer for Malaysian women is 1 in 19. This is lower than the global estimate of 1 in 8, likely due to differences in demographics, lifestyle, and genetic factors. Whether inherited or acquired, breast cancer is recognised as a genetic disease.[1] Numerous genes contribute to the tumorigenesis of breast cancer, with the BRCA being extensively studied among them.[2] Within the realm of inherited familial breast cancer syndromes, the BRCA demonstrates a founder effect, wherein the mutated gene is transmitted across generations.[2,3,4] This phenomenon is notably evident in populations with European ancestry, such as the Ashkenazi Jews. Uncovering founder mutations in Asian nations, Malaysia included, remains an ongoing endeavour. The importance of these founder mutations stems from the specificity of the variants within a particular population, simplifying the process of genetic screening for that specific group.

In Malaysia, the approach to genetic screening for breast cancer involves cascade testing. A study revealed that implementing population screening for BRCA variants among Ashkenazi Jews, as opposed to cascade testing strategies, led to a 21.6-fold increase in Quality-Adjusted Life Years (QALY) and a reduction in the occurrence of three breast cancer cases per one thousand women.[5] Distressingly, 51% of breast cancer patients in Malaysia succumb within five years of diagnosis.[6] The substantial 21.6-fold improvement in QALY implies a considerable reduction in cancer-related mortality.

Since the advent of next-generation sequencing in 2000, our comprehension of breast cancer and genetic mutations has advanced significantly. The extensive bioinformatic data incorporated into

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genetic variant databases based on populations has facilitated standard references. Polymerase chain reaction (PCR) and DNA sequencing have become the gold standard for identifying mutations associated with breast cancer, including BRCA.[7] While PCR and DNA sequencing are sensitive and specific, they pose challenges due to their expense, labour-intensive nature, the requirement for trained personnel, and limited accessibility to the broader population. To enable widespread screening for mutation carriers, we need a method that is not only equally sensitive and specific but also a point-of-care testing solution that is affordable, portable, and easily accessible.

To achieve this, many researchers work on developing a concise DNA biosensor. These devices convert biorecognition events into measurable electrochemical signals by integrating biological recognition elements with physical transducers.[8,9] The efficiency of a DNA electrochemical biosensor relies on precisely specifying the oligonucleotide target sequence of the gene of interest.

The challenge is to use the existing database of BRCA variants in Malaysia to create sensitive and specific biorecognition elements for an electrochemical biosensor. This review will delve into the distribution of ethnic-specific BRCA variants in Malaysia and assess how the current database can guide gene selection for biorecognition elements in biosensor detection.

DNA ELECTROCHEMICAL BIOSENSOR

Biosensors are defined as having both a biorecognition element and a transducer. The biosensor assembly comprises analytes, receptors, signals, transducers, and a data analysis system. The DNA sensing component involves a bioreceptor, where biorecognition reactions occur between the DNA target analyte and DNA biorecognition elements. Molecular repulsion resulting from the hybridisation of the two DNA strands is transformed into measurable electrochemical signals. To facilitate this process, nucleic acid biorecognition elements are fixed onto a fabricated electrode surface. The careful selection of nucleic acid sequences as biorecognition elements is crucial in determining the quality of the DNA biosensor.

SELECTING BIORECOGNITION ELEMENT OF BRCA VARIANTS FOR DNA BIOSENSOR

In selecting the target biorecognition element for the BRCA A gene, it is essential to adhere to the principles guiding effective DNA biosensors, covering both the technical intricacies of genetic screening and considerations for clinical management. An ideal biorecognition element for a DNA biosensor targeting a DNA bioanalyte is a natural nucleic acid. The chosen nucleic acid must meet five critical criteria for an efficient biosensor that is sensitivity, specificity, reproducibility, reusability, and the ability to establish linearity in the detection equation.[10] Significantly, this needs to align with technical standards and extends to diagnostic gene panels. As outlined by the American College of Medical Genetics and Genomics (ACMG), the selection of genes for inclusion in diagnostic gene panel testing should meet standardised technical standards, demonstrating sensitivity, specificity, and validity of the said gene to the development of disease. Furthermore, the gene testing panel must encompass all confirmed variants proven to be pathogenic and causative of disease.[11]

BRCA FUNCTION AND CONTRIBUTION TO TUMORIGENESIS

The two BRCAs, BRCA 1 and BRCA 2, have been known to contribute to inherited breast cancer syndrome since their first discovery in the year 1994 and 1995 respectively.[12] BRCA1 is a large and complex gene containing 22 exons, about 100kb long, with a transcript size of 7.8kb, situated at chromosome 17. BRCA2 contains 27 exons and the cDNA has 10,254 base pairs coding for a protein of 3418 amino acids, situated at chromosome 13.[13,14,15]

Mutations in BRCAs can occur across the entire nucleotide base pair sequence. Dysfunction of BRCA can manifest in three ways, germline mutations within gametes, somatic mutations within the target organ and alteration in gene function involving upregulation and downregulation.

BRCAs play a role in the repair of double-stranded DNA through the Homologous Recombinant Repair (HRR) System.[13] In the HRR process, BRCA1 in particular, participates in the E2 ubiquitination of cellular proteins at lysine residues, initiating proteolysis.[14,15] BRCA2 facilitates the formation of RAD51 filaments, searching for homologous, undamaged sister

chromatids during DNA repair.[16] In embryonic cells lacking BRCA1, cellular aberrations are observed, characterised by numerical and structural chromosome abnormalities. Similarly, a study on mouse embryonic cells deliberately depleted on BRCA2 shows a phenotype akin to BRCA1 null embryos.[15]

Both genes exhibit type 3 haploinsufficiency, resulting from frameshift mutations, deleterious mutations and large genomic rearrangements[17,18] Notably, haplotype analysis reveals the inherited nature of these occurrences. For instance, the mutation of 185delAG in exon 3 causes a defective ring structure in the BRCA1 , disrupting BRCA1's ubiquitination process. The loss of phenotypic protein function renders the genotype to be pathogenic, underscoring a deficiency in double-stranded DNA repair. However, not all variants predispose to cancer, while some missense variants may affect local protein structure, the degree of pathogenicity depends on the specific functional domain affected. Gene alterations can be categorised as pathogenic, likely pathogenic, of uncertain significance, likely benign or benign. Only pathogenic and likely pathogenic variants requiring medical management.

FOUNDER VARIANTS FOR GERMLINE BRCA

A total of seventy thousand germline BRCA variants have been documented, with the presence of the BRCA dating back 1-2 billion years ago when prehistoric populations acquired it in their genome.[17-19] The BRCAs underwent positive selection, with scholars suggesting it provided an evolutionary advantage in terms of reproduction, immunity and neural development. Pathogenic variants of BRCA emerged around 5000 years ago.[19,20] Due to diverse living conditions, inherited genes experienced genetic bottlenecks and genetic drifting, evolving into founder variations around two thousand years later. Founder variations are uniquely attributed to specific ethnic groups. One of the most recognised instances is observed in the high population frequency of three founder mutations (BRCA1 185delAG, BRCA1 5382insC, and BRCA2 6174delT) in Ashkenazi Jews, traced back to their ancestors through haplotype studies. Founder variations also exhibit unique molecular characteristics in different ethnicity; for instance, European Caucasoid BRCA variants are more associated with breast and ovarian cancer, whereas Asian BRCA variants in Northern China contribute to oesophageal cancer and, in Russia, lead to gastric cancer.[20] The frequency of differences in BRCA mutations, concerning distribution and variation, rises proportionally with the distance from the European continent. Therefore, this evidence suggests that the prolonged dispute over the European database does not accurately reflect the situation for the Asian population.

MAJOR DATABASE FOR BRCA

Numerous databases collect volunteered information on BRCA mutations from global research and studies, including the BRCA Exchange, ClinVar, the Human Gene Mutation Database (HGMD), the Leiden Open Variation Database (LOVD), the Consortium of Investigators of Modifiers of BRCA (CIMBA), and the Evidence-based Network for the Interpretation of Germline Mutant Allele (ENIGMA). This wealth of information is consolidated into ClinGen, an organisation funded by the National Institute of Health that defines the clinical relevance of genes and variants for use in precision medicine and research. For a gene variant to be considered clinically relevant, it must meet four criteria: Gene-disease validity, Dosage Sensitivity, Clinical Actionability, and Variant Pathogenicity.[21]

These international efforts are mirrored in Malaysia through the Malaysian Node of the Human Variome Project (MyHVP), which aims to consolidate local variant data and make it accessible to researchers, clinicians, and developers. The MyHVP provides an ethnic-specific genomic resource that complements MyBrCa by expanding the capture of germline variants across diverse Malaysian populations, thus strengthening the foundation for precision diagnostics.[7,22,23,24]

MALAYSIAN BRCA DISTRIBUTION

The Malaysian BRCA database is predominantly derived from the Malaysia Breast Cancer (MyBrCa) Project, which commenced in 2002.[25] MyBrCa is a hospital-based control study located in Kuala Lumpur, with participants recruited from the government teaching hospital, University Malaya Medical Center (UMMC), and a privately owned hospital, Subang Jaya Medical Center (SJMC). Prior to 2002, there was no existing database.[25] Additionally, other records of BRCA variants have been contributed

by various smaller studies conducted throughout the different states of Malaysia.

A series of studies were published over 10 years, from 2008 to 2018.[7,22,23,24,25] The cohort predominantly comprised 89% Chinese, 6% Malay, and 3.4% Indian women. The cohort composition does not match the ethnic proportions in the Malaysian population. This discrepancy is due to the reliance on hospital-based data from UMMC and SJMC, where the patient population is disproportionately Chinese. The number of confirmed breast cancer cases among study participants increased from 187 to 2575 over the course of the research. In certain publications, controls were included and selected from volunteers attending breast cancer screening at the respective centres. The age range of BRCA carriers varied from 25 to 65 years, with a median age of 51. Approximately 15% of carriers reported a family history of breast or ovarian cancer. The studies also documented lifestyle habits related to the risk of developing breast cancer, such as the use of oral contraceptives and hormone replacement therapy. Genomic DNA was extracted from blood samples, and targeted next-generation sequencing was utilised for analysis. Variant calling was conducted using established bioinformatics tools, and rigorous quality control measures were applied.

MyBrCa has documented 10 Variant of Unknown Significance (VUS) for BRCA1, 22 BRCA2 VUS, 41 deleterious variants for BRCA1, and 56 deleterious variants for BRCA2. With additional BRCA variants being contributed through various small studies, a total of 63 deleterious BRCA1 and 73 deleterious BRCA2 variants were documented in Malaysia. These variants of BRCA1 and BRCA2 are demonstrated in Figure 1.

Approximately 5% to 9% of these variants are intronic. The majority of mutations are located within exon 11, the largest exon in both genes. The most recurring variants include BRCA1 185delAG (exon 2), BRCA1 c.2635G>T (exon 11), and BRCA2 c.262_263delCT (exon 3).

Whilst some genotypes can be seen as recurrent in some ethnicities, there is no clear dominance of the pattern. Variant distribution varies among ethnicities, with specific genotypes being more prevalent in certain groups.[26,27,28,29,30] For example, BRCA1 185delAG was observed at a higher frequency in the Indian population, while BRCA2 c.262_263delCT was more prevalent in the Malay population. Some variants only occur

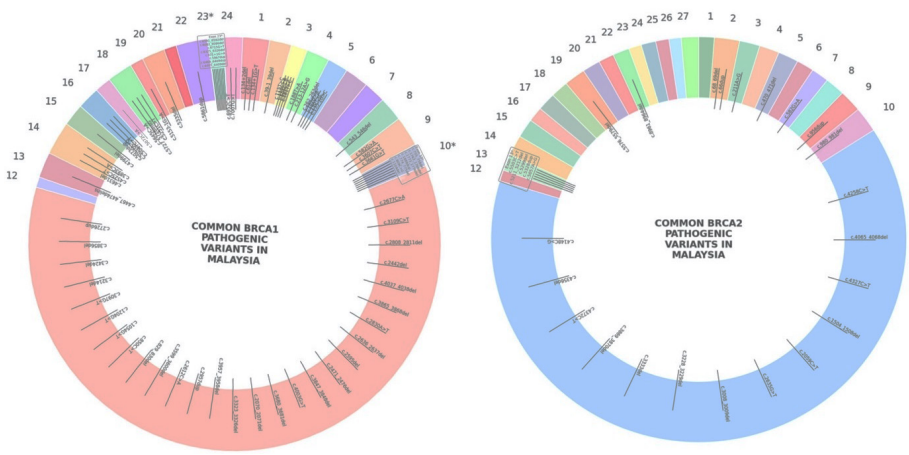

Figure 1: Exon-specific distribution of pathogenic BRCA1 and BRCA2 variants among Malaysian breast cancer patients. The doughnut charts illustrate the localisation of reported pathogenic variants across exons of BRCA1 and BRCA2, with each exon scaled proportionally to its nucleotide length. Pins denote individual pathogenic mutations, and variant names are labelled directly except for high-density exons (BRCA1: Exons 10 and 23; BRCA2: Exon 13), which are grouped externally to maintain readability. This exon-centric visualisation reveals a marked accumulation of pathogenic variants in BRCA1 Exon 11, which spans over 3 kb, as well as in Exons 10 and 23, while BRCA2 Exon 11—the largest exon—also demonstrates substantial mutational clustering. These findings support a rational design framework for biosensor development by prioritising exon coverage based on mutational burden. Targeting exons with high pathogenic variant density may enhance diagnostic efficiency, sensitivity, and cost-effectiveness in population-specific BRCA screening strategies, especially within the Malaysian cohort.

once or twice in different ethnic with several overlapping.

Discoveries indicate a higher prevalence of BRCA2 mutations compared to BRCA1 mutations in Malaysia. Among non-breast cancer patients, the rate of BRCA mutation carriers is 0.4%, consistent with findings reported in studies based on Caucasian populations. Notably, the BRCA accounts for only 2-5% of inherited breast cancer cases, thus leaving the majority of other occurrences unexplained.[30]

When comparing BRCA variants across Asian continents, it was observed that some variants found in Malaysia are also prevalent in other Asian countries.[31] Notably, the BRCA1 68_69delAG, identified in the Malaysian Indian population, is prevalent across all Asian nations. Similarly, BRCA2 c.262_263delCT is shared with Singapore, China, and Iran. The intersection of BRCA1 and BRCA2 variants between Malaysia and other Asian countries is depicted in Figure 2. Approximately 60% of the BRCA variants observed in Malaysia are also found in Southeast Asia and East Asia, followed by South Asia and Central Asia. Interestingly, none of the Malaysian variants are present in West Asia. For example, BRCA1 c.582G>A, exclusive to Malaysia and can be traced back to Homo sapiens fossils discovered in Glazkovo, Siberia, Tungus, and the Eastern Steppe, dating back 4162 years ago.[31]

Malaysians carrying pathogenic variants in BRCA exhibit distinctive characteristics, including onset at a young age. The majority of patients were diagnosed at later stages, richness in HER2 positivity and higher immune scores.[30] Even though many studies reported an association between BRCA mutations with triple-negative breast cancer, this might not be true for the Malaysian population. Additionally, these carriers of pathogenic BRCA variants are often associated with other mutations such as in PTEN loss, TP53, PLAB2, PIK3CA and APOBECB.[27,32,33,34,35,36]

Recent studies on ancestry-informative markers (AIMs) have provided deep insights into the genetic structure of the Malay population and other major ethnic groups in Malaysia. Yahya *et al*. demonstrated that Malays exhibit significant sub-ethnic genetic stratification, and developed AIM panels capable of classifying individuals with over 90% accuracy. These findings reinforce the idea that BRCA variant distribution is not random, but rather follows underlying ancestral patterns. The presence of overlapping but distinct variant profiles across Chinese, Malay, and Indian populations in the MyBrCa dataset is thus biologically plausible and supports the development of ethnically tuned biosensors.[20,21]

The variant panel proposed for biosensor development in Malaysia draws strength not only from locally documented BRCA mutations but also from substantial overlap with variants identified across other regions of Asia. As visualised in Figure 2, A and B, a number of BRCA1 and BRCA2 variants present in Malaysian patients are also observed in South, East, Southeast, Central, and West Asia. This overlap supports two important conclusions: first, that Malaysian BRCA data, although still incomplete, can be augmented by regional datasets to inform biosensor design; and second, that a variant panel designed using Malaysian and Asian data has the potential for broader trans-ethnic screening applications. This makes the NACOTS platform scalable and adaptable for future deployment across populations with shared variant profiles, particularly in resource-limited Asian contexts where local BRCA databases remain underdeveloped.

EXPERIMENTAL VALIDATION (BRCA1 981_982DEL)

To explore the feasibility of utilising Malaysian-specific BRCA variants in biosensor development, we conducted a preliminary validation study involving 70 confirmed breast cancer patients. The cohort consisted predominantly of Malays (66 individuals), with additional representation from the Indian (1) and Chinese (3) ethnic groups, including three sibling pairs. Variant-specific Loop Amplification Mediated Polymerase (LAMP) primers and biosensor probes were designed to detect a locally documented BRCA1 pathogenic variant: BRCA1 981_982del. This variant was chosen because it was found repeatedly in MyBRCA cohort.

Genomic DNA was extracted from blood samples of breast cancer patients and amplified by PCR targeting BRCA1 981_982del. Amplicons were purified and subjected to Sanger sequencing to confirm the presence or absence of the target mutations. In parallel, LAMP reactions were performed using colorimetric indicators for visual detection of DNA amplification. The LAMP products were then deposited onto screen-printed gold electrodes for electrochemical detection using the NACOTS platform. Redox signals were recorded and interpreted to assess

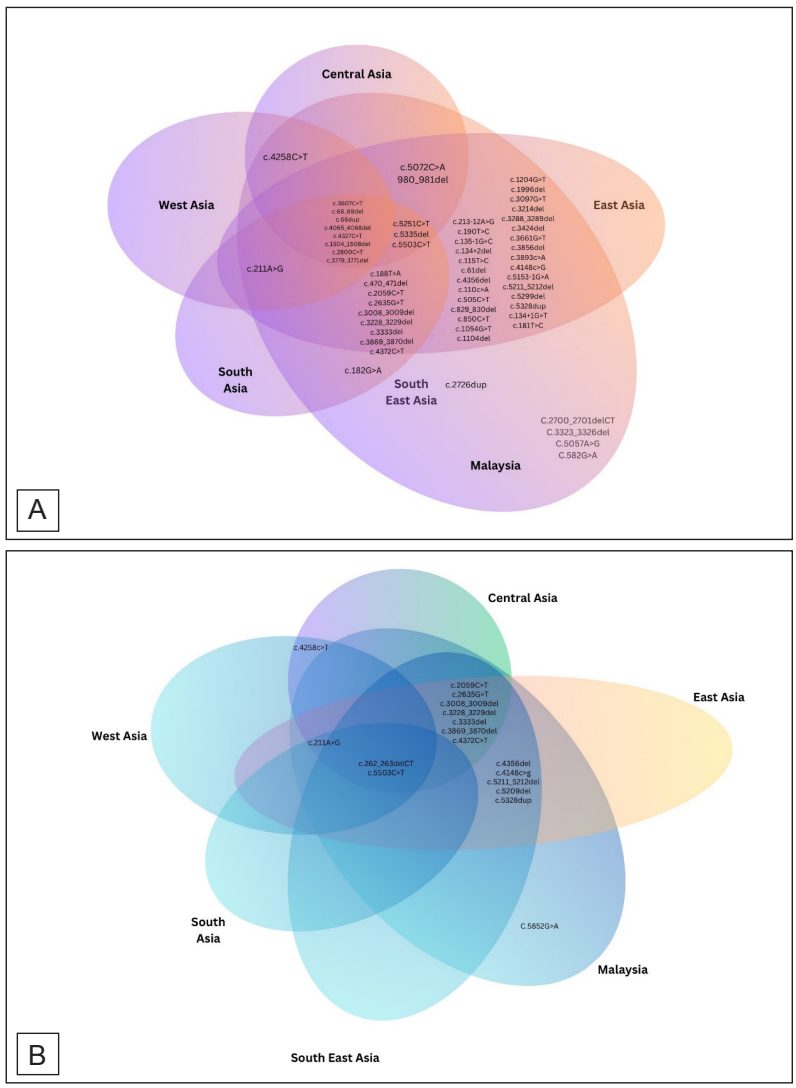

Figure 2: Geographical distribution of Malaysian BRCA1 (A) and BRCA2 (B) mutations across Asia. The Venn diagrams illustrate the overlap of pathogenic and likely pathogenic BRCA1 (A) and BRCA2 (B) variants reported in Malaysia with those found in other Asian regions, including East Asia, Southeast Asia, South Asia, Central Asia, and West Asia. The figures highlight both shared and unique mutations, such as BRCA1 c.68_69delAG and BRCA2 c.262_263delCT, which are recurrent across Asia, as well as variants exclusive to Malaysia, including BRCA1 c.2700_2701delTC and BRCA2 c.5852G>A. These distributions emphasise the importance of regional variant mapping in guiding population-specific screening strategies and biosensor target selection.

mutation presence based on changes in potential (Figure 3). No BRCA1 mutations were detected by Sanger sequencing in any of the 70 patients (Figure 4). Consistently, no LAMP amplification was observed, and no electrochemical potential was detected by the biosensor, indicating an absence of the targeted BRCA1 variant in this cohort (Figure 5 and 6). While these results do not demonstrate positive variant detection, they reinforce the importance of selecting highly prevalent and ethnically representative variants when designing biorecognition elements for population-level screening tools. This finding highlights the critical role of comprehensive local BRCA databases in guiding future biosensor design and underscores the need for broader sequencing efforts to refine target selection and ensure inclusivity across Malaysia's diverse population.

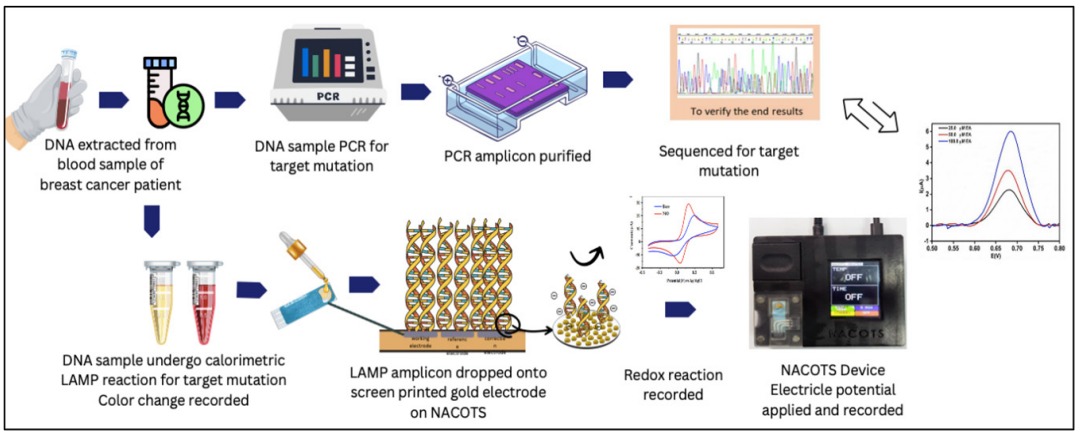

Figure 3: Schematic overview of the experimental workflow for validation of BRCA1 981_982del mutations using colorimetric LAMP, DNA sequencing, and the NACOTS biosensor platform.

CONCLUSION

The BRCA Database in Malaysia offers a comprehensive overview of BRCA variants within the population. It aligns its data with ClinGen and ClinVar databases and categorises variants based on their contribution to breast cancer development. This serves as a valuable foundation for selecting biorecognition elements for DNA biosensors. This can potentially be developed into microfluidic biosensor systems capable of detecting multiple mutations simultaneously.

However, there are challenges when it comes to practical applications within Malaysia. Foremost, a database of BRCA variants is by convenient sampling; hence, generalisation of findings to the entire population is hindered by disparities in ethnic proportions and study locations. The presence of unique variations in mutations among different ethnicities suggests that a universal gene panel may not be suitable for diverse ethnic groups. The possibility of a very low carrier rate exists because a significant number of variants have not yet been determined or have been labelled as variants of unknown significance (VUS), with potential emerging data in the future. Adhering to ACMG recommendations by including VUS in gene panel testing, is crucial to prevent dropouts and false negatives in DNA biosensors.

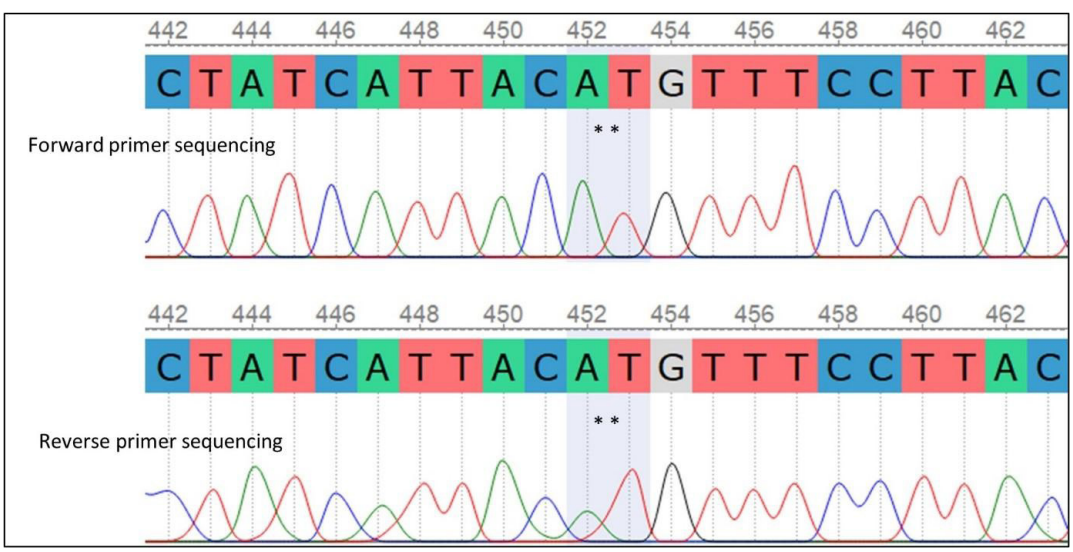

Figure 4: Sanger sequencing results for all 70 DNA samples from breast cancer patients showing there is no deletion of c.981_982del

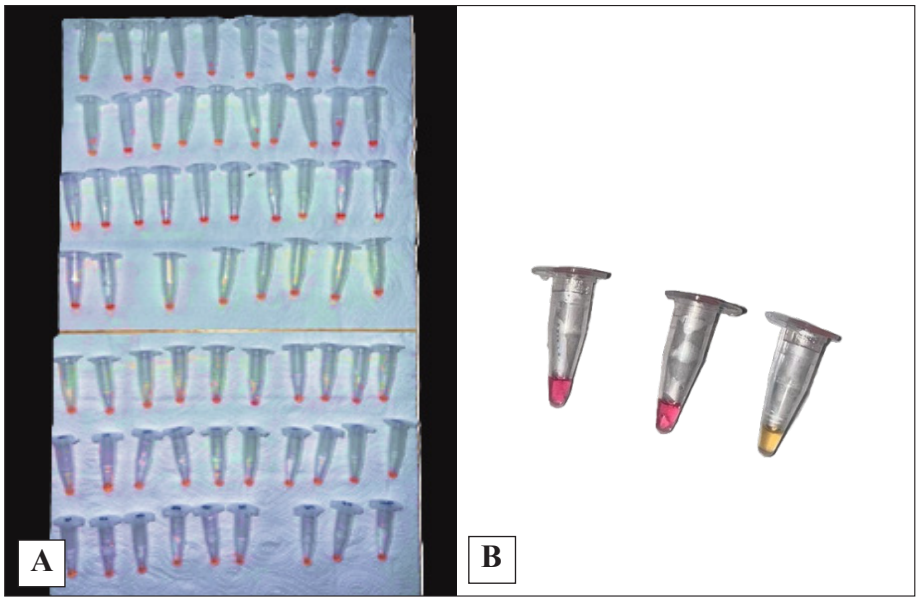

Figure 5: DNA samples from 70 breast cancer patients run with BRCA1 981_982del Loop MP Assay. The results are all negative, where the samples' colour remains pink (A). The positive control sample turn colour from pink to yellow (B)

In comparison, population screening may not be as cost-effective as cascade screening. Improving functionality could be attainable by expanding the study to include the entire Malaysian population and establishing a database that accurately reflects its diversity. Biosensor effectiveness could be heightened by identifying founder mutations specific to each ethnic group in Malaysia. A positive aspect is, the observed regional overlap in BRCA mutations further supports the feasibility of constructing gene panels that are not only locally relevant but also regionally translatable, addressing the limitations posed by Malaysia's currently incomplete BRCA variant database.

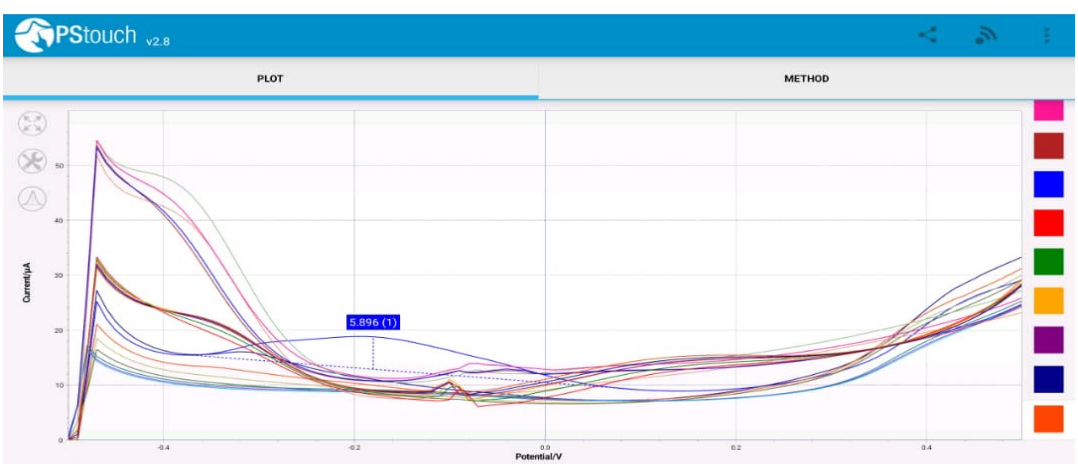

Figure 6: Electrochemical signal plots from the screen printed gold electrode and biosensor with LAMP products of positive control and 70 samples showed no measurable potential shift, consistent with negative detection. These results collectively confirmed the absence of BRCA1 981_982del mutations in the tested clinical samples, validating the specificity of both the LAMP assay and the biosensor-based NACOTS detection system.

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REFERENCES

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, *et al*. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74(3):229–263.
2. Antoniou A, Pharoah PDP, Narod S, Risch HA, Eyfjord JE, Hopper JL, *et al*. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case Series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet. 2003;72(5):1117–30.
3. Zayas-Villanueva OA, Campos-Acevedo LD, Lugo-Trampe J de J, Hernández-Barajas D, González-Guerrero JF, Noriega-Iriondo MF, *et al*. Analysis of the pathogenic variants of BRCA1 and BRCA2 using next-generation sequencing in women with familial breast cancer: a case–control study. BMC Cancer. 2019;19(1):722.
4. Ford D, Easton DF, Stratton M, Narod S, Goldgar D, Devilee P, *et al*. Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The Breast Cancer Linkage Consortium. Am J Hum Genet. 1998;62(3):676–89.
5. Michaelson-Cohen R, Cohen MJ, Cohen C, Greenberg D, Shmueli A, Lieberman S, *et al*. Real-World Cost-Effectiveness Analysis of Population Screening for BRCA Variants among Ashkenazi Jews Compared with Family History-Based Strategies. Cancers (Basel). 2022;14(24):6113.
6. Abdullah NA, Wan Mahiyuddin WR, Muhammad NA, Ali ZM, Ibrahim L, Ibrahim Tamim NS, *et al*. Survival rate of breast cancer patients in Malaysia: a population-based study. Asian Pac J Cancer Prev. 2013;14(8):4591–4.
7. Nik Hassan NN, Plazzer JP, Smith TD, Halim-Fikri H, Macrae F, Zubaidi ALA, *et al*. Harmonizing the interpretation of genetic variants across the world: the Malaysian experience. BMC Res Notes. 2016;9(1):125.
8. Park JW. Principles and Applications of Loop-Mediated Isothermal Amplification to Point-of-Care Tests. Biosensors. 2022;12(10):857.
9. Putzbach W, Ronkainen NJ. Immobilization Techniques in the Fabrication of Nanomaterial-Based Electrochemical Biosensors: A Review. Sensors. 2013;13(4):4811–40.
10. Morales MA, Halpern JM. Guide to Selecting a Biorecognition Element for Biosensors. Bioconjugate Chem. 2018;29(10):3231-3239.
11. Bean LJH, Funke B, Carlston CM, *et al*. Diagnostic gene sequencing panels: from design to report—a technical standard of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2020;22(3):453–461.
12. Somasundaram K. BRCA1 and BRCA1 Genes and Inherited Breast and/or Ovarian Cancer: Benefits of Genetic Testing. Indian J Surg Oncol. 2010;1(3):245–9.
13. Stewart MD, Merino Vega D, Arend RC, Baden JF, Barbash O, Beaubier N, *et al*. Homologous recombination deficiency: concepts, definitions, and assays. Oncologist. 2022;27(3):167–174.
14. Clark SL, Rodriguez AM, Snyder RR, Hankins GDV, Boehning D. Structure-Function of the Tumor Suppressor BRCA1. Comput Struct Biotechnol J. 2012;1:e201204005.
15. Deng CX, Brodie SG. Roles of BRCA1 and its interacting proteins. Bioessays. 2000;22(8):728–37.
16. Andreassen PR, Seo J, Wiek C, Hanenberg H. Understanding BRCA2 Function as a Tumor Suppressor Based on Domain-Specific Activities in DNA Damage Responses. Genes (Basel). 2021;12(7):1034.
17. Clinical Genome Resource. https://search.clinicalgenome.org/kb/gene-dosage/HGNC:1100 [10 February 2024].
18. Clinical Genome Resource. https://search.clinicalgenome.org/kb/gene-dosage/HGNC:1101. [10 February 2024].
19. Rebbeck TR, Friebel TM, Friedman E, Hamann U, Huo D *et al*., Mutational spectrum in a worldwide study of 29,700 families with BRCA1 or BRCA2 mutations. Hum Mutat. 2018;39(5):593-620.
20. Li J, Zhao B, Huang T, Qin Z, Wang SM. Human BRCA pathogenic variants were originated during recent human history. Life Sci Alliance. 2022;5(5):e202101263.
21. Rehm HL, Berg JS, Brooks LD, Bustamante CD, Evans JP, Landrum MJ, *et al*. ClinGen- The Clinical Genome Resource. New England Journal of Medicine. 2015;372(23):2235–42.
22. Yahya P, Sulong S, Harun A, Isa HW, Rajab NSA, Wangkumhang P, *et al*. Analysis of the genetic structure of the Malay population: Ancestry-informative marker SNPs in the Malay of Peninsular Malaysia. Forensic Science International: Genetics. 2017;30:152–9.
23. Yahya P, Sulong S, Harun A, Wangkumhang P, Wilantho A, Ngamphiw C, *et al*. Ancestry-informative marker (AIM) SNP panel for the Malay population. Int J Legal Med. 2020;134(1):123–34.
24. Kalidasan V, Theva Das K. Is Malaysia Ready for Human Gene Editing: A Regulatory, Biosafety and Biosecurity Perspective. Front Bioeng Biotechnol. 2021;9:649203.
25. Tan M-M, Ho W-K, Yoon S-Y, Mariapun S, Hasan S N, Lee DS-C, *et al*. A case-control study of breast cancer risk factors in 7,663 women in Malaysia. PLoS One. 2018;13(9):e0203469.

26. Thirthagiri E, Lee SY, Kang P, Lee DS, Toh GT, Selamat S, *et al*. Evaluation of BRCA1 and BRCA2 mutations and risk-prediction models in a typical Asian country (Malaysia) with a relatively low incidence of breast cancer. Breast Cancer Res. 2008;10(4):R59.
27. Lee DSC, Yoon SY, Looi LM, Kang P, Kang IN, Sivanandan K, *et al*. Comparable frequency of BRCA1, BRCA2 and TP53 germline mutations in a multi-ethnic Asian cohort suggests TP53 screening should be offered together with BRCA1/2 screening to early-onset breast cancer patients. Breast Cancer Res. 2012;14(2):R66.
28. Ng PS, Wen WX, Fadlullah MZH, Yoon SY, Lee SY, Thong MK, *et al*. Identification of germline alterations in breast cancer predisposition genes among Malaysian breast cancer patients using panel testing. Clin Genet. 2016;90(4):315–23.
29. Lai KN, Ho WK, Kang IN, Kang PCE, Phuah SY, Mariapun S, *et al*. Characterization of BRCA1 and BRCA2 variants in multi-ethnic Asian cohort from a Malaysian case-control study. BMC Cancer. 2017;17(1):149.
30. Wen WX, Allen J, Lai KN, Mariapun S, Hasan SN, Ng PS, *et al*. Inherited mutations in BRCA1 and BRCA2 in an unselected multiethnic cohort of Asian patients with breast cancer and healthy controls from Malaysia. J Med Genet. 2018;55(2):97–103.
31. Wang SM. A global perspective on the ethnic-specific BRCA variation and its implication in clinical application. J Natl Cancer Cent. 2023;3(1):14–20.
32. Amini F, Hou W-F, Chye E N S, Omar R, Rejab SM, Noor I W M, *et al*. Mutation profile of breast cancer in Malaysian patients. J Health Transl Med (JUMMEC). 2021;24(1):37–44.
33. Phuah SY, Looi LM, Hassan N, Rhodes A, Dean S, Taib NAM, *et al*. Triple-negative breast cancer and PTEN (phosphatase and tensin homologue) loss are predictors of BRCA1 germline mutations in women with early-onset and familial breast cancer, but not in women with isolated late-onset breast cancer. Breast Cancer Res. 2012;14(6):R142.
34. Wen WX, Soo JSS, Kwan PY, Hong E, Khang TF, Mariapun S, *et al*. Germline APOBEC3B deletion is associated with breast cancer risk in an Asian multi-ethnic cohort and with immune cell presentation. Breast Cancer Res. 2016;18(1):56.
35. Yang XR, Devi BCR, Sung H, Guida J, Mucaki EJ, Xiao Y, *et al*. Prevalence and spectrum of germline rare variants in BRCA1/2 and PALB2 among breast cancer cases in Sarawak, Malaysia. Breast Cancer Res Treat. 2017;165(3):687–97.
36. Pan JW, Zabidi MMA, Chong BK, Meng MY, Ng PS, Hasan SN, *et al*. Germline APOBEC3B deletion increases somatic hypermutation in Asian breast cancer that is associated with Her2 subtype, PIK3CA mutations and immune activation. Int J Cancer. 2021;148(10):2489–501.