Appendix A

Reagents and Solutions

PCR

Sterile distilled water (sdH_2O)	Up to 25µl
reaction buffer (1X)	2.5µl of 10X
$MgCl_2$ (1.5mM)	1.5µl of 25mM
dNTPs (10mM)	4.0µl
Forward primer (0.4pmole/µl)	1.0µl of 10pmole
Reverse primer (0.4pmole/µl)	1.0µl of 10pmole
Taq DNA polymerase (1U)	0.2µl of 5U/µl

Agarose Gel Electrophoresis

5X Tris Borate EDTA (TBE)	54.0g Tris-base (445mM) 27.5g Borate (445mM) 20ml of 0.5M EDTA (10mM, pH 8.0)
6X loading dye	30% glycerol, 0.25% bromophenol blue, 0.25% xylene cyanol FF

SSCP

MDE gel	25% MDE gel solution 0.6% TBE 0.1% APS 0.1% TEMED
Bind solution	80% Absolute ethanol 20% Acetic Acid 0.003% Bind Silane
Fix-Stop solution	10% Absolute ethanol 5% Acetic Acid
Silver staining solution	1g/L $AgNO_3$ powder
Developing solution	30g/L NaOH powder 37% Formaldehyde
2X SSCP loading dye	95% Formamide 10mM NaOH 0.05% Bromophenol blue 0.05% Xylene cyanol

Cloning into *Escherichia coli*

Luria Bertani (LB) Broth	20g/L LB broth powder
Luria-Bertani (LB) Agar with Ampicillin	35g /L LB broth powder 50mg/ml Ampicillin 0.08mg/ml X-gal 0.5mM IPTG

Plasmid Extraction

Solution I	50mM glucose, 10mM EDTA and 25mM Tris-Cl (pH 8.0)
Solution II	0.2N NaOH and 1% SDS
Solution III	3M KOAc and 10% acetic acid

Restriction enzyme digestion

*Eco*RI digestion	1X restriction enzyme buffer 0.1µg/ µl acetylated BSA 1µg DNA template 1U *Eco*RI restriction enzyme

Competent cells preparation

RF1 solution (pH 5.8)	1.2g $RbCl_2$ 0.99g $MnCl_4$•4H2O 0.3g KOAc 0.15g $CaCl_2$•4H20 15% Glycerol
RF2 solution (pH 6.8)	0.21g MOPS 0.12g $RbCl_2$ 1.10g $CaCl_2$•4H20 15% Glycerol

Cell culture

Cryosolution	90% FBS 10% DMSO
1X DMEM growth medium	10% DMEM 10% FBS 1% Ampicillin
10X DMEM growth medium	26.8g DMEM powder 6.0 g $NaCO_3$ 9.56g HEPES buffer

Appendix B

List of primers used in this study.

Table B1: *APC* gene primers used in this study (taken from Groden *et al*., 1991).

Exon	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	Expected size (bp)
1	AGGTCCAAGGGTAGCCAAGG	TAAAAATGGATAAACTACAATTAAAAG	197
2	AAATACAGAATCATGTCTTGAAGT	ACACCTAAAGATGACAATTTGAG	152
3	TAACTTAGATAGCAGTAATTTCCC	ACAATAAACTGGAGTACACAAGG	252
4	ATAGGTCATTGCTTCTTGCTGAT	TGAATTTTAATGGATTACCTAGGT	194
5	CTTTTTTTGCTTTTACTGATTAACG	TGTAATTCATTTTATTCCTAATAGCTC	244
6	GGTAGCCATAGTATGATTATTTCT	CTACCTATTTTTATACCCACAAAC	204
7	AAGAAAGCCTACACCATTTTTGC	GATCATTCTTAGAACCATCTTGC	238
8	ACCTATAGTCTAAATTATACCATC	GTCATGGCATTAGTGACCAG	184
9	AGTCGTAATTTTGTTTCTAAACTC	TGAAGGACTCGGATTTCACGC	341
9a	TCATTCACTCACAGCCTGATGAC	GCTTTGAAACATGCACTACGAT	196
10	AAACATCATTGCTCTTCAAATAAC	TACCATGATTTAAAAATCCACCAG	216
11	GATGATTGTCTTTTTCCTCTTGC	CTGAGCTATCTTAAGAAATACATG	215
12	TTTTAAATGATCCTCTATTCTGTAT	ACAGAGTCAGACCCTGCCTCAAAG	179
13	TTTCTATTCTTACTGCTAGCATT	ATACACAGGTAAGAAATTAGGA	306
14	TAGATGACCCATATTCTGTTTC	CAATTAGGTCTTTTTGAGAGTA	308
15-A	GTTACTGCATACACATTGTGAC	GCTTTTTGTTTCCTAACATGAAG	372
15-B	AGTACAAGGATGCCAATATTATG	ACTTCTATCTTTTTCAGAACGAG	347
15-C	ATTTGAATACTACAGTGTTACCC	CTTGTATTCTAATTTGGCATAAGG	398
15-D	CTGCCCATACACATTCAAACAC	TGTTTGGGTCTTGCCCATCTT	382
15-E	AGTCTTAAATATTCAGATGAGCAG	GTTTCTCTTCATTATATTTTATGCTA	430
15-F	AAGCCTACCAATTATAGTGAACG	AGCTGATGACAAAGATGATAATG	435
15-G	AAGAAACAATACAGACTTATTGTG	ATGAGTGGGGTCTCCTGAAC	382
15-H	ATCTCCCTCCAAAAGTGGTGC	TCCATCTGGAGTACTTTCTGTG	421
15-I	AGTAAATGCTGCAGTTCAGAGG	CCGTGGCATATCATCCCCC	515
15-J	CCCAGACTGCTTCAAAATTACC	GAGCCTCATCTGTACTTCTGC	318
15-K	CCCTCCAAATGAGTTAGCTGC	TTGTGGTATAGGTTTTACTGGTG	352
15-L	ACCCAACAAAAATCAGTTAGATG	GTGGCTGGTAACTTTAGCCTC	415
15-M	ATGATGTTGACCTTTCCAGGG	ATTGTGTAACTTTTCATCAGTTGC	251
15-N	AAAGACATACCAGACAGAGGG	CTTTTTTGGCATTGCGGAGCT	339
15-O	AAGATGACCTGTTGCAGGAATG	GAATCAGACGAAGCTTGTCTAGAT	292
15-P	CCATAGTAAGTAGTTTACATCAAG	AAACAGGACTTGTACTGTAGGA	411
15-Q	CAGCCCCTTCAAGCAAACATG	GAGGACTTATTCCATTTCTACC	373
15-R	CAGTCTCCTGGCCGAAACTC	GTTGACTGGCGTACTAATACAG	362
15-S	TGGTAATGGAGCCAATAAAAAGG	TGGGAGTTTTCGCCATCCAC	307
15-T	TGTCTCTATCCACACATTCGTC	ATGTTTTTCATCCTCACTTTTTGC	302
15-U	GGAGAAGAACTGGAAGTTCATC	TTGAATCTTTAATGTTTGGATTTGC	402
15-V	TCTCCCACAGGTAATACTCCC	GCTAGAACTGAATGGGGTACG	276
15-W	CAGGACAAAATAATCCTGTCCC	ATTTTCTTAGTTTCATTCTTCCTC	334

Table B2: List of primers used for *APC* minigene construction via SOE-PCR method.

Primer name	Overlapping primer sequence (5'-3')	Expected size (bp)
Ex7F	GTAGACGCG**GAATTC**AGTCGACCGCCAATCGTACTGGAG	440
Ex7R	AGTATGTTGGTACTGAATGCTTCTGG	
Ex8F	TACCAACATACTTAGTAAGCGTATAGGT	424
Ex8R	AGTTGGAACTCCTGGCCTCAAGTGATCCAC	
Ex9F	GGAGTTCCAACTTATCTAGGCAAACAGCAC	692
Ex9IntR	GTCCATGCCTCGTTCATGAGCTTCCTGCCA	
Ex9IntF	TGGCAGGAAGCTCATGAACCAGGCATGGAC	330
Ex9R	ACGTTCACAA**GAATTC**TCTAGCTCTACTAAGGCCCTAC	

Note: Bold letters represent the cut site for Eco*RI incorporated into the primers*

Table B3: List of primers used for site-directed mutagenesis method.

Primer name	Primer sequence (5'-3')
SDM-F	TAGGGTTCAACTACA**T**GAATGGACCATGAAACAGC
SDM-R	GCTGTTTCATGGTCCATTC**A**TGTAGTTGAACCCTA

Note: Bold letters represent the mutated nucleotide incorporated into the primers

Appendix C

Genomic sequences of exon 8 and exon 13 of the *APC* gene.

(a) Exon 8

```
127801 catacttagt aagcgtatag gtaaaaaata ttttgaacag ttataatggt catactttta

127861 tgatgtattt aattgtttat catacagaca cttcatttgg agtaccttaa catgatgtta

127921 tctgtattta cctatagtct aaattatacc atctataatg tgcttaattt ttagggttca

                c.847C>T                              c.875_876InsT
127981 actacaC/Tgaa tggaccatga aacagccagt gttttTgagtt ctagtagcac acactctgca

128041 cctcgaaggc tgacaagtca tctgggaacc aaggtaacag aagattacaa accctggtca

128101 ctaatgccat gactactttg ctaagacatt cttggccagg tgcagtggct cacacctgta

128161 atcccagcat tttgggaggc caaggcaggt ggatcacttg aggccaggag ttcaagacca
```

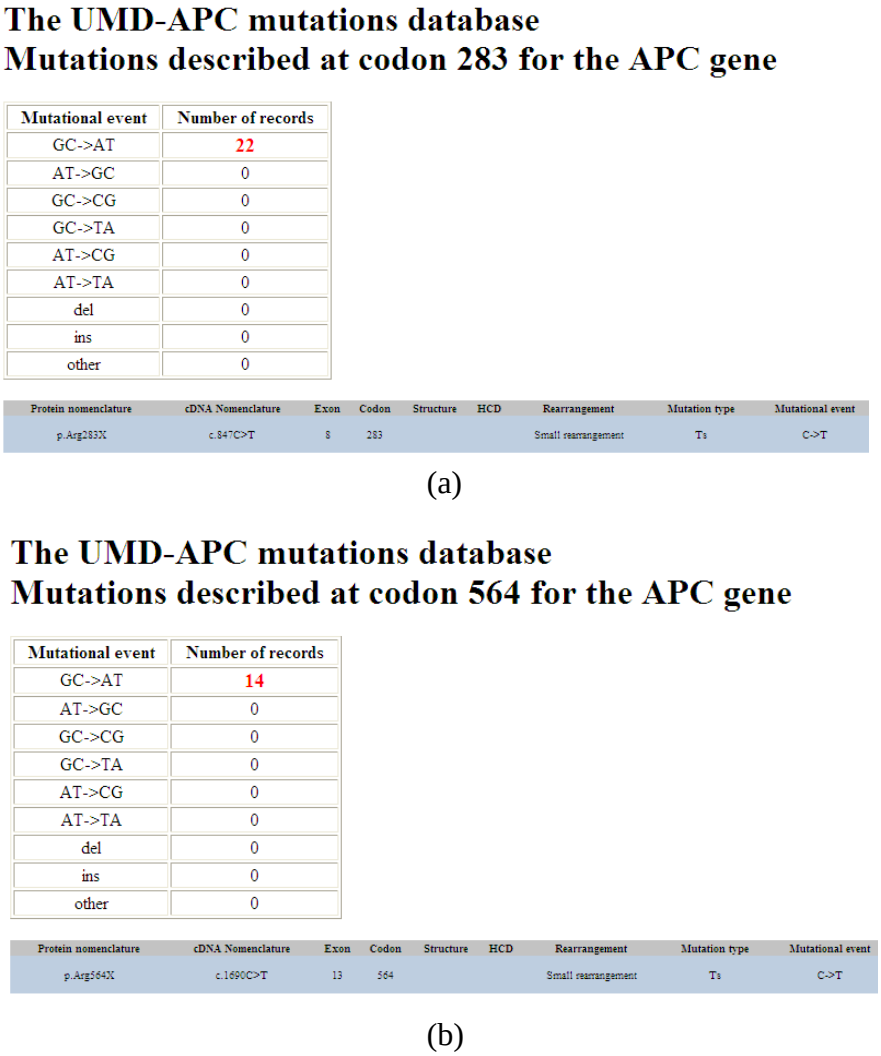
(b) Exon 13

```
141121 ggtctcactg tgttacccag aaggtcttga actcctggtc tcaggagatc ctcctgcctc

141181 agcctcccaa agtgatagga ttacaggcgt gagtcaccac ggctagccag aatttctttc

141241 ttaatagatt tctattctta ctgctagcat taaaaacaaa aaagcaacta gtatgatttt

                                                      c.1635G>A
141301 atgtataaat taatctaaaa ttgattaatt tgcaggttat tgcG/Aagtgtt ttgaggaatt

                                                  c.1690C>T
141361 tgtcttggcg agcagatgta aatagtaaaa agacgttgC/Tg agaagttgga agtgtgaaag

141421 cattgatgga atgtgcttta gaagttaaaa aggtaccttt gaaaacattt agtactataa

141481 tatgaatttc atgtttggct ttttttgct gccttctttt agccatgaga tttcctaatt

141541 tcttacctgt gtattattca gtactataat atgaatttca tgtttagctt tttttgctgc

141601 cttcttttag ccatgagatt ccctaatttc ttttttgaga tggggtctct ttctctcgcc
```

Figure C1:Excerpts from NCBI Genbank (NCBI Reference Sequence: NG_008481.4) showing genomic sequences of (a) exon 8 and (b) exon 13 of the *APC* gene. The coding regions are underlined while mutations and SNP found in this study are highlighted in red with their respective mutation nomenclatures.

Appendix D

Query results from online *APC* mutation databases.



The UMD-APC mutations database
Mutations described at codon 283 for the APC gene

Mutational event	Number of records
GC->AT	22
AT->GC	0
GC->CG	0
GC->TA	0
AT->CG	0
AT->TA	0
del	0
ins	0
other	0

Protein nomenclature	cDNA Nomenclature	Exon	Codon	Structure	HCD	Rearrangement	Mutation type	Mutational event
p.Arg283X	c.847C>T	8	283			Small rearrangement	Ts	C->T

(a)

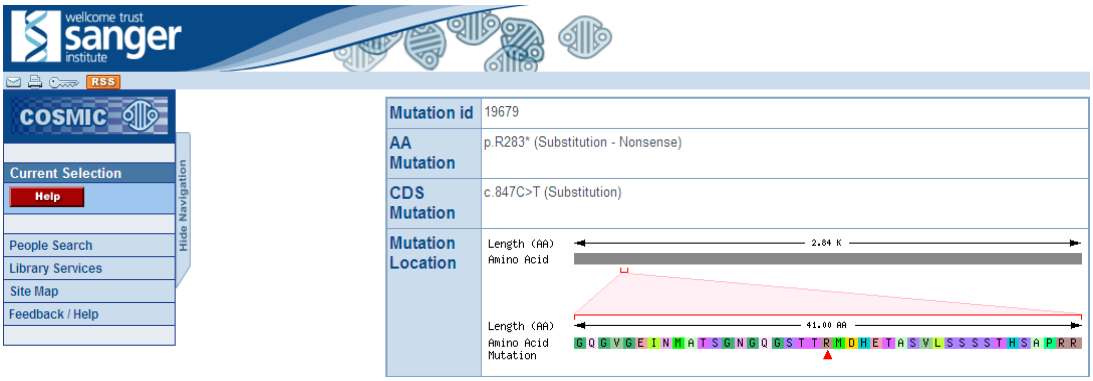
The UMD-APC mutations database
Mutations described at codon 564 for the APC gene

Mutational event	Number of records
GC->AT	14
AT->GC	0
GC->CG	0
GC->TA	0
AT->CG	0
AT->TA	0
del	0
ins	0
other	0

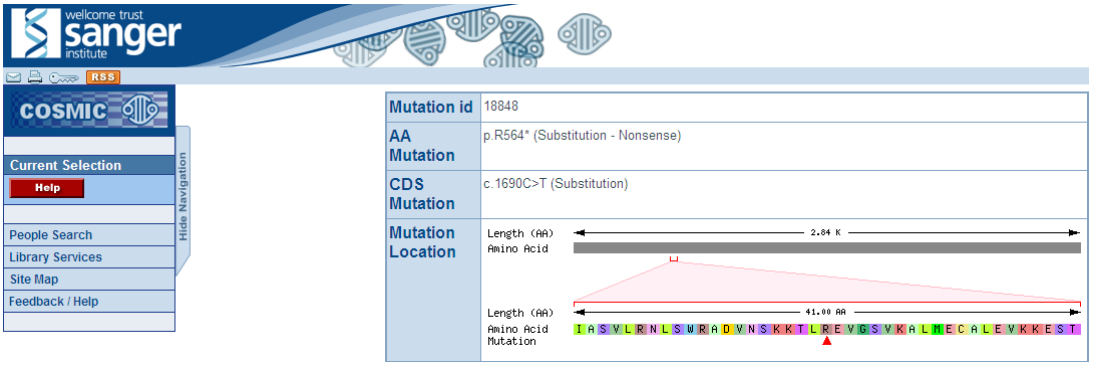
Protein nomenclature	cDNA Nomenclature	Exon	Codon	Structure	HCD	Rearrangement	Mutation type	Mutational event
p.Arg564X	c.1690C>T	13	564			Small rearrangement	Ts	C->T

(b)

Figure D1: Images from the Universal Mutation Database webpage show the result of queries made for (a) mutation c.847C>T and (b) mutation c.1630C>T. The number of reports for each mutation is 22 and 14 respectively. Characterization data of the mutation such as mutational event, mutation type and rearrangement type are also given.



(a)



(b)

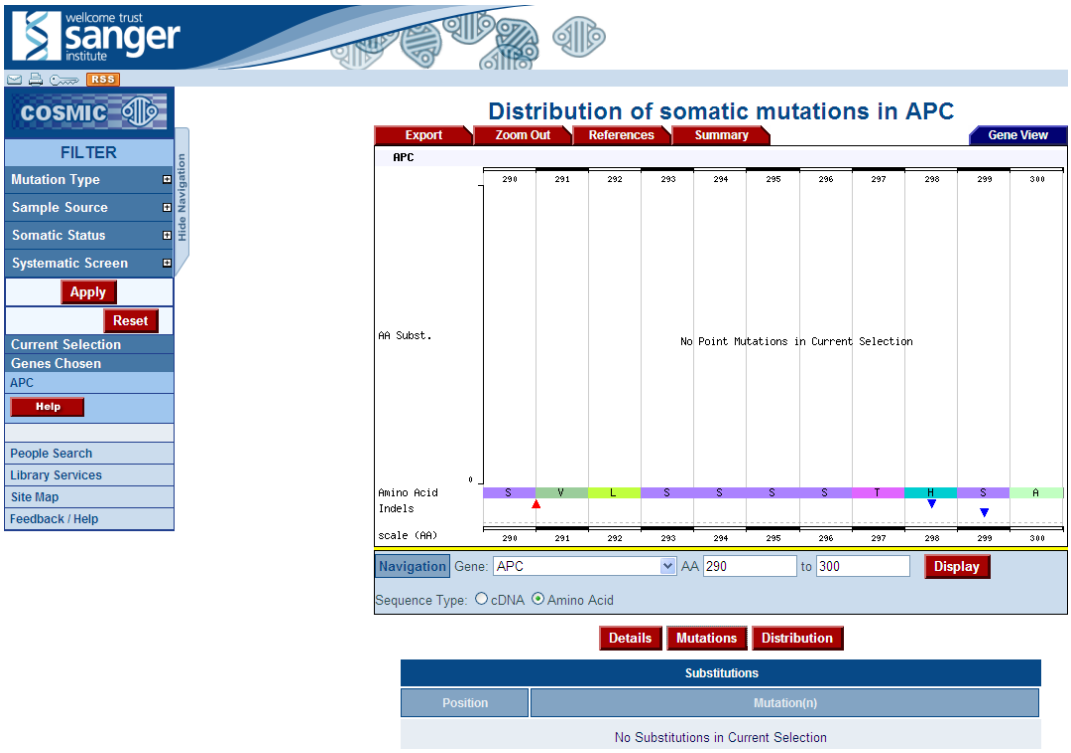
Figure D2: Images from the Catalogue of Somatic Mutation in Cancer database webpage show the results of queries made for (a) mutation c.847C>T and (b) mutation c.1630C>T.

The UMD-APC mutations database
Mutations described at codon 292 for the APC gene

Mutational event	Number of records
GC->AT	0
AT->GC	0
GC->CG	0
GC->TA	0
AT->CG	0
AT->TA	1
del	0
ins	0
other	0

Protein nomenclature	cDNA Nomenclature	Exon	Codon	Structure	HCD	Rearrangement	Mutation type	Mutational event
p.Leu292X	c.875T>A	8	292			Small rearrangement	Tv	T->A

(a)



(b)

Figure D3: Images from (a) Universal Mutation Database webpage and (b) Catalogue of Somatic Mutation in Cancer database webpage show the result of a query made for mutation c.875_876InsT. No record of this mutation was found in both databases.

Appendix E

Proceedings and awards

PROCEEDINGS

Khaidizar, F. D., & Mohamed, Z. (2009, December). *Mutation screening of* APC *gene in Malaysian FAP patients and analysis of the splicing enhancer region.* Oral presentation made at the 14th Biological Sciences Graduate Congress, Bangkok, Thailand.

Khaidizar, F. D., & Mohamed, Z. (2009, May). *Mutation within the splicing enhancer region: Effect of Arg283Ter mutation on the normal splicing as the basis of variable expressivity of FAP.* Oral presentation made at the 3rd Regional Conference on Molecular Medicine, Kota Bharu, Kelantan, Malaysia.

Mohamed, Z., **Khaidizar, F. D.**, & Ng, C. (2008, September). *Mutation in the* APC *gene in families with Familial Adenomatous Polyposis (FAP)*. Poster presented at the Human Genome Meeting 2008, Hyderabad, India.

Khaidizar, F. D., Kam, P. V., & Mohamed, Z. (2008, June). *Mutation screening of the Adenomatous Polyposis Coli gene in FAP-diagnosed patients using Single-Strand Conformation Polymorphism (SSCP) analysis.* Poster presented at the 17th scientific meeting of the Malaysian Society for Molecular Biology and Biotechnology, Kuala Lumpur, Malaysia.

AWARD

Best poster presentation award
Khaidizar, F. D., Kam, P. V., & Mohamed, Z. (2008, June). *Mutation screening of the Adenomatous Polyposis Coli gene in FAP-diagnosed patients using Single-Strand Conformation Polymorphism (SSCP) analysis.* Poster presented at the 17th scientific meeting of the Malaysian Society for Molecular Biology and Biotechnology, Kuala Lumpur, Malaysia.

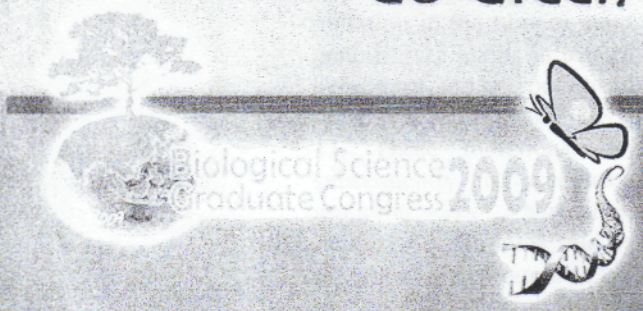
Chulalongkorn University
จุฬาลงกรณ์มหาวิทยาลัย
Pillar of the Kingdom

NUS National University of Singapore

UNIVERSITY OF MALAYA
KUALA LUMPUR

The 14th Biological Sciences Graduate Congress

" Go Green ,Grow Science Together "



Program and Abstracts

December 10-12, 2009

Chulalongkorn University, Bangkok, THAILAND

Hosted by :

Faculty of Science,
Chulalongkorn University, Bangkok, THAILAND

In Collaboration with :

Department o Biological Sciences,
National University of Singapore, SINGAPORE

Institute of Biological Sciences, Faculty of Science,
University of Malaya, MALAYSIA

Sponsored by :

สวทช NSTDA *National Science and Technology Development Agency, THAILAND*

CBB-OR 28

Mutation screening of *APC* gene in Malaysian FAP patients and analysis of the splicing enhancer region

Khaidizar F. D.[1] and Mohamed Z.[1]

[1]Genetics and Molecular Biology Unit, Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia **E-mail: fiqri@um.edu.my**

A set of patients diagnosed with Familial Adenomatous Polyposis (FAP) along with their undiagnosed family members were subjected to genetic screening for mutation in the tumour suppressor gene *Adenomatous Polyposis Coli* (*APC*). The *APC* gene has 15 exons in total that codes for a protein of 2834 amino acid long, in which hereditary and/or sporadic mutations could result in colorectal cancer. Screening was done using PCR-SSCP-DNA sequencing method and revealed mutations in exons 8 and 13. These mutations formed premature transcription termination signals and subsequently produced truncated dysfunctional APC proteins. The exon 8 truncating mutation was of a particular interest of ours due to an earlier case where we observed a man having the same said mutation but was diagnosed as asymptomic, eventhough *APC* mutations was thought to have 100% penetrance. Further investigations revealed that the mutation most probably had abolished an exonic splicing enhancer (ESE) motif, hypothetically causing exon 8 to be excluded from the mature *APC* mRNA transcript. Proof-of-concept experiment was attempted by means of *in vitro* expression of the *APC* minigene in cancer cells lines. A multiple degree of splicing was observed but none exhibited the expected exon exclusion result. Further analysis of this is currently ongoing by using other splicing assays.

MSMBB
MALAYSIAN SOCIETY FOR MOLECULAR BIOLOGY & BIOTECHNOLOGY

MALAYSIAN SOCIETY FOR MOLECULAR BIOLOGY AND BIOTECHNOLOGY

This certificate is awarded to

Fiqri Dizar Khaidizar, Kam Pei Voon and Zulqarnain Mohamed

University of Malaya

as the

BEST POSTER

For poster entitled:

Mutation screening of the Adenomatous Polyposis Coli gene in FAP-diagnosed patients using Single Strand Conformation Polymorphism (SSCP) analysis.

at the 17th Scientific Meeting of MSMBB
(The Saujana Kuala Lumpur Hotel, 23-25th June 2008)

"THE COLOURS OF BIOTECHNOLOGY:
Harnessing The Spectrum For Economic Prosperity"

Associate Professor Dr Fong Mun Yik
President
Malaysian Society for Molecular Biology & Biotechnology

against the cardiovascular, respiratory and neuromuscular depressant effects of *Naja naja sputatrix* (NNS) venom. MPE pretreatment may have a direct effect on rat heart rendering the heart more resistant to venom-induced cardiovascular depressant effects. Alterations of gene expression in the rat heart as a result of MPE pretreatment was examined using microarray and real time PCR. Alpha synuclein, natriuretic peptide precursor, calsequestrin and triadin were found to be upregulated as a result of MPE pretreatment and may contribute to the direct protective action of MPE as they are either involved in intracellular calcium regulation or maintenance of cardiovascular homeostasis. The genes related to energy supply that were up-regulated include desmin, phosphofructokinase, branched chain amino acid transferase I, basigin, lysyl oxidase and aminolevulinic acid synthase. The up-regulation of genes related to energy production and metabolism probably also plays a role in maintaining the viability of the heart

R8) **GENE EXPRESSION PROFILING OF BRONCHIAL SMOOTH MUSCLE CELLS AND HUMAN LUNG FIBROBLASTS AFTER STIMULATION WITH A CYTOKINE COCKTAIL**

Froemming GA, Jaafar F, Harun R and Mohamed Said MS
Institute of Medical Molecular Biotechnology (IMMB), Faculty of Medicine, UiTM,
UKM Medical Molecular Biology Institute (UMBI), HUKM & Faculty of Applied Sciences, UiTM

Introduction: Structural airway cells like bronchial smooth muscle cells (BSMC) and lung fibroblasts (NHLF) are able to secrete inflammatory molecules e.g. cytokines or express adhesion molecules which attract inflammatory cells into the airways. Instead of looking at the effect of single cytokines we looked at a cocktail of cytokines indicated in the progression of asthma. **Objectives:** To evaluate overall changes in gene expression in cytokine stimulated BSMC and NHLF using microarray and to apply different strategies in data analysis to discover expression patterns causing airway remodeling. **Material and Methods:** Microarray analysis was conducted on BSMC and NHLF stimulated for 1 and 24h with a cocktail of IL1β, IL4, IL5 and IL13 (10ng/ml each). After confirmation through real time PCR, sets of significant genes were subjected to pathway analysis. **Results:** Analysis of the overall change in BSMC and NHLF showed 594 and 472 genes with at least a 2 fold change. Up-regulated were chemokines like CCL11, CCL5, CCL7 and 8, inflammatory cytokines (IL1, IL6 and IL8)) and cell adhesion molecules (ICAM-1, VCAM-1). A significant difference in expression could be seen between 1 and 24h as well as between the two cell types. **Conclusions:** The expression of cytokines, chemokines and adhesion molecules by BSMC and NHLF indicate an active involvement in airway remodeling whereby ICAM-1 and VCAM-1 play a critical role by offering docking possibilities for lymphocytes.

R9) **Mutation screening of the Adenomatous Polyposis Coli gene in FAP-diagnosed patients using Single Strand Conformation Polymorphism (SSCP) analysis.**

Fiqri Dizar Khaidizar, Kam Pei Voon and Zulqarnain Mohamed
Unit of Genetics and Molecular Biology, Institute of Biological Sciences, Faculty of Science,
University of Malaya, 50603 Kuala Lumpur, Malaysia.

Mutations found in the Adenomatous Polyposis Coli (*APC*) gene are the primary causative factors for Familial Adenomatous Polyposis (FAP). The effects of these mutations are manifested in the form of polyp formation, hundreds to thousands in numbers, carpeting the lumen of the colon and rectum. Failure in early detection would result in the polyps progressing into malignant tumors. Thus, presymptomic diagnosis of FAP by mutation screening of the *APC* gene would greatly benefit high-risk family members of affected patients. *APC* gene was isolated from DNA samples of clinically diagnosed FAP patients by PCR. The isolated *APC* gene fragments were then subjected to Single Strand Conformation Polymorphism (SSCP) analysis. In principle, DNA strands with different nucleotide composition will have different conformation structure that will be visualised in different banding patterns on a non-denaturing acrylamide gel. Out of 15 exons analysed, SSCP mobility shifts were observed for exons 8 and 13 of the FAP patients compared to a non-FAP control. Potentially, these exons may harbor mutations that alter the transcript of the gene rendering it dysfunctional. Sequence analysis would further corroborate current results obtained.

THE COLOURS OF BIOTECHNOLOGY

Harnessing the Spectrum for Economic Prosperity

23 - 25 June 2008

The Saujana Kuala Lumpur

Officiated by

Y.Bhg Academician Tan Sri Emeritus Professor Datuk Dr. Augustine S. H. Ong
Senior Fellow, Academy of Sciences Malaysia

MSMBB

The present study was carried out to characterize the causative genetic mutation in several medium sized Malaysian families affected with FAP. Mutation screening was performed using the SSCP analysis technique, after which the exons showing mobility shifts (variant bands) were sequenced to determine the nature of the sequence change. SSCP results showed mobility shifts various exon, including 8, 11, 13 and 15. Sequence analysis revealed polymorphisms (exon 15) and patient specific mutations (in exons 11, 13 and 15) which have proved valuable for presymptomatic diagnosis of at-risk family members.

POSTER NO: 296
Gene expression profiling of the hepatic transcriptome in the presence of TNF α

Neha Munjal, Amit Pandey, Malabika Datta
Institute of Genomics and Integrative Biology, Near Jubilee Hall, Mall Road, Delhi-110 007, India

Diabetes mellitus, often simply termed Diabetes, is a syndrome characterized by disordered metabolism and high blood sugar. It is caused due to low levels of insulin hormone or from abnormal resistance to insulin in its target tissues. World Health Organization estimates that India will alone have 79.4 million diabetic patients in 2030. One of its major form Type 2 diabetes, is often associated with obesity, hypertension, elevated cholesterol and metabolic syndrome. Changes in life style, such as consumption of high-calorie diet and lack of exercise, have increased the global prevalence not only of diabetes but also of obesity. Type 2 diabetes is characterized by insulin resistance in target tissue, occurs due to several reasons and one of them being the proinflammatory cytokine, TNFα. It is also known as the link between diabetes and obesity. High levels of TNFα interfere with insulin signaling to cause the effect and to further investigate into the situation, gene transcription profiling was examined in control and TNFα treated HepG2 cells. Results indicated that TNFα could significantly alter the expression of a significant number of genes that were identified to be related to lipid and fat metabolism on one hand and to immunoglobulin receptor activity and IgE binding thereby on the other thereby indicating global dysregulation of fat metabolism and compromise in immune defense mechanism(s) within the hepatocyte by TNFα. Pathway analysis revealed 'biosynthesis of steroids' to be most effected. All these indicate TNFα to be significantly altering the transcriptome profiling within HepG2 cells with genes involved in lipid and steroid metabolism being the most favoured and this could explain one of the underlying mechanisms of TNFα action in the liver.

POSTER NO: 297
Differntially expressed transcripts in the adipose tissue of 'sumo rats' (WNIN/Ob)

Srivani Muthuswamy, Sudip Ghosh, NV Giridharan, NZ Ehtesham
National Institute of Nutrition (ICMR), Jamai Osmania PO, Tarnaka, Hyderabad, 500007, India

The etiology of obesity and associated disorders involve very complex gene-gene and gene-environment interactions, making it a formidable challenge to study their molecular mechanisms in human. Therefore, the best way to study the pathophysiology of these disorders is to employ various animal models of genetic and non-genetic animal models of obesity. A rat model of obesity has been developed at the National Institute Nutrition, Hyderabad. These 'sumo rats' (WNIN/Ob), originally identified from an inbred Wistar rat line, attain bodyweight more than twice of their lean littermates, are also hyperphagic, euglucaemic. They also show hyperinsulinaemia, hypertrigleridaemia and hypercholerolaemia characteristic to human obesity. To identify the transcripts that are over or under expressed in the adipose tissue of these rats, we carried out forward and reverse subtraction hybridization PCR using the RNA isolated from the adipose tissue of the sumo rats and their lean littermates. Genes identified to be overexpressed in the adiopose tissue of these obese rats include members from a wide range of gene families like lipid and carbohydrate metabolism, electrolyte transporters, general and specific transcription factors, signal transducers etc. Some of these genes were previously known to be over expressed in obesity whereas others are so far not implicated in the development of obesity. Functional characterization of these genes are in progress using siRNA and transient over expression studies.

POSTER NO: 298
Attenuation of hepatic insulin sensitivity by TNFα

Amit K Pandey, Gaurav Verma, Malabika Datta
Institute of Genomics and Integrative Biology, Mall Road, Delhi-110 007, India

Type 2 diabetes is almost invariably associated with obesity and the adipose tissue that was originally identified as an inert storage organ, is now appropriately classified as an endocrine organ. Several factors have been identified as being released from the adipocytes and their circulatory levels have been found to correlate proportionately to the adipocyte mass and the associated status of insulin resistance. Tumor necrosis factor alpha (TNF- alpha) is one such factor that is increased under these conditions and it inhibits insulin signaling by interfering at several points of the signaling cascade. Of the several insulin target tissues, the liver is critical in maintaining circulatory glucose levels through hepatic glucose output and alterations in this phenomenon aggravates an already existing hyperglycemic status as observed in obese diabetics. Using the human hepatoma (HepG2) cell line, we studied the role of TNF alpha in the regulation of this pathway and determined the molecular mechanisms underlying the effect(s) of TNF-alpha in insulin action on hepatic gluconeogenesis. TNF-alpha significantly attenuated insulin induced inhibition of the expression of gluconeogenic enzymes and hepatic glucose production. Since the transcription factor, Foxa2 has in part been implicated in the regulation of gluconeogenic gene's transcription, we studied the effects of TNF-alpha and/or insulin on its cellular status in the hepatocyte. Preincubation of HepG2 cells with TNF-alpha followed by insulin significantly narrowed down insulin mediated exclusion of Foxa2 from the nucleus thereby substantially increasing its nuclear concentration and this possibly is responsible for the varied effects on gluconeogenesis and hepatic glucose output. TNF-alpha thus significantly abrogates insulin signaling in HepG2 cells leading to an increased nuclear presence of Foxa2 and subsequent elevated expression of gluconeogenic gene and glucose production. These results explain one of the mechanisms behind unrestrained hepatic glucose output that exaggerates an existing hyperglycemic status as observed in diabetic individuals.

POSTER NO: 299
Balancing the role of gene and environment: High-altitude adaptation and mal-adaptation

[1]MA Qadar Pasha, [1]Tsering Stobdan, [2]Tsering Norboo, [3]Gulam Mohammad, [3]Mohammad Iqbal, [3]Tashi Thinles
[1]*Institute of Genomics and Integrative Biology, Functional Genomics Unit, Institute of Genomics and Integrative Biology, Delhi, India,* [2]*Ladakh Institute of Prevention, Ladakh Inst of Prevention, Leh, J and K, India,* [3]*SNM Hospital, SNM Hospital, Leh, J and K, India*

High-altitude (HA) adaptation/mal-adaptation is a multifactor trait to which genetic and environmental factors contribute interactively. The traditional candidate gene approach for identifying molecular variants having functional role and associating with HA adaptation and disorders such as High-altitude pulmonary edema (HAPE) have achieved considerable success in elucidating individual's susceptibility. Identification of candidate genes still poses a great challenge. Since at HA, the adaptation/mal-adaptation is mainly characterized by induced pulmonary vasoconstriction, endothelial dysfunction and intra-vascular fluid retention, the genes involved in maintaining pulmonary vascular tone could be possible candidates.

In a comparative study of highland (HL), lowland (LL) natives, and case-control i.e. HAPE patients-HAPE resistant sojourners, we investigated the polymorphisms insertion/deletion (I/D) (GenBank accession no X62855) of ACE, G-6A (rs5049); T174M (rs4762) and M235T (rs699) of AGT; the G894T (rs1799983), 27 base pair 4b/4a (Ensembl Gene ID-ENSG00000164867), -922 A/G (rs1800779) and -786 T/C (rs3918161) of NOS3; the -344T/C (rs1799998), intron-2 conversion (Iw/Ic) (NCBI accession No. NW_924018) and Lys173Arg (rs4539) of CYP11B2 and (CT)n-(CA)n repeat (GenBank accession No. J05008), -3A/-4A (rs10478694), G2288T (rs2070699) and Lys198Asn (rs5370) of EDN1. Individual allele/genotype, combinations of genotypes, haplotypes, gene-gene interactions and relevant biomarkers were analyzed.

The allele/genotype distribution at the same locus varied significantly between different groups. The I, 894G and 4b, 2288G and longer repeats of -3A/-4A, and -344T allele frequencies were higher in HL than the LL ($p<0.05$). Whereas, over-

POSTER NO: 292
CYP17A1 (T-34C), CYP19A1 (Trp39Arg), and FGFR2 (C-906T) polymorphisms and the risk of Breast Cancer in South Indian Women Population

Samson Mani, Rama Ranganathan, Swaminathan Rajaraman, Sridevi Veluswami, Nirmala nancy Karunakaran, Rajkumar Thangarajan
Cancer Institute (WIA), # 38 Sradar Patel Road, Chennai 600036, India

Breast Cancer is initiated by exposure to endogenous and exogenous estrogens. Genes involved in biosynthesis of estrogens and growth factor receptors play an important role in breast cancer development. A case (n=250) - control (n=500) study was undertaken to investigate the role of Single Nucleotide Polymorphisms (SNP's) in CYP17A1 (T-34C), CYP19A1 (Trp39Arg) and FGFR2(C-906T). Genotyping was done using Taqman Allelic discrimination assay for CYP17A1 (T-34C) and FGFR2 (C-906T) and PCR-CTPP for CYP19A1 (Trp39Arg). There was a significant association of heterozygous (TT/CC) genotype of CYP17A1 gene with the risk of developing breast cancer (OR=0.68, 95% CI: 0.49-0.96). And the same genotype of the CYP17A1 gene was significantly associated with deceased risk in postmenopausal women (OR=0.56, 95% CI: 0.35-0.89) (p=0.015). CYP19A1 (Trp39Arg) is a rare polymorphism, all the cases were homozygous for wild type Trp allele (100%); in controls 99.2% were homozygous for wild type and 0.8% was heterozygous. We are unable to detect the variant form of the CYP19A1 gene in south Indian women population. There was no significant association between the risk of breast cancer and FGFR2 (C-906T), which is supposed to be a newly identified gene linked with breast cancer incidence in western population. These results suggest that CYP17A1 TT/CC genotype was associated with decreased risk for breast cancer especially in post menopausal women. CYP19A1 (Trp39Arg) and FGFR2 (C-906T) have no role to play with breast cancer risk in South Indian women population, further studies with more cases and control are needed to evaluate the role of these genes risk in South Indian women population.

POSTER NO: 293
Clinical, biochemical and genetic analysis of Leigh syndrome patients with atypical presentation

[1]Shalini Mani, [2]Karuna Madap, [1]Kranthi Kumar Kranthi Kumar, [2]S Narsimha Rao, [1]GR Chandak
[1]*Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad, India,* [2]*Niloufer Hospital for Women and Children, Red Hills, Hyderabad, India*

Leigh syndrome is a progressive neuro-metabolic mitochondrial disorder usually presenting in infancy or early childhood with varied clinical features and evidence of genetic heterogeneity. Leigh syndrome in India has hardly been explored and hence, we attempted to understand its clinical, biochemical, imageological, and genetic basis in 165 patients from South India. All the patients were infants, presented in acute life threatening condition and responded dramatically towards thiamine supplementation. Electron microscopic and histochemical analyses revealed the structural and functional defects in mitochondria. All the investigations, suggested a different phenotype of Leigh syndrome in Indians.

To investigate the genetic basis of this phenotype, complete mitochondrial genome and nuclear genes encoding components of respiratory complexes were screened in the patients and 94 normal infants. Based on bioinformatics analysis using Clustal W, SIFT and PolyPhen, seven mutations in different genes were investigated using in-vitro assays to understand likely mechanism responsible for the phenotype. Effect of mitochondrial mutations were analysed by generating cybrids while SURF1 mutations were analyzed using wild and mutant SURF1 cDNA constructs followed by preparation of stable clone in COS-7 cells. Two mutations, G6036A (G45S) in MT-CO2, found in three patients and (exon 9, C>T) P298L in SURF1, present in 6 patients were predicted to affect the conserved residues and affect the stereochemical property of the protein. Both mutations showed ~50% decrease in Complex IV activity suggesting that defect in complex IV may be one of the major causes for such a phenotype.

Since majority of patients responded to thiamine supplementation, estimation and comparison of thiamine level in blood samples of patients and randomly selected normal individuals from same geographical region suggested that thiamine deficiency alone could not cause the disease but may add to the severity of the phenotype.

To conclude, our study involving the largest cohort of LS patients gives an idea about the atypical presentation of Leigh Syndrome patients in Indian population and shows a variable genetic basis. It also suggests that thiamine deficiency could be an additional factor, influencing the phenotype in the presence of genetic abnormality. In addition, it also alerts the clinicians towards the importance of thiamine supplementation for such a phenotype, as timely thiamine supplementation can save several precious lives.

POSTER NO: 294
Multiple HLA-DR3 haplotypes associated with autoimmunity in North Indians

[1]NK Mehra, [1]Neeraj Kumar, [1]Gurvinder Kaur, [2]Nikhil Tandon
[1]*Dept Transplant Immunology and Immunogenetics, All India Institute of Medical Sciences, New Delhi, India,* [2]*Dept Endocrinology and Metabolism, All India Institute of Medical Sciences, New Delhi, India*

The aim of the study was to define HLA-DR3 positive extended haplotypes associated with susceptibility to Type 1 diabetes in North India. We evaluated multiple SNPs and microsatellites located between HLA-A and DQB1 locus in the MHC region in 145 North Indian T1D patients. The MHC halotypes were deduced from family pedigrees and compared with those prevalent in healthy Indian population. A comparison of frequencies of HLA class I alleles among T1D patients and healthy controls showed a significant increase in HLA-A*02, A*26, B*08, B*50 and B*58 in the patient group. Similarly, analyses of class II genes revealed a strong association of DR3-DQ2 among the patients (75.9% vs 14.6% in controls, p=7.53E-11). Further molecular analyses revealed that there are multiple DRB1*03 positive haplotypes (predominantly B8-DR3, B50-DR3 and B58-DR3) that are associated with T1D in the Indian population. These haplotypes differ significantly from the classical Caucasian AH8.1 (HLA-A1-B8-DR3), associated with several autoimmune diseases. The Caucasian AH8.1 is rare in the Indian population and has been replaced by a variant AH8.1v and other DR3 positive haplotypes, A26-B8-DR3 (AH8.2), HLA-A24-B8-DR3 (AH8.3), A3-B8-DR3 (AH8.4), A31-B8-DR3 (AH8.5), A2-B8-DR3 (AH8.6), A11-B8-DR3 (AH8.7) and A33-B8-DR3 (AH8.8). Among these, the AH8.2 is the most common haplotype and represents 43% of the total B8-DR3 haplotypes in this population. The Indian B8-DR3 haplotypes differ significantly from Caucasian AH8.1 at multiple loci. For example, the Indian haplotypes have HLA-Cw*0702, HLA-DRB3*0202, HSP70-21267A, TNFA-308G, TNFa 105, Bf- F, C4A-1, MIB 352 as compared to Caucasian HLA-Cw*0701, HLA-DRB3*0101, HSP70-21267G, TNFA-308A, TNFa 99, Bf-S, C4A-0, MIB 350. These differences suggest that B8-DR3-DQ2 haplotypes in the Asian Indian population might have originated independently of Caucasian AH8.1 selectively through recombination and multiple mutations. Among these B8-DR3 haplotypes, a significant association of AH8.2 (p=2.91E-06), AH8.3 (p= 2.01E-05) and AH8.6 (p= 1.13E-07) was observed with T1D. These findings have important implications in understanding disease associations along with their evolutionary significance.

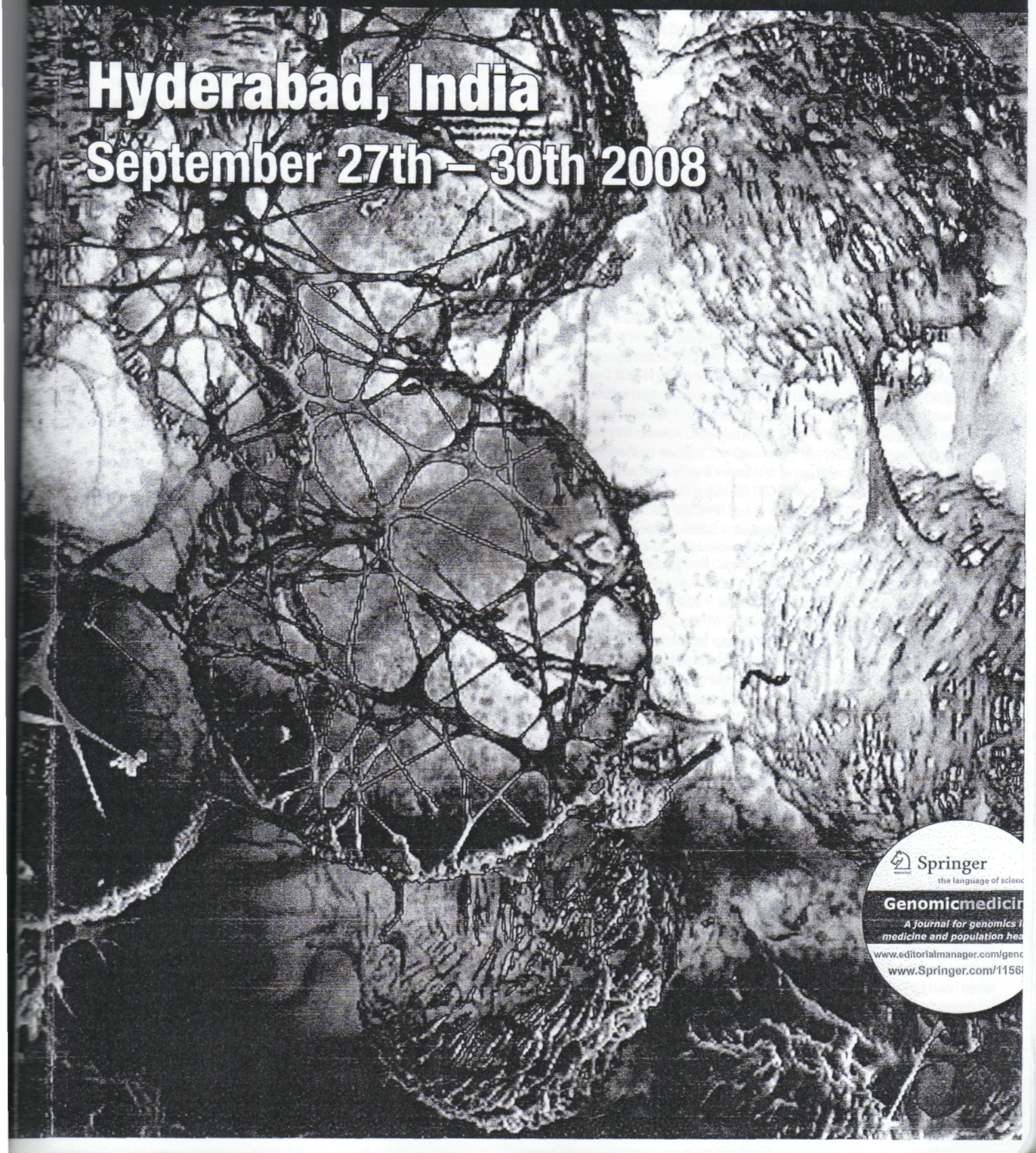
POSTER NO: 295
Mutations in the APC gene in families with Familial Adenomatouos Polyposis (FAP)

[1]Zulqarnain Mohamed, [1]Fiqri-Dizar Khaidizar, [2]Colin Ng
[1]*Institute of Biological Sciences, Faculty of Science, University of Malaya, Malaysia,* [2]*Department of Surgery, University of Malaya Medical Center, Malaysia*

Colorectal cancer has been recognized as one of the more common causes of early death due to malignancy worldwide. About 20% of all colon cancer cases are thought to be hereditary. Two well defined forms of hereditary colon cancer are familial adenomatous polyposis coli (FAP) and hereditary non-polyposis colon cancer (HNPCC). Germline mutations in the tumour-suppressor APC gene, localized on 5q in 1991, are associated with FAP. The vast majority of these mutations are nonsense or frameshifts resulting in non-functional, truncated APC protein products.

HGM2008
HUMAN GENOME MEETING

Programme and Abstract book



Mutation Within The Splicing Enhancer Region: Effects Of Arg283Ter Mutation On Normal Splicing As The Basis Of Variable Expressivity Of FAP

Fiqri Dizar Khaidizar and Zulqarnain Mohamed

Genetics and Molecular Biology Unit, Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.

Introduction: We have observed two unrelated families in which the truncating Arg283Ter mutation in the eighth exon of the Adenomatous Polyposis Coli (*APC*) gene is segregating. One of them is affected with Familial Adenomatous Polyposis (FAP), while the other is declared asymptomic after a thorough clinical diagnosis. This event is quite intriguing, as mutations in the APC gene were regarded as 100% penetrant. Further investigation revealed that the mutation potentially affects an exonic splicing enhancer (ESE) motif, hypothetically causing exon 8 to be excluded from the mature *APC* mRNA transcript. Even though missing the exon, the reading frame is expected to still be retained and producing fuctional APC proteins as the 25 amino acids lost were in a region of unknown obvious function. Similar cases have been reported where aberration of the splicing mechanism influences the manifestation of diseases (Cogan JD, 1997, Barbaux S, 1997 and D'Souza 1999).

Aim: To study the effect of the aforementioned mutation on exon splicing by means of *in vitro* expression.

Methodology: We constructed two minigenes (with and without the mutation) spanning exons 7, 8 and 9 of the *APC* gene. Both constructs were cloned into pTarget expression vectors and were transfected into cultured HepG2 cells. Cells were either left to grow for 48h or were treated to low pH, cold temperature or hyperosmotic culture condition 24h post-transfection mRNAs were collected 48h post-transfection and then subjected to reverse transcription PCR (RTPCR).

Results and Conclusions: Both constructs yielded similar DNA banding patterns on agarose, but varied for each treatment. DNA sequencing revealed the transcripts were indeed the products of different degrees of alternative splicing as predicted. At the moment, work is ongoing to test the reproducibility of the results.

Keywords: *Arg283Ter mutation, Adenomatosus polyposis coli, Constructs*

3rd REGIONAL CONFERENCE ON MOLECULAR MEDICINE 2009

Genomics, Proteomics and ... The 'omics' : DREAMS vs REALITY

• 2 - 4 May 2009 •
• Renaissance Hotel, Kota Bharu, Kelantan •
• Malaysia •

PROCEEDINGS

Organized by :
Institute for Research in Molecular Medicine (INFORMM), USM
In collaboration with
Universiti Teknologi MARA (UiTM)
Malaysian Society for the Advancement of Pharmacogenetics (MSAP)

USM
Universiti Sains Malaysia

iNFORMM
Institute for Research in Molecular Medicine

Universiti Teknologi MARA

MSAP

UNIVERSITI MALAYA

PERAKUAN KEASLIAN PENULISAN

Nama: (No. K.P/Pasport:)

No. Pendaftaran/Matrik:

Nama Ijazah:

Tajuk Kertas Projek/Laporan Penyelidikan/Disertasi/Tesis ("Hasil Kerja ini"):

Bidang Penyelidikan:

Saya dengan sesungguhnya dan sebenarnya mengaku bahawa:

(1) Saya adalah satu-satunya pengarang/penulis Hasil Kerja ini;
(2) Hasil Kerja ini adalah asli;
(3) Apa-apa penggunaan mana-mana hasil kerja yang mengandungi hakcipta telah dilakukan secara urusan yang wajar dan bagi maksud yang dibenarkan dan apa-apa petikan, ekstrak, rujukan atau pengeluaran semula daripada atau kepada mana-mana hasil kerja yang mengandungi hakcipta telah dinyatakan dengan sejelasnya dan secukupnya dan satu pengiktirafan tajuk hasil kerja tersebut dan pengarang/penulisnya telah dilakukan di dalam Hasil Kerja ini;
(4) Saya tidak mempunyai apa-apa pengetahuan sebenar atau patut semunasabahnya tahu bahawa penghasilan Hasil Kerja ini melanggar suatu hakcipta hasil kerja yang lain;
(5) Saya dengan ini menyerahkan kesemua dan tiap-tiap hak yang terkandung di dalam hakcipta Hasil Kerja ini kepada Universiti Malaya ("UM") yang seterusnya mula dari sekarang adalah tuan punya kepada hakcipta di dalam Hasil Kerja ini dan apa-apa pengeluaran semula atau penggunaan dalam apa jua bentuk atau dengan apa juga cara sekalipun adalah dilarang tanpa terlebih dahulu mendapat kebenaran bertulis dari UM;
(6) Saya sedar sepenuhnya sekiranya dalam masa penghasilan Hasil Kerja ini saya telah melanggar suatu hakcipta hasil kerja yang lain sama ada dengan niat atau sebaliknya, saya boleh dikenakan tindakan undang-undang atau apa-apa tindakan lain sebagaimana yang diputuskan oleh UM.

Tandatangan Calon Tarikh

Diperbuat dan sesungguhnya diakui di hadapan,

Tandatangan Saksi Tarikh

Nama:
Jawatan:

UNIVERSITI MALAYA

ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: FIQRI DIZAR BIN KHAIDIZAR (I/C No: 840724-14-6155)

Registration/Matric No: SGR 080132

Name of Degree: MASTER OF SCIENCE

Title of Project Paper/Research Report/Dissertation/Thesis ("this Work"):

MUTATION SCREENING AND ANALYSIS OF THE *APC* GENE IN MALAYSIAN FAP PATIENTS

Field of Study: MOLECULAR GENETICS

I do solemnly and sincerely declare that:

(1) I am the sole author/writer of this Work;
(2) This Work is original;
(3) Any use of any work in which copyright exists was done by way of fair dealing and for permitted purposes and any excerpt or extract from, or reference to or reproduction of any copyright work has been disclosed expressly and sufficiently and the title of the Work and its authorship have been acknowledged in this Work;
(4) I do not have any actual knowledge nor do I ought reasonably to know that the making of this work constitutes an infringement of any copyright work;
(5) I hereby assign all and every rights in the copyright to this Work to the University of Malaya ("UM"), who henceforth shall be owner of the copyright in this Work and that any reproduction or use in any form or by any means whatsoever is prohibited without the written consent of UM having been first had and obtained;
(6) I am fully aware that if in the course of making this Work I have infringed any copyright whether intentionally or otherwise, I may be subject to legal action or any other action as may be determined by UM.

Candidate's Signature Date

Subscribed and solemnly declared before,

Witness's Signature Date

Name:
Designation: