UNIVERSITI SAINS MALAYSIA
GERAN PENYELIDIKAN UNIVERSITI
PENYELIDIKAN
LAPORAN AKHIR

IDENTIFICATION PREDISPOSITION GENOTYPES THAT CONTRIBUTE TO COLORECTAL CANCER SUSCEPTIBILITY IN MALAYSIA

PENYELIDIK

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DR. ZAIDI ZAKARIA
SITI NURFATIMAH SHAHPUDIN
AHMAD AIZAT ABDUL AZIZ

2012

FINAL REPORT
FUNDAMENTAL RESEARCH GRANT SCHEME (FRGS)

Laporan Akhir Skim Geran Penyelidikan Asas (FRGS) IPT
Pindaan 1/2009

A **RESEARCH TITLE** : **IDENTIFICATION OF PREDISPOSITION GENOTYPES THAT CONTRIBUTE TO COLORECTAL CANCER SUSCEPTIBILITY IN MALAYSIA**
Tajuk Penyelidikan

PROJECT LEADER : **PROF. DR RAVINDRAN ANKATHIL**
Ketua Projek

PROJECT MEMBERS : 1. Prof Biswa Mohan Biswal
(including GRA) 2. Dr Muhammad Radzi Abu Hassan
Ahli Projek 3. Assoc.Prof Gurjeet Kaur
4. Dr Venkatesh Naik
5. Prof Dr Zilfalil Alwi
6. Dr Hoh Boon Peng
7. Dr Ahmad Shanwani Mohd Sidek
8. Dr Zaidi Zakaria
9. Siti Nurfatimah Shahpudin (MSc student)
10. Ahmad Aizat B Abdul Aziz (Msc student)

PROJECT ACHIEVEMENT (*Pencapaian Projek*)

B

ACHIEVEMENT PERCENTAGE			
Project progress according to milestones achieved up to this period	**0 - 50%**	**51 - 75%**	**76 - 100%**
Percentage			X

RESEARCH FINDINGS		
Number of articles/ manuscripts/ books	**Indexed Journal**	***Non-Indexed Journal***
	5	7
Paper presentations	**International**	***National***
	5	6
Others (Please specify)		

HUMAN CAPITAL DEVELOPMENT

Human Capital	Number		Others (Please specify):
	On-going	Graduated	
PhD Student			
Masters Student		2	
Undergraduate Students			
Temporary Research Officer			
Temporary Research Assistant			
Total			

EXPENDITURE (*Perbelanjaan*)

C

Budget Approved *(Peruntukan diluluskan)* : **RM 149,500.00**
Amount Spent *(Jumlah Perbelanjaan)* : **RM 149,499.77**

Balance *(Baki)* : **RM 0.23**
Percentage of Amount Spent : **99.99 %**
(Peratusan Belanja)

D ADDITIONAL RESEARCH ACTIVITIES THAT CONTRIBUTE TOWARDS DEVELOPING SOFT AND HARD SKILLS

International

Activity	Date (Month, Year)	Organizer
Conference:		
1. Human Genome Meeting, Pan Arab Human Genetics Conference	14-17 March 2011, Dubai	Human Genome Organization
2. International Conference on Advancement in Science and Technology, 2010	26-29 November 2010, Vistana Hotel, Pahang.	
3. Asia Pacific Digestive Week 2010	9-22 September 2010, Kuala Lumpur Convention Centre, Malaysia	WILEY-BLACKWELL and Journal of Gastroenterology and Hepatology Foundation
4. Asia Pacific Conference on Human Genetics	30th Nov- 3rd Dec 2010, Hong Kong	Hong Kong Academy of Medicine
5. 3rd Regional Conference on Molecular Medicine	2-4 May 2009, Kota Bharu, Kelantan, Malaysia.	USM, INFORMM, UITM & MSAP

National

Activity	Date (Month, Year)	Organizer
Conference:		
1. 1st National Postgraduate Conference in Molecular Medicine, 2011	13-14 April 2011,Kota Bharu, Kelantan	USM, INFORMM & UMBI
2. 16th National Conference on Medical and Health Sciences	22-23 June 2011, USM, Health Campus, Kelantan	School of Medical Sciences
3. 2nd National Conference on Environment and Health 2010	17-18 March 2010 (Renaissance Hotel, Kota Bharu, Kelantan)	School of Health Sciences and PERSALA, USM
4. 15th National Conference on Medical and Health Sciences	21-22 July 2010 (Grand Riverview Hotel, Kota Bharu, Kelantan)	School of Health Sciences, USM
5. 8th Malaysia Genetics Congress, 2009	4-6 August 2009, Awana Genting, Pahang	Persatuan Genetik Malaysia, UKM, UPM and MOSTI

PROBLEMS / CONSTRAINTS IF ANY *(Masalah/ Kekangan sekiranya ada)*

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Although this is the first comprehensive study on the association of SNP in xenobiotic metabolizing and DNA damage repair genes in Malaysian population, the study has been limited by the small sample size due to short time period and budget. Because of this, large number of samples could not be included. For the same reason, the frequency of variant allele observed for certain SNPs were too small or sometimes nil which must have resulted in inadequate in statistical power.

During risk analysis of combination of genotypes, the infrequent presence or rarity of at-risk genotype or allele, for some of the combination SNPs studied resulted in deriving risk association values with high ORs values, but with extremely wide range of 95% confidence intervals. Such combinations genotypes were not considered as high risk predisposition genotypes despite the high ORs obtained.

Malaysian population comprises 3 major ethnic groups; Malay, Chinese and Indian with a ratio of approximately 60:30:10, respectively. Different the ethnic races have different genetic background. The difference in genetic background of the study subjects which comprised these 3 ethnic races was not taken into account and this was another limitation. A stratified analysis based on ethnicity with equal number of the study subjects in each ethnic group, could have given better results with adequate power.

The study subjects in this study were recruited from differing collaborating hospitals in Malaysia. From some of the hospitals, it was difficult and unable to get many of the epidemiological and clinicopathological details of the case subjects. So the interaction of confounding factors like lifestyle habits (smoking, alcohol consumption), dietary habits etc with the SNP included in the study could not be examined.

RECOMMENDATION

Even though the incidence of CRC is increasing in Malaysia, there is paucity of information on the nature of genetic predisposition factors that contribute to susceptibility to CRC in Malaysian patients, as no previous studies have been undertaken in Malaysia. Moreover, report on the association of SNPs in Xenobiotic metabolizing and DNA repair genes as genetic predisposition factors for CRC susceptibility from other population are inconsistent. To the best of knowledge, this is first study from Malaysia on this aspect. From this study, few Xenobiotic metabolizing and DNA repair SNPs and combination SNPs associated with CRC susceptibility risk in Malaysian population have been identified.

In future, advances in SNP mapping utilizing high throughout genotyping methods could facilitate the analysis of multiple polymorphisms within DNA repair genes and also the analysis of multiple gene within DNA repair pathways.This may lead to the advancement of knowledge on genetic predisposing factors related to CRC susceptibility in Malaysian population. This, in turn, may help to identify the high risk individuals with CRC susceptibility risk genotype in the population and devise appropriate preventive screening strategies for them. Regular, frequent and effective screening for CRC is not currently available at the population level. The public perception of genetic determinism is that, if the polymorphic at risk, genotype status of an individual is known, the cancer predisposition status of that individual can be predicted and appropriate surveillance programs can be initiated by allowing more effective prevention strategies. By this, morbidity and mortality of CRC can be reduced at the population level.

Furthemore, the detection of protective nature of few SNPs, is an interesting observation which may have a great impact in evaluating the treatment response of CRC patients carrying the specific genotypes. So it would be ideal to associate the genotype pattern with the treatment response and survival of these CRC patients.

The incidence of sporadic colorectal cancer (CRC) is increasing in Asian countries, including Malaysia. Although several factors have been implicated in CRC etiology, CRC develops as a result of interaction between environmental factors and genetic predisposition. Exposure to environmental carcinogens through dietary components and cigarette smoke are associated with an increased risk of CRC. However, the genetic predisposition factors associated with CRC development remains still undetermined. It was hypothesized that genetic variations such as single nucleotide polymorphisms (SNPs) in xenobiotic metabolism and DNA damage repair genes could have effects on the sensitivity of individuals to environmental genotoxins and may influence CRC susceptibility. In order to clarify the role of xenobiotic metabolizing and DNA repair genes in colorectal carcinogenesis, a case-control study was designed and undertaken to investigate the genotype frequencies of 10 polymorphisms from 6 genes encoding enzymes involved in xenobiotic metabolism (*GSTT1*, *GSTM1*, *GSTP1* Ile105Val, *CYP1A2* G3860A, *CYP1A2* T739G,*CYP1A2* C729T, *NAT1* C1095A, *NAT2* G191A, *NAT2* A803G, *NAT2* G857A) and 4 polymorphisms in genes involved in DNA damage repair (*XRCC1* Arg399Gln, *XRCC3* Thr241Met, *XPD/ERCC2* Lys751Gln and *P53* Arg72Pro) and to determine their influence, either singly or as combination genotypes, in CRC susceptibility risk. After getting informed consent, peripheral blood of all study subjects were collected, DNA extracted and genotyping carried out using PCR- RFLP and multiplex PCR methods. Genotypes were categorized into homozygous major, heterozygous variant and homozygous variant. The risks of CRC associated with these polymorphisms were estimated by calculating Odds Ratios (ORs) and 95% confidence intervals using unconditional logistic regression. For the risk association of xenobiotic metabolism genes and CRC susceptibility, *CYP1A2* A3860A, *CYP1A2* T739G, *GSTP1* val/val genotypes and *GSTT1* null showed significant risk association with CRC predisposition. When analyzed in 2 way combinations, remarkably increased risk was observed for carriers of *CYP1A2* A3860A/T739T, *GSTT1* (-/-)/ *GSTM1*(-/-), (*GSTT1* (-/-)/ *GSTP1* Ile/Ile), (*GSTT1* (-/-)/ *GSTP1* Ile/Val) genotype combinations. In triple genotype combination analysis, the *GSTM1* (-/-)/*GSTT1* (-/-)/ *GSTP1* Ile/Ile) genotype combination emerged as high risk predisposition genotype associated with CRC susceptibility risk. In case of DNA damage repair genes and CRC susceptibility risk, homozygous variant *P53* Pro72Pro showed significantly higher risk

association with CRC susceptibility. When analyzed in 2 way combinations, the genotype combinations of *XRCC3* Thr/Thr+*P53* Pro/Pro emerged as high risk combination genotypes associated with CRC susceptibility. It is reasonable to suggest that SNPs studied in xenobiotic metabolism genes might be promoting CRC susceptibility through their capability of increased activation of chemical carcinogens and/or decreased ability of cells to detoxify carcinogens. So also, whereas SNPs in DNA damage repair might be promoting colorectal carcinogenesis by altering the respective DNA repair gene expression and modulating the DNA repair function and thereby enhancing the CRC susceptibility risk. In conclusion, the SNPs in xenobiotic metabolism and DNA damage repair genes, showing significant risk association with CRC predisposition, either singly or in combination, may be considered as candidate genetic predisposition factors associated with CRC susceptibility risk in Malaysian population.

COMMENTS, IF ANY/ ENDORSEMENT BY RESEARCH MANAGEMENT CENTER (RMC)
(Komen, sekiranya ada/ Pengesahan oleh Pusat Pengurusan Penyelidikan)

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Name:
Nama:

Signature:
Tandatangan:

Date:
Tarikh:

UNIVERSITI SAINS MALAYSIA
JABATAN BENDAHARI
KUMPULAN WANG PENYELIDIKAN FUNDAMENTAL
PENYATA PERBELANJAAN SEHINGGA 31 JUL 2011

Jumlah Geran	RM149,500.00	Ketua Projek	PROF. RAVINDRAN ANKATHIL
Peruntukan (Tahun 1) Okt 07-Okt 08	RM49,400.00	Tajuk Projek	IDENTIFICATION OF PREDISPOSITION GENOTYPES THAT CONTRIBUTE TO COLORECTAL CANCER SUSCEPTIBILITY IN MALAYSIA
Peruntukan (Tahun 2) Okt 08-Okt 09	RM57,000.00		
		Tempoh	42 BULAN (15/10/2007-15/04/2011)
Peruntukan (Tahun 3) Okt 09-Okt 10	RM43,100.00	No. Akaun	203/PPSP/6171112

Kwgan	Akaun	PTJ	Projek	Peruntukan Projek	Perbelanjaan Terkumpul sehingga Tahun lalu	Peruntukan Semasa	Tanggungan Semasa	Bayaran Tahun Semasa	Belanja Tahun Semasa	Baki Projek
203	11000	PPSP	6171112	36,000.00	45,627.45	(9,627.45)	-	5,345.10	5,345.10	(14,972.55)
203	14000	PPSP	6171112		-	-	-	-	-	-
203	15000	PPSP	6171112	-	1,000.00	(1,000.00)	-		-	(1,000.00)
203	21000	PPSP	6171112	3,800.00	5,331.30	(1,531.30)	-		-	(1,531.30)
203	22000	PPSP	6171112	2,100.00	-	2,100.00	-	-	-	2,100.00
203	23000	PPSP	6171112	1,400.00	410.85	989.15	-	52.76	52.76	936.39
203	24000	PPSP	6171112	-	80.00	(80.00)	-		-	(80.00)
203	26000	PPSP	6171112			-	-	-	-	-
203	27000	PPSP	6171112	88,300.00	52,030.00	36,270.00		885.06	885.06	35,384.94
203	28000	PPSP	6171112	900.00	-	900.00		-	-	900.00
203	29000	PPSP	6171112	17,000.00	35,682.30	(18,682.30)		3,054.95	3,054.95	(21,737.25)
203	35000	PPSP	6171112	-	-	-	-	-	-	-
203	A11559	PPSP	6171112	-	-	-		-	-	-
203	A11102	PPSP	6171112	-	-	-		-	-	-
				149,500.00	140,161.90	9,338.10	-	9,337.87	9,337.87	0.23