UNIVERSITI SAINS MALAYSIA
GERAN PENYELIDIKAN UNIVERSITI
PENYELIDIKAN
LAPORAN AKHIR

SCREENING AND CHARACTERIZATION OF 10398A POLYMORPHISM IN INVASIVE BREAST CANCER IN MALAY POPULATION OF PENINSULAR MALAYSIA

PENYELIDIK

PROF. MADYA DR. ZAFARINA ZAINUDDIN

PENYELIDIK BERSAMA

DR. RAPEAH SUPPIAN
DR. HASMAH ABDULLAH

2013

FINAL REPORT FOR SHORT TERM GRANT

TITLE: Screening and characterization of 10398A polymorphism in invasive breast cancer in Malay population of Peninsular Malaysia (304/PPSK/6131564)

Principal Investigator : Prof Madya Dr Zafarina Zainuddin

Co-Researchers : Dr Rapeah Suppian

Dr Hasmah Abdullah

SCHOOL OF HEALTH SCIENCES

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PEJABAT PENGURUSAN & KREATIVITI PENYELIDIKAN

LAPORAN AKHIR PROJEK PENYELIDIKAN JANGKA PENDEK

FINAL REPORT OF SHORT TERM RESEARCH PROJECT

Sila kemukakan **dua (2) salinan** laporan akhir ini melalui Jawatankuasa Penyelidikan di Pusat Pengajian dan Dekan/ Pengarah/ Ketua Jabatan kepada Pejabat Pengurusan dan Kreativiti Penyelidikan (RCMO)

1. **Nama Ketua Penyelidik: DR ZAFARINA ZAINUDDIN**
   Name of Research Leader

   ☑ Profesor Madya/ *Assoc. Prof.* ☐ Dr./ *Dr.* ☐ Encik/Puan/Cik *Mr/Mrs/Ms*

2. **Pusat Tanggungjawab (PTJ):** **School of Health Sciences**
   School/Department

3. **Nama Penyelidik Bersama: i) Dr Rapeah Suppian**
   Name of Co-Researcher **ii) Dr Hasmah Abdullah**

4. **Tajuk Projek:** **Screening and characterization of 10398A polymorphism in invasive breast cancer in Malay population of Peninsular Malaysia**
   Title of Project

5. **Ringkasan Penilaian/***Summary of Assessment*:	Tidak Mencukupi *Inadequate*		Boleh Diterima *Acceptable*	Sangat Baik *Very Good*	
	1	2	3	4	5
i) **Pencapaian objektif projek:** *Achievement of project objectives*	☐	☐	☐	☐	☑
ii) **Kualiti output:** *Quality of outputs*	☐	☐	☐	☑	☐
iii) **Kualiti impak:** *Quality of impacts*	☐	☐	☐	☑	☐
iv) **Pemindahan teknologi/potensi pengkomersialan:** *Technology transfer/commercialization potential*	☐	☐	☑	☐	☐
v) **Kualiti dan usahasama :** *Quality and intensity of collaboration*	☐	☐	☐	☑	☐
vi) **Penilaian kepentingan secara keseluruhan:** *Overall assessment of benefits*	☐	☐	☐	☑	☐

6. *Abstrak Penyelidikan*

(Perlu disediakan di antara 100 - 200 perkataan di dalam **Bahasa Malaysia dan juga Bahasa Inggeris.** Abstrak ini akan dimuatkan dalam Laporan Tahunan Bahagian Penyelidikan & Inovasi sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti & masyarakat luar).

Abstract of Research

(An abstract of between 100 and 200 words must be prepared in Bahasa Malaysia and in English).
This abstract will be included in the Annual Report of the Research and Innovation Section at a later date as a means of presenting the project findings of the researcher/s to the University and the community at large)

The mitochondrial DNA (mtDNA) 10398 polymorphism is hypothesised to alter a mitochondrial subunit of the electron transfer chain and is associated with several neurodegenerative disorders and cancers. In this study, a mtDNA polymorphism at nucleotide position 10398 was screened in 101 Malay female patients with invasive breast cancer and 90 age-matched healthy female controls using minisequencing analysis. The Malay women with the 10398G variant showed a significantly increased risk of invasive breast cancer (OR = 2.29, 95% CI 1.25–4.20, $P = 0.007$). Immunohistochemistry analysis was conducted to investigate the effect of this polymorphism on the levels of apoptosis in breast cancer cells. The level of Bax (a pro-apoptotic protein) expression was significantly higher than that of Bcl-2 (an anti-apoptotic protein) in patients carrying the G allele ($P = 0.016$) but not in those carrying the A allele ($P = 0.48$). Based on these findings, we propose that the mtDNA 10398 polymorphism may be a potential risk marker for breast cancer susceptibility in the Malay population.

7. Sila sediakan laporan teknikal lengkap yang menerangkan keseluruhan projek ini.
[Sila gunakan kertas berasingan]
Applicant are required to prepare a Comprehensive Technical Report explaning the project.
(This report must be appended separately)

As attached

Senaraikan kata kunci yang mencerminkan penyelidikan anda:
List the key words that reflects your research:

Bahasa Malaysia	**English**
Kanser payudara, penjujukan DNA, SNP, penanda genetik, DNA mitokondria	Breast cancer, DNA sequencing, SNP, genetic marker, mitochondrial DNA

8. Output dan Faedah Projek
Output and Benefits of Project

(a) * **Penerbitan Jurnal**
Publication of Journals
(Sila nyatakan jenis, tajuk, pengarang/editor, tahun terbitan dan di mana telah diterbit/diserahkan)
(State type, title, author/editor, publication year and where it has been published/submitted)

1. Publication:

Nadiah, T.B., Hasnan,J and Zafarina, Z. (2012) Association of Mitochondrial DNA 10398 Polymorphism in Invasive Breast Cancer in Malay Population of Peninsular Malaysia. *Malaysian Journal of Medical Sciences.* Jan-Mar 2012; 19(1): 36-42

2. Conference presentation

Nadiah, T.P. and Zafarina, Z. (2010) Mitochondrial DNA 10398 polymorphism and breast cancer risk in Malay females of Peninsular Malaysia. 15th National Conference on Medical and Health Sciences. 21-22 July 2010, Kelantan, Malaysia

(b) **Faedah-faedah lain seperti perkembangan produk, pengkomersialan produk/pendaftaran paten atau impak kepada dasar dan masyarakat.**
State other benefits such as product development, product commercialisation/patent registration or impact on source and society.

Not relevant -

* Sila berikan salinan/*Kindly provide copies*

(c) **Latihan Sumber Manusia**
Training in Human Resources

i) Pelajar Sarjana:
Graduates Students
(Perincikan nama, ijazah dan status)
(Provide names, degrees and status)

Tengku Puteri Nadiah Tengku Baharudin Shah

MSc in Biomedicine, graduated in 2011

ii) Lain-lain:
Others

9. **Peralatan yang Telah Dibeli:**
Equipment that has been purchased

1 unit air condition for Genetic Anayzer room

Komen Jawatankuasa Penyelidikan Pusat Pengajian/Pusat
Comments by the Research Committees of Schools/Centres

Interesting finding with one article being published.

one MSc student has been trained.

UNIVERSITI SAINS MALAYSIA
JABATAN BENDAHARI
KUMPULAN WANG PENYELIDIKAN GERAN USM(304)
PENYATA PERBELANJAAN SEHINGGA 30 JUN 2013

Jumlah Geran:	RM	29,879.00	Ketua Projek:	Zafarina Zainuddin, Dr
Peruntukan 2008 (Tahun 1)	RM	16,170.00	Tajuk Projek:	Screening and Characterization of 10398A Polymorphism in Invasive Breast Cancer in Malay Population of Peninsular Malaysia
Peruntukan 2009 (Tahun 2)	RM	13,709.00		
			Tempoh:	1 Mac 2008 - 31 Ogos 2010
			No.Akaun:	304/PPSK/6131564

Kwg	Akaun	PTJ	Projek	Donor	Peruntukan Projek	Perbelanjaan T'kumpul Hingga Tahun Lalu	Peruntukan Semasa	Tanggungan Semasa	Bayaran Tahun Semasa	Belanja Tahun Semasa	Baki Projek
304	11000	PPSK	6131564		-	-	-	-	-	-	-
304	14000	PPSK	6131564		-	-	-	-	-	-	-
304	15000	PPSK	6131564		-	-	-	-	-	-	-
304	21000	PPSK	6131564		3,420.00	1,512.00	1,908.00	-	-	-	1,908.00
304	22000	PPSK	6131564		-	-	-	-	-	-	-
304	23000	PPSK	6131564		150.00	260.51	(110.51)	-	-	-	(110.51)
304	24000	PPSK	6131564		-	-	-	-	-	-	-
304	25000	PPSK	6131564		-	9.80	(9.80)	-	-	-	(9.80)
304	26000	PPSK	6131564		-	-	-	-	-	-	-
304	27000	PPSK	6131564		22,709.00	22,249.91	459.09	-	-	-	459.09
304	28000	PPSK	6131564		-	-	-	-	-	-	-
304	29000	PPSK	6131564		3,600.00	2,000.00	1,600.00	-	-	-	1,600.00
304	32000	PPSK	6131564		-	-	-	-	-	-	-
304	35000	PPSK	6131564		-	3,600.00	(3,600.00)	-	-	-	(3,600.00)
304	A11559	PPSK	6131564		-	-	-	-	-	-	-
304	A11102	PPSK	6131564		-	-	-	-	-	-	-
					29,879.00	29,632.22	246.78	-	-	-	246.78

Original Article
Provisional PDF

Association of Mitochondrial DNA 10398 Polymorphism in Invasive Breast Cancer in Malay Population of Peninsular Malaysia

Tengku Baharudin Nadiah[1], Jaafar Hasnan[2], Zainuddin Zafarina[1]

Submitted: 27 Feb 2011
Accepted: 31 Aug 2011

[1] *School of Health Sciences, Universiti Sains Malaysia Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia*

[2] *Department of Pathology, School of Medical Sciences, Universiti Sains Malaysia Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia*

Abstract

Background: **The mitochondrial DNA (mtDNA) 10398 polymorphism is hypothesised to alter a mitochondrial subunit of the electron transfer chain and is associated with several neurodegenerative disorders and cancers.**

Methods: **In this study, an mtDNA polymorphism at nucleotide position 10398 was screened in 101 Malay female patients with invasive breast cancer and 90 age-matched healthy female controls using minisequencing analysis.**

Results: **The Malay women with the 10398G variant showed a significantly increased risk of invasive breast cancer (OR = 2.29, 95% CI 1.25–4.20, P = 0.007). Immunohistochemistry analysis was conducted to investigate the effect of this polymorphism on the levels of apoptosis in breast cancer cells. The level of Bax (a pro-apoptotic protein) expression was significantly higher than that of Bcl-2 (an anti-apoptotic protein) in patients carrying the G allele (P = 0.016) but not in those carrying the A allele (P = 0.48).**

Conclusion: **Based on these findings, we propose that the mtDNA 10398 polymorphism may be a potential risk marker for breast cancer susceptibility in the Malay population.**

Keywords: *breast cancer, DNA sequencing, genetic marker, mitochondrial DNA, oncology, single nucleotide polymorphism*

Introduction

Mitochondria are a major site and target of intracellular reactive oxygen species (ROS), which are a natural by-product of electron transport chain activity (1,2). Mitochondrial DNA (mtDNA) is vulnerable to the effect of these molecules and has a limited ability to repair itself. Therefore, the excessive formation and continuous accumulation of ROS could lead to a cellular stress response and the inhibition of apoptosis (3). Several findings (4–6) show that both defects and reduction in the apoptosis threshold can extend the life span of the cell, contributing to continuous proliferation that may lead to cancer development. However, the exact role of mtDNA mutations in inhibiting apoptosis, either by suppression of pro-apoptotic genes or by activation of anti-apoptotic genes, has not been defined.

Several mutations, including single nucleotide polymorphisms in certain genes in both the nuclear and mitochondrial genomes, are implicated in breast cancer susceptibility (7,8). An A to G polymorphism at nucleotide position 10398 in the mitochondrial genome causes a non-conservative amino acid substitution from threonine to alanine within the NADH dehydrogenase (ND3) subunit of Complex I (9,10). This particular polymorphism has also been reported to alter both mitochondrial pH and intracellular calcium levels (11,12); these alterations have been associated with the modulation of ATP production and apoptosis (13). The structural alteration and impairment of Complex I may lead to increased production of free radicals and has been associated with increased risk of several mitochondrial disorders, such as Parkinson's disease (14,15) and bipolar disorders (16).

The association of the mtDNA 10398 polymorphism in Complex I with breast cancer was first studied by Canter et al. (17), which showed that the risk of invasive breast cancer was significantly higher in African-American women carrying the 10398A allele compared with non-carriers. This polymorphism is also associated

