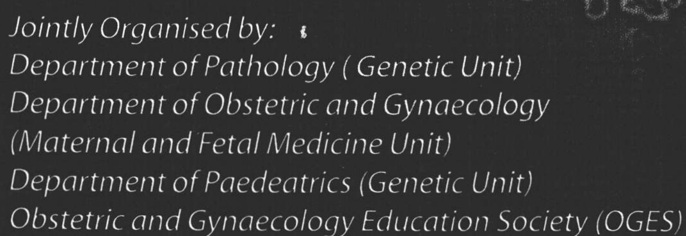
Medical Genetic Conference 2007

Genetic Service: A Team Approach

ABSTRACTS

16th-19th NOV 2007
Colmar Tropicale, Bukit Tinggi
Pahang, Malaysia


Jointly Organised by:
Department of Pathology (Genetic Unit)
Department of Obstetric and Gynaecology
(Maternal and Fetal Medicine Unit)
Department of Paedeatrics (Genetic Unit)
Obstetric and Gynaecology Education Society (OGES)

P4

IS FRAGILE X SYNDROME UNDERDIAGNOSED THROUGH CYTOGENETIC ANALYSIS ALONE?

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The Fragile X syndrome (FRAXA) is the most common form of inherited mental retardation. The prevalence is approximately 1 in 4000 males and 1 in 8000 females. Fragile X syndrome is caused by an unstable mutation in the FMR1 gene located on the X q27.3. Fragile X q27.3 can be detected cytogenetically, but not in all cases. This report presents the data on cytogenetic analysis carried out at the Human Genome Center, USM, from 2006 to 2007, on 25 male patients suspected to have Fragile X syndrome. The phenotypic features were variable. Out of these 25 patients, a fragile site at chromosome Xq27.3 was detected cytogenetically in 3 patients (12%). The low percentage of detection by cytogenetic analysis could be attributed to the high degree of variability in fragile X 27.3 expression between individuals, variability among the scorer and the lab technologists. Hence, Fragile X syndrome may be under diagnosed by doing cytogenetic analysis alone. This warrants the need to undertake molecular studies for the accurate diagnosis of Fragile X syndrome.

P8

METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) POLYMORPHISMS IN NON SYNDROMIC CLEFT LIP/PALATE.

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MTHFR is an important enzyme in folic acid metabolism and polymorphisms C677T and A1298C of the MTHFR gene are being studied for causations of non syndromic cleft lip/palate. Our aim was to investigate its prevalence and role in Malay patients as no previous studies are available in this population. Thirty patients with non syndromic cleft lip/palate and their mothers and 30 healthy controls were included in the study. DNA was extracted from blood followed by PCR and RFLP. Genotyping and statistical analysis (fisher exact) was done to compare incidence of the polymorphisms among cases and the mothers of cleft lip/palate with a control population. The results on genotyping for C677T polymorphism in patients having cleft lip/palate showed 20% heterozygote as compared to 16.6% among the controls. Of the mothers 14.4% were heterozygous and 4% homozygous for TT variants. Fisher exact test revealed p value of 1 and 0.651 respectively on comparing cases with controls and mothers with controls for heterozygous genotype. These results being statistically insignificant, indicates possible absence of association of C677T polymorphism in patients as well as in their mothers with occurrence of cleft lip/palate. The A1298C polymorphism showed a similar prevalence rate and possibly no association with cleft lip/palate. The study indicates the prevalence of the MTHFR variants in cleft lip/palate patients and controls and is similar to the prevalence of this polymorphism quoted for other Asian races. However, the causation of non syndromic cleft lip/palate in the Malaysian population due to either 677 or 1298 polymorphisms, could not be proved.

P9

SCREENING FOR *BETA-GLOBIN* MUTATIONS BY PCR-RFLP AMONG KELANTAN MALAYS WITH BETA-THALASSEMIA MAJOR

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Beta-thalassemia major is an autosomal recessive disorder characterized with severe microcytic hypochromic anemia. Beta-thalassemia is known to occur due to the mutation in β-globin gene (*HBB*) located on chromosome 11. Over 200 mutations in this gene were reported to be associated with thalassemia. Six common mutations in the Malay population were studied which were IVS-1 nt5 (G>C), IVS-1 nt1 (G>T), codon 26 (G>A), codon 19 (A>G), codon 15 (G>A) and codon 41-42 (4 bp del). A cross sectional study was conducted with 35 Kelantan Malay β-thalassemic patients who attended the Hospital Universiti Sains Malaysia. DNA was extracted from blood and subjected to PCR amplification. The amplicons were then digested with six restriction enzymes, *Cac*8I, *Bsl*I, *Alu*I, *Sfc*I, *Mnl*I and *Taq*I to detect the presence of mutations. Five mutations were detected namely, IVS-1 nt5 (G>C), IVS-1 nt1 (G>T), codon 26 (G>A), codon 41-42 (4 bp del) and codon 19 (A>G). One mutation in codon 15 (G>A) was not detected. Among these, the two most common mutations were codon 26 (G>A) and IVS-1 nt5 (G>C) which accounted for 74.3% and 48.6% respectively. None of the mutations were detected in two patients. Our results add to the existing data on the common β-globin gene mutations among the Kelantan Malays. A larger sample size is needed to confirm the spectrum of β-thalassemia mutations and its clinical implications among this Malay ethnic group.

P10

MUTATION ANALYSIS OF THE NON-DELETED SAMPLES OF SURVIVAL MOTOR NEURON1 GENE IN SPINAL MUSCULAR ATROPHY PATIENTS.

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Spinal Muscular Atrophy (SMA) is the second most frequent autosomal recessive disease with a prevalence of 1 out of 6000 newborn and the carrier frequency is 1 in 35. SMA is characterized by degeneration of the motor neurons in the anterior horns of the spinal cord. Survival Motor Neuron gene (SMN) is a candidate gene for SMA disease. SMN gene is present in two highly homologous copies SMN1 and SMN2. SMN1 and SMN2 genes are composed of nine exons. Ninety five percent of patients showed a homozygous deletion of exon 7 and 8. SMN1 subtle mutation was reported in non-deleted patients and only about 4% of SMA patients bear one SMN1 copy with an intragenic mutation. DNA was extracted from blood samples using DNA extraction kit and subjected to SMN1 gene deletion analysis according to the method described by van der Steege et al, 1995. Mutation analysis was performed in 4 non-deleted patient and one control using long-range PCR to distinguish between SMN1 and 2. Following that, nested PCR for exon 3, 4 and 6 were done. We found substitution G to A in exon 3 in region 273 in one patient and no mutation were found in the other 3 patients. Quantitative (Real Time) PCR was done to confirm the copy number of SMN1 gene in this patient.

P12

MOLECULAR CHARACTERIZATION OF THE PROMOTER REGION OF *SMN* GENE USING BIOINFORMATIC TOOLS.

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Computational prediction of eukaryotic promoters is one of the most challenging problems in sequence analysis. *SMN* (Survival of Motor Neuron) gene is about 28 kb in size with a total of 9 exons. The two isotypes of *SMN* gene; *SMN*1 and *SMN*2 encodes for FL-SMN protein (functional SMN) and Δ7SMN protein (non functional). The lack of *SMN*1 produces spinal muscular atrophy (SMA) and the increasing gene dosage of *SMN*2 has been shown to decrease the severity of the SMA. The core promoter region of the *SMN* genes has not been identified but a number of literatures suggest this gene to be regulated by about 4.6 kb region up stream of the transcription start site (TSS). The computational analysis of this region is needed to detect the presence of important features, which can help in developing a strategy for gene therapy against spinal muscular atrophy (SMA). In this analysis, the open reading frames, Pribnow box sequences, restriction sites and the transcriptional factor binding sites were identified. A total of 6003 restriction sites were determined within 15 open reading frames. A total of 15 ORFs and 24 nested ORFs were determined with 7 and 11 ORFs on the complementary strand. These ORFs contained 15 TATA box sequences reflecting the diverse function integrity of *SMN* promoter region. To the best of our knowledge this is the first reported study in the literature describing the ORFs, restriction enzyme recognition sites and TATAA box sequences within the promoter region of *SMN* gene.

P 13

MUTATION ANALYSIS OF *CYP2C8* IN CARDIOVASCULAR PATIENTS USING DHPLC

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CYP2C8 plays a major role in the metabolism of several therapeutically important drugs and endogenous substance such as EETs. It has been less well characterized with little information on the substrates and inhibitors as well as population genetic data. Assay methods available are either too costly, small scale or do not allow detection of new variants. The objectives of the study are to develop a higher through-put method to investigate the genetic background of this gene in Malaysian population and to correlate between mutations of this gene with the incidence of cardiovascular disease. Two hundred blood samples were collected from cardiovascular patients (100) and healthy subjects (100). The DNA was retrieved from the blood samples by lysis DNA extraction method. Positive controls and wild type samples were used to develop the DHPLC method for exon specific homoduplex or heteroduplex detection. The DHPLC method was optimized with regards to the buffer and oven temperature used. The chromatographic peaks were compared between the samples and the reference wild type or positive controls. Samples were sent for sequencing for confirmation. We had found that the method was able to identify the mutation sites of known positive controls and detected variation at sites which had not been reported. Higher occurrence of mutation in *CYP2C8* exon 9 was detected in cardiovascular patients (40%) compared to healthy volunteers (40%). This shows that mutations which occurred in *CYP2C8* are related to the incidence of the cardiovascular disease.

P14

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

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Dermal fibroblasts play a central role in skin tissue regeneration. Fibroblasts appear at the injury site at a very early stage and proliferate rapidly as wound healing progresses. Deep dermal injuries will disturb normal healing process and allow an uncontrolled overproduction of fibroblasts, collagen synthesis and connective tissue to form hypertrophic scars (hSC). The resulting hSC fibroblasts (hSCF) can reach size of more than 4 times that of normal skin. The present study sought to compare the growth characteristic of primary human skin fibroblasts derived from normal and hypertrophic scar culture. Fibroblasts were maintained in DMEM supplemented growth medium in a humidified condition at 37^0C with 5% CO_2. Cells were collected by centrifugation and normal human dermal fibroblasts (nHDF) and hSCF were seeded at 1×10^4 cells/ T-25 flask in 5 ml medium. Flasks were incubated for 2, 4, 6, 8 and 10 days and the growth of nHDF and hSCF were counted with haemacytometer by using Trypan Blue Exclusion Test assay. Mean cell count for nHDF at day 2, 4, 6, 8, 10 was 2×10^4 cells/ml, 5×10^4 cells/ml, 8×10^4 cells/ml, 13×10^4 cells/ml and 30×10^4 cells/ml respectively. In contrast to nHDF, mean cell count for hSCF at day 2, 4, 6, 8 and 10 were 17×10^4 cells/ml, 36×10^4 cells/ml, 58×10^4 cells/ml, 80×10^4 cells/ml and 100×10^4 cells/ml. The calculation of cells growth showed there was a significant difference between nHDF and hSCF at each experimental time point. Samples of nHDF and hSCF were used to display a contact inhibition pattern and hSCF exhibited linear growth and sustained a higher cellular viability compared to nHDF.

P 15

THE ROLE OF SINGLE NUCLEOTIDE POLYMORPHISM 153, 104 (A TO G) OF RB1 GENE IN THE CLINICAL PRESENTATION OF RETINOBLASTOMA PATIENTS

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The single nucleotide polymorphism (SNP) 153, 104 of the RB1 gene was reported to be found exclusively among Asian population. We studied the distribution of this SNP in Malaysian children, their parents and healthy unrelated control subjects and its association with the staging and laterality of the disease. Forty-six Malaysian retinoblastoma patients and their parents and 46 ethnic-matched controls were recruited. SNP genotyping was performed by PCR-RFLP. No significant association between this SNP and staging and laterality of retinoblastoma was observed. However, we found significant association between the paternal polymorphism and the affected children ($p=0.002$). The presence of paternal SNP may play an important role in the susceptibility of Malaysian children to develop retinoblastoma.

P16

GENE EXPRESSION ANALYSIS OF THE EPIDERMAL GROWTH FACTOR RECEPTOR AMONG OPERATED ORAL CANCER PATIENTS AT HUSM: A PRELIMINARY REPORT

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Epidermal Growth Factor Receptor (EGFR) gene, located on chromosome 7p12, encodes for a 170 kDa cell surface glycoprotein. EGFR was found to be expressed in 90% of all oral cancer cases. A study was conducted to evaluate the EGFR expression using Real-Time Polymerase Chain Reaction. The study samples consisted of five oral cancer patients who underwent surgical treatment at Hospital Universiti Sains Malaysia (HUSM). Their medical reports were reviewed and the relevant clinical data were extracted based on the questionnaire developed by the Oral Cancer Research and Coordinating Centre. Total RNA was extracted from both tissue samples (normal and tumor) using a commercial RNA extraction kit. The total RNA was then reverse transcribed to cDNA. All assays were performed using real-time RT-PCR, which yields a value (Ct) denoting the threshold cycle of PCR amplification at which product is first detected by fluorescence. The Ct is dependent on the quantity of the target molecule in the sample. To control for variation in RNA quantity and quality, we used 18S ribosome RNA as an internal control to calculate a relative Ct for the target molecule of interest, EGFR. Paired normal and cancerous tissue samples from five oral cancer patients were assayed to ascertain the relative levels of the EGFR. EGFR was found to be increased in tumor tissue as compared to normal tissue in all samples. Average level of the over expression was 4.77 fold (Highest: 12.61, Lowest: 0.47). Although with a limited number of samples, our data suggested that EGFR may play a role in oral carcinogenesis by promoting uncontrolled cell proliferation.

P17

DETECTION OF HOMOLOGOUS DELETION OF THE SURVIVAL OF MOTOR NEURON GENE IN SPINAL MUSCULAR ATROPHY PATIENTS; CONVENTIONAL VS ALELLE-SPECIFIC PCR

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Spinal Muscular Atrophy (SMA) is an autosomal recessive disease characterized by degeneration of the anterior horn cell of the spinal cord. Survival of Motor Neuron (SMN) gene has been identified as a candidate gene for SMA. The SMN gene is located at chromosome 5q13 and encoding 9 exons. The SMN protein is important for the survival of the motor neurons. About 90% of SMA patients have a deletion in exon 7 of the SMN1 gene. Conventionally the homozygous deletion of SMN1 gene is detected using the Polymerase Chain Reaction-Restriction Enzyme (PCR-RE) digestion method. This method is time consuming, expensive and require considerable high amount of DNA. An alternative application has been developed in this study for the rapid detection of the homozygous deletion of the SMN1 gene and for the confirmation of the clinical diagnosis in SMA. One hundred and twenty samples have been analyzed using PCR-RE digestion method and Allele-Specific PCR (AS-PCR) method. An internal control (beta globin gene) have been derived and seeded in the AS-PCR mixture for the validation of false-negative result. The results from both methods were then compared. All the samples (100%) showed the same result. We therefore conclude that, the AS-PCR method is more rapid and cost effective, because compared to PCR-RE method, it requires only a single PCR step and it has been developed upon a single nucleotide polymorphism in the exon 7 of the *SMN1* and *SMN2*.

P 20

MOLECULAR GENETIC DIAGNOSIS OF SMA: 5 YEARS EXPERIENCE OF HUMAN GENOME CENTER (2003-2007)

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The deletion of Survival Motor Neuron 1 (*SMN1*) gene causes the disease in about 95% of SMA patients irrespective of clinical severity. Intragenic mutations were reported in the remaining 5% of the patients. Molecular genetic testing is now provided as an alternative tool for the muscle biopsy and EMG. Blood samples were obtained after getting the informed consent, and DNA was extracted. Deletion analysis of exons 7 & 8 of the *SMN1* gene was performed according to van der Steege *et al* (1995). Exons 7 and 8 of the *SMN1* gene were amplified and digested with restriction enzymes; *Dra I* and *Dde I* respectively. One hundred and sixteen samples from government hospitals, medical academic institutions and private hospitals from various parts of Malaysia were received from August 2003 till November 2007. Most of the samples came from HUSM (27 cases) and HKL (12 cases). Fifty six percents of these cases fulfilled the diagnostic criteria described by the International SMA Consortium (1998). A total of 31 type I, 25 type II and 9 type III SMA patients were grouped as highly suggestive of clinical SMA. The deletion of at least exon 7 of the *SMN1* gene was found in 80% of these patients. The deletion of the *SMN1* gene was found to be a major cause for SMA in Malaysian population. The molecular diagnosis of SMA has become the preferred diagnostic tool for SMA as the number of samples received increased by the year.