

Volume 15 Supplement 1

ISSN 13-94-195X

2008

**MJMS**

THE MALAYSIAN JOURNAL OF  
**MEDICAL  
SCIENCES**

PENERBIT  
**U&M**

**OB-14**

**GENETIC RELATIONSHIP & DISTRIBUTION OF ANCESTRAL GENETIC COMPONENT AMONG PENINSULAR MALAYSIA MALAY SUB-ETHNIC GROUPS**

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**Objectives:** To determine a probabilistic genetic structure of 4 Malay sub-ethnic groups in Peninsular Malaysia which are *Melayu Kelantan*, *Minang*, *Jawa* and *Bugis* as well as to infer the population ancestry based on the single nucleotide polymorphism (SNP) microarray multilocus genotype data.

**Patients and Method:** The analysis of genotype data of these 4 Malay sub-ethnic groups were compiled together with 11 other populations' genotype data from Indonesia, China, India, Africa and *Orang Asli* sub-groups in Peninsular Malaysia obtained from the Pan Asian SNP Initiative (PASNPI) Database. Two major approaches which are distance-based clustering method and model-based clustering method were implemented by several specific bioinformatics tools such as PEAS v1.0, MEGA 4 and STRUCTURE v2.2. Phylogenetic tree for 4 genetic distances algorithm were constructed by Neighbor Joining method using all 54,794 autosomal SNPs encompassing the entire genome which are shared by 434 individuals. Bootstrapping was done for 1000 replications and clades with bootstrap values less than 80% were condensed. STRUCTURE analysis for 5 dataset running with 20,000 burn-in period and 20,000 MCMC iterations from K=2 to K=9 were done using admixture model and assuming that allele frequencies were correlated.

**Results:** All resulted phylogenetic tree performed more than 95% of bootstrap value at each node with very similar topologies. The phylogenies showed that *Jawa*, *Bugis* and *Minang* have a very close relationship and tend to cluster together with Indonesians, meanwhile the position of *Melayu Kelantan* is far apart on the tree indicated that they are not sharing the most recent common ancestral. According to the distribution of Ln Probability and estimated membership coefficient (Q) from STRUCTURE, the most probable and appropriate number of clusters in the 15 populations should be 6 (K=6) and all the studied Malay and Indonesian population sub-groups are in the same cluster but the *Melayu Kelantan* are still slightly different from the other modern Malays.

**Discussion and Conclusion:** The individual and population ancestry of all studied population also been inferred from the Q plot and knowledge of individual ancestry will be important for biomedical studies.

**OB-15**

**GROWTH CHARACTERISTICS OF HYPERTROPHIC SCAR FIBROBLASTS AND NORMAL HUMAN SKIN CULTURE CELLS**

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**Objective:** To compare the growth characteristics of hypertrophic scar fibroblasts and normal human skin culture cells.

**Patients and Method:** Hypertrophic scar fibroblasts were cultured near the excised scar and maintained in DMEM supplemented with 10% FBS. Normal human skin culture fibroblasts (nHDF) were cultured in 5ml medium in 25cm<sup>2</sup> flasks for 7 days and the number of cells was counted using a haemocytometer according to the manufacturer's instructions.

**Results:** Mean cell count of hypertrophic scar fibroblasts was  $1.2 \times 10^4$  cells/ml, 12.0% respectively. In contrast, normal human skin culture cells were  $7.3 \times 10^4$  cells/ml and  $26.4 \times 10^4$  cells/ml respectively.

**Discussion and Conclusion:** There is a significant difference in growth characteristics between hypertrophic scar fibroblasts and normal human skin culture cells. The growth characteristics of hypertrophic scar fibroblasts are significantly different from normal skin culture cells.

**OB-15**

**GROWTH CHARACTERISTIC PATTERN OF NORMAL DERMAL FIBROBLASTS AND HYPERTROPHIC SCAR FIBROBLASTS IN PRIMARY HUMAN SKIN CULTURE**

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**Objective:** To compare the growth characteristic of human skin fibroblasts derived from normal and hypertrophic scar culture.

**Patients and Method:** Discarded tissues from surgical procedures in 5 patients with hypertrophic scars were sampled. In addition, a small excision biopsy of normal skin near the excised scar was obtained as a control from the same patient. Fibroblasts were maintained in DMEM supplemented with 10% Fetal Bovine Serum (FBS) and 1% Penicillin in humidified condition at 37°C with 5% CO<sub>2</sub>. Normal human dermal fibroblasts (nHDF) and hypertrophic scar fibroblasts (hSCF) were seeded at  $1 \times 10^4$  cells in 5ml medium in T75 culture flasks. Flasks were incubated for 1, 2, 3, 4 and 5 days and the number of viable cells of nHDF and hSCF were counted with haemocytometer according to the Trypan Blue Exclusion Test method.

**Results:** Mean cell count for nHDF at day 1, 2, 3, 4 and 5 were  $5.9 \times 10^4$  cells/ml,  $8.1 \times 10^4$  cells/ml,  $12.0 \times 10^4$  cells/ml,  $14.7 \times 10^4$  cells/ml and  $20.4 \times 10^4$  cells/ml respectively. In contrast to nHDF, mean cell count for hSCF at day 1, 2, 3, 4 and 5 were  $7.3 \times 10^4$  cells/ml,  $12.9 \times 10^4$  cells/ml,  $18.1 \times 10^4$  cells/ml,  $21.8 \times 10^4$  cells/ml and  $26.4 \times 10^4$  cells/ml respectively.

**Discussion and Conclusion:** The calculation of cells growth showed a significant difference in growth characteristic between nHDF and hSCF culture. Samples of nHDF and hSCF were used to display a contact inhibition pattern and fibroblasts from hypertrophic scar exhibited linear growth and sustained a higher cellular viability compared to normal skin fibroblasts.

**OB-16**

**MUTATION ANALYSIS OF DYSTROPHIN GENE IN MALAYSIAN DUCHENNE MUSCULAR DYSTROPHY (DMD) PATIENTS**

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**Objective:** To develop a multiplex PCR to detect the distal hotspot of dystrophin gene in Malaysian DMD patients.

**Patients and Method:** The blood of 25 clinically diagnosis DMD patients from varies hospitals in Peninsula Malaysia were taken and sent to Human Genome Center. Two hundred  $\mu$ l of DNA were extracted using QIAGEN kit and analyzed using multiplex PCR to detect deletion in seven exons. Two sets of multiplex PCR were developed to screen these exons, which were exon 43, 44, 45, 46 and 50 for set 1, and exon 49 and 51 for set 2. These exons represent for the distal hotspots of the dystrophin gene.

**Results:** We detected deletions of the distal hotspot of the DMD gene in 13 patients (52%). Out of these, 4 had no family history of DMD. The most frequently deleted exons were exons 49, 50 and 51 with 20% of deletion for each exon. The remaining 12 patients did not show any deletion for these exons. The samples that had no mutations detected using our developed method are planned for further analysis of the mutation in the proximal hotspot, or the mutation might be duplication or point mutation.

**Discussion and Conclusion:** Multiplex PCR assay allows a rapid molecular diagnosis for DMD patients in the country. The deletion frequency of the distal hotspot in our Malaysian DMD patients is similar to the frequency of other population.

**OB-17**

**FRAGILE X SYNDROME IS UNDERDIAGNOSED THROUGH CYTOGENETIC ANALYSIS ALONE: 6 YEARS OF HUSM EXPERIENCE**

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**Objectives:** To detect chromosomal abnormalities of Fragile Xq27.3 in individuals suspected to have Fragile X Syndrome using cytogenetic technique and to investigate the phenotype (clinical characters) correlates with the Fragile X Syndrome.

**Patients and Method:** Cytogenetic analysis were done by employing the standard method of blood culture for fragile site using folic acid deprived culture media, followed by harvesting, slide preparation, staining and karyotyping. Minimum of 20 metaphases were screened for the slides stained with G banding technique and 100 metaphases were screened for the slides stained with Unbanded-Giemsa Stain technique.

**Results:** This report presents the data on cytogenetic analysis carried out in Human Genome Center, USM, during 2002-2007 periods, on 39 male patients and 1 female patient suspected of Fragile X Syndrome. The phenotypic features were variable. Out of these 40 patients, Fragile Xq27.3 was detected cytogenetically in 5 patients (12.5%) only.

**Discussion and Conclusion:** The low percentage of Fragile Xq27.3 detection by cytogenetic analysis could be attributed to the high degree of variability in fragile Xq27.3 expression between individuals, variability among the cytogeneticist and the laboratories. Hence, Fragile X Syndrome may go under diagnosed by cytogenetic analysis alone. This report warrants the importance of the molecular studies to be performed for the accurate diagnosis of Fragile X Syndrome.



**OB-18**

**MOLECULAR ANALYSIS TEST OF SURVIVAL MOTOR NEURON GENE IN 118 MALAYSIAN SPINAL MUSCULAR ATROPHY (SMA) PATIENTS**

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**Objectives:** To determine the frequency of SMN1 exon7 deletion in Malaysian SMA Patients and to establish the molecular analysis method for diagnosing SMA in Malaysia

**Patients & Method:** A total of 118 blood samples were received from August 2003 until April 2008. The samples were taken from clinically suspected SMA patients (86 Malay, 17 Chinese, 6 Indian and 9 other,) from various hospitals in Malaysia. DNA were extracted from blood samples using DNA extraction kit and deletion analysis of exon 7&8 of SMN1 gene were done using the method described by van der Steege et al (1995). PCR product was digested with *Dra I* and *Dde I* restriction enzymes respectively and digested PCR product were analyzed by electrophoresis in 3% agarose gel.

**Results:** Fifty eight percent (68) of the patients fulfilled the criteria for SMA described by the International SMA consortium (1998). Out of these 68, 46% were type I SMA, 40% were type II and 14% type III SMA. Seventy eight percent (53) of these patients ( 22 type I, 23 type II, 8 type III) were found to have homozygous deletion of exon 7 SMN1 gene and 22% of patients ( 9 type I, 4 type II, 1 type III) showed the presence of exon of the SMN1 gene.

**Discussion and Conclusion:** SMN1 deletion has been found to be a major cause for SMA in Malaysia. SMN1 deletion analysis has been proven to be useful for establishing the diagnosis of SMA and can be used as alternative method for diagnosing SMA compared to the more invasive clinical investigations of muscle biopsy and EMG.

**OB-24**

**DEVELOPMENT OF ALLELE SPECIFIC PCR FOR THE DETECTION OF HOMOZYGOUS DELETION OF THE SMN1 GENE IN SPINAL MUSCULAR ATROPHY (SMA) PATIENTS IN MALAYSIA.**

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**Objective:** To develop an alternative method using allele-specific PCR for the rapid detection of homologous deletion of the survival of motor neuron 1 (SMN1) gene and for the confirmation of the clinical diagnosis in SMA.

**Patients and Method:** A total of 125 blood samples were obtained from patients clinically suspected to have SMA in various hospitals in Malaysia. Genomic DNA was extracted and analyzed by PCR-RE digestion method using *Dra I* (exon 7) and *Dde I* (exon 8). Both deleted and non-deleted samples resulted from PCR-RE methods were then analyzed using Allele-Specific PCR (AS-PCR). An internal control has been derived and seeded in the AS-PCR mixture for the validation of false negative result. The PCR products were analyzed using 2% agarose gel. The cost and time of running the tests for both methods were compared.

**Results:** Results from both methods were compared. All samples (100%) showed the same result. The cost for running PCR-RE was RM286 while the cost for AS-PCR was RM118. The AS-PCR method was able to complete within 3 hours while PCR-RE took longer than 5 hours.

**Discussion and Conclusion:** The AS-PCR method is more rapid and cost effective which the cost was reduced by about 58%, compared to PCR-RE method. This method only required a single PCR step and it has been developed upon a single nucleotide polymorphism in the exon 7 of the SMN1 and SMN2 gene.

**OB-25**

**MUTATIONAL ANALYSIS OF CREB BINDING SITES IN SMN2 GENE**

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**Objectives:** To analyze the bioinformatics characteristics of the SMN2 (Survival of Motor Neuron) promoter region and to determine the mutational analysis of the CREB binding sites within the promoter region of the healthy individual and clinical types of SMA (Spinal Muscular Atrophy).

**Patients and Method:** Three SMA patients (Type I, II and III) with homozygous deletion of the SMN1 genes were subjected to the mutational analysis. The cloned amplified PCR products with in the pTOPO 2.1e cloning vector were subjected to direct sequencing. The sequences from the samples of healthy and the clinical types were aligned through CLUSTALX and were presented as Gene Doc file.

**Results:** Total of 39 ORFs (Open Reading Frames) contained 15 TATA box sequences reflecting the diverse function integrity of SMN promoter region. Out of these 15 TATA boxes, 11 were TATA, 2 tata and 2 Goldberg-Hogness sequences. The positive strands', essential Cis element binding sites were LSF, ERE, Tef, Sp1 and CRE and on the negative strand were Mef2, ERE, Ets, AP1 and SRF. The mutational analysis of the CREB binding sites in healthy control and the clinical types of SMA revealed that there were no mutations detected in any of the clinical types.

**Discussion and Conclusion:** We characterized SMN2 promoter region using bioinformatics soft wares. There was no mutation detected in the CREB binding sites within any of the clinical types of SMA in our HUSM SMA patients' promoter regions.



**OS-16****CHARACTERISTIC AND TRENDS OF NASOPHARYNGEAL CARCINOMA (NPC) IN HOSPITAL UNIVERSITI SAINS MALAYSIA (HUSM)**

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**Objective:** To evaluate the characteristic and trend of NPC in patients registered for treatment at Hospital Universiti Sains Malaysia (HUSM) from January 1999 to December 2007.

**Patients and Method:** 106 patients with confirmed NPC were reviewed at HUSM, Kelantan over the time period from January 1999 to December 2007. These patients were from Kelantan, Terengganu, Pahang, Perak, Johor, Kedah and Sabah. The patients included in this study had histologically proven NPC according to the World Health Organisation (WHO) classification and the Tumor, Node, Metastasis (TNM) staging. We observed great difference in time in trend and characteristic of NPC in the populations. Their clinical records were reviewed and clinical data collected.

**Results:** The trends of NPC patients in HUSM are not constant. The number of patients shows a continuous rise and sudden drop. The Malay ethnic group showed highest number that attended HUSM. There were twice as many males as females. The highest mean age was in year 2000 which is 54.5 years. Majority of patients (46.2%) were from WHO type III classification which is different from previous study done in HUSM. Based on the TNM staging, 63.2% patients had reached stage IV. Most of the Kelantan patients (63.2%) were from Kota Bharu district which is the main district in Kelantan.

**Discussion and Conclusion:** Our result indicates that majority of the NPC patients attending HUSM were Malays. Over all number of new cases of NPC reporting to HUSM have significantly dropped from 2005-2007. The mean age for every year is between 40-55 years which is similar to many previous studies.

**PB-16**

**ALLELIC IMBALANCE IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL): APPLICATION OF SINGLE NUCLEOTIDE POLYMORPHISMS ARRAY (SNPA)**

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**Introduction:** Allelic imbalance is a common genetic event in many types of malignancy. The recent introduction of high-density SNPA for the concurrent analysis of loss of heterozygosity (LOH) and changes in chromosome copy number (CN) in small amounts of DNA has facilitated a comprehensive, genome-wide analysis of tumour cell populations. This method is of value in malignancies where standard cytogenetic analysis is difficult to perform.

**Objective:** To characterise LOH and CN alterations in leukaemic samples using SNPA.

**Patients and method:** A cohort of 86 patients presenting with childhood ALL within the Northern Region of the UK (Sept 1986-Jan 2005) were analysed using GeneChip<sup>®</sup> Human Mapping 10K Array (Affymetrix, Ltd.). Good quality of DNA (250ng) was sent to MRC Geneservice (Cambridge) for sample analysis. Results from SNPA analysis were analysed using proprietary software (Affymetrix). The results spreadsheets obtained from MRC contained information about the identity of each SNP and its chromosomal location were exported to Excel spreadsheets for in house analysis.

**Results:** Sixty-nine of the 86 samples (80%) showed one or more significant areas of LOH. Consideration of the CN of the regions affected suggested that this was of 2 types: LOH associated with CN reduction (deletion) and LOH associated with no CN change or copy neutral-LOH (acquired isodisomy, AID). Allelic imbalances have been frequently identified on chromosome 9p, 12p and 6q. Loss of 9p has been associated with tumourigenesis and to be progressive in some cases at relapse. A tumor suppressor gene, p16<sup>INK4a</sup> is always included in 9p deleted region and has been suggested to be involved in leukaemogenesis.

**Discussion and conclusion:** This study indicated that application of SNPA is very useful to characterize allelic imbalance in childhood ALL. In addition, unlike other whole-genome screening methods, it can readily detect LOH associated with the preservation of the normal CN (AID).

**PM-41**

**LIPID PROFILES AND PREVALENCE OF ASSOCIATED CARDIOVASCULAR RISK FACTORS AMONG DYSLIPIDEMIC PATIENTS IN HUSM: A RETROSPECTIVE STUDY**

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**Objective:** The aim of the present study is to analyze the lipid parameters among dyslipidemic patients who have been admitted to HUSM and to identify the prevalence of associated cardiovascular risk factors.

**Patients & Methods:** A retrospective design was adopted and 144 patients (57males and 87 females) records with dyslipidemia who had been admitted to HUSM at 2007 were collected. Information about age, gender, smoking status, values of the lipid profiles, presence or absence of associated cardiovascular risk factors and antihyperlipidemic treatment were obtained. Lipid profiles were classified according to the NCEP ATP III guidelines.

**Results:** Age range was 35 to 89 years ( $58.9 \pm 9.9$ ) years. Mean  $\pm$  SD for TC, TG, LDL-C HDL-C, were  $6.16 \pm 1.12$  mmol/l,  $3.8 \pm 0.8$  mmol/l,  $3.9 \pm 4.8$  mmol/l and  $1.7 \pm 1.0$  mmol/l respectively. Of the dyslipidemic patients 43% had high TC, 13% high TG, 18.7% very high LDL -C and 13.8% low HDL-C. The older the patient the higher TC ( $P=0.04$ ), the lower HDL-C ( $P=0.05$ ), Females had a higher HDL than males ( $P<0.05$ ). Our results showed that 72.9 % had hypertension, 22.2 % diabetes, 22.2 % ischemic heart disease, 4.2% stroke, 8.3% renal impairment, 76% were smokers.

**Discussion & Conclusion:** Large proportion of our dyslipidemic patients had high TC. Female comprise the larger proportion of our dyslipidemic patients. The mean TC increases with age as oppose to HDL-C level. Majority of the patients were smoker and hypertensive.