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'When' and 'Where': acquisition of spatial navigationYS Chan

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Spatial navigation requires integration of inputs from both the external environment and internal cues of balance. Emergence of the adult pattern of spatial navigation involves differential processing of sensory cues of balance with postnatal expansion of experience. Signals about head orientations are transmitted from the inner ear to neurons in the vestibular nucleus within the brainstem. We found that functional maturation of these central neurons occurred after developmental acquisition of specific glutamate receptor subtypes in postsynaptic sites such that functionally silent synapses are converted into functional ones. During this process, long-term potentiation (LTP), a known read-out of plastic changes in synaptic efficacy for learning and memory, was expressed. With the use of a short peptide that selectively inhibits the insertion of excitatory AMPA receptors in postsynaptic membrane during LTP formation, we provide evidence that the blockade is sufficient to delay developmental emergence of a gravity-triggered orienting behaviour in rats. Altogether, we demonstrate that vestibular LTP plays a causal role in the realisation of sensorimotor function.

Blockade of glutamate receptors in the vestibular nucleus during a postnatal critical period of susceptibility resulted in a cascade of consequences in mature animals. The establishment of a spatial reference map of 3-dimensional orientations was deterred in the inferior olive. The olivo-cerebellar connectivity was also disturbed, resulting in the attenuation of synaptic plasticity in cerebellar Purkinje cells. As a consequence, the cerebellar output signals are disrupted and the animals exhibited motor learning deficits. Furthermore, the 3-dimensional space map in the vestibular thalamus of mature animals was deterred. Deficits in spatial navigation and home-reckoning ability were also evidenced. Notably, similar deficits in spatial navigation were observed with selective ablation of specific vestibular thalamic relays, indicating that vestibular information is important for spatial navigation. All in all, our findings imply that postnatal expansion of experience is important for maturation of the vestibular circuitry and expression of spatial recognition behaviors.

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Prospect of individualised therapy in breast cancerW Yeo

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Breast cancer is the commonest malignancy among Hong Kong women. Despite the rise in breast cancer incidence worldwide, there has been a reduction in breast cancer mortality based on data from the West. Adjuvant therapies significantly improve disease-free and overall survival for breast cancer patients. Traditionally, these include the use of locoregional radiation therapy, as well as systemic therapy involving hormonal therapy and conventional cytotoxic chemotherapy. More recently, targeted therapy has been included in the therapeutic armamentarium of breast cancer; the identification of molecular targets enables biological therapies to be tailored for individual patient.

Conventional risk assessment has provided important indicators that enable clinicians to select adjuvant systemic therapy in early breast cancer, and these incorporate both patient-related and tumour-related prognostic factors. These prognostic factors aid in the characterisation of the background level of risk of disease relapse against the benefits and treatment-associated toxicities of adjuvant therapies. Patient-related factors include age, menopausal status and co-morbidities. Tumour-related factors include lymph node involvement, tumour size, tumour grade, estrogen receptor and progesterone status and more recently, HER2 status. However, a more sensitive way to differentiate patients at high risk versus those at low risk of disease relapse will enable proper determination of appropriate therapy for the former, while sparing the latter from unnecessary treatment-related toxicity.

Over the past decade, one of the major areas of breast cancer researches has focused in developing more sensitive means to identify patients at risk of disease relapse, based on molecular classification and multigene expression signatures; prospective randomised studies are currently evaluating these tools in a prospective manner, whereby appropriate adjuvant therapies can be offered.

Bone mineral density and serum osteoprotegerin levels in pre- and post-menopausal women

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Introduction: Osteoprotegerin (OPG) is an essential regulator of bone turnover through its suppression on osteoclastogenesis. Findings from previous studies of serum OPG and bone mineral density (BMD) in humans have been conflicting. The objective of this study was to identify factors associated with serum OPG levels and to determine its effect on BMD in pre- and post-menopausal women.

Methods: This is a part of the Hong Kong Osteoporosis Study. A total of 2343 community-dwelling, treatment- and hormonal therapy-naïve female subjects aged 18 years or above were recruited (679 pre-menopausal women, mean age 36.7±8.8 years; 1664 post-menopausal women, mean age 62.6±8.5 years). Baseline demographic characteristics, serum biochemistry, hormonal profile, and fasting serum OPG levels were obtained. Baseline BMD at the spine and hip were measured.

Results: Serum OPG level was correlated with age in both pre- and post-menopausal women (pre-menopause $r=0.208$, post-menopause $r=0.258$; both $P<0.0001$). After adjusting for age, OPG levels were positively correlated with serum estradiol ($r=0.100$, $P<0.05$) and negatively correlated with follicular stimulating hormone (FSH, $r=-0.114$, $P<0.01$) in pre-menopausal but not post-menopausal women. In pre-menopausal women, higher serum OPG levels were associated with higher age- and BMI-adjusted BMD (spine $r=0.147$, $P<0.05$; femoral neck $r=0.138$, $P<0.05$; total hip $r=0.148$, $P<0.05$). In post-menopausal women, age-adjusted OPG showed no correlation with BMD in the linear regression model. However, a negative correlation was observed between OPG in quartiles and hip BMD (P -trend <0.01), but not spine BMD.

Conclusions: Serum OPG level is an independent factor associated with higher BMD in pre-menopausal women. However its protective effect on BMD is not significant in postmenopausal women with low bone mass.

Acknowledgement: This study was supported by the Bone Health Fund of the Hong Kong University Foundation and Osteoporosis Research Fund of the University of Hong Kong.

Conflict of interest: The authors have no conflict of interest.

Fractures in Chinese men: the Hong Kong Osteoporosis Study

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Introduction: Clinical risk factors with or without bone mineral density (BMD) measurements are increasingly recognised as reliable predictors of fracture risk. Prospective data on fracture incidence in Asian men remain sparse. This prospective study aimed to determine the risk factors and the 10-year absolute fracture risk in Southern Chinese men.

Methods: This is a part of the Hong Kong Osteoporosis Study. A total of 1810 community-dwelling, treatment-naïve men aged 50 years or above were evaluated. Baseline demographic characteristics, clinical risk factors and BMD were recorded. Ten-year risk of osteoporotic fracture was calculated using Cox proportional hazards models.

Results: The mean age of subjects was 68.0±10.3 years. After a mean follow-up period of 3.5±2.9 (range, 1-14) years, 37 incident low-trauma fractures were recorded. The incidence for all osteoporotic fractures and hip fractures was 635/100 000 person-years and 123/100 000 person-years, respectively. The most significant predictors of osteoporotic fracture were history of fall (RR=14.5), femoral neck BMD T score <-2.5 (RR=13.8), and history of fracture (RR=4.4). Each SD reduction in BMD was associated with a 1.8-to-2.6-fold increase in fracture risk. Subjects with seven clinical risk factors and BMD T-score of -1 had an absolute 10-year risk of osteoporotic fracture of 8.9%, but this increased to 22.7% if they also had a femoral neck BMD T-score of -2.5.

Conclusions: These findings show substantial population differences in fracture incidence and risk prediction. The addition of BMD information to clinical risk factor assessment improved fracture risk prediction in Chinese men.

Acknowledgement: This study was supported by the Bone Health Fund of the Hong Kong University Foundation and Osteoporosis Research Fund of the University of Hong Kong.

Conflict of interest: The authors have no conflict of interest.

Ac-SDKP ameliorates the pro-inflammatory response of proximal tubular epithelial cell induced by TGF-beta and pathogenic albumin loading

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Introduction: The endogenous tetra-peptide, N-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP), is known to inhibit TGF-beta signal transduction in glomerular mesangial cells. This study investigated the potential for Ac-SDKP to exert similar beneficial effects on the renal proximal tubular epithelial cell (PTEC), the key cell type that orchestrates tubulointerstitial inflammation, in response to TGF-beta and pathogenic albumin loading.

Methods: PTEC incubated in serum-free medium, with or without Ac-SDKP (1, 10 or 100 nM), were challenged with human serum albumin (HSA) or TGF-beta at 5 mg/mL and 2.5 ng/mL respectively. Western blotting was performed to determine the effects on intracellular signalling whilst qPCR and ELISA were performed to determine the level of cellular gene and protein expressions respectively.

Results: HSA-induced ERK signalling in human primary PTEC was inhibited by Ac-SDKP in a dose-dependent fashion. Furthermore, HSA and TGF-beta induced IL-6 and MCP-1 mRNA expressions were attenuated by Ac-SDKP treatment at 100 nM. While HSA and TGF-beta significantly induced IL-6 secretion by 5- and 3-fold respectively, Ac-SDKP almost completely abrogated these responses.

Conclusion: We conclude that Ac-SDKP reduces the pro-inflammatory response of PTEC in response to TGF-beta and albumin challenge.

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Dermatitis flammeus — an emerging infection-related complication of atopic dermatitis

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Objective: To investigate the clinical, microbiological, immunological and pathological features of a proposed novel complication of atopic dermatitis (AD) related to infection.

Design: Case series and retrospective analysis.

Setting: A tertiary university hospital and a private specialist dermatology clinic in Hong Kong.

Patients: Twenty patients were included between January 2008 and September 2010.

Main outcome measures: Clinical characteristics, microbiological findings, therapeutic strategy and prognosis of the proposed condition.

Results: Patients' ages ranged from 17 months to 52 years (mean, 23 years). The male-to-female ratio was 1:1. AD was the single major predisposing factor. Skin lesions followed an identical triphasic progression in all cases. A symmetric and predominant flexural involvement was observed. *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* were most frequently cultured from superficial skin swabs. Second-generation cephalosporins or anti-pseudomonal antibiotics were the preferred first-line antimicrobial regimen. Intravenous immunoglobulins were used with significant improvement in refractory cases. Complete resolution was evident in all 20 cases and recurrence was common (35%).

Conclusions: A unique clinical pattern was observed in our series. No conclusive data were identified from the microbiological and histopathological findings. Further microbiological and molecular studies are required to identify the role of culprit micro-organisms in its pathogenesis.

Optimal Timing on re-Vascularisation and Outcome in Acute Coronary Syndromes (OPTIVO-ACS)

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Introduction: Several earlier studies have shown that early invasive intervention strategy could improve outcome in patients admitted for non-ST segment elevation myocardial infarction (NSTEMI) and acute coronary syndromes (ACS), especially in some high-risk subsets. However, the optimal timing for arranging such intervention remains uncertain. This study aimed to determine whether coronary intervention (percutaneous coronary intervention [PCI] or bypass surgery) performed as early as less than 48 hours after admission for NSTEMI-ACS patients can result in a reduction of major adverse cardiac events (MACE) when compared with delayed intervention done after 48 hours.

Methods: Patients admitted to Queen Mary Hospital from the period of 1 January 2004 to 30 June 2009 were identified retrospectively, all with the diagnosis of NSTEMI, unstable angina or ACS. Patients were then separated into two main time groups according to their time of receiving coronary intervention, that is <48-hour time group and >48-hour time group, for statistical analysis. The primary endpoint was MACE at 6 months' follow-up. MACE was defined as a composite endpoint comprising death from any cause, myocardial infarction, stroke and urgent target vessel/lesion re-vascularisation.

Results: A total of 1296 patients admitted for NSTEMI-ACS were identified and 164 of them received coronary angiography. Of these, 142 patients had revascularisation treatment, in which 128 patients had PCI and 14 patients had coronary artery bypass graft (CABG). At 6 months, MACE occurred in one patient in the <48-hour time group and 15 patients in the >48-hour time group (OR=0.083; 95% CI, 0.01-0.65; P=0.005). Major secondary endpoints also showed that the outcome was better in patients with age <60 years subgroup (P=0.02), as well as a shorter length of hospital stay in the <48-hour group (mean 4.26 vs 8.21 days; P<0.0001).

Conclusion: In patients admitted for NSTEMI-ACS, a strategy of early coronary intervention by either angioplasty or bypass surgery, as short as less than 48 hours after admission, when compared to delayed intervention, reduced MACE at 6 months and the average length of hospital stay for the index hospitalisation.

Implementation of acute myocardial infarction (AMI) clinical pathway for uncomplicated AMI at Queen Mary Hospital: three years' experience, outcomes, and impacts

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Introduction: Development of acute myocardial infarction (AMI) clinical pathway was aiming at incorporating evidence-based medicine into clinical practice in the management of uncomplicated AMI patients. The use of clinical pathways has increased over the past decade in many developed countries. Less regular adoption of clinical pathways is noted in developing countries and in Asia. There are no published data on the implementation and the subsequent impacts of clinical pathways in Hong Kong. This study explored the logistics, results and impacts of such development in a university teaching hospital receiving acute patients in Hong Kong. The parameters focused included the magnitude of improvement in quality care of patients with AMI, the length of hospital stay, the in-patient and 30-day mortality, the door-to-needle time (DNT) in thrombolytic therapy (TT) and door-to-balloon time (DBT) in primary percutaneous coronary intervention (primary PCI) in ST-segment elevation myocardial infarction (STEMI) patients.

Methods and Results: AMI clinical pathway was implemented at Queen Mary Hospital since January 2007. A total of 535 AMI patients were recruited and managed according to the pathway protocol. A total of 402 patients had successfully completed the pathway and 72.9% had a diagnosis of STEMI. At the beginning, the mean length of hospital stay was 4.0±2.6 days, the in-patient and 30-day mortality were 6.0% and 9.6% respectively, the mean DNT was 105.9±66.4 minutes and the DBT was 161.3±95.6 minutes. In 2009, after 3 years of implementation of the clinical pathway, the mean length of hospital stay was shortened to 3.9±1.8 days, the in-patient and 30-day mortality improved to 0.9% and 0.9% respectively as well, the mean DNT reduced to 39.6±17.3 minutes and the DBT 107.2±24.9 minutes.

Conclusions: AMI clinical pathway helps streamline and standardise patient care in those with uncomplicated AMI. All parameters—including the DNT, DBT, mortality rate and use of medications—have improved since its implementation at Queen Mary Hospital in 2007.

Treatment with dual wavelength 1550-nm and 1927-nm fractional laser for facial skin rejuvenation in Asians

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Background: The objective of this study was to assess the efficacy and side-effects of dual wavelength treatment with fractional 1550-nm erbium-doped fiber laser and a novel 1927-nm thulium fiber laser for facial skin rejuvenation in Asians. The combination wavelengths aimed to achieve both deep and superficial resurfacing within one treatment.

Method: Each patient received one full-face treatment with two passes of 1550-nm laser followed by two passes of 1927-nm laser. Standardised photos were taken with the Canfield Visia CR system at baseline, 1 month and 3 months post-treatment, and were assessed by two independent physicians.

Results: Eight Chinese female patients were included. The energy levels for 1550-nm laser and 1927-nm laser were 40 mJ and 10 mJ, respectively. The total densities (MTZ/cm²) ranged from 66 to 114 for 1550-nm laser; and 144 to 222 for 1927-nm laser. Preliminary results demonstrated statistically significant improvement in skin texture (P=0.01), skin laxity (P=0.01), wrinkles (P=0.01), enlarged pore (P=0.026), and overall pigmentation irregularity (P=0.017) at last follow-up. The degree of improvement was graded as slight to moderate. Erythema was seen in one patient at last follow-up. No hyperpigmentation was recorded. All patients were satisfied with the treatment at 3 months' follow-up.

Conclusions: A single treatment with the dual wavelength lasers achieved significant clinical improvement with a good safety profile for facial skin rejuvenation in Asians.

Clinical study of transcutaneous focused ultrasound for lower facial and submental skin tightening in Asians

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Background: The objective of this study was to determine the clinical efficacy of a focused ultrasound device for the treatment of lower facial and submental skin laxity in Asians.

Methods: Each patient received one treatment with the transcutaneous focused ultrasound device. Two transducers (7.5 MHz, 3.0 mm depth; 4.0 MHz, 4.5 mm depth) were used to deliver two passes of microthermal areas of coagulation over the lower facial and submental regions at two different depths. Standardised photos and three-dimensional images were taken with the Canfield Visia CR and Vectra systems respectively at baseline, 1 month and 3 months post-treatment. They were assessed by two independent physicians.

Results: Sixteen Chinese patients were included. Erythema and edema were the only temporary adverse effects, which resolved by 1 month. Preliminary objective assessment of standardised photos showed statistically significant improvement for skin laxity along the jawline (P=0.046), and cheek (P=0.008) at last follow-up. The degree of improvement was graded as slight to moderate. Vectra 3D images showed positive skin tightening in submental region.

Conclusions: The dual-plane approach of energy delivery with transcutaneous high-intensity focused ultrasound appeared effective for lower facial and submental skin laxity in Asians. Further studies to optimise treatment parameters may enhance clinical outcomes.

A correlation of mobilisation regimens and outcome during autologous haematopoietic stem cell mobilisation and transplantation

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Introduction: Autologous haematopoietic stem cell transplantation (ASCT) is the mainstay of treatment for plasma cell myeloma and selected cases of lymphoma. Haematopoietic stem cell (HSC) can be mobilised using combination of chemotherapy and granulocyte-colony stimulating factor (G-CSF). However it is associated with treatment toxicity and the need for prolonged hospitalisation. HSC can also be mobilised using G-CSF alone, but the yield may be lower. Plerixafor, a CXC chemokine receptor 4 (CXCR-4) antagonist, was recently approved by the US FDA in patients who failed G-CSF HSC mobilisation. In the present study, we compared the HSC yield and engraftment after ASCT in patients who underwent HSC mobilisation with these regimens.

Methods: Consecutive patients who underwent ASCT for plasma cell myeloma between 2009 and 2010 were retrospectively analysed. Total CD34⁺ cells collected, number of apheresis sessions and haematopoietic recovery after ASCT was correlated with their HSC mobilisation regimens. Numerical data were compared using Mann-Whitney *U* test and categorical data was evaluated using χ^2 test. A *P* value of less than 0.05 was considered statistically significant.

Results: A total of 28 patients were analysed; 18 patients have received cyclophosphamide (3 g/m²) and G-CSF for mobilisation (Cy+G-CSF), 10 received G-CSF of whom five required plerixafor (G-CSF+P) to achieve target cell dose of 2x10⁶/kg body weight. Patients receiving Cy+G-CSF required longer duration of hospitalisation when compared with G-CSF±Mozobil group. Most patients in the Cy+G-CSF group (n=15) required only one harvest whereas all patients in the G-CSF required more than one (*P*<0.01). There was no significant difference in neutrophil engraftment as well as hospital stay during ASCT in these patients.

Conclusion: The use of plerixafor improves the stem cell yield when added to G-CSF as mobilisation, and has a significant less hospital stay when compared with cyclophosphamide as mobilisation.

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A newly derived small synthetic compound alleviated ventricular fibrillation in a pig model with chronic myocardial infarction as revealed by optical mapping

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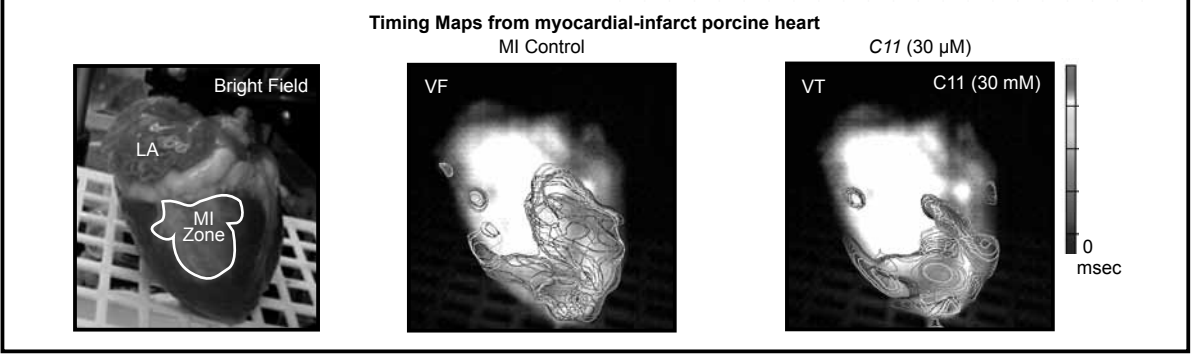
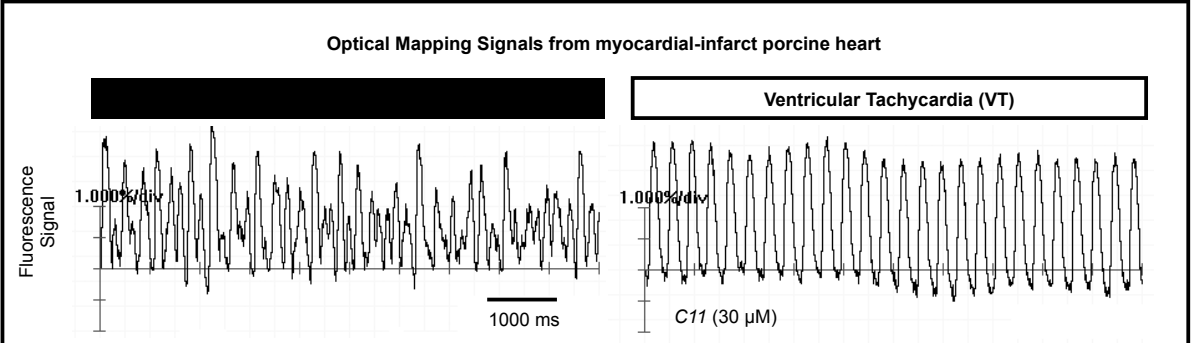
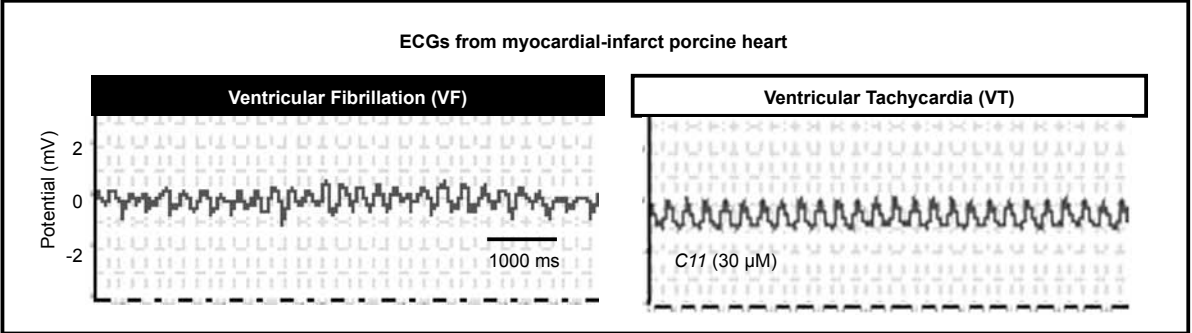
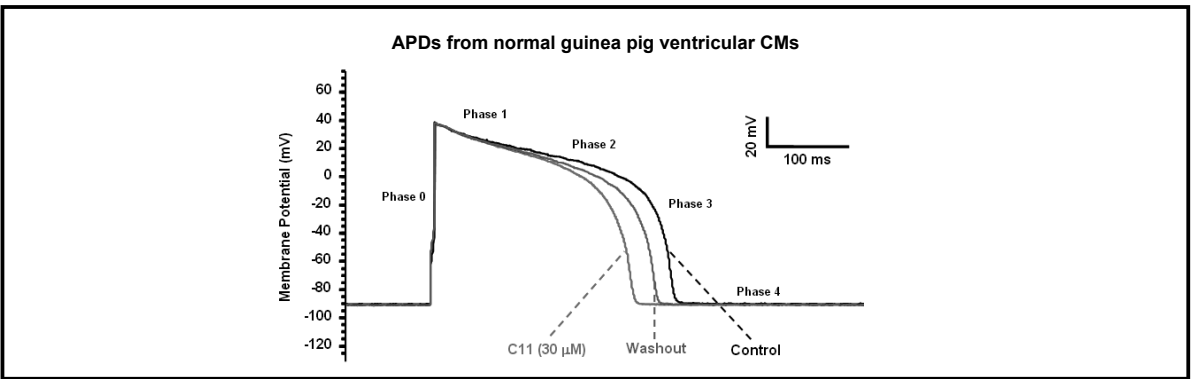
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The electrophysiological hallmark of cells and tissues isolated from failing hearts is prolongation of action potential duration (APD), resulted from down-regulation of repolarising K⁺ currents and/or alterations in depolarising Na⁺ and Ca²⁺ currents, which predisposes the failing heart to lethal ventricular tachyarrhythmia (ventricular tachycardia [VT] and ventricular fibrillation [VF]). C11, a small synthetic Cl⁻ channel, exhibits membrane-repolarising power. Therefore, we hypothesise C11 corrects the delayed repolarisation and shortens APD at cellular level, thus modifying ventricular arrhythmogenic substrate at whole heart level. First, we demonstrated APD reduction upon C11 application (30 μ M) at 37°C to isolated guinea pig ventricular cardiomyocytes with patch-clamp experiments in whole cell configuration (*Figure: upper panel*).

To examine whether C11 works in disease model, pig hearts with chronic myocardial infarction (MI) were optically mapped. Electrocardiograms (ECGs) [*Figure: middle panel*] and the optical mapping signals with optical timing maps [*Figure: lower panel*] displayed the attenuation of VF to VT in the presence of C11 (30 μ M).

In conclusion, C11 alleviated VF in our ex-vivo pig heart model with chronic MI. Further investigation in the ionic properties of C11 will be worthy to further dissect the underlying mechanism of function posing potential use of C11 in clinical prospect.



Absence of *NPM1* promoter hypermethylation in human myelodysplastic syndrome

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Introduction: *Npm1*^{+/-} heterozygous mice develop a haematological disorder with features resembling human myelodysplastic syndrome (MDS). Promoter hypermethylation of *NPM1* gene may lead to suppressed gene transcription and hence functional haploinsufficiency, which contributes to myelodysplasia. Furthermore, reversal of gene promoter hypermethylation by demethylating agents has been shown to be of therapeutic benefit. Therefore, we hypothesised that epigenetic alterations of *NPM1* might lead to *NPM1* haploinsufficiency, thereby contributing to the development of MDS.

Methods: A comprehensive methylation analysis of *NPM1* was evaluated in 31 MDS patients and eight normal individuals for promoter methylation and mRNA expression of *NPM1*. Methylation-specific polymerase chain reaction (MSP), combined bisulfite restriction analysis technique (COBRA) and bisulfite sequencing were used to examine the *NPM1* methylation status. Quantitative polymerase chain reaction was employed to assess the expression of *NPM1*.

Results: *NPM1* promoter methylation was rare, occurring in one of 31 cases as determined by MSP, but no significant methylation was found using COBRA and bisulfite sequencing. Furthermore, real-time quantitative RT-PCR showed that there was no significant difference in *NPM1* mRNA expression between MDS and normal blood samples. These results revealed that promoter methylation and functional haploinsufficiency of *NPM1* do not contribute to the development of human MDS.

Conclusion: Our findings suggested that *NPM1* methylation was infrequent in MDS and did not play a major role in its pathogenesis.

Circulating pigment epithelium-derived factor levels and the risk of hypertension in a community-based study

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Introduction: Pigment epithelium-derived factor (PEDF), a serine protease inhibitor, is secreted from the adipose tissue and circulates at high concentrations. A recent study found that PEDF played a causal role in obesity-induced insulin resistance and metabolic dysfunctions in mice. Previous cross-sectional studies had demonstrated a positive association of PEDF with increased systolic blood pressure, pulse pressure and lower small artery elasticity. The objective of this study was to determine whether high circulating PEDF levels predicted the development of hypertension in a 10-year prospective study.

Methods: Baseline plasma PEDF levels were measured by ELISA in 520 non-diabetic subjects recruited from the Hong Kong Cardiovascular Risk Factor Prevalence Study. Multiple logistic regression was used to analyse whether PEDF was an independent factor related to hypertension at baseline. The role of PEDF in predicting the development of hypertension over 10 years was analysed using Cox regression analysis.

Results: At baseline, sex-adjusted PEDF levels were significantly higher in subjects with hypertension ($P < 0.001$) and the association remained significant (odds ratio=1.203; 95% confidence interval [CI], 1.065-1.359; $P = 0.003$) after adjustment for covariates. Of the 386 subjects with normal blood pressure at baseline, 132 developed hypertension over 10 years. High baseline PEDF was predictive of hypertension, independent of the effects of age, sex, baseline obesity parameters and blood pressure (hazard ratio=1.135; 95% CI, 1.039-1.241; $P = 0.005$).

Conclusion: Our data suggest that plasma PEDF is significantly associated with both prevalent and incident hypertension, and may be involved in the pathogenesis of hypertension in humans.

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Ubiquitination is indispensable for the insulin-sensitising activity of the adaptor protein APPL1

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Background: Insulin inhibits hepatic glucose production through activation of the protein kinase Akt. Our previous work has identified the multi-domain adaptor protein APPL1 as a positive modulator of insulin-evoked Akt activation in hepatocytes. However, the detailed mechanisms remain elusive. This study aimed to address the role of post-translational modification in APPL1-mediated potentiating effects on hepatic actions of insulin.

Methods: Rat hepatocytes were infected with adenovirus encoding luciferase control or FLAG-tagged APPL1 together with HA-tagged ubiquitin, followed by serum starvation and stimulation with insulin (10 nM). Total cell lysate was subjected to immunoprecipitation, Western blot analysis, and real-time quantitative PCR analysis. Ubiquitination of APPL1 was detected by Western blot analysis. Ultracentrifugation was employed to separate the hepatocytes into cytosolic and plasma membrane fractions.

Results and Conclusion: In rat hepatocytes, APPL1 undergoes ubiquitination upon insulin stimulation in a time-dependent manner. APPL1 ubiquitination is lysine 63-linked but not lysine 48-linked, indicating that this post-translational modification may regulate its cellular localisation and functions, but is not responsible for proteasomal degradation. Our mutagenesis experiment identified that APPL1 residue lysine 160 is the site for its ubiquitination. Mutation of lysine 160 to arginine abolishes the potentiating effects of APPL1 on insulin sensitivity. Further analysis reveals that an E3 ubiquitin ligase, tumour necrosis factor receptor associated factor (TRAF6), is responsible for ubiquitination of APPL1. This E3 ligase is associated with APPL1 upon insulin treatment. Over-expression of TRAF6 further enhances insulin-stimulated APPL1 ubiquitination. Moreover, knockdown of TRAF6 expression attenuates insulin-mediated translocation of APPL1 from cytosol to cellular membrane, which in turn inhibits the potentiating actions of APPL1 on insulin signalling. Taken together, these results support the notion that APPL1 ubiquitination is a vital step for hepatic functions of insulin through modulating the intracellular trafficking of Akt.

Acknowledgement: This work was supported by General Research Fund (HKU 779707M).

Increasing prevalence of hypertension in Hong Kong Cardiovascular Risk Factor Prevalence Study: role of general and central obesity

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Introduction: General obesity and central obesity are well-known risk factors of hypertension. We investigated the change in the prevalence of hypertension in the population-based prospective Hong Kong Cardiovascular Risk Factor Prevalence Study (CRISPS) and the relationship of change in blood pressure with change in body mass index (BMI) and waist circumference over a follow-up period of 11.9 years.

Methods: A total of 2888, 1942 and 1798 subjects in CRISPS-1 (1995-1996), CRISPS-2 (2000-2004) and CRISPS-3 (2005-2008) were included in this analysis respectively. Hypertension was defined as blood pressure $\geq 140/90$ mm Hg or taking anti-hypertensive medication. General obesity was defined as BMI ≥ 27.5 kg/m² and central obesity was defined as waist circumference ≥ 90 cm in men or ≥ 80 cm in women.

Results: The prevalence of hypertension increased from 18.1% to 39.4% ($P < 0.001$ after adjusting for age and sex). The prevalence of central obesity increased from 25.4% to 41.4%, but that of general obesity decreased from 16.8% to 14.8% (both $P < 0.001$ after adjusting for age and sex). Among 1347 subjects who did not take any anti-hypertensive medication at both CRISPS-1 and CRISPS-3, the change in waist circumference, but not that in BMI, was associated with the changes in both systolic and diastolic blood pressures ($\beta = 0.087$, $P = 0.015$ and $\beta = 0.122$, $P < 0.001$ respectively).

Conclusions: The increase in prevalence of hypertension might be explained by the increase in central obesity. Our findings further confirm the importance of waist circumference in this population; calculating the BMI alone may give a false sense of security.

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Role of genetic variants in gene encoding lipocalin-2 in the development of elevated blood pressure

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Introduction: Lipocalin-2 is recently recognised as a biomarker of obesity and inflammation, which are both risk factors for hypertension. We therefore investigated the association of common single nucleotide polymorphisms (SNPs) in the gene encoding lipocalin-2 (*LCN2*) with elevated blood pressure in Hong Kong Chinese.

Methods: Five tagging SNPs were genotyped in 1936 subjects from the Hong Kong Cardiovascular Risk Factor Prevalence Study-2 (CRISPS-2) with a median follow-up period of 6.4 years. Elevated blood pressure was defined as $\geq 130/85$ mm Hg or taking anti-hypertensive medication.

Results: There were only two haplotypes with frequency of $>5\%$, namely AGATC (45.5%) and GGTC (41.2%). Haplotype GGTC was associated with elevated blood pressure at follow-up (OR=1.17 compared to haplotype AGATC, $P=0.031$ after adjusting for age and sex). Among 1381 subjects without elevated blood pressure at baseline, 321 subjects developed elevated blood pressure at follow-up. Haplotype GGTC was associated with the development of elevated blood pressure at follow-up (OR=1.30 compared to haplotype AGATC, $P=0.011$ after adjusting for age, sex, systolic blood pressure, and follow-up duration; OR=1.44, $P=0.0015$ after further adjusting for other covariates). Among subjects not taking anti-hypertensive medication, carriers of the haplotype GGTC had higher systolic blood pressure than non-carriers (119.7 ± 16.4 mm Hg vs 117.9 ± 17.3 mm Hg, $P=0.043$).

Conclusion: Our findings suggest, for the first time, that genetic variants in *LCN2* may affect blood pressure. Further studies on the role of lipocalin-2 in blood pressure regulation are warranted.

Acknowledgement: This study was funded by Hong Kong Research Grant Council grants (HKU7229/01M and HKU7626/07M) and the Sun Chieh Yeh Heart Foundation.

Association of genetic variants in gene encoding lipocalin-2 with plasma alanine aminotransferase and aspartate aminotransferase

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Introduction: Lipocalin-2 is a biomarker for obesity, inflammation and insulin resistance, which are all risks factors for non-alcoholic fatty liver disease (NAFLD). Subjects with NAFLD have elevated circulating levels of lipocalin-2 and liver enzymes such as alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and γ -glutamyl transaminase (GGT). We therefore investigated the relationship of genetic variants in the gene encoding lipocalin-2 (*LCN2*) with plasma ALP, ALT, AST and GGT.

Methods: Five tagging single nucleotide polymorphisms (SNPs) were genotyped in 1337 subjects in the Hong Kong Cardiovascular Risk Factor Prevalence Study-2 (CRISPS-2) who had plasma liver enzymes measured.

Results: The minor T allele of the SNP rs10987900 was significantly associated with 9.6% (95% CI, 2.7-16.0%) lower plasma ALT level ($P=0.0069$) and 6.2% (95% CI, 1.6-10.6%) lower plasma AST ($P=0.0092$) after adjusting for age and sex. The geometric mean (95% CI) of plasma ALT in subjects with CC, CT and TT genotypes were 21.6 (20.9-22.3), 19.9 (18.4-21.5) and 16.4 (12.2-22.1) U/L respectively and those of plasma AST were 22.9 (22.4-23.4), 21.5 (20.6-22.4) and 20.7 (17.6-24.3) U/L respectively. The association remained significant after excluding regular drinkers ($P=0.0092$ and 0.0035 for ALT and AST, respectively) and after further adjusting for body mass index, triglycerides, high-density lipoprotein cholesterol, 2-hour glucose level, insulin resistance index, C-reactive protein, fibrinogen, regular drinking and current smoking ($P=0.022$ and 0.014 respectively).

Conclusion: This study provides further evidence for the role of lipocalin-2 in the development of NAFLD.

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Mendelian randomisation analysis suggests that plasma interleukin-6 is raised in hypertension but does not cause its development

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Introduction: Interleukin-6 (IL-6) plays a central role in inflammation and insulin resistance as well as atherogenesis. We investigated the associations of plasma IL-6 and its genetic variants with hypertension in both cross-sectional and prospective study designs.

Methods: Plasma IL-6 was measured in 648 normotensive and 294 hypertensive subjects from the Hong Kong Cardiovascular Risk Factor Prevalence Study-2 (CRISPS-2) in 2000-2004 and three tagging SNPs in the IL-6 gene (*IL6*) were genotyped. Among subjects normotensive in CRISPS-2, 515 subjects were followed up in CRISPS-3 in 2005-2008 and 100 of them had developed hypertension.

Results: Plasma IL-6 correlated with systolic blood pressure ($r=0.128$, $P<0.001$), pulse pressure ($r=0.144$, $P<0.001$), and mean arterial pressure ($r=0.086$, $P=0.008$). Hypertensive subjects have significantly higher plasma IL-6 level after adjusting for age and sex (geometric mean [95% CI]=0.60 [0.54-0.65] vs 0.47 [0.44-0.50] pg/mL, $P=0.021$). In stepwise logistic regression, plasma IL-6 was associated with hypertension in women ($P=0.004$), but not in men. The SNP rs1800796 was associated with plasma IL-6 ($\beta=-0.098$, $P=0.002$) in stepwise linear regression. However, this SNP was not associated with hypertension or blood pressure. Among subjects normotensive in CRISPS-2, plasma IL-6 was not associated with the development of hypertension in CRISPS-3.

Conclusion: Elevated plasma IL-6 is associated with hypertension, especially in women. Plasma IL-6 is influenced by the SNP rs1800796. However, this SNP is not associated with hypertension, suggesting that hypertension is caused by other factors that elevate plasma IL-6.

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Association of a genetic variant in the apolipoprotein A5 gene with the metabolic syndrome in Chinese

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Introduction: We previously reported that the single nucleotide polymorphism (SNP) rs662799 (-1131T>C) in the apolipoprotein A5 gene (*APOA5*) was an important determinant of plasma triglycerides in both Hong Kong and Guangzhou Chinese. We, therefore, investigated the association of SNPs in *APOA5* with the metabolic syndrome (MetS) in the Hong Kong and Guangzhou Chinese.

Methods: MetS was defined according to the consensus criteria proposed jointly by several organisations in 2009. Five tagging SNPs were genotyped in 1330 unrelated subjects from the Hong Kong Cardiovascular Risk Factor Prevalence Study cohort with follow-up after a median interval of 6.4 years. A total of 1952 subjects from the Guangzhou Biobank Cohort Study-Cardiovascular Disease Subcohort were used to replicate the findings.

Results: The minor allele of rs662799 was significantly associated with higher odds for the MetS in Hong Kong subjects at both baseline (OR=1.47, $P=0.00082$) and follow-up (OR=1.30, $P=0.010$). A similar association was found in Guangzhou subjects (OR=1.27, $P=0.0041$). In a pooled sample of Hong Kong subjects at follow-up and Guangzhou subjects, this SNP was also associated with HDL and LDL cholesterol ($P<0.001$ and 0.010, respectively). All these associations disappeared after further adjusting for plasma triglycerides ($P>0.05$). In a meta-analysis of six studies, the combined OR (95% CI) was 1.38 (1.25-1.52) for the TC + CC genotype compared with the TT genotype ($P<0.00001$).

Conclusion: The association of -1131T>C polymorphism in *APOA5* with the MetS was mainly due to its strong effect on plasma triglycerides. Further studies are needed to assess the utility of this genetic marker in risk stratification.

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Association of a genetic variant in adrenomedullin gene with its plasma level

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Introduction: Adrenomedullin is an adipokine with vasodilatory property. It plays a role in both acute and chronic inflammatory responses. However, there are no studies on the relationship of common single nucleotide polymorphisms (SNPs) in the gene encoding adrenomedullin (ADM) with plasma adrenomedullin. We, therefore, investigated the relationship of plasma adrenomedullin with other biomarkers related to inflammation and obesity, and SNPs in ADM.

Methods: Plasma adrenomedullin was measured by radioimmunoassay in 476 unrelated Hong Kong Chinese subjects, randomly selected from the population-based Hong Kong Cardiovascular Risk Factor Prevalence Study-2. Four SNPs (rs3814700, rs11042725, rs34354539 and rs4910118) in ADM were genotyped. Plasma C-reactive protein (CRP), fibrinogen, interleukin-6 (IL-6) and adiponectin were also measured.

Results: There was a marginally significant trend of decreasing age with increasing tertiles of plasma adrenomedullin (beta= -0.089, P=0.049). Each tertile of plasma adrenomedullin was associated with a plasma IL-6 level 11.9% (95% CI, 2.6-20.3%) lower (beta= -0.116, P=0.014). Plasma adrenomedullin level was not related to other clinical characteristics, including plasma CRP, fibrinogen and adiponectin levels. The four SNPs—rs3814700, rs11042725, rs34354539 and rs4910118—had minor allele frequencies of 31.1%, 28.7%, 33.8% and 23.4%, respectively. Carriers of the minor allele of rs4910118 had plasma adrenomedullin level 10.5% (95% CI, 2.5-17.8%) lower than the non-carriers (beta= -0.115, P=0.011). Haplotype analysis revealed a similar significant association with plasma adrenomedullin (overall P=0.040).

Conclusions: Plasma adrenomedullin is influenced by its genetic variants and is associated with plasma IL-6, but not other plasma biomarkers related to inflammation and obesity in Hong Kong Chinese.

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Gamma-glutamyl transferase level predicts the development of hypertension in Hong Kong Chinese

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Introduction: Liver enzymes are elevated in cardiometabolic diseases, particularly when there is non-alcoholic fatty liver disease. We therefore investigated if hypertension is associated with elevated levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase and γ -glutamyl transferase (GGT).

Methods: We included 235 hypertensive and 708 normotensive subjects from the Hong Kong Cardiovascular Risk Factor Prevalence Study-2 (CRISPS-2) in 2000-2004 who had fewer than one alcoholic drink a week. In the follow-up study in 2005-2008 (CRISPS-3), 126 out of the 708 subjects had developed hypertension.

Results: In CRISPS-2, plasma ALT (OR=1.31 per SD of log-transformed level, P=0.005) and GGT (OR=1.52 per SD of log-transformed level, P<0.001) were significantly associated with prevalent hypertension after adjusting for age, sex and body mass index (BMI). Among subjects not on anti-hypertensive medication, plasma ALP and GGT were significantly associated with both systolic blood pressure (beta=0.141, P<0.001 for ALP and beta=0.096, P=0.004 for GGT) and diastolic blood pressure (beta=0.131, P<0.001 for ALP and beta=0.102, P=0.004 for GGT). In forward stepwise logistic regression analysis of subjects normotensive at CRISPS-2, the highest tertile of plasma GGT level was an independent predictor of the development of hypertension in CRISPS-3 (OR=2.40, P=0.010), together with age, BMI, systolic blood pressure and plasma CRP at baseline, and change in BMI. The other liver enzymes were not significantly predictors of new-onset hypertension.

Conclusions: Among the four liver enzymes, elevated GGT level is the strongest risk factor for hypertension in Hong Kong Chinese.

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Using glycosylated haemoglobin to define the metabolic syndrome in adults in the United States

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Introduction: Recently, the American Diabetes Association has proposed the use of glycosylated haemoglobin (GHb) in the definition of diabetes and the category of increased diabetes risk. We therefore investigated whether GHb can be used instead of fasting plasma glucose in identifying individuals with the metabolic syndrome, which is associated with increased risk of cardiovascular diseases.

Methods: Participants of the US National Health and Nutrition Examination Survey (NHANES) 1999-2006 who had fasting blood glucose were included (n=3551 in 1999-2002 and n=3412 in 2003-2006). The metabolic syndrome was defined using International Diabetes Federation criteria in 2009. Raised blood glucose was defined either as fasting glucose ≥ 100 mg/dL (5.6 mmol/L), or as GHb $\geq 5.7\%$.

Results: In 2003-2006, there was 91.3% agreement between GHb and fasting glucose when either is used to define the metabolic syndrome, although the use of GHb slightly lowered the syndrome's prevalence (34.8% vs 38.8%, $P=0.012$). The agreement was good ($\geq 87\%$) irrespective of age, sex, race/ethnicity and body mass index. Only 2.3% of the sample population had the metabolic syndrome defined using GHb but not using fasting glucose. The syndrome, defined using GHb alone, was associated with cardiovascular diseases (ischaemic heart disease, heart failure or stroke) [OR=1.95, $P=0.002$]. Similar results were found in 1999-2002.

Conclusions: Using GHb instead of fasting glucose to define the metabolic syndrome is feasible. The syndrome defined in this way also identifies individuals with increased cardiovascular risk.

Association of the *KCNJ11* genetic variant (rs5219) with progression of glycaemia in a 12-year prospective study

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Objective: The potassium inwardly rectifying channel, subfamily J, member 11 (*KCNJ11*) genetic variant, rs5219, has been found to be associated with type 2 diabetes mellitus (T2DM) in various populations. This project aimed to examine whether this genetic variant could predict the progression of glycaemia in a 12-year prospective study in Southern Chinese.

Methods: We conducted a 12-year prospective study in the population-based Hong Kong Cardiovascular Risk Factors Prevalence Study (CRISPS) cohort. We genotyped rs5219 in 427 subjects who showed progression of glycaemia (NGT \rightarrow IGT/IFG/T2DM or IGT/IFG \rightarrow T2DM) from baseline to 12-year follow-up assessment (CRISPS-3) and 901 subjects who were NGT at baseline and remained NGT at CRISPS-3.

Results: We observed significant association of rs5219 with the progression of glycaemia ($P=0.004$; OR=1.28; 95%CI, 1.08-1.52). Multivariate logistic regression analysis showed that rs5219 was independently associated with the progression of glycaemia, after adjustment for age, sex, body mass index (BMI) and the insulin resistance index HOMA-IR ($P=0.027$; OR=1.24; 95%CI, 1.03-1.51). Similar findings were obtained if waist circumference was included in the model instead of BMI ($P=0.019$; OR=1.26; 95%CI, 1.04-1.53). If HOMA-IR was replaced by fasting glucose level or 2-hour post-OGTT glucose level in the model, rs5219 remained a significant independent predictor of glycaemic progression, whether BMI or WC was included in the model.

Conclusions: These results suggested that the *KCNJ11* genetic variant rs5219 may be useful for prediction of the progression of glycaemia in Southern Chinese.

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Binding of anti-dsDNA antibodies to human mesangial cells is mediated by annexin II

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Introduction: Production of anti-dsDNA antibodies is a cardinal feature of lupus nephritis. The mechanism through which anti-dsDNA antibodies bind to resident renal cells remains to be fully elucidated. This study characterised the cross-reactive antigen(s) that mediates anti-dsDNA antibody binding to the surface of human mesangial cells (HMC).

Methods: Human polyclonal anti-dsDNA antibodies were isolated from patients with lupus nephritis by affinity chromatography. Binding of anti-dsDNA antibodies to HMC was assessed by flow cytometry and cellular ELISA. Purified plasma membrane proteins from HMC were separated by SDS-PAGE followed by Western blotting and membranes probed with anti-dsDNA antibodies. Proteins detected by anti-dsDNA antibodies were excised from polyacrylamide gels and subjected to MALDI-TOF spectrometry to identify 'cross-reactive' membrane proteins. Renal biopsies were also assessed by immunohistochemistry.

Results: Addition of chromatin material to HMC or removal of DNA from the surface of HMC had no effect on anti-dsDNA antibody binding, whilst removal of cell surface proteins with limited trypsin significantly reduced anti-dsDNA antibody binding. Anti-dsDNA antibodies predominantly bound to a cell surface antigen on HMC with a molecular weight of approximately 36kDa. MALDI-TOF identified the protein as annexin II. Glomerular expression of annexin II was significantly increased in patients with active lupus nephritis compared to controls or non-lupus kidney diseases, and the binding of anti-dsDNA antibodies to annexin II correlated with disease activity.

Conclusions: Our data demonstrated that annexin II is a cross-reactive antigen on the cell surface of HMC that binds anti-dsDNA antibodies. Binding of anti-dsDNA antibody to annexin II correlated with disease activity suggesting that annexin II may play a role in the pathogenesis of lupus nephritis. The functional consequence of anti-dsDNA antibody-annexin II interaction is currently being investigated.

Diagnostic value of a novel ultrasonographic scoring system of the salivary glands in Chinese patients with primary Sjogren's syndrome

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Objective: To evaluate the diagnostic value of a novel ultrasonographic scoring system of the salivary glands in a cohort of Chinese patients with primary Sjogren's syndrome.

Methods: A total of 29 patients with primary Sjogren's syndrome and 33 age- and sex-matched controls were recruited. Ultrasound examination of the parotid and submandibular glands was performed by a radiologist and a rheumatologist, and it was graded according to scoring system suggested by Hocevar et al. The sensitivity, specificity and the inter-observer variability of this ultrasonographic scoring system were determined.

Results: A cutoff value of 5.5 out of 48 reflected the best accuracy of diagnosis of 88%, which offered the sensitivity of 83% and the specificity of 94%. Incorporating the result of the serologic test and the ultrasound examination into analysis offered a better sensitivity and specificity of 94% and 100% respectively in the diagnosis of primary Sjogren's syndrome. There was only a fair inter-observer agreement of 0.361 between the radiologist and the rheumatologist.

Conclusion: Ultrasound examination of the salivary glands has been shown to be a sensitive and specific test for the diagnosis of primary Sjogren's syndrome.

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Introduction: Hospital admission is a risk factor for delirium and disorientation to elderly patients especially for those with underlying cognitive impairment. This study aimed to examine the effectiveness of an innovative orientation programme in enhancing the cognitive and orientation function of elderly patients in a regional geriatric hospital.

Methods: All patients admitted to the Geriatric Unit in Grantham Hospital during February to September 2010 were recruited. Subjects with mini-mental state examination (MMSE) score 10 or above and the MMSE time- and place-orientation subscore of 3 or below for each were included. Eligible subjects were randomly assigned to the intervention or control group. Verbal consent was obtained. Intervention consisted of an orientation programme, in which each session lasted for 30 minutes, conducted by an occupational therapist. The intervention group attended the programme 5 days a week for up to 10 sessions or stopped when both the MMSE time- and place-orientation subscores reached 4 or above. Both groups would receive usual occupational therapy. Outcome measures were MMSE total score and time- and place-orientation subscores. Demographic data and dementia history were collected. Independent *t* test and Chi-square test were used to compare the outcomes in the two groups before and after the programme as appropriate. A *P* value of less than 0.05 was regarded as statistically significant.

Results: There were 105 subjects in each group. There were no significant differences in age (82.33 vs 82.30 years, *P*=0.967), gender (male 23.8% vs 13.3%, *P*=0.051), place of residence (home 82.9% vs 85.6%, *P*=0.468) and prevalence of dementia (17.1% vs 14.3%, *P*=0.569) in the intervention and control groups, respectively. There was also no significant difference in baseline MMSE total score and time- and place-orientation subscores in the two groups. After the orientation programme, the intervention group showed significant improvement in MMSE total score (19.61 vs 16.48, *P*=0.000), MMSE time-subscore (2.93 vs 1.48, *P*=0.000) and MMSE place-subscore (3.90 vs 2.52, *P*=0.000). Subgroup analysis on patients with dementia (18 in intervention group and 15 in control group) showed similar finding that the MMSE time- and place-subscores were significantly higher in the intervention group after the programme.

Conclusion: An orientation programme is effective in improving the cognitive function of geriatric in-patients including those with dementia. Future studies may consider including subjects with more advanced cognitive impairment.

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Objective: Do-not-resuscitation (DNR) orders are commonly used in hospitals for providing compassionate end-of-life care. Factors associated with mortality is lacking locally. We aimed to study factors associated with mortality in geriatric patients designated for DNR.

Methods: All patients admitted to the geriatric unit in Grantham Hospital and who had a DNR order in 2009 were reviewed. Demographic information including age, gender, admission diagnosis, comorbid diseases, and functional state were obtained. Outcome measures were survival or death. Descriptive analysis was used to review the demographic features of studied population. Independent *t* test and Chi-square test were used to compare the mortality and survival groups on pre-selected variables. A *P* value of less than 0.05 was regarded as statistical significant.

Results: During the 1-year period, 142 patients were designated a DNR order. Of these, 101 (71.1%) were female. Mean age (\pm SD) was 87.3 \pm 7.9 years; 62.0% were living in old-age home, 57.7% were immobile, and 82.4% were dependent in activities of daily living. The admission diagnosis included pneumonia (34.5%), stroke (12.0%), urinary tract infection (10.6%), renal failure (8.5%), heart failure (6.3%), ischaemic heart disease (4.9%), gastroenteritis (4.2%), diabetes mellitus (2.8%), and malignancy (2.1%). Among the studied population with DNR order, mortality was 33.1% (47/142). There was no significant difference in age, functional and residential status, timing between DNR order and death or discharge, length of stay in hospital between the survival and mortality groups. However, it was found that being a male was significantly at a higher risk of mortality (46.3% vs 27.7%, *P*=0.033). Mortality was higher in those admitted for gastroenteritis (83.3% vs 30.4%, *P*=0.014), but lower in those admitted for urinary tract infection (6.7% vs 35.7%, *P*=0.017) and those who had dementia (21.3% vs 41.3%, *P*=0.012).

Conclusion: Being a male or those admitted for gastroenteritis were found to be at a higher risk of mortality. Those admitted for urinary tract infection and those with dementia seemed to do better. Future prospective studies are suggested to include the reasons of DNR, attitudes, perception and knowledge of medical staff and family members towards DNR and to verify the findings of current study.

Supraventricular ectopic activity and new occurrence of atrial fibrillation

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Introduction: Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia encountered in clinical practice. Although patients often with symptoms including palpitation preceding the first-diagnosed AF, AF is often diagnosed when its complications such as ischaemic stroke occur. While various risk factors have been identified for increasing risk of AF, predicting occurrence of new AF remains challenging. Here, we tested the hypothesis whether a 24-hour ECG-derived parameter, excessive supraventricular ectopic activity (SVE), could be exploited to predict new-onset AF.

Methods and Results: From 2002 to 2004, 428 patients without pre-existing AF or structural heart disease undergoing 24-hour ECG monitoring for palpitation, dizziness, and syncope were recruited. Of these, 107 patients with SVE at the top quartile (ie >100 SVE/day) were defined to have excessive SVE. After a mean follow-up of 6.1 ± 1.3 years, 31 (29%) patients with excessive SVE developed AF whereas only 29 (9%) patients with SVE ≤ 100 /day developed AF ($P < 0.01$). Likewise, patients with excessive SVE were more likely to develop ischaemic stroke compared with those with SVE ≤ 100 /day ($P < 0.01$). Furthermore, Cox regression analysis revealed that excessive SVE was an independent predictor of new occurrence of AF (HR=3.32; 95% CI, 1.96-5.65; $P < 0.01$).

Conclusion: Excessive SVE predicts subsequent development of AF.

Anti-tumour efficacy of recombinant human arginase in combination with chemotherapeutic agents in human hepatocellular carcinoma

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Introduction: Systemic chemotherapy of hepatocellular carcinoma (HCC) relies on few drugs and the response rates are low especially in patients with advanced HCC. HCC is considered as auxotrophic for arginine due to the lack of expression of argininosuccinate synthetase (ASS). Several recombinant human arginases were found to be effective in inhibiting liver, pancreatic and leukaemic cell proliferation. Recently, a new recombinant human arginase, BCT-100, has been synthesised to deprive arginine and inhibit arginine-dependent tumour growth. In this study, the efficacy of BCT-100 and the combined used of BCT-100 with cisplatin and fluorouracil on the inhibition of in-vitro cell proliferation of HCC cell lines and in-vivo tumour growth was studied.

Methods: The anti-tumour efficacy of BCT-100, cisplatin and fluorouracil, alone or in combination, on cell proliferation, cell cycle distribution and cellular apoptosis were determined in human hepatoma HepG2, Hep3B and PLC/PRF/5 cells. Protein phosphorylation and expression in the Wnt/ β -catenin pathways, and expression of cyclin D1, eIF4E, survivin and XIAP, were also analysed by Western blotting. For in-vivo animal studies, subcutaneous tumours were established by subcutaneous injections of 1×10^6 cells into nude mice. BCT-100 in combination with cisplatin or fluorouracil was administered. Mice were sacrificed at week 16 or when tumour sizes exceeded 30% of their body weight.

Results: Treatment with BCT-100 alone was found to inhibit cell proliferation and enhance cellular apoptosis. Additive effect of BCT-100 with cisplatin or fluorouracil was found in inhibiting cell proliferation and increasing apoptosis of HepG2, Hep3B and PLC/PRF/5 cells. Cell cycle arrest at G1/S phase was also observed with BCT-100 treatment. A significant reduction in β -catenin, cyclin D1, phosphorylated eIF4E, survivin and XIAP expression was observed. Furthermore, repeated sequential use of BCT-100 and chemotherapy with either cisplatin or fluorouracil demonstrated synergistic effect of inhibition of tumour growth compared with BCT-100 or chemotherapy alone.

Conclusion: These preclinical data suggested additive/synergistic effect of BCT-100 with other chemotherapeutic agents in HCC, which suggested the rationale of combining BCT-100 and chemotherapy in clinical treatment of HCC.

Detection of CD44⁺ cancer initiating cells in pleural effusion of a liver cancer patient

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Introduction: Advanced hepatocellular carcinoma (HCC) is always accompanied with multiple lung metastasis which may result in an accumulation of fluid in the pleura. Recently, the concept of cancer-initiating cells in HCC has been established. By studying the cells isolated from the pleural effusion (PE) may provide a model to study the presence of cancer-initiating cells which lead to metastatic disease in HCC. The objective of this case study was to detect a subpopulation of CD44⁺ cancer-initiating cells in PE.

Methods: PE-derived cells were isolated from PE using ficoll density gradient and primary culture was sustained in DMEM with 30% v/v sterilely filtered PE-fluid to provide the microenvironment for the growth of the isolated cells. Flow cytometry was performed to detect the amount of CD44⁺ subpopulation. CD44⁺ and CD44⁻ cells were then isolated by fluorescent-activated cell sorting (FACS) and spheres formation was performed using 1000 cells/well on an ultra-low attachment plate. CD44⁺ cells were also subjected to differentiate into osteocytes using the StemPro osteogenesis differentiation kit. PCR analysis was performed to detect the stem cell and liver cell marker of the CD44⁺ subpopulation.

Results: Flow cytometry analysis demonstrated that over 90% of the cells isolated were CD44⁺ cells. PE-derived cells were able to be expanded in culture for several weeks. CD44⁺ but not CD44⁻ cells were able to form spheres in vitro. CD44⁺ cells were also able to be differentiated into osteocytes with positive staining of calcium accumulation using Alizarin Red S staining. PCR analysis demonstrated the expression of markers for stem cell (Lin28, Oct4, Nanog, Sox2 and Msi1) and liver cell (alpha-fetoprotein, albumin and asiaglycoprotein receptor).

Conclusion: This is a case study demonstrating the presence of cancer-initiating cells in PE by detection of CD44⁺ cells. The isolation of PE-derived cells might provide a model for subsequent molecular and cellular analyses, which allows the study of cancer-initiating cells in metastatic HCC patients and provides a direction for the preclinical development of rational therapeutics.

Plasma pigment epithelium-derived factor as a mediator of insulin resistance associated with estrogen deficiency

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Objective: Pigment epithelium-derived factor (PEDF), a serine protease inhibitor secreted from adipose tissue and present at high concentrations in the circulation, has been found in mice to play a pathogenic role in obesity-induced insulin resistance and metabolic dysfunctions. Serum PEDF levels are lower in women and treatment of cultured human ovarian surface epithelial cells with 17 β -estradiol inhibited the expression of PEDF at a transcriptional level. To further delineate the relationship between estrogen and PEDF, we investigated the change in PEDF levels in pre-menopausal women following bilateral salpingo-oophorectomy (BSO).

Methods: We measured body mass index (BMI), waist circumference (WC), percentage body fat, plasma PEDF and estradiol levels in a group of pre-menopausal women before and after BSO for benign gynaecological conditions. Repeated measure ANOVA was used to analyse the changes (Δ) in PEDF, estradiol, homeostatic model assessment of insulin resistance (HOMA-IR) and quantitative insulin-sensitivity check index (QUICKI) following BSO.

Results: Twenty-one pre-menopausal women (age, 49.57 \pm 3.54 years) were recruited. The mean duration between pre- and post-operative assessment was 4.01 \pm 0.72 months. BMI was marginally reduced in the postoperative period (25.24 \pm 3.98 kg/m² preop vs 24.80 \pm 4.34; P=0.048). However, no significant difference between WC or percentage body fat was observed. The marked reduction in plasma estradiol levels postoperatively (225 [109-418] pmol/L preop vs 32 [19-50]; P<0.001) was accompanied by an increase in plasma PEDF levels (7.66 \pm 1.61 ng/mL preop vs 8.77 \pm 1.53; P=0.001, after controlling for change in BMI). An inverse relationship was found between Δ estradiol and Δ PEDF (r =-0.497; P=0.022). There was also a significant increase in insulin resistance postoperatively, as indicated by HOMA-IR (1.06 [0.73-1.93] preop vs 1.63 [1.06-2.26]; P=0.002) and QUICKI (0.38 \pm 0.04 preop vs 0.36 \pm 0.03; P=0.006). Δ HOMA-IR and Δ QUICKI became insignificant (P=0.101 and 0.141 respectively) after adjusting for Δ PEDF.

Conclusions: We have demonstrated that the reduction in plasma estradiol levels after BSO was significantly associated with an increase in plasma PEDF levels and insulin resistance in a group of pre-menopausal women. The loss of statistical significance in Δ HOMA-IR and Δ QUICKI after adjusting for Δ PEDF suggests that PEDF may play a role in mediating insulin resistance in these women with surgical menopause, and its expression is likely regulated by estradiol, as evidenced by the correlation between Δ estradiol and Δ PEDF.

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Introduction: Transplantation of adult donors'-derived mesenchymal stem cells (MSCs) is able to improve cardiac function in experimental acute myocardial infarction models. However, the poor survival rate of adult MSCs in ischaemic environment has limited its therapeutic efficiency. Previous investigations have indicated that MSCs derived from early embryonic stage such as embryonic stem cells or induced pluripotent stem cells (iPSCs) have greater potential of proliferation and differentiation. Nevertheless, up to date, therapeutic capacity of mouse MSCs derived from iPSCs remains elusive. This ongoing study therefore aimed to derive and isolate mouse MSCs from iPSCs for attenuation of tissue ischaemia. We hypothesise that transplantation of mouse-induced pluripotent stem cell-derived mesenchymal stem cells (iPSC-MSCs) will achieve a better therapeutic efficiency by enhanced retention or survival rate compared to adult bone marrow-derived mesenchymal stem cells.

Methods: We utilised a three stepwise method to derive and isolate mesenchymal-like stem cells from mouse iPSCs. Firstly, feeder cells and leukaemia inhibitory factor (LIF) were removed to induce iPSCs into spontaneous differentiation. The subsequent step is enrichment of MSCs by conditioned medium with basic fibroblast growth factor (FGF2) and epidermal growth factor (EGF) supplements. Lastly, isolation of enriched MSCs was analysed by fluorescence-activated cell sorting (FACS) to isolate subpopulation CD90+/CD133-. The single cell-derived MSC-like colonies were expanded by limiting dilution. These cells were subjected to surface markers profiling and multipotent differentiation studies, namely adipogenesis, osteogenesis and chondrogenesis.

Results: The iPSC-derived cells were morphologically similar to adult bone marrow-derived MSCs. These cells were negative for CD34, anti Oct4, anti TRA-1-60 and CD133, while being positive for mesenchymal markers, CD44, CD73 and CD90. These cells were further induced into adipocytes, osteocytes and chondrocytes under differentiation-conditioned medium.

Conclusion: Derivation of mouse mesenchymal stem cells from mouse-induced pluripotent stem cells was successful. These cells hold potential therapeutic properties for ischaemic disease regeneration in near future.

Enhanced therapeutic efficacy of transarterial chemoembolisation treatment in hepatocellular carcinoma (HCC) by mTOR inhibitor RAD001: implication for a novel treatment regimen in HCC

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Introduction: Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide. Transarterial chemoembolisation (TACE) is commonly used for the treatment of locally advanced HCC by dual actions of chemotherapy and ischaemic hypoxia. In this study, the therapeutic efficacy of RAD001, a novel mTOR inhibitor and its potential combinatory use with TACE for treatment of HCC using orthotopic HCC models was investigated.

Methods: Therapeutic efficacy of RAD001 and TACE was investigated in vivo and in vitro. For in-vivo studies, equal portions of established xenografts were orthotopically implanted into mice to generate HCC tumours. RAD001 was administered orally or by intraportal vein injection, hepatic artery ligation was performed to mimic transarterial chemoembolisation treatment, and chemotherapeutic effect was achieved by intraportal injection of cisplatin. For in-vitro studies, MHCC97L cells were treated with cisplatin and RAD001 under normoxic and hypoxic conditions to investigate their biological effects on HCC cells, including cell proliferation and regulation of different targets in mTOR pathway.

Results: Significant inhibition of tumour growth was observed when MHCC97L was treated with RAD001 and cisplatin in orthotopic mouse models. Combination of hepatic artery ligation combined with RAD001 treated through portal vein led to >90% tumour shrinkage when compared with the non-treated HCC controls. In addition, synergetic inhibitory effect was observed when RAD001 combined with cisplatin treated through TACE in orthotopic HCC model. In-vitro studies also showed inhibition of cell proliferation by 52% and 40% in normoxic and hypoxic condition, respectively, when treated with 10 nm RAD001 alone. Cell proliferation was further inhibited when treated in combination with 2 mg/mL of cisplatin by 56% under normoxia and 36% by hypoxia. Western blot analysis revealed inhibition of mTOR, p70S6K(thr389) and PRAS40 phosphorylation by RAD001, and also downregulation of HIF-1 α via the mTOR pathway under hypoxic conditions.

Conclusion: RAD001 used in combination with TACE could effectively inhibit tumour growth via the mTOR pathway and also enhances the cisplatin-induced toxicity towards HCC, providing the basis for use of RAD001 in combination with TACE + cisplatin as an effective regimen for treatment of HCC.

Distinct roles of microRNA-1 and -499 in ventricular specification and maturation of human embryonic stem cells

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Aims and Methods: MicroRNAs (miRs) negatively regulate transcription and are important determinants of normal heart development and heart failure pathogenesis. Despite the significant knowledge gained in mouse studies, their functional roles in human (h) heart remain elusive. We hypothesised that miRs that figure prominently in cardiogenesis are differentially expressed in differentiating, developing, and terminally mature human cardiomyocytes (CMs).

Results: As a first step, we mapped the miR profiles of human (h) embryonic stem cells (ESCs), hESC-derived (hE), fetal (hF) and adult (hA) ventricular (V) CMs. 63 miRs were differentially expressed between hESCs and hE-VCMs. Of these, 29, including the miR-302 and -371/372/373 clusters, were associated with pluripotency and uniquely expressed in hESCs. Of the remaining miRs differentially expressed in hE-VCMs, 23 continued to express highly in hF- and hA-VCMs, with miR-1, -133, and -499 displaying the largest fold differences; others such as miR-let-7a, -let-7b, -26b, -125a and -143 were also significantly expressed in h fibroblasts, indicating non-cardiac specificity. Functionally, LV-miR-499 transduction of hESC-derived cardiovascular progenitors significantly increased the yield of hE-VCMs (to 72% from 48% of control; $P < 0.05$) and contractile protein expression without affecting their electrophysiological properties ($P > 0.05$). By contrast, LV-miR-1 transduction did not bias the yield ($P > 0.05$) but decreased ADP and hyperpolarised RMP/MDP in hE-VCMs due to increased I_{to} , I_{Kr} and I_{Kv} and decreased I_f ($P < 0.05$) as signs of maturation. Also, LV-miR-1 but not -499 augmented the immature Ca^{2+} transient amplitude and kinetics.

Conclusion: Based on these and additional molecular pathway analyses, we conclude that miR-1 and -499 play differential roles in human cardiogenesis, and their effects are context dependent. While miR-499 promotes ventricular specification of hESCs, miR-1 serves to facilitate electrophysiological maturation.

An event-related potential study on the information flow during prospective memory interference

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Background: Electroencephalography (EEG) can examine the temporal sequence of brain activity related to a specific event, which is referred to as event-related potential (ERP). Prospective memory (PM) interference presents the interference effect of an embedded PM task on other ongoing task. In the previous functional MRI study, we found that the fusiform gyrus, left inferior parietal lobe and left frontal lobe play an essential role in PM interference effect. However, little temporal information could be retrieved from the functional MRI data.

Methods: Six young subjects participated in the present ERP study on PM task. The PM task was the same as that in fMRI. Briefly, there were ongoing and PM condition, both of them had four blocks of 42s task and 18s rest. The ERP data were collected by NeuroSCAN machine. Preliminary average data of each participant were preprocessed by SCAN software, then they were further processed by the EEG function in SPM software.

Results: Behavioural data showed that the reaction time of pure ongoing trial, contaminated ongoing trial and PM trial were 354, 391, 467 ms, and their accuracy were 99%, 99% and 95%, respectively. ERP data showed that no significant difference of EEG between pure and contaminated ongoing was found in the occipital lobe at about 150 ms after stimuli presentation, then it spread to the temporo-occipital lobe at around 332-350 ms, to the prefrontal area at 566 ms, then to the posterior parietal lobe at 619 ms, finally to the posterior frontal lobe at 725-746 ms.

Conclusion: The ERP data are largely concurred with fMRI result with regard to location. Additionally, it showed that brain activity related to PM interference may initiate at the temporo-occipital lobe, then spread to the prefrontal area, posterior parietal lobe, finally to the posterior frontal lobe. Such ERP data demonstrated the information flow of PM interference in young adults.

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The effect of normal and abnormal ageing on prospective memory showed increased cognitive conflict: a functional MRI study

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Introduction: Prospective memory (PM) is memory for planned intention, which needs to be executed appropriately in the future. PM task is usually embedded in ongoing activities. The neural correlates of PM have not been elucidated. To date, no functional imaging study has been conducted to examine the relevant functional change of PM during the processes of ageing and dementia.

Methods: Twelve patients with mild AD, 12 age-matched old adults, and 11 young adults were recruited. A laboratory event-based PM paradigm was revised and applied to this study. It was block-designed. Basically, there were three conditions conducted sequentially: baseline ongoing condition, no-go condition, and PM condition. Each condition had four alternations of 42s activation and 18s rest, which lasted for 4 minutes. The fMRI study was conducted with a 3T Philips MRI scanner in MRI centre of the University of Hong Kong. Three conditions were scanned separately. TR=2000 ms, TE=40 ms, FOV: 230×230×128 mm, temporal resolution: 1.8×1.8×4 mm, flip angle: 90°, slice thickness=4 mm. The data were processed by standard procedure of statistic parametric mapping (SPM8).

Results: Behavioural data showed that accuracy of three groups in three conditions were all higher than 90%. fMRI results showed that the activation of brain during task performance were larger in old adults and AD patients than in the young adults, including supplementary motor area, precentral and postcentral gyrus, inferior parietal lobe. PM task activates a similar network of bilateral, especially left inferior frontal lobe, angular gyrus and inferior temporal gyrus among three groups.

Conclusion: There is obvious compensation of the old adults and AD patients when performing PM task, although PM task activates a similar brain network for action observation and execution among the groups. Interestingly, PM-specific activations in frontal lobe of young, old adults and patients with AD demonstrate a rostral-caudal activation. This may imply increasing cognitive conflict among the three groups.

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Fibroblast growth factor 21 promotes glucose uptake through transactivation of glucose transporter-1 gene in adipocytes

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Introduction: Fibroblast growth factor-21 (FGF-21) is a liver-secreted hormone with multiple beneficial effects on obesity-related disorders. Recent studies indicated that FGF-21 induces glucose uptake through a pathway independent of insulin in adipocytes. However, the molecular mechanism underlying this FGF-21 action remains elusive. Our study aimed to investigate the signalling transduction pathway by which FGF-21 upregulates glucose transporter (GLUT)1 in adipocyte.

Method: 3T3-L1 adipocytes were stimulated with recombinant FGF-21 and the mRNA level of GLUT1 was quantified by real-time PCR. The activity of the protein kinases Erk1/2 was assessed by Western blotting against phospho-Erk1/2. The transcriptional activity of the GLUT1 promoter was measured by the luciferase reporter assay and chromatin immunoprecipitation (ChIP) was performed to evaluate the association of the transcription factors with the promoter. Suppression of Erk1/2 was achieved by its pharmacological inhibitor PD98059.

Results: FGF-21 induced GLUT1 expression through transcriptional activation. This effect was mediated by Erk1/2, which promoted the recruitment of Ets-like protein 1 (Elk-1) and serum response factor (SRF) to the highly conserved E-Twenty Six (ETS) and Serum Response Element (SRE) binding motifs located within the distal region of the GLUT1 promoter. Furthermore, FGF-21-evoked phosphorylation of Erk1/2, transcriptional activation of the GLUT1 gene and induction of glucose uptake were markedly attenuated in the diet-induced obese mice.

Conclusion: FGF-21 induces GLUT1 expression through Erk1/2, which in turn activates SRE/ETS signalling cascade to enhance glucose uptake in adipocytes. The present findings suggest that FGF-21 signalling pathway may represent an appealing therapeutic target for the treatment of obesity-related metabolic diseases.

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Non-gastric marginal zone B-cell lymphoma: clinicopathologic features and outcome

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Introduction: The optimal treatment strategy and outcome of non-gastric marginal zone lymphoma (MZL) remains undefined. The role of rituximab and fludarabine in MZL has not been critically appraised and compared with conventional chemotherapy. Our objective was to review the clinicopathologic features and determine treatment outcome using rituximab- and fludarabine-based regimens in non-gastric MZL.

Methods: We retrospectively analysed 81 consecutive patients with non-gastric MZL (mucosa-associated lymphoid tissue lymphoma, n=66; splenic MZL, n=11; nodal MZL, n=4) between January 1998 and January 2010.

Results: As a group, treatment results were favourable, with an overall response rate of 87%, and a complete response (CR) rate of 73%. The CR rate was similar for conventional chemotherapy, and rituximab- and fludarabine-containing regimens. However, the relapse rates were significantly reduced in rituximab- and fludarabine-containing regimens (13% vs 41%, P=0.015 and 10% vs 42%, P=0.03 respectively). The use of rituximab and fludarabine was associated with acceptable treatment toxicity. For splenic MZL, splenectomy was significantly associated with a superior CR rate. Favourable prognostic factors for CR were early-stage (I/II) disease (odds ratio=3.11, P=0.039), good performance status (≤ 1) [odds ratio=9.36, P<0.001] and low IPI risk score (<3) [odds ratio=4.17, P=0.011]. The 5-year overall survival was 84.6% (95% CI, 0.73-0.92) and the 5-year disease-free survival was 82.2% (95% CI, 0.70-0.90). During the follow-up period, 14 patients (non-gastric MZL, n=9; splenic MZL, n=5) died—10 from refractory diseases, two from treatment-related causes, one from therapy-related leukaemia, and one from unrelated causes. Prognostic factors associated with a lower mortality included stage I/II disease (odds ratio=0.08, P<0.001) and IPI risk score <3 (odds ratio=0.09, P<0.001).

Conclusion: Rituximab and fludarabine were safe and efficacious for non-gastric MZL and resulted in more durable remissions.

Factors influencing heart rate recovery after exercise training among patients with prior myocardial infarction

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Aims: Heart rate recovery (HRR), defined as the heart rate (HR) decrease through the first minute after peak exercise, has been shown to predict cardiac death after myocardial infarction (MI). Previous studies have found that HRR improved with exercise training, but whether there exist other factors that affect post-training HRR remains uncertain. This study sought to evaluate the relationship between clinical and exercise stress test parameters and HRR after exercise training.

Methods: Consecutive patients with MI who enrolled into our cardiac rehabilitation programme in Tung Wah Hospital and completed an 8-week exercise training programme were recruited. The relationship between clinical and exercise stress test parameters and post-exercise training HRR were analysed in a multivariate linear regression model. P<0.05 was considered as clinically significant.

Results: A total of 334 patients (mean age 63.7±11.2 years, 76.6% male) were included in the analysis. HRR was shown to significantly increase after exercise training (17.5±10.0 to 19.0±12.3 bpm, P=0.011). Multivariate linear regression analysis showed that the age of enrolment (P=0.014), hypertension (P=0.037), and post-training resting HR (P>0.001) negatively correlated with HRR after exercise training, while pre-training parameters including peak HR (P=0.015), exercise capacity (P=0.008) and HRR (P<0.001), and post-training parameters including peak HR (P<0.001) and exercise capacity (P<0.001), positively correlated with HRR after exercise training. On the contrary, diabetes mellitus, renal disease, the use of beta-blockers and calcium channel blockers, were not shown to correlate with post-exercise training HRR.

Conclusion: HRR, which improved with exercise training, was found to be influenced by various clinical and exercise parameters, but not the use of beta-blockers, which makes it a useful prognostic marker among MI patients who treated with beta-blockade.

High attainment rate of low-density lipoprotein cholesterol among high-risk patients treated with lipid-lowering agents — experience from the Cardiac Clinic, Queen Mary Hospital

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Aim: It has been shown that maintenance of satisfactory low-density lipoprotein cholesterol (LDLC) level effectively reduced morbidity and mortality associated with cardiovascular disease. Nevertheless, previous studies have demonstrated a uniformly low LDLC goal attainment rate across different geographic areas. This study sought to evaluate the LDLC goal attainment rate among high-risk patients treated in our centre and to identify the determinants of effective LDLC management.

Methods: A cross-sectional study was conducted in the cardiology clinics of Queen Mary Hospital between April and December 2008. Patients aged ≥ 18 years with ≥ 2 cardiovascular risk factors, who had been treated with lipid-lowering agents for at least 3 months and no dose adjustment in recent 6 weeks were recruited. Demographic data and other relevant clinical information were collected, and full lipid profiles were measured and compared with therapeutic targets. All definitions and criteria set by the updated 2004 Adult Treatment Panel III guidelines of the National Cholesterol Educational Program (NCEP ATP III) on cholesterol management were applied.

Results: A total of 561 patients (mean age 65.2 ± 10.6 years, 76.9% male) were identified; 534 of them (95.2%) had coronary artery disease (CAD). In all, 465 (82.9%) patients achieved their LDLC goals, including 83.1% of those with target LDLC < 70 mg/dL, 100% of those with target LDLC < 100 mg/dL, and 53.8% of those whose LDLC target < 130 mg/dL. Univariate logistic regression analyses revealed that patient's baseline LDLC (OR=0.98; 95% CI, 0.97-0.99; $P < 0.001$ for each 100 mg/dL), gender (female: OR=0.62; 95% CI, 0.39-0.99; $P = 0.043$), systolic (OR=0.98; 95% CI, 0.97-0.99; $P = 0.002$) and diastolic blood pressure (OR=0.97; 95% CI, 0.95-0.99; $P = 0.002$), physician's gender (female: OR=0.31; 95% CI, 0.14-0.70; $P = 0.009$) and years of practice (OR=1.37; 95% CI, 1.01-1.87; $P = 0.046$), prescription of lipid-lowering drugs only to patients who adhered to life-style modification (OR=5.64; 95% CI, 3.00-10.63; $P < 0.001$), patients informed of their high-density lipoprotein cholesterol (OR=1.90; 95% CI, 1.10-3.28; $P = 0.026$) and triglyceride levels (OR=1.91; 95% CI, 1.08-3.39; $P = 0.03$) were significant predictors of LDLC goal attainment.

Conclusion: High-risk patients treated with lipid-lowering agents in our centre were able to achieve a very high LDLC attainment rate with majority of patient achieved LDLC < 70 mm Hg. Both patients' understandings of their lipid profile and physicians' practice were shown to have a major impact on treatment outcomes, reflecting the importance of patient's and doctor's education and their communication in implementing effective lipid-lowering therapy.

Role of heme oxygenase-1 in intermittent hypoxia-induced inflammation and oxidative stress in EAhy 926 endothelial cell line

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Introduction: Intermittent hypoxia (IH) is a hallmark feature in obstructive sleep apnoea (OSA), which is increasingly recognised as an independent risk factor for atherosclerosis. Although the underlying mechanisms have not been fully understood, inflammation and oxidative stress have been suggested as major pathological events initiating or accelerating atherogenesis. Heme oxygenase-1 (HO-1) plays a regulatory role in the inflammatory response by modulating production of pro-inflammatory cytokines, and its expression is mediated by nuclear translocation of transcription factor Nrf-2. This study was to address whether IH would upregulate the expression of pro-inflammatory cytokines via HO-1 expression in endothelial cells in vitro.

Methods: EAhy 926 cells were exposed to intermittent normoxia (IN as control) or IH (a 10-min hypoxia [5% O₂] followed by a 5-min normoxia [21% O₂] for 64 cycles using the BioSpherix OxyCycler C42 system [BioSpherix, Redfield, NY]). IL-6 and IL-8 mRNA expressions were measured by RT-PCR, and protein secreted was measured by ELISA. Cellular activities of GSH-related enzymes such as glutathione peroxidase (GPx) and glutathione reductase (GR) were analysed. Whole cell lysates, cytosolic and nuclear fractions were extracted to perform Western blot for HO-1, Nrf-2, and phospho-ERK.

Results: IH increased the production of IL-6 and IL-8 protein without changing the mRNA levels. IH also enhanced the enzyme activities of GPx and GR. On the other hand, IH suppressed HO-1 and Nrf-2 expression, accompanied by the inhibition of ERK phosphorylation.

Conclusions: These data suggest an underlying mechanism for OSA subjects on the process of atherogenesis and a potential role of HO-1 in the therapeutic benefit for OSA-related atherosclerosis.

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Activation of c-Rel mediates neuroprotection via mitochondrial uncoupling protein-4 under NF- κ B pathways

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Objectives: To examine the role of the cRel, subunit of NF- κ B, involved in the protective mechanism of uncoupling protein-4 (UCP4) in promoting survival signals and preventing activation of death signals during mitochondrial dysfunction.

Methods: c-Rel construct was co-transfected with UCP4-promoter-luciferase-fusion expression vector into SH-SY5Y cells. Luciferase activity was measured to determine the promoter activity of UCP4. Changes in protein expression of UCP4 were measured by Western blot and real-time RT-PCR. LDH release assay was performed to measure cell death induced by mitochondrial toxin, MPP⁺. Nuclear binding of c-Rel on UCP4 promoter region was shown by gel-shift assay (EMSA).

Results: Our data showed that UCP4 mRNA was induced by MPP⁺. Blockage of NF- κ B caused severe cell death after MPP⁺ exposure. Overexpression of c-Rel subunit had no effect on the p50 and p65 expression in SH-SY5Y cells. UCP4 promoter activity and protein expression were significantly increased upon overexpression of c-Rel. Moreover, EMSA showed that up-regulation of c-Rel causes an increased binding to the putative NF- κ B site in the promoter region of UCP4.

Conclusions: Our group has characterised the critical promoter region of human UCP4 gene and identified several transcription factors regulating UCP4 gene expression, including NF- κ B. We have reported that UCP4 is transcriptionally regulated by NF- κ B, via a functional NF κ B site in its promoter region, and that UCP4 has a significant role in NF- κ B pro-survival signalling, mediating its protection against MPP⁺ toxicity. NF- κ B was shown to have dual role in regulating neuronal survival and death by specific activation of its diverse NF- κ B complexes. We have identified the effect of c-Rel in regulating UCP4 expression in neuronal cells. Elucidation of the pro-survival effects of c-Rel via regulating UCP4 will provide useful information to understand the physiological importance of neuroprotection of mitochondrial uncoupling proteins under NF- κ B pathways.

Endothelial progenitor cells derived from human-induced pluripotent stem cells, embryonic stem cells, and bone marrow stem cells in attenuation of limb ischaemia in mice

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Background: Human-induced pluripotent stem cells (hiPSC) have the capacity to differentiate into multiple lineages and provide an unlimited pool of cells for autologous cell replacement therapies. Differentiation of endothelial cell (EC) from human embryonic stem cell (hES) holds great promise on the therapeutics efficacy. The present study aimed to differentiate and isolate EC from hiPSC and hES to compare their respective properties and efficacy.

Methods and Results: We previously established a protocol to differentiate hES into EC.¹ We applied the same protocol to hiPSC to examine their directed differentiation. Three hESC cell lines and six hiPSC were successfully differentiated into CD34⁺CD31⁺ EC revealed by flow cytometry. EC population was then enriched by CD34⁺CD31⁺ cell sorting and further expanded. The morphology of iPSC-derived EC (hiPSC-EPC) and hESC-derived EC (hESC-EPC) morphologically resemble adult EC. These ECs possess the common endothelial-specific function, such as uptake of acetylate LDL and ability to form vascular tube like networks on Matrigel. Furthermore, key proangiogenic cytokines were present in all established ECs conditioned-medium suggesting their ability to promote angiogenesis in a paracrine manner. Transplantation of hESC-EC and hiPS-EC significantly improve neovascularisation in a mouse model of hindlimb ischaemia. The benefits of hiPSC-EPCs on limb ischaemia were comparable with those of hESC-derived EPCs and adult bone marrow EPCs.

Conclusion: Our results suggest that EPC could be differentiated from hiPSC and might be useful as an alternative source for angiogenic therapy.

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Objectives: Neuronal differentiation is involved in brain development. Stimulation of all-trans-retinoic acid (RA) in neuroblastoma cells results in growth inhibition, increased neuron-specific enolase (NSE) activity, and promoted axonal growth. Mitochondrial uncoupling protein-4 (UCP4) is specifically expressed in brain. UCP4 overexpression is neuroprotective against mitochondrial dysfunction by increasing ATP supply. We aimed to determine changes of UCP4 expression, and its role in modulating energy supply during neuronal differentiation.

Methods: We stably overexpressed human UCP4 in SH-SY5Y cells. Neuronal differentiation was induced by RA for 14 days in both UCP4 overexpressing and vector control cells. Neurite growth was visualised by immunostaining of neuronal tubulin (Tuj1), and changes in UCP4 expression were determined by Western blot. Number of neurite-bearing cells and neurite length were determined by confocal microscopy. The total ATP levels were measured by luciferase bioluminescent assay.

Results: Treatment of RA for 14 days induced neuronal differentiation in SH-SY5Y cells, as visualised by morphological changes and induction of neurite projection. The lengths of neurites in UCP4 overexpressing cells were significant longer compared with the controls. During the time course, UCP4 expression was gradually increased in parallel with Tuj1. The pattern of changes of ATP levels in both UCP4 overexpressing and vector cells were similar. However, the ATP level in UCP4 overexpressing cells was consistently higher than the controls.

Conclusions: This study revealed potential role of UCP4 in promoting neuronal differentiation, possibly via increasing energy supply. Higher ATP level in UCP4 overexpressing cells is likely rendering the cells better energy supply for the differentiation processes. Knowledge in UCP4 which modulate neuronal energy supply and differentiation has shed light on potential cell therapies against various neurodegenerative diseases.

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Background: Obesity is a major risk factor for non-alcoholic fatty liver disease (NAFLD). Our recent study showed that circulating level of adipocyte fatty acid binding protein (A-FABP) is elevated in patients with NAFLD and correlated with hepatic inflammation and fibrosis. We also demonstrated that both acute and chronic liver injuries are accompanied by elevated A-FABP expression in Kupffer cells. This study aimed to examine whether pharmacological inhibition of A-FABP acts as a promising strategy for the treatment of NAFLD using a diet-induced obese mouse model.

Methods: C57 male mice were fed with a high-fat high-cholesterol (HFHC) diet to induce chronic liver injury and administered with an A-FABP selective inhibitor, BMS309403 (BMS), for 16 weeks. Serum alanine aminotransferase (ALT), aspartate transaminase (AST), triglyceride and cholesterol levels, and hepatic fat content, were measured by biochemical methods. Quantitative-PCR was performed to determine the hepatic expression levels of A-FABP and several pro-inflammatory cytokines, as well as endoplasmic reticulum (ER) stress and fibrotic markers. H&E staining and oil red O staining were performed to determine the inflammatory status and necrosis in the liver.

Results: Oral administration of BMS alleviated glucose intolerance, insulin resistance and liver injury in HFHC-diet-induced obese mice. Treatment with BMS markedly reduced the hepatic expression of pro-inflammatory cytokines (TNF- α , MCP-1, IL-6), endogenous A-FABP, fibrotic (pro-collagen and TIMP-1) and ER stress (CHOP, GRP78 and XBP-1) markers. H&E staining and oil red O staining indicated the alleviation of inflammatory status and steatosis.

Conclusion: A-FABP may play an aetiological role in obesity-induced NAFLD by inducing ER stress and inflammation. The selective inhibitor of A-FABP may represent a promising drug candidate for the treatment of NAFLD.

Acknowledgement: This study was supported by a GRF grant (#768209) from the RGC, Hong Kong.

Functional expression of transient receptor potential channels in human preadipocytes

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Background: Preadipocytes are extensively used as a type of proliferative cell culture model to investigate proliferation and differentiation of adipocytes and lipodystrophy (eg obesity)-related metabolic dysfunctions and disease. However, cell biology is not well understood in human preadipocytes. The present study was to investigate the expression of transient receptor potential (TRP) channels in human preadipocytes.

Methods: Human adipocytes were cultured in DMEM medium, and expression of TRP channels was using whole-cell patch voltage-clamp and RT-PCR approaches.

Results: A small background current was inhibited by the TRPC channel blocker La^{3+} . Removal of Mg^{2+} of pipette solution or bath solution induced a Mg^{2+} -sensitive current, which was suppressed by 2-aminoethoxydiphenyl borate. In addition, an intracellular calcium-activated current was inhibited by the TRPV channel blocker capsazepine. RT-PCR revealed that mRNAs of TRPC1, TRPC4, TRPV1, TRPV2, TRPV4, and TRPM7 are significant in human preadipocytes.

Conclusion: Our results demonstrate the novel information that multiple TRP channels, TRPC1/4, TRPV1/2/4, and TRPM7, are present in human preadipocytes. Roles of these channels in cell proliferation and adipogenesis are being investigated.

Evaluation of airway wall thickness with high-resolution computed tomography in severe stable asthmatics with sputum bacteria load

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Background: Studies have shown that potentially pathogenic bacteria in asthma is associated with neutrophilic inflammation. It is unclear whether this contributes to structural airway changes. This study used high-resolution computed tomography (HRCT) to compare changes in large and small airways against positive sputum culture in severe stable asthmatics.

Methods: Airway structure was assessed by a validated method using wall thickness (WT), wall area (WA), and WA corrected by body surface area (WA/BSA). WT and WA were corrected for total airway diameter and area (WT% and WA% respectively). We divided the bronchi into large airways with a lumen diameter (L) of ≥ 2 mm and small airways with $L < 2$ mm. Using HRCT at five set levels, WT and WA were recorded in severe stable asthma patients (n=56) separately with positive sputum culture (n=29) and negative culture (n=27).

Results: The commonest strains cultured in these asthmatic patients included *H influenzae*, *P aeruginosa*, and *S aureus*. In small airways, WA% and WT% showed no significant difference between the sputum culture-negative and -positive groups (79.1 \pm 6.8 vs 80.4 \pm 7.1) and (29.0 \pm 8.0 vs 28.6 \pm 4.3) respectively (P>0.05). Similarly, in large airways, there was no significant difference between the culture-negative vs culture-positive groups (WA% 68.1 \pm 5.7 vs 66.8 \pm 5.5) and (WT% 21.9 \pm 2.8 vs 21.5 \pm 2.5). WA and WA/BSA in the small airways showed a significant negative correlation with FEV₁% ($r = -0.37$ and $r = -0.37$, P<0.05). In contrast, WA/BSA in the large airways showed a significant positive correlation with FEV₁% ($r = 0.32$, P=0.03).

Conclusions: These findings suggest that bacterial load may not be related to airway wall thickness in asthma.

Assessment of the clinical efficacy of the portable exhaled nitric oxide analyser (FeNO NObreath) in monitoring asthma control and predicting asthma exacerbation

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Background: Asthma is characterised by chronic airways inflammation that is mediated by eosinophils, T-lymphocytes and mast cells. Clinical symptom questionnaires, pulmonary function test, and blood tests are easy to perform, and may be used to diagnose or assess asthma control; but their sensitivity and specificity may be low. Bronchial hyperresponsiveness to methacholine challenge and induced sputum testing is typically sensitive in assessing airway inflammation directly, but such tests are invasive, time-consuming and difficult to perform safely in all subjects. Fractional exhaled nitric oxide (FeNO) is elevated in asthma. Studies have shown that FeNO levels correlate well with eosinophils in induced sputum, airway hyperresponsiveness and FEV₁. In this study, we aimed to investigate whether daily monitoring of FeNO using a new portable FeNO NObreath analyser could be useful in asthma control, and whether it could be used as a reliable predictor of asthma exacerbations.

Methods: Healthy subjects were first recruited to measure 5x FeNO measurements diurnally for 2-week period each (n=10); further studies will be undertaken on patients with mild/moderate and severe asthma (according to GINA guidelines) recruited in groups of n=10.

Results: Interim results were examined to assess whether reliable daily FeNO measurements are feasible in the home setting using a portable device; we then examined intra-day and day-to-day variation in this cohort. Across the 700 measurements taken to date, the repeatability of the FeNO measurements was found to be ± 6.5 pp (mean coefficient of variability=15.5%). Intra-class correlation coefficient values (*r*) were consistently >0.7 (significant).

Conclusion: FeNO measurements using this novel portable analyser appear to be feasible and reproducible.

Oxidative stress and proliferation of human airway smooth muscle in asthma and chronic obstructive pulmonary disease: the role of hydrogen sulfide

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Background: Hydrogen sulfide (H₂S) is synthesised from L-cysteine by cystathionine β -synthase (CBS) or cystathionine γ -lyase (CSE). It is evolutionarily preserved across a wide range of mammalian tissues. H₂S contributes to the pathogenesis of inflammatory disease, demonstrates direct antioxidant activity, and up-regulates indirect antioxidant pathways such as glutathione-s-transferase. There are over 300 million patients with asthma, and >210 million patients with chronic obstructive pulmonary disease (COPD) worldwide. Inflammation, airway remodelling, and oxidative stress are key features that are localised to airway smooth muscle cells (ASMCs). This project studied the role of H₂S in oxidative stress, inflammation and proliferation in ASMCs in these diseases.

Methods: Endobronchial biopsy and surgical samples (healthy transplant donors) provided tissue from five defined groups; including healthy non-smokers, smoking controls, COPD (GOLD stage II), mild/moderate asthmatic, and severe asthmatic patients. Cells were cultured to confluence and stimulated $\pm 2.5\%$ foetal calf serum (FCS); \pm methaemoglobin (MetHb) 10 μ M (endogenous H₂S scavenger); and \pm sodium hydrogen sulfide (NaHS) 100 μ M (exogenous H₂S donor) in variable combination. Cell proliferation was measured using BrdU ELISA and MTT cell viability assays. Supernatants were studied for CXCL-6 and CXCL-8 pro-inflammatory cytokines. Further studies will use Western blot to quantify MnSOD, CSE, and CBS, and study knockdown models of CSE and CBS expression.

Results: Control and asthma samples showed that removal of endogenous H₂S with MetHb in the presence of FCS increased cell DNA synthesis by a factor of 1.80 (n=6, P<0.05), and CXCL-8 release by a factor of 2.83 (n=6, P<0.001). These effects were significantly abrogated when exogenous H₂S was reintroduced with NaHS.

Conclusion: These results suggest that H₂S plays a role in ASMC proliferation and inflammation. Further studies will examine antioxidant effects in asthma and COPD; and establish clearer understanding of the mechanisms.

Adipocyte fatty acid binding protein potentiates lipids-induced endothelial dysfunction through induction of endoplasmic reticulum stress: implication in vascular disease

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Objective: Adipocyte fatty acid binding protein (A-FABP) is a lipid-binding chaperone that is implicated in obesity-related cardiometabolic syndrome through integrating lipid metabolism and inflammation. However, the precise mechanism whereby A-FABP potentiates lipids-induced inflammation remains elusive. This study investigated the interplay between A-FABP and endoplasmic reticulum (ER) stress in lipids-induced vascular inflammation in both cell culture system and animals.

Methods: Human umbilical vein endothelial cell (HUVEC) was stimulated with palmitic acid and the expression of A-FABP was quantified by real-time PCR and immunoassay. Induction of ER stress was evaluated by xbp-1 splicing and Western blotting analysis for multiple ER stress markers. C57BL/6J mice or Toll-like receptor 4 deficient mice (TLR4 -/-) and its wild type controls were fed with high-fat high-cholesterol diet alone or with oral administration of the A-FABP inhibitor. The vasoreactivity was measured by myograph. Suppression of TLR4 and de-novo ceramide synthesis were achieved by either siRNA-mediated knocking down or pharmacological inhibitors.

Results: Palmitic acid induced ER stress in a time- and dose-dependent manner in HUVEC, which was accompanied by a significant increase in A-FABP expression. Chemical chaperone, 4-Phenyl butyric acid (PBA), or selective inhibitor of A-FABP alleviated palmitic acid induced ER stress in HUVEC and sensitised cells to insulin evoked eNOS signalling. In addition, the activities of JNK and NFκB were also substantially suppressed. Oral administration of the A-FABP inhibitor improved insulin-induced vasodilatation in mice on high-fat high-cholesterol diet. Knocking down of TLR4 or blockade of de-novo ceramide synthesis attenuated the induction of A-FABP expression and ER stress in HUVECs. Finally, the essential role of TLR4 in mediating lipids-related ER stress and endothelial dysfunction was verified in TLR4-deficient mice which were resistant to high-fat diet-mediated impairment on endothelium-dependent vasodilatation.

Conclusion: Toxic lipids induce A-FABP expression through TLR4 and ceramide synthesis, and elevated A-FABP in turn potentiates lipids-induced endothelial dysfunction and vascular inflammation via ER stress. Pharmacological inhibition of A-FABP represents a promising strategy for treating vascular diseases.

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Effect of clinical and virological parameters on the level of neutralising antibody against pandemic influenza A virus H1N1 2009*

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Background: Little is known about the antibody response from natural infection by the novel 2009 influenza A (H1N1) virus and its relationship with the clinical and virological parameters. The relatively lack of background neutralising antibody against this novel virus provides a unique opportunity for understanding this issue.

Methods: Cases presenting with influenza-like illness positive for the pandemic H1 gene by RT-PCR were identified. The serum antibody response was assayed by neutralising antibody titer (NAT) against the virus in 881 convalescent donors. We retrospectively analysed their clinical parameters and viral load.

Results: Ninety percent of convalescent donors had seroprotective titer of 1 in 40 or above. The geometric mean titer of donors with convalescent NAT measured between day 21 and 42 is 1:101.1. Multivariate analysis by ordinal regression showed that pneumonia (P=0.004, odds ratio [OR]=3.39) and sputum production (P=0.046, OR=1.75) were the two independent factors associated with a higher level of convalescent NAT. Afebrile upon influenza presentation was associated with subsequent poor NAT (<1:40) response (P=0.04). A positive correlation between the NPS viral load upon presentation and the convalescent NAT was demonstrated (Spearman's: 0.238 [P=0.026]).

Conclusions: About 10% of these convalescent patients do not have a seroprotective NAT and may benefit from vaccination to prevent reinfection. The convalescent NAT correlated well with the initial viral load and was independently associated with severity of the viral illness including pneumonia. The findings provide both the clinical and virological markers for identifying potential convalescent plasma donors with high serum NAT, which can be used to produce hyperimmune intravenous immunoglobulin in randomised treatment trial for patients with severe pandemic H1N1 infection.

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Gastrointestinal follicular lymphoma: a retrospective analysis of 16 cases

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Objective: To investigate the clinical behaviour, treatment response, and prognosis of primary gastrointestinal follicular lymphoma.

Method: Clinical records of all follicular lymphoma diagnosed at Queen Mary Hospital from 1988 to 2008 were reviewed. Cases with gastrointestinal tract as the only or predominant site of involvement at presentation were considered to be primary gastrointestinal follicular lymphoma. Patient demographics, disease staging, treatment and outcome were analysed.

Results: There were 155 cases of follicular lymphoma diagnosed from 1988 to 2008, of which 123 cases were nodal and 32 cases were extranodal. Sixteen cases of primary gastrointestinal follicular lymphoma were identified, constituting 10.3% (16/155) of all follicular lymphomas, and 50% (16/32) of extranodal follicular lymphomas. There was a slight male predominance (male:female=1.67:1), presenting at a median age of 60.5 (28-85) years. The majority of patients (12/16, 75%) presented with advanced Ann-Arbor stage (stage III or IV). The Follicular Lymphoma International Prognostic Index was evaluated in 13 cases, and seven (53.8%) patients were classified as high-risk. Common primary sites were ileum (n=6), colon (n=5), and duodenum (n=3). The complete remission rate after first-line chemotherapy was 43.7% and the median duration of progression-free survival was 19 (1-156) months. The median overall survival was 54.5 (8-264) months. At a median follow-up of 93 (16-267) months, five patients had died—four from unrelated diseases and one from refractory lymphoma. The median progression-free survival of nodal follicular lymphoma in this cohort was 23.5 (1-228) months and median overall survival was 76 (5-790) months.

Conclusions: The gastrointestinal tract was the most common site of extranodal follicular lymphoma. In this cohort, primary gastrointestinal follicular lymphoma had a worse clinical outcome as compared with nodal follicular lymphoma.

microRNA-21 promotes survival but not functional maturation of human embryonic stem cell-derived cardiomyocytes (hESC-CMs)

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Purpose: microRNAs (miRNAs) are naturally occurring small non-encoding RNAs (22 nt) which negatively modulate gene expression via mRNA degradation and translational repression. Studies suggested that miRNAs are important in both normal cardiogenesis and pathogenesis of the failing heart. miRNA-21 is among those that are differentially expressed, according to our miRNAs profiling, and we hypothesised that its expression level may play a part in functional maturation of human embryonic stem cell-derived cardiomyocytes (hESC-CMs).

Methods: miRNA profiling was performed to identify differential expression of miRNAs in human embryonic stem cells (hESCs), hESC-derived, fetal (hF) and adult (hA) ventricular cardiomyocytes (VCMs). Overexpression and knockdown of miRNA-21 were achieved by lentivector (LV)-based system. To functionally characterise maturity, whole-cell patch-clamp probing for the action potential (AP) profile, and cytosolic Ca²⁺ transients measurement were performed in control versus miRNA-21 overexpressed/knockdown hESC-CMs. Contractile protein and sarcoplasmic reticulum (SR) Ca²⁺ handling protein expression were quantified with qPCR.

Results: miRNA-21 was highly expressed in hESC and hESC-VCMs; repressed in hF-VCMs and hA-VCMs. We hypothesised its expression level might be associated with functional maturity. However, we did not find any significant functional changes of hESC-CMs by manipulating its expression. The expression of contractile protein and SR Ca²⁺ handling protein was not affected, consistent with the functional data. Previous studies suggested pro-survival effects of miRNA-21 overexpression during compensatory cardiac hypertrophy. We performed TUNEL assay and found that overexpressing miRNA-21 in hESC-CMs significantly protected the cells against apoptosis induced by hydrogen peroxide challenge.

Conclusions: Differential expression of miRNA-21 in hESCs, hESC-, hF- and hA-VCMs is apparently unrelated to global functional maturity of hESC-CMs. High level of miRNA-21 expression during embryonic stage might confer survival advantages to developing myocytes.

Generation of patient-specific induced pluripotent stem cells as a diagnostic and research platform of rare diseases

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Introduction: Induced pluripotent stem cells (iPSC) generated from human adult somatic cells through reprogramming hold great promises for future regenerative medicine. In addition, because of their pluripotent potential and unlimited quantity of cell supply, they can be derived into multiple cell types such as cardiomyocytes, endothelial cells, liver cells and neuronal cells. For some diseases—such as Brugada syndrome, Friedreich's ataxia, atypical Werner syndromes (aWS) and Hutchinson–Gilford progeria syndromes (HGPS)—understanding of their underline mechanism was limited by the scarcity of patients and inaccessibility of patients' tissues for diagnosis and researches. Therefore, generation of the patient-specific iPSC provides a novel platform in understanding the disease mechanism.

Methods: Skin biopsies were obtained from the patients with Brugada syndrome (1 cases), Friedreich's ataxia (1 case), laminopathy (2 cases), aWS (1 case) and HGPS (1 case). Fresh human dermal fibroblasts obtained from the patients were reprogrammed with a defined set of transcription factors using lentiviral vectors in a feeder-independent cell culture system with defined culture medium.¹ Then, the hiPS were subsequently differentiated into cardiomyocytes,² endothelial cells,³ fibroblast and neuronal cells respectively.

Results: Six disease-specific human-induced pluripotent stem cell lines were generated from dermal fibroblasts of different patients under feeder-independent culture system with defined factors. The resultant cells maintained normal karyotypes and expressed a panel of pluripotency markers including stage-specific embryonic antigen (SSEA)-4, tumour-rejection antigen (TRA)-1-60 and (TRA)-1-81, and alkaline phosphatase. Their pluripotency was further characterised by the teratoma formation in SCID mice. Cardiomyocytes were successfully generated with satisfied quantity from the Brugada syndrome iPS for electrophysiology and calcium handling studies. Endothelial cells and fibroblasts were generated from the iPS of laminopathy patients and can be used in ageing experiments. In addition, neuronal cells were well cultured from the iPS of Friedreich's ataxia and subsequently used in the studies of mitochondrial dysfunction of this disease.

Conclusion: Six different patient-specific human-induced pluripotent stem cells were generated and subsequently derived into different cell types respectively. Tissue-specific disease platforms were set up for diseases diagnosis and pathway delineation.

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The role of microRNA 885-5p in regulating tumour metastasis in colorectal cancer

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Introduction: MicroRNAs (miRNAs) are a class of small non-coding RNAs that play a crucial role in cellular proliferation, differentiation and apoptosis by regulating gene expression, and have been implicated in the regulation of human diseases as well as colorectal cancer (CRC). CRC is one of the commonest cancers in the world and liver is the most common site of distant metastasis, which marks poor prognosis for CRC patients. Recent evidences suggested that miRNAs affect the cell invasiveness of various cancers and regulate key steps in metastatic cascade. This study aimed to identify miRNAs involved in colorectal liver metastasis and the molecular mechanisms involved.

Methods: Microarrays were used for screening the expression differences of miRNAs between normal colon, CRC and liver metastasis. Lentiviral-transduced colon cell lines were sorted with flow cytometer high-speed sorter by green fluorescent protein signal. Orthotopic implantation of mi-885-5p-expressing CRC cells into the cecal wall of nude mice was performed to study their tumourigenic and metastatic capacity in vivo. Functional assays such as wound healing and invasion chamber were used to study the metastatic property (migration and invasion) in vitro. GeneChip expression was used to study the mRNA expression differences between gain-of-function and control.

Results: We quantified the expression levels of miRNAs in 14 liver metastasis samples and 12 CRC tissues, and identified hsa-miR-885-5p whose expression levels were significantly up-regulated in liver metastasis samples compared with the CRC samples. Over-expressed miR-885-5p cell line showed more invasive and migratory when comparing with control. 67% of the mice developed liver metastasis with orthotopic implantation of a CRC cell line overexpressing miR-885-5p, as compared with 33% of metastatic incidence in mice injected with the vector-expressing CRC cell line. GeneChip array showed that VCAM1 may be one of a possible target of miR-885-5p, which was comparable with the online database.

Conclusion: We conclude that overexpression of miR-885-5p is observed in liver metastasis and has an important role in regulating the metastatic capacity of CRC cells in vitro and in vivo.

Expression of nicotinic acetylcholine receptor in human bronchial epithelium in relation to airflow obstruction

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Background: The expression of non-neuronal nicotinic acetylcholine receptors (nAChR) has been demonstrated in cultured human bronchial epithelial cells (HBEC). Activation or down-regulation of nAChR is thought to play a role in airway inflammation. Understanding the pattern and regulatory mechanisms of nAChR expression in human airway may give insight into their roles in inflammatory airway disease.

Aim: The aim of this study was to determine the pattern of nAChR expression in human bronchial epithelium and the correlations with respiratory symptoms, smoking status and pulmonary function.

Methods: Patients presenting atypical cells in sputum cytology and undergoing diagnostic fiberoptic bronchoscopy with autofluorescence examination were recruited. Respiratory Health Questionnaires were administered and spirometry was performed before bronchoscopy. Endobronchial biopsy was taken from normal-appearing areas in one lobar bronchial orifice. Immunohistochemical staining on bronchial biopsy was performed with specific nAChR antibodies (Santa Cruz, CA, US).

Results: Twenty-five endobronchial biopsy specimens were scored, 12 (mean age, 60.9±12.2 years; male:female=9:3; 10 smokers, 2 non-smokers) showed positive cytosolic staining of nAChR α 3 with accentuation at the subplasma membrane of the ciliated cells and 10 (mean age, 65.5±10.0 years; all males; 8 smokers, 2 non-smokers) showed no staining (negative). The nAChR α 3-positive group showed significantly higher mean pre-bronchodilator and post-bronchodilator FEV₁/FVC ratios (P=0.019 and 0.038 respectively) compared to the negative group. No significant difference in age, amount of tobacco smoking and respiratory symptoms was found between the nAChR α 3-positive and -negative groups.

Conclusion: nAChR α 3 expression in human bronchial epithelium was associated with a lesser degree of airflow limitation. Further recruitment to increase the sample size would be warranted to confirm such association. The pattern of nAChR expression and their roles in airway inflammation deserve further investigation.

Serum-advanced glycation end products is associated with insulin resistance in non-diabetic subjects

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Introduction: In addition to the important role of advanced glycation end products (AGEs) in the pathogenesis of diabetic vascular complications, recent data suggest that AGEs can also impair insulin action in vitro. We have investigated whether circulating AGEs are associated with insulin resistance in human subjects independent of metabolic parameters.

Methods: A total of 207 healthy non-obese non-diabetic subjects (97 male, 110 female) were recruited from the community. Serum levels of AGEs, adiponectin, malondialdehyde (MDA) and high-sensitivity C-reactive protein were assayed. Insulin resistance was determined by the homeostasis model assessment index (HOMA-IR).

Results: Male subjects had significantly higher body mass index, waist circumference and lower adiponectin level than female subjects and were more insulin resistant. Serum AGEs (3.67 ± 1.15 unit/mL vs 3.23 ± 1.15 ; $P < 0.05$) and MDA levels ($P < 0.05$) were also higher in male than female subjects. Serum AGEs correlated with HOMA-IR in both male ($r = 0.32$, $P = 0.004$) and female ($r = 0.28$, $P = 0.003$) subjects. Serum adiponectin inversely correlated with HOMA-IR in female ($r = -0.38$, $P < 0.001$) but not in male. On multiple regression analysis, serum AGEs remained an independent determinant of HOMA-IR even after adjusting for age, gender, waist, smoking, adiponectin and markers of oxidative stress and inflammation.

Conclusions: Formation and accumulation of AGEs progress during normal ageing. We have demonstrated that circulating level of AGEs is associated with insulin resistance even in non-obese, non-diabetic subjects independent of adiponectin.

Poor finger dexterity is associated with increased risk of peritonitis in patients on peritoneal dialysis

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Introduction: Peritoneal dialysis (PD)-related peritonitis is the major cause for technique failure and mortality. Despite different educational programmes and repeated training, the peritonitis rate has not been much reduced. Impairment of hand function (IHF) is a possible cause for peritonitis. Herein, we prospectively studied IHF and its association with peritonitis risk in patients on long-term PD treatment.

Methods: All prevalent and incident PD patients were recruited except those required helper to perform dialysis exchange. Single occupational therapist performed all hand function assessment and was blinded to patients' clinical information. IHF was defined by the power grip strength, tripod pinch, lateral pinch and finger dexterity (unimanual and bimanual). Nerve conduction test was performed to look for underlying peripheral neuropathy (PN) and carpal tunnel syndrome (CTS). Patients after assessment were followed up for 2 years and censored for onset of peritonitis. Cox regression model and Kaplan Meier analysis were used to analyse the risk factors for peritonitis risk and peritonitis-free survival, respectively.

Results: A total of 152 (female=76) patients with age 57.6 ± 12.5 years were studied. Of these, 48 (32%) and 16 (11%) patients had DM and CVA, respectively; 81 (53.3%) and 72 (47.4%) patients were found to have PN and CTS, respectively. The mean follow-up time was 14.6 ± 7.5 months. Throughout this period, 52 (34.2%) patients developed peritonitis. The peritonitis-free survival at 12 months and 24 months were 74% and 61%, respectively. Better finger dexterity conferred 12% protection (95% CI, 0.79-0.99; $P = 0.03$) from developing peritonitis by multivariate analysis. Patients with better higher finger dexterity were also found to have higher peritonitis-free survival ($P = 0.03$).

Conclusion: PD patients with IHF may associate with a higher risk of developing peritonitis. Hand function should be assessed regularly throughout PD treatment so that a better surveillance on peritonitis risk could be achieved.

Early endothelialisation of Genous (EPC capture) stent by optical coherence tomography: the EGO study

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Introduction: While able to reduce restenosis, drug-eluting stents (DES) cause poor stent healing and incomplete endothelialisation (after more than 1 year), and lead to the development of late stent thrombosis which could be fatal. The Genous endothelial-progenitor-cell (EPC) capture stents emphasise on the pro-healing concept with a bio-engineered coating of anti-CD34 antibody in the inner stent surface to capture circulatory EPCs. Documenting endothelialisation and stent coverage in vivo have never been possible until the availability of the ultra-high resolution of the optical coherence tomography (OCT). While animal models and autopsy findings are available, in-vivo documentation on the extent of neointimal coverage shortly (within 1 month) after stent implant in human coronary artery has not been reported. This is the first study conducted to document complete neointimal stent coverage by OCT within 1 month of implantation.

Methods: In this prospective, open-label study, 30 consecutive patients with acute coronary syndrome requiring PCI were treated with the Genous stents. Restudy angiograms and OCT analyses were randomised from 15 days to 30 days after the index PCI. All OCT frames and all stent struts would be analysed, targeting on the degree of stent coverage and the neointimal hyperplasia. Analyses would be categorised into the well-apposed struts, mal-apposed struts, and struts on the opening of the side-branches. The findings would be plotted against the time, in order to generate the graphs and trend of endothelialisation and coverage.

Results: All patients had uneventful PCI procedure and were discharged. Until this abstract, 28 of the 30 patients have completed the restudy and all the OCT results were prepared for analysis. Good endothelialisation has already been documented by 15 days and near-complete stent coverage by 28 days.

Conclusion: OCT with its ultra-high resolution represents a new novel imaging technology in evaluation of endothelialisation and healing after stenting at a very early stage. The Genous stent allows for very early endothelialisation with its pro-healing EPC capturing capacity, as disclosed by OCT.

A review of thrombolytic therapy in patients with ST-elevation myocardial infarction: demographics, intracranial haemorrhage, and other outcomes

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Introduction: Thrombolytic therapy remains an important reperfusion strategy for eligible patients with acute ST-elevation myocardial infarction (STEMI). Commonly used thrombolytic agents are streptokinase and fibrin-specific agents (FSA), including tenecteplase (TNK-tPA) and alteplase.

Methods: A total of 342 patients who received thrombolytic therapy for management of STEMI in 2001 to 2009 were analysed. Cases were identified through Hospital Pharmacy Record, Hospital Authority Clinical Management System (CMS) and Acute Myocardial Infarction Clinical Pathway. Clinical records were retrieved. Data were analysed using software SPSS 16.0.

Results: A total of 266 patients (77.78%) and 76 patients (22.22%) were given streptokinase and FSA (TNK-tPA/Alteplase), respectively. The median door-to-needle time and pain onset-to-needle time was 68 minutes and 3 hours 27 minutes, respectively. The overall rate of intracranial haemorrhage was 1.46% (5 out of 342). The rate of intracranial haemorrhage of FSA (TNK-tPA/Alteplase) and streptokinase was 5.26% (4 of 76) and 0.38% (1 of 266) respectively ($P=0.002$; odds ratio=14.71). Rate of intracranial haemorrhage was significantly higher in patients with admission systolic blood pressure ≥ 160 mm Hg ($P=0.043$, odds ratio=5.33). Higher rates of intracranial haemorrhage were observed in subgroups of age ≥ 75 years, female, history of stroke/transient ischaemic attack ($P=0.0114$, 0.266, 0.155 respectively). Streptokinase was associated with more adverse events (34.96%) at the time of thrombolytic administration which included hypotension, bradycardia and allergic reactions ($P=0.000$). The success rate of reperfusion was higher with TNK-tPA (78.67%) compared to streptokinase (71.97%), but not reaching statistically significant level ($P=0.246$).

Conclusion: This study showed the reperfusion rates after thrombolysis in STEMI were only marginally better by FSA (tenecteplase and alteplase) than by streptokinase, and the difference was statistically insignificant. The rate of intracranial haemorrhage, however, was much higher statistically with FSA. These findings warrant extreme caution to be taken when selecting thrombolytic agents in Chinese patients with STEMI.

Identification of serotonin analog in aqueous-phase cigarette smoke may lead to activation of 5-HT_{2A} receptors in human bronchial epithelial cells

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Introduction: Cigarette smoking is a major risk factor for chronic obstructive pulmonary disease (COPD), in which it is associated with a disruption of serotonin (5-hydroxytryptamine [5-HT]) homeostasis. Cigarette smoke has been shown to interfere with the serotonergic system via nicotinic pathway in the central nervous system¹ and the 5-HT pool of blood platelet in the peripheral.² However, little is known whether any substances in cigarette smoke may directly interact with the serotonin receptors (5-HTRs). 5-HTR₂ has been found to be expressed in human lung epithelial cells and exposure to 5-HT elevated IL-8 release.³ The present study aimed at investigating whether CS-induced IL-8 release via activation of 5-HTR₂ in human bronchial epithelial cells is due to the presence of 5-HT analog in aqueous-phase cigarette smoke.

Methods: BEAS-2B cells were treated with or without various concentrations of cigarette smoking medium (CSM) or 5-HT. IL-8 levels were measured by ELISA. Expression of 5-HTR₂ subtypes was determined by RT-PCR and Western blot. Purification and measurement of 5-HT were done by HPLC. The molecular weights of the analytes were determined by mass spectrometry and the linear structure of the analytes by NMR.

Results: Our results showed that blockage of 5-HTR_{2A} by ketanserin (10 nM) significantly attenuated CSM or 5-HT-induced IL-8 release. Expression of 5-HTR_{2A} was induced by CSM. A 5-HT analog in aqueous-phase cigarette smoke with molecular weight 179.086 was detected compared to 179.092 of the 5-HT standard. An identical NMR spectrum was observed from the analyte compared to 5-HT standard. There was a concentration-dependent increase of 5-HT analog in aqueous-phase cigarette smoke.

Conclusion: These data indicate the presence of a 5-HT analog in aqueous-phase cigarette smoke, which may be responsible for the activation of 5-HTR_{2A} leading to the release of proinflammatory cytokine IL-8 from human bronchial epithelial cells.

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Effect of low-dose and high-dose atorvastatin on carotid intima-media thickness, high-sensitivity C-reactive protein and endothelial progenitor cells in statin naïve patients

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Introduction: Statin has been shown to retard the progression of or even reverse atherosclerosis. Besides, it has anti-inflammatory properties and reduces cardiovascular events. This study aimed to assess the effect of low-dose and high-dose statin on carotid intima-media thickness (CIMT), high-sensitivity C-reactive protein (hs-CRP) and endothelial progenitor cells (EPC), in statin-naïve patients undergoing percutaneous coronary intervention (PCI).

Methods: Under the IVUS-Virtual Histology (VH-IVUS) study protocol, 40 Chinese patients treated by PCI were randomised to receive either atorvastatin 10 mg daily or 40 mg daily for 6 months. The effects on CIMT, hs-CRP and EPC in each arm were analysed apart from the changes in VH.

Results: Baseline demographics, low-density lipoprotein cholesterol (LDL-C), degree of coronary artery disease, baseline medications, CIMT, hs-CRP and EPC were similar between two groups (P=NS) before statin treatment. Over a follow-up period of 6 months, both groups showed significantly decrease in LDL-C and hs-CRP (P<0.01). When compared with low-dose group, high-dose group had a significantly higher circulating CD34⁺ EPC count (15.23±58.75 cells/μL vs 21.93±45.59 cells/μL; P=0.04). There was a reduction of CIMT in the high-dose group (low-dose 0.003±0.124 mm, P=0.903; high-dose 0.04±1.076 mm, P=0.116) although it was not statistically significant.

Conclusion: This study demonstrates a significant reduction in LDL-C and hs-CRP after statin treatment. High-dose statin treatment for 6 months is associated with elevation of circulating EPC. Importantly, high-dose statin treatment appears to induce regression on CIMT while low dose is observed to have CIMT progression (although not statistically significant).

Virtual histology intravascular ultrasound findings and Effects on clinical outcomes in Native coronary disease Under varying doses of Statin: the VENUS study

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Introduction: While statin treatment can induce plaque regression, the simultaneous effects of varying doses of statin on plaque volume and plaque composition, and clinical outcomes are unknown. This study was the first of its kind in design assessing such effects by serial virtual histology intravascular ultrasound (VH-IVUS). The primary endpoint was the difference in VH-IVUS findings at 6-month restudy and the secondary endpoints the major adverse cardiac events. The overall effects of statin treatment were also examined.

Methods: In this prospective, randomised, double-blinded study, 40 consecutive statin-naïve patients with stable angina requiring percutaneous coronary intervention (PCI) were randomised in two arms (20 each) to receive 6 months of either atorvastatin 10 mg or 40 mg daily. VH-IVUS was performed in all non-PCI lesions at baseline and 6 months; all analyses were performed by core laboratory.

Results: A total of 54 VH-IVUS lesions were studied in 10-mg group and 57 in 40-mg group. Overall, plaque volume was reduced by 4.28% ($-5.10 \pm 14.93 \text{ mm}^3$, $P < 0.001$), absolute VH-IVUS fibrous volume 10.54% ($-4.87 \pm 10.74 \text{ mm}^3$, $P < 0.001$) and relative percentage fibrous component $3.29 \pm 7.84\%$ ($P < 0.001$), while relative percentage dense calcium increased by $1.50 \pm 3.08\%$ ($P < 0.001$) and necrotic core $3.19 \pm 7.82\%$ ($P < 0.001$). Beneficial effects were more substantial in 40-mg group with significantly more percentage plaque volume regression ($-1.50 \pm 3.85\%$ vs $0.38 \pm 4.05\%$ increase in 10-mg group; 95% CI, 0.39-3.36; $P = 0.014$), and less percentage necrotic core expansion ($1.68 \pm 7.57\%$ vs $4.78 \pm 7.82\%$ in 10-mg group; 95% CI, 0.19-5.99; $P = 0.037$), without occurrence of acute coronary syndrome or target vessel revascularisation event (versus 6 patients in 10-mg group, $P = 0.020$).

Conclusion: In statin-naïve patients requiring PCI, 6-month atorvastatin treatment induced significant percentage plaque volume reduction and extensive modulation of VH-IVUS components (in particular limitation of percentage necrotic core expansion), in a dose-dependent manner. Significant correlations between individual plaque composition changes and clinical outcomes required further studies.

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Calcium handling in human-induced pluripotent stem cell-derived cardiomyocytes

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Rationale: Cardiomyocytes generated from human-induced pluripotent stem cells (hiPSCs) are suggested as the most promising candidate to replenish cardiomyocyte loss in regenerative medicine. Little is known about their calcium homeostasis, the key process underlying excitation-contraction coupling.

Objective: We investigated the calcium handling properties of hiPSC-derived cardiomyocytes and compared with those from human embryonic stem cells (hESCs).

Methods and Results: We differentiated cardiomyocytes from hiPSCs (IMR90) and hESCs (H7) with established protocols. Beating outgrowths from embryoid bodies were typically observed 2 weeks after induction. Cells in these outgrowths were stained positively for troponin and sarcomeric alpha-actinin. Reverse-transcription polymerase chain reaction studies demonstrated the expressions of cardiac-specific markers in both hiPSC- and hESC-derived cardiomyocytes. Calcium handling properties of 20-day-old hiPSC- and hESC-derived cardiomyocytes were investigated using fluorescence confocal microscopy. Compared with hESC-derived cardiomyocytes, spontaneous calcium transients from hiPSC-derived cardiomyocytes were of significantly smaller amplitude and with slower maximal upstroke velocity. In addition, spatial inhomogeneity in temporal properties of calcium transients across the width of cardiomyocytes was more pronounced in hiPSC-derived cardiomyocytes than their hESC counterpart as revealed line-scan calcium imaging. Furthermore, in contrast with hESC-derived cardiomyocytes, ryanodine did not reduce the amplitudes, maximal upstroke and decay velocity of calcium transients of hiPSC-derived cardiomyocytes. Ryanodine receptor was expressed in both hiPSC- and hESC-derived cardiomyocytes, expressions of other key calcium-handling proteins including sodium-calcium exchanger, sarcoplasmic reticulum calcium-ATPase, triadin, were significantly lower in hiPSC than in hESCs.

Conclusions: The results indicate the calcium handling properties of hiPSC-derived cardiomyocytes are relatively immature to hESC counterparts.

The use of acupuncture in relieving dizziness, nausea, and vomiting after cerebellar stroke: a case report

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Introduction: Acupuncture is found to be effective in relieving chemotherapy-induced and postoperative nausea and vomiting. We sought to explore whether similar acupuncture technique is helpful in reducing dizziness, nausea, and vomiting after acute cerebellar stroke.

Methods: We report on a 66-year-old man who presented with sudden collapse in mid-August 2009 and was found to have acute bilateral cerebellar infarcts. He ran a very stormy acute course and was complicated by obstructive hydrocephalus and respiratory failure due to aspiration pneumonia. He was transferred to our rehabilitation unit at week 5 after stroke eventually. Patient remained bed- and chair-fast because of severe dizziness and nausea whenever he changed his positions. Acupuncture was applied on Neiguang (PC6) of both forearms. Even reinforcing-reducing needling technique was employed to achieve soreness, numbness and distension in the local areas. Needles were retained for 30 minutes and manipulated once every 10 minutes to intensify the needling sensation. The treatment was given once every other day, 3 times a week, for 2 weeks. Dizziness, nausea and vomiting were measured by global self-rating (nil or minimal, mild, moderate, and severe) and visual analogue scale (VAS) before and after treatment.

Results: The patient reported remarkable improvements with dizziness and nausea reduced from severe grade to mild grade and VAS from 8 to 3. No complication was observed.

Conclusion: Acupuncture by needling the Neiguang points is potentially beneficial. Further large-scale, randomised and controlled studies are warranted.

Endothelium-specific activation of AMP-activated protein kinase alleviates diabetes-induced impairment in endothelial repair in mice

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Introduction: Endothelial injury, an initiating step in various cardiovascular diseases, can be repaired in part by endothelial progenitor cell (EPC). Impaired functionality and reduced number of EPC are commonly observed in diabetic patients. AMP-activated kinase (AMPK) is a well-known target of several anti-diabetic and cardiovascular drugs. The objective of this study was to test whether or not activation of AMPK in endothelium alone is sufficient to prevent diabetes-induced impairment in EPC function and endothelial repair using a tissue-specific transgenic mouse model.

Methods: The transgenic mice with endothelium-selective expression of a constitutively active AMPK (AMPK-Tg) were generated using the T-cadherin gene promoter. Diabetes was induced by injection with streptozotocin (STZ). Bone marrow-derived EPCs (BM-EPCs) were assessed for (i) adhesion function and (ii) tubular formation capacity in vitro. Wire-mediated injury was introduced to the right common carotid artery of the mice which were allowed to recover for 3 days, and vascular repair was assessed using Evans blue staining. Post-injury circulating EPC numbers were quantified by flow cytometry analysis.

Results: In healthy mice, adhesion and tube formation were enhanced in BM-EPCs isolated from the AMPK-Tg mice when compared to wild type (WT) mice. These were, however, diminished in response to high glucose (25 mM) treatment in WT mice but not in AMPK-Tg EPCs. Re-endothelialisation after wire-mediated carotid injury was accelerated in AMPK-Tg mice and this was associated with a reduced number of circulating EPCs during the recovery process. In mice rendered diabetic with STZ, adhesion and tube formation of BM-EPCs were further impaired in wild type but not the AMPK-Tg mice in response to glucose (25 mM) treatment. Re-endothelialisation after wire injury was much less in WT mice compared to the AMPK-Tg mice, concomitant with a higher number of circulating EPCs after carotid injury.

Conclusion: These findings collectively suggest that endothelial activation of AMPK can prevent diabetes-induced endothelial injury through improving the EPC function. AMPK may represent an appealing therapeutic target for prevention of cardiovascular disease in diabetes.

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Background: The natural history of viral shedding from the upper respiratory tract of the new pandemic 2009 influenza A (H1N1) and the effect of oseltamivir treatment were uncertain.

Methods: A retrospective cohort study involving 145 consecutive patients with specimens positive by reverse transcriptase–polymerase chain reaction for the matrix and new H1 genes was conducted.

Results: The non-treated and oseltamivir-treated patients were comparable in their viral load at presentation, demography, and the presenting symptoms. No correlation was observed between viral load with age and number of symptoms. Viral load of nasopharyngeal aspirate (NPA) was significantly lower in treated than in non-treated patients at day 5 after symptom onset. When oseltamivir was initiated ≤ 2 days after symptom onset, a greater rate of viral load reduction in NPA of treated patients than that of non-treated patients was observed (-0.638 [95% CI, -0.809 to -0.466] vs -0.409 [95% CI, -0.663 to -0.185] log(10) copies/mL/d post-symptom onset), and the viral load was undetectable at day 6 after oseltamivir initiation, which was 1 day earlier than that of those whose treatment was initiated >2 days of symptom onset. The viral load was inversely correlated with concomitant absolute lymphocyte count in non-treated patients (Pearson correlation coefficient $[r] = -0.687$, $P = 0.001$) and treated patients (Pearson $r = -0.365$, $P < 0.001$). Resolution of fever was 1.4 days later in non-treated than treated patients ($P = 0.012$).

Conclusions: The natural viral load profile was described. Oral oseltamivir suppresses viral load more effectively when given early in mild cases of pandemic 2009 influenza A (H1N1) infections.

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Introduction: Diabetic nephropathy (DN) is characterised by a considerable inflammatory component of tubulointerstitial lesions. Toll-like receptors (TLRs) have been implicated in the regulation of immune responses and inflammatory diseases, and their role in DN is unclear.

Methods: Human kidney biopsy with proven DN was used to localise and quantify TLR4, and CD68+ cell infiltrates. Cultured human proximal tubular epithelial cells (PTEC) were used to explore the impact of high glucose (HG) on TLR expression and the effect of abolishing TLR on the downstream proinflammatory events.

Results: TLR4 was highly expressed in renal tubules of DN kidneys compared with diabetic non-nephropathy controls and normal controls, and correlated positively with infiltration of CD68+ cells in the interstitial space ($r = 0.729$; $P < 0.001$) and HbA1C, and negatively with eGFR at the time of biopsy. In vitro, HG-induced TLR4 overexpression was in a time- and dose-dependent manner, resulting in up-regulation of IL-6 and CCL-2 expression via I κ B/NF- κ B activation. Knocking down TLR4 in PTEC with siRNA-attenuated HG-induced I κ B/NF- κ B activation, the associated downstream IL-6 and CCL-2 mRNA synthesis ($P < 0.01$) and protein secretion ($P < 0.05$).

Conclusion: Our findings suggest a novel TLR4-mediated pathway through which HG may contribute to tubular inflammation in the diabetic kidney.

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High prevalence of sleep-disordered breathing in Chinese patients with difficult-to-control hypertension

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Aim: To evaluate the prevalence of sleep disordered breathing (SDB) in Chinese subjects with difficult-to-control hypertension.

Methods: Medical records of patients attending the hypertension clinic at a university teaching hospital were screened over 2 years. Inclusion criteria were age 18 to 65 years with hypertension requiring three or more anti-hypertensive medications. Patients with known secondary causes of hypertension, unstable medical illnesses in the past 3 months, on drugs (other than anti-hypertensives) that might influence blood pressure were excluded. Eligible subjects were invited to undergo 24-hour ambulatory blood pressure measurement and polysomnography (PSG).

Results: A total of 124 subjects were interviewed and 101 consented for the study. Two are awaiting PSG, six had defaulted the appointment and 93 underwent PSG, with the mean age of 51.6 ± 8.5 years, male:female=1.7:1, mean body mass index (BMI) 29.7 ± 5.2 kg/m², mean systolic blood pressure 140.9 ± 16.1 mm Hg, mean diastolic blood pressure 81.8 ± 10.2 mm Hg. Of the 93 subjects who completed the study, SDB (apnoea hypopnoea index AHI >5/hour) was diagnosed in 71 subjects (76.3%) [mean age 51.6 ± 8.9 years, male:female=2.5:1, mean BMI 30.7 ± 4.9 kg/m²]. Fifty-three (53/93=57%) had moderate or severe SDB (AHI ≥ 15 /hour). Among those with SDB, obstructive apnoea was the predominant type of SDB seen. Central apnoea predominance was observed in two subjects (2.6%).

Conclusion: SDB, in particular obstructive sleep apnoea, was found to be highly prevalent in Chinese with difficult-to-control hypertension, and high awareness of this association is warranted.

Rehabilitation outcomes 6 months after discharge from the geriatric day hospital in older Chinese patients

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Introduction: Whether rehabilitation outcome can be maintained after discharge from geriatric day hospital (GDH) has not been thoroughly investigated. This study was conducted to examine the rehabilitation outcome and its predictors 6 months after discharged from GDH.

Methods: It was a retrospective study performed in the GDH of Fung Yiu King Hospital. Cognitive status was assessed with Cantonese version of Mini-Mental State Examination (C-MMSE). Functional Independence Measure (FIM) upon GDH admission (FIM admission), discharge (FIM discharge) and 6 months after discharge (FIM post 6 months) were measured. FIM gain was FIM discharge – FIM admission while FIM efficiency was FIM gain divided by number of GDH visits.

Results: A total of 418 patients attended post 6 months GDH assessment. Of these, 164 (39.2%) showed a drop of FIM after 6 months. There was a significant drop of FIM post 6 months as compared with FIM discharge (91.5 ± 17.1 vs 93 ± 20.5 , $P < 0.001$). However, the FIM post 6 months remained significantly higher than FIM admission (91.5 ± 17.1 vs 86.8 ± 17.4 , $P < 0.001$). Univariate analysis showed that living in old-age home, incontinence, having musculoskeletal problems or Parkinsonism as the main complaint, C-MMSE score, FIM admission, FIM discharge and FIM efficiency were significant factors related to FIM drop post 6 months. Multivariate analysis revealed that FIM discharge was a negative predictor (odds=0.96; 95% CI, 0.95-0.98; $P < 0.001$) while Parkinsonism was a positive independent predictor for FIM drop post 6 months (odds=3.2; 95% CI, 1.35-7.5; $P = 0.008$).

Conclusion: A proportion of functional gain can still be maintained 6 months after discharged from GDH. More studies are needed to look for strategies in maintaining functional gain in GDH-discharged patients, especially those with Parkinsonism.

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Introduction: The effect of cognition on rehabilitation outcomes has been controversial. This study was conducted to examine the effect of cognition on rehabilitation outcomes in older patients undergoing geriatric day hospital (GDH) training.

Methods: It was a retrospective study performed in the GDH of Fung Yiu King Hospital. Cognitive status was assessed with Cantonese version of Mini-Mental State Examination (C-MMSE). Patients were stratified into three C-MMSE groups: <10, ≥10-19, and ≥20. Functional Independence Measure (FIM) upon GDH admission (FIM admission) and discharge (FIM discharge) were measured. FIM efficacy was FIM discharge – FIM admission while FIM efficiency was FIM efficacy divided by number of GDH visits. FIM discharge ≥90 was defined as satisfactory outcome of rehabilitation.

Results: A total of 547 patients attended GDH were studied. A significant positive correlation was observed between C-MMSE admission and FIM discharge ($P<0.001$). There were significant differences in the FIM admission and FIM discharge among the three C-MMSE groups, with lower discharge scores in low C-MMSE groups ($P<0.001$). The FIM efficacy and FIM efficiency during GDH rehabilitation were not different among different C-MMSE groups. Multivariate analysis showed that C-MMSE admission (odds=1.08; 95% CI, 1.0-1.15; $P=0.03$) and FIM admission (odds=1.33; 95% CI, 1.25-1.41; $P<0.001$) were both positive independent predictors for a satisfactory rehabilitation outcome (FIM discharge ≥90).

Conclusion: In GDH, patients with poor cognition had lower FIM discharge. Cognitive function was an independent predictor for satisfactory rehabilitation outcome. However, cognitive function was not associated with FIM efficacy and efficiency. This suggested that selected patients with impaired cognition could still benefit from GDH rehabilitation.

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Objective: To investigate the relationship between admission serum albumin and rehabilitation outcome of older Chinese patients with medical illnesses.

Methods: A retrospective study performed in two geriatric convalescence hospitals. Admission albumin levels (g/L) were measured and segregated into three groups: <30 g/L; 30 to <35 g/L; ≥35 g/L. Absolute functional and motor gain were determined by Barthel Index (BI) and Elderly Mobility Scale (EMS), and expressed as BI efficacy and EMS efficacy. BI and EMS efficiencies were deduced by the efficacy divided by the length of hospital stay (LOS). Satisfactory motor and functional outcomes were defined as discharge EMS ≥15 and BI ≥75.

Results: A total of 1604 patients were included in the study. Significant improvement in EMS and BI scores across all three albumin groups on discharge was observed, with lower scores in low-albumin groups. The EMS and BI efficacies were the same in three albumin groups. However, EMS and BI efficiency were higher in the high-albumin groups. Admission albumin was not independent predictors for satisfactory motor and functional outcomes. For satisfactory motor outcome (EMS ≥15), female gender (odds=0.51, $P=0.0004$), age (odds=0.96, $P=0.0009$) and urinary incontinence (odds=0.59, $P=0.0076$) were negative predictors while living at home (odds=1.98, $P=0.0028$), admission EMS (odds=1.38, $P<0.001$) and BI score (odds=1.02, $P=0.0004$) were positive predictors. For satisfactory functional outcome (BI ≥75), age (odds=0.96, $P=0.015$) and urinary incontinence (odds=0.31, $P<0.001$) were negative predictors while female gender (odds=1.89, $P=0.0024$), LOS (odds=1.02, $P=0.033$), C-MMSE (odds=1.07, $P<0.001$), admission EMS (odds=1.07, $P=0.003$) and BI (odds=1.1, $P<0.001$) were positive predictors.

Conclusion: Admission albumin levels were related to functional and motor efficiency, but not with absolute functional and motor gain. Albumin was not an independent predictor for satisfactory motor and functional outcome.

Systemic sclerosis is an independent risk factor for coronary atherosclerosis

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Background: Endothelial dysfunction and inflammation are pathogenic mechanisms common to scleroderma (SSc) and atherosclerosis.

Objectives: To examine in SSc patients the relationship of coronary atherosclerosis, as assessed by coronary artery calcium score (CACS), with conventional cardiovascular and disease-specific risk factors.

Methods: CACS, as measured by computed tomography, and cardiovascular risk factors were examined in SSc patients and compared with age-, sex- and glycaemic status-matched controls. Disease activity score, antiphospholipid antibodies, high-sensitivity C-reactive protein (hsCRP) and ESR were measured in SSc patients.

Results: A total of 53 SSc (50 female and 3 male) patients and 106 controls were recruited. These patients were 53.1±12.8 years in age with the median disease duration of 9 years. Compared to controls, SSc patients had significantly lower LDL-cholesterol (P=0.001), HDL-cholesterol (P=0.01), diastolic blood pressure, waist circumference and body mass index, and were more likely receiving vasodilators (all P<0.001). There was a significantly higher proportion of SSc patients among subjects with more severe coronary atherosclerosis (CACS ≥101), compared to those with lesser severity (CACS ≤100) [56.5 vs 29.4%; P=0.01]. Multiple logistic regression analysis revealed SSc to be an independent risk factor for CACS ≥101 (odds ratio [OR]=5.98; 95% confidence interval [CI], 1.29-27.78; P=0.02) together with age and LDL-cholesterol after adjustment for other cardiovascular risk factors. Among disease specific factors, only disease duration (OR=1.13; 95% CI, 1.01-1.26; P=0.04) was independently associated with coronary atherosclerosis (CACS ≥101).

Conclusion: SSc was an independent predisposing factor of coronary calcification, in addition to conventional risk factors of coronary atherosclerosis, such as age and hypertension.

A needs assessment and review curriculum of content of teaching on systemic lupus erythematosus

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Objective: To evaluate student knowledge on systemic lupus erythematosus (SLE) and their feedback on teaching on SLE-related topics at a medical school in Hong Kong.

Methods: Senior medical students were recruited to complete a self-administered questionnaire regarding learning, teaching and subjects regarded as important in SLE.

Results: A total of 124 (109 MBBS IV, 15 MBBS V) medical students from the University of Hong Kong were recruited. Majority of students acquired knowledge on SLE from standard sources provided by curriculum teaching including lectures (98.4%), textbook (96.7%), patient contact (86.1%), and problem-based learning sessions (64.8%). A significant proportion of students (77.0%) also obtained knowledge from internet and e-learning. An additional 36.4% of students also preferred learning from extra-curricular sources including educational leaflets from professional societies, family contact, and television program. Most students regarded knowledge on clinical presentations (45.4%), diagnosis (49.6%) management and treatment (76.5%) as the most fundamental knowledge an average doctor should know more about SLE. Renal disease was considered most important (36.7%) among all organ involvement in SLE teaching. Only 8.4% of students regarded epidemiology and pathogenesis as essential but overall importance of subjects on epidemiology and pathogenesis, management and complications ranked 4.0/5, 4.3/5 and 4.2/5, respectively. These senior medical students graded their confidence in knowledge in SLE as 3.4/5. In general, the quality (3.9/5) and quantity (3.5/5) of teaching regarding SLE is good. Majority (85.1%) of students preferred to be taught by rheumatologists whereas only 15.8% regarded rheumatology nurse as an important source.

Conclusions: Majority of students were satisfied with current curriculum teaching and valued clinical management with higher priority than epidemiology and pathogenesis among the taught subjects. Extra-curricular sources of learning including information from professional societies and rheumatology nurse may be considered as adjunct to teaching.

Constitutive expression of human apoA-I enhances cardiac differentiation and functional maturation of embryonic stem cells

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Introduction: The cardioprotective effects of high-density lipoprotein (HDL) and apolipoprotein A1 (apoA-I) are well documented, but their effects in the direction of the cardiac differentiation of embryonic stem cells are unknown. We evaluated the effects of exogenous apoA-I expression on cardiac differentiation of ESCs and maturation of ESC-derived cardiomyocytes.

Method and Results: We stably over-expressed full-length human APOA1 cDNA with lentivirus (LV)-mediated gene transfer in undifferentiated D3 mouse ESCs. Upon cardiac differentiation, we observed a significantly higher percentage of beating embryoid bodies, increased number of cardiomyocytes as determined by flow cytometry, as well as expression of cardiac markers including α -myosin heavy chain, β -myosin heavy chain and myosin light chain 2 ventricular transcripts in LV-APOA1 transduced ESCs compared with control (LV-GFP). Activation of BMP4-SMAD signalling cascade in APOA1-transduced ESCs, while application of BMP4 antagonist, noggin completely abolished the APOA1 stimulated cardiac differentiation, suggesting the pro-cardiogenic APOA1 is mediated via the BMP4-SMAD signalling pathway. Functionally, cardiomyocytes derived from the APOA1-transduced cells exhibited improved calcium handling properties in both non-caffeine and caffeine-induced calcium transient, suggesting the role of APOA1 in enhancing the cardiac maturation.

Conclusion: We present for the first time direct experimental evidence that APOA1 enhances cardiac differentiation of ESCs and promotes maturation of calcium handling property of ESC-derived cardiomyocytes via BMP-4/Smad signalling pathway.

BCT-100: a recombinant arginase which is cytotoxic to leukaemia cells

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Background: BCT-100 is a recombinant human arginase comprising 329 amino acid residues. Previous studies suggested that cancer cells were auxotrophic for arginine due to absence of argininosuccinate synthetase (ASS) expression, and cancer cells need to absorb arginine from blood. Thus, we hypothesised that recombinant arginase, BCT-100, is cytotoxic to human leukaemia cells by depleting the arginine level in blood.

Methods: Effect of BCT-100 on proliferation of 10 leukaemia cell-lines was determined by wst-1 assay. The IC₅₀ value of the 10 leukaemia cell-lines was calculated in order to compare their sensitivity to BCT-100. Its effect on cell cycle progression was studied by flow cytometry. The molecular effect of BCT-100 was examined by Agilent Affymetrix 3'Expression microarray and the result was analysed by Genespring software.

Results: Among the 10 leukaemia cell-lines used in this study, K562 cell-line which was the most responsive model showed an IC₅₀ value of 30 mU/mL. Cell cycle analysis showed that 48- and 72-hour treatment of 30 mU/mL BCT-100-induced K562 cell cycle arrest at G₀/G₁-phase and reduction of cells entering the S- and G₂-phase. IL5 and TCR pathways were altered by BCT-100 based on Genespring software analysis.

Conclusions: BCT-100, which depletes arginine with recombinant arginase, was cytotoxic to leukaemia cells in vitro by inducing cell cycle arrest.

Actopaxin: a novel regulator of cell migration and invasion in human hepatocellular carcinoma

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Background: The mortality rate of hepatocellular carcinoma (HCC) is high due to tumour recurrence and development of metastasis, involving cell spreading, lamellipodia formation and cell migration. Formation of focal adhesions is a key regulator of cell motility and cell invasion. Actopaxin, a focal adhesion and cytoskeleton-associated protein, is required for such processes. The aim of this study was to examine the role of Actopaxin in HCC cell migration, invasion and development of metastasis.

Methods: The expression of Actopaxin in primary and metastatic liver cancer cell-lines was examined by Western blot and RT-PCR. The functional and molecular effects of Actopaxin expression were studied by enforced expression or down-regulation in HCC cell lines. Immunofluorescence staining for actin cytoskeleton and focal adhesion was performed to examine the effect of Actopaxin expression on cell shape, stress fiber organisation, and focal adhesion. The clinicopathological significance of Actopaxin expression in tumourous tissues of 119 HCC patients was also investigated.

Results: High protein level of Actopaxin was found in the metastatic HCC cell lines compared with other non-metastatic HCC cell lines. Expression of a shorter form of Actopaxin was also detected in some of these cell lines. Comparing to Actopaxin (LF-Actopaxin), the unreported short form (SF-Actopaxin) lacks a fragment in the C-terminal and hence resulted in an incomplete second CH domain which consists of binding sites for its downstream activation targets. Expression of LF-Actopaxin but not SF-Actopaxin in HCC cell lines was positively correlated with their migration and invasion potential *in vitro*, accompanied by altered expressions of several focal adhesion proteins as well as E-cadherin which is associated with EMT process. Study in clinical samples showed that LF-actopaxin expression was positively correlated with tumour size ($P < 0.05$) while SF-actopaxin expression was lower in HCC tumour compared with corresponding non-tumorous liver ($P < 0.001$) and lower in HCC with metastasis compared with HCC without metastasis ($P < 0.05$).

Conclusion: This study demonstrated for the first time the pro-migratory effects of Actopaxin in human HCC, and the existence of a short form which lacks a complete CH domain that is critical for cell migration, re-organisation of cytoskeletal events and turnover of focal adhesions.

Transient axonal glycoprotein-1 polymorphism and its correlation with clinical features and prognosis in chronic inflammatory demyelinating polyradiculoneuropathy

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Introduction: Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is a heterogeneous group of acquired disorders characterised by peripheral nerve demyelination. Previous studies have shown a good response to immunotherapy but a significant proportion of patients continue to require maintenance therapy. A recent study identified a potential genetic marker for responsiveness to intravenous immunoglobulin (IVIG). In this study, patients with CIDP were examined to look for associations between clinical, electrophysiologic, genetic factors and prognosis.

Methods: Case records of 32 Chinese CIDP patients diagnosed between 1995 and 2010 were examined for clinical features, electrophysiologic parameters, disease course, and outcome. Blood samples were collected from 22 patients and 147 controls and the transient axonal glycoprotein-1 (TAG-1) genotype with regard to the single nucleotide polymorphism (SNP) rs2275697 was determined.

Results: The overall response rate to prednisolone, IVIG or plasma exchange was 80% with no difference among the three therapies. Clinical features and electrophysiologic parameters were not associated with treatment response. Fifty-eight percent of the patients were dependent on maintenance therapy after a mean follow-up period of 5.8 years. Patients with more prolonged distal motor latencies (DML) in the upper limbs had a higher risk of treatment dependence (OR=1.03; $P=0.03$). TAG-1 genotypes were not different between CIDP patients and controls. Patients who were homozygous for G had more prolonged DML ($P=0.02$); patients with the G allele also had lower compound muscle action potential amplitude in the upper limbs ($P=0.04$) and more prolonged DML in the lower limbs ($P=0.04$).

Conclusions: The treatment response rate in CIDP is high; however, many patients require long-term maintenance therapy to prevent relapses. Patients with more prolonged DML initially had a higher risk of treatment dependence. The TAG-1 G allele was associated with more severe demyelination on presentation, which suggests a role of TAG-1 in the pathogenesis of demyelination.

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Introduction: Cardiomyocytes (CM) withdraw from the cell cycle when they enter adulthood and mature in terms of their electrophysiological and Ca²⁺ handling properties. However, the mechanisms underlying human CM cell cycle exit and maturation are poorly understood. We previously demonstrated that miR-29b is upregulated in human adult CM relative to fetal CM and human embryonic stem cells (hESC)-derived CM. Here, we aimed to test our hypothesis that miR-29b is important for hESC-CM cell cycle exit and maturation.

Methods: We overexpressed miR-29b in hESC-CM by lentiviral transduction. We then compared the proliferation potential of control and miR-29 overexpressing hESC-CM by immunostaining for the presence of Ki67, a proliferation marker. The mRNA expression of cyclins and cyclin-dependent kinases were examined by quantitative real-time PCR. We also performed functional assays including patch-clamp and Ca²⁺-imaging to assess the maturation of control and miR-29 overexpressing hESC-CM.

Results: MiR-29b overexpression inhibited hESC-CM proliferation by decreasing the mRNA expression of multiple cyclins and cyclin-dependent kinases. However, electrophysiological and Ca²⁺ handling properties were unchanged, indicating that hESC-CM maturation was not affected by miR-29b overexpression.

Conclusion: We conclude that miR-29b is a negative regulator of hESC-CM cell cycle, but miR-29b induced cell cycle exit does not promote hESC-CM maturation.

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Introduction: Obesity is a growing problem worldwide. We set out to investigate the use of anti-obesity drugs in the United States in recent years.

Methods: We included 2630 men and 2702 women who took part in the National Health and Nutrition Examination Survey (NHANES) in 2007-8. We analysed their demographic and anthropometric data, and their weight and drug history. Sampling weights were used to adjust for non-response bias and the oversampling of blacks, Mexican Americans, and the elderly.

Results: 46.0% of men and 44.9% of women were candidates for anti-obesity medication (initial body mass index ≥ 30 kg/m², or ≥ 27 kg/m² in the presence of other risk factors [eg hypertension, diabetes or dyslipidemia]). Among these participants, 85.1% considered themselves overweight and 90.1% would like to lose weight. However, only 61.9% had dietary changes, 36.5% exercised, 3.7% took non-prescription diet pills and 2.2% took prescription diet pills to control weight during the preceding year. During the preceding month, 0.4% and 0.1% of participants were taking phentermine and orlistat, respectively. There were no participants on sibutramine.

Conclusions: Obesity is highly prevalent in the United States, but only a very small percentage is on anti-obesity medication. The withdrawal of sibutramine in October 2010 would have minimal impact on the general population. While improvements in pharmacological treatment of obesity are needed, our study revealed that there is also a need for more lifestyle changes in the majority of obese individuals.

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Introduction: Cell replacement therapy holds great potential for brain tissue repair following intracerebral haemorrhage (ICH). Haematoma evacuation alleviates the mass effect and prevents the secondary pathological processes. This study was conducted to investigate the survival and differentiation of neural stem cells (NSCs) after transplantation into the brain cavity following haematoma aspiration in adult male Sprague-Dawley rats.

Methods: Experimental ICH was induced by local injection of bacterial collagenase IV into the basal ganglia. Haematoma was removed 3.5 hours after ICH onset. Following the removal, E13.5-derived NSCs were injected into the lesion. Two weeks after transplantation, the survival and differentiation of transplanted cells was assessed immunohistochemically. The concentration of trophic factors in the transplanted brain areas was measured using ELISA. Function recovery was evaluated 1, 3, 7 and 14 days after ICH.

Results: Transplanted NSCs survived along the wall of haematoma cavity and partially migrated into the brain parenchyma 2 weeks after transplantation. One third of the survived cells (30.0±9.7%) remained undifferentiated and others could differentiate into astrocytes (45.7±14%) and neurons (0.6±0.3%). NSCs transplantation group showed a trend toward increased secretion of NGF, BDNF and GDNF in the ipsilateral striatum. NSCs-transplanted group showed improved functional recovery when compared to the control group 2 weeks after transplantation.

Conclusion: The results provided evidence that the NSCs transplantation into the brain cavity after haematoma aspiration may be a potential treatment strategy in ICH.

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Objective: The significance of early HBV DNA suppression during telbivudine treatment in predicting long-term outcomes needs further investigation.

Methods: We determined the cumulative rates of HBeAg seroconversion, ALT normalisation, HBV DNA suppression (<12 IU/mL) and telbivudine-resistant mutations (using the highly sensitive line probe assay) for 117 treatment-naïve chronic hepatitis B (CHB) patients (61.5% HBeAg-positive) on telbivudine for 3 years. The significance of serum HBV DNA at week 12 and 24 was compared.

Results: The median age and duration of follow-up were 39 years and 24.2 months, respectively. A total of 117, 105, 69 and 43 patients had been followed up for at least 6 months and 1, 2, and 3 years respectively. The cumulative rates of HBeAg seroconversion, ALT normalisation, HBV DNA undetectability were 46.8%, 80.5% and 51.2% respectively at 3 years. There was an incremental increase in virologic breakthroughs to 39.5% by year 3. The cumulative rate of telbivudine-resistant mutations was 4.8%, 17.6% and 34.0% for year 1, 2 and 3 respectively. Week 12 HBV DNA of <200 IU/mL was predictive of a higher chance of HBV DNA undetectability (P=0.022) and lower chance of resistance (P=0.001) by year 3. Undetectable HBV DNA at week 24 was predictive of viral suppression at year 2 (P<0.001) but not at year 3 (P=0.241).

Conclusion: Continuous telbivudine resulted in improved biochemical and virologic outcomes, although there was an incremental increase in cumulative rate of resistance up to year 3. Week 12 HBV DNA of <200 IU/mL was predictive of favourable long-term outcomes.

Acknowledgement: The assays used to determine HBV DNA levels and viral resistance were supported by an unrestricted grant from Novartis Pharmaceuticals.

A large population histology study of clinical parameters for the prediction of significant fibrosis in chronic hepatitis B

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Objective: We determined the association between various clinical parameters and significant liver injury in both hepatitis B e antigen (HBeAg)-positive and HBeAg-negative patients.

Methods: From 1994 to 2008, liver biopsy was performed on 319 treatment-naïve chronic hepatitis B (CHB) patients. Histologic assessment was based on the Knodell histologic activity index for necroinflammation and the Ishak fibrosis staging for fibrosis. Liver biochemistry, HBeAg status and HBV DNA levels were checked.

Results: A total of 211 HBeAg-positive and 108 HBeAg-negative patients were recruited, with a median age of 31 and 46 years respectively. Nine (22.5%) out of 40 HBeAg-positive patients with normal ALT had significant histologic abnormalities (necroinflammation grading ≥ 7 or fibrosis score ≥ 3). There was a significant difference in fibrosis scores among HBeAg-positive patients with an ALT level within the Prati criteria (30 U/L for men, 19 U/L for women) and patients with a normal ALT but exceeding the Prati criteria ($P=0.024$). Age, aspartate aminotransferase and platelet count were independent predictors of significant fibrosis in HBeAg-positive patients with an elevated ALT by multivariate analysis ($P=0.007$, 0.047 and 0.045 respectively). Serum HBV DNA and platelet count were predictors of significant fibrosis in HBeAg-negative disease ($P=0.020$ and 0.015 respectively). An elevated ALT was not predictive of significant fibrosis for both HBeAg-positive ($P=0.345$) and HBeAg-negative ($P=0.544$) disease. There was no significant difference in fibrosis staging among ALT 1-2 x upper limit of normal (ULN) and >2 ULN for both HBeAg-positive ($P=0.098$) and HBeAg-negative ($P=0.838$) disease.

Conclusion: An elevated ALT does not accurately predict significant liver injury. Decisions on commencing antiviral therapy should not be heavily based on a particular ALT threshold. HBV DNA and platelet count are more important determinants of significance fibrosis in HBeAg-negative disease.

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Hepatitis B virus suppression, viral resistance and clinical safety of continuous 3-year entecavir therapy in treatment-naïve chronic hepatitis B patients

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Background and Aims: Long-term data on entecavir 0.5 mg for treatment-naïve chronic hepatitis B (CHB) patients are lacking. Our aims were to determine the anti-viral potency, viral resistance rate and clinical safety of 3-year entecavir treatment.

Methods: We determined the cumulative rates of undetectable HBV DNA levels (<12 IU/mL), HBeAg seroconversion, ALT normalisation and entecavir signature mutations (using highly sensitive line probe assay) and monitored side-effects for 222 treatment-naïve CHB patients (40.5% HBeAg positive) for 3 years.

Results: The median age and follow-up duration were 45 years and 25.1 months, respectively. A total of 222, 188 and 101 patients had been followed up for at least 1, 2 and 3 years, respectively. There were incremental increases in the rates of HBV DNA undetectability, HBeAg seroconversion, and ALT normalisation reaching to 92.1%, 43.9% and 90.4% at year 3 respectively. 100% and 76.5% of patients with baseline HBV DNA levels $<$ and ≥ 8 logs copies/mL respectively had undetectable HBV DNA at year 3. The cumulative rate of entecavir resistant mutations was 1.2% at year 3. Three patients experienced virologic breakthroughs. Two patients with baseline rt204I mutations responded to entecavir treatment. There were no serious adverse events observed up to 3 years.

Conclusions: Even using very sensitive assays for HBV DNA and viral resistance measurements, continuation of entecavir treatment 0.5 mg for treatment-naïve CHB patients for 3 years is associated with more than 90% chance of undetectable HBV DNA level and only 1.2% chance of emergence of entecavir resistant mutations.

Acknowledgement: The assays used to determine HBV DNA levels and viral resistance were supported by an unrestricted grant from Bristol-Myers Squibb Company.

Efficacy of 1927-nm thulium fiber laser for the treatment of melasma in Chinese patients

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Background: 1927-nm thulium fiber laser contains two fiber lasers in one system. It is FDA approved for non-ablative skin resurfacing and rejuvenation. It provides a higher wavelength with higher absorption.

Objective: The objective of the study was to assess the efficacy of 1927-nm thulium fiber laser for the treatment of melasma in Chinese patients.

Methods: Twelve female subjects aged 44 to 58 years with mild-to-severe melasma were recruited. All subjects received a single treatment. They were assessed at baseline, 1 day, 7 days, 1 month and 2 months post-treatment. Adverse effects were noted and clinical photos were taken using Canfield Visia CR System. A questionnaire was given at the follow-ups for the subjects to rate the pain level, swelling, dryness, wrinkles, pore size, pigmentation, any hyper-/hypo-pigmentation as well as overall satisfaction. Two independent physicians assessed the clinical photos, giving the MASI score, rating the skin texture and identifying any adverse effects.

Results: All subjects experienced pain and swelling, all of which subsided within 7 days post-treatment. 54.5% and 58.3% subjects noticed an improvement in their melasma at 1 and 2 months post-treatment respectively. A decrease in wrinkles was observed by 36.4% at 1-month post-treatment but the effect only lasted in 16.7% by 2 months post-treatment. 62% noticed an improvement in pore size. Physician assessment supported the above with a statistically significant improvement in MASI score ($P=0.018$), pore size ($P=0.011$) and skin texture ($P=0.005$). The improvement in fine lines was also significant at 1-month follow-up ($P=0.014$) but was not enough to be statistically significant at 2-month follow-up.

Conclusion: 1927-nm thulium fiber laser is temporarily effective for treating melasma in Chinese patients. The treatment also improves the overall skin texture.

Multiple treatments, non-invasive cryolipolysis for body contouring in Chinese patients

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Background: Non-invasive cryolipolysis has been demonstrated to be effective in body contouring. The objective of this study was to determine the efficacy of multiple treatments with a cryolipolysis device for body contouring in Chinese patients.

Methods: This was a retrospective study where 12 patients, aged 35 to 60 years, with discreet bulges were treated with two treatments of cryolipolysis at their own cost. On average, the two treatments were 3 months apart. The cryolipolysis system was used and the treatment parameters were CIF 41.6 (-73 milliwatts/cm²) for 60 minutes per site at the desired anatomical region. The areas treated included the abdomen and love handles. At baseline visit, their weight was measured and caliper measurement was taken at the maximum area of fat when standing. Standard photographs were taken with Canfield Visia CR system. They were followed up 2 months after, when the physician would perform assessment. Subjects were assessed prior to the second treatment and were followed up again 2 months post-second treatment.

Results: During the study, the weight of the subjects remained relatively constant. For both the abdomen and love handles, there was a statistically significant improvement after one treatment ($P<0.001$ and $P=0.003$ respectively) with a further improvement after a second treatment ($P=0.02$ and $P=0.084$ respectively). However, the decrease in measurement with the caliper was of lesser extent after the second treatment compared to the first. Control sites had no significant difference post-treatment.

Conclusion: Multiple treatments with a cryolipolysis device for body contouring in Chinese patients demonstrated a cumulative effect.

Delayed clearance of viral load and marked cytokine activation in severe cases of pandemic H1N1 2009 influenza virus infection*

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Background: Infections caused by the pandemic H1N1 2009 influenza virus range from mild upper respiratory tract syndromes to fatal diseases. However, studies comparing virological and immunological profile of different clinical severity are lacking.

Methods: We conducted a retrospective cohort study of 74 patients with pandemic H1N1 infection, including 23 patients who either developed acute respiratory distress syndrome (ARDS) or died (ARDS-death group), 14 patients with desaturation requiring oxygen supplementation and who survived without ARDS (survived-without-ARDS group), and 37 patients with mild disease without desaturation (mild-disease group). We compared their pattern of clinical disease, viral load, and immunological profile.

Results: Patients with severe disease were older, more likely to be obese or having underlying diseases, and had lower respiratory tract symptoms, especially dyspnoea at presentation. The ARDS-death group had a slower decline in nasopharyngeal viral loads, had higher plasma levels of proinflammatory cytokines and chemokines, and were more likely to have bacterial coinfections (30.4%), myocarditis (21.7%), or viremia (13.0%) than patients in the survived-without-ARDS or the mild-disease groups. Reactive haemophagocytosis, thrombotic phenomena, lymphoid atrophy, diffuse alveolar damage, and multiorgan dysfunction similar to fatal avian influenza A H5N1 infection were found at postmortem examinations.

Conclusion: The slower control of viral load and immunodysregulation in severe cases mandate the search for more effective antiviral and immunomodulatory regimens to stop the excessive cytokine activation resulting in ARDS and death.

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Serum metformin level is not prognostic in patients with metformin-associated lactic acidosis: a retrospective review

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Introduction: While metformin-associated lactic acidosis (MALA) has been well described in the literature, its prognosis in modern practice of critical care needs to be reviewed. The value of serum metformin level as a prognostic marker has not been described.

Methods: MALA was diagnosed by: (i) arterial blood pH <7.30 with concomitant lactate level >4.0 mmol/L, and (ii) recent intake of metformin, and (iii) exclusion of other causes of lactic acidosis. From January 2006 to September 2010, we retrospectively identified patients who either (i) presented to the Accident and Emergency Department (A&E) or Adult Intensive Care Unit with suspected MALA or (ii) had a request to the Biochemistry Laboratory for assay of metformin level, and the above diagnostic criteria were met.

Results: Twenty-three patients were identified with the age of 72.1±10.0 years (mean±SD); 17% of subjects had intentional overdoses, 52% had gastrointestinal symptoms at presentation including 22% having hyper-amylasemia. Hypoglycaemia at the A&E (haemoglucostix <4.0 mmol/L) were noted in 63.6% of subjects, among whom 28.6% had no concomitant use of insulin or other oral hypoglycaemic agents. Eighty-two percent of patients required renal replacement therapy. Overall hospital mortality rate was 30.4%. Compared to the survivors, the subjects who died had lower systolic blood pressure (SBP) [79.1±27.1 vs 116.8±25.4 mm Hg; P=0.004, paired T-test] and higher lactate level at presentation (21.7±5.2 vs 16.1±5.8 mmol/L; P=0.04, paired T-test). Metformin levels were available in 22 subjects and there were no significant differences between the groups (median±IQR: 63.0±93.0 vs 45.0±51.0 mmol/L; P=0.244, Mann Whitney test). On multiple logistic regressions, only SBP was associated with hospital mortality.

Conclusion: We noted some unique features in our patients with MALA. Haemodynamic status seemed to be more prognostic than serum markers.

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Introduction: Neuromyelitis optica spectrum disorders (NMOSD) are typified by longitudinally extensive transverse myelitis (LETM) and/or optic neuritis (ON), a significant proportion of patients are seropositive for aquaporin-4 autoantibodies (AQP4 Ab). Cerebral involvement is increasingly recognised.

Aim: To study clinical, neuroradiological features of cerebral involvement documented by MRI in local NMOSD patients.

Methods: NMOSD patients diagnosed (according to Wingerchuk's criteria) and followed up in Queen Mary Hospital from 1988 to 2008 were studied. All have MRI brain and spinal cord with gadolinium performed on initial presentation and repeated

6 to 12 months later or during relapse. Since 2002, all had repeated MRI brain yearly for 3 years even without relapse. AQP4 Ab was assayed by cell-based immunofluorescence using HEK293 cells transfected with human AQP4 gene.

Results: Thirty-three NMOSD patients (18 NMO, 9 relapsing myelitis [RM], 6 relapsing ON) were studied, mean onset age was 39.0 years (range, 17-70), 30 (91%) were female; mean follow-up duration was 6.0 years (range, 2-16 years). AQP4 Ab were detected in 23 (69.7%). MRI brain lesions were detected in 19 (58%) in (i) brainstem (14 patients, 42%) in medulla, midbrain, pons, cerebellar peduncles, peri-ependymal regions around third, fourth ventricles, periaqueductal region, (ii) peri-ventricular regions around lateral ventricles (7, 21%), (iii) frontal/temporal/parietal lobes as small white matter lesions (7, 21%), (iv) corpus callosum (4, 12%), (v) parietal and occipital lobes as large confluent (>3 cm) white matter lesions (2, 6%), (vi) hypothalamus (1, 3%). Gadolinium-enhancing lesion were detected in 3 (9%), and 2 (6%) had MRI abnormalities fulfilling criteria for MS. Ten (53%) of the 19 patients had clinical manifestations due to cerebral involvement including (i) brainstem encephalitis with diplopia, ataxia, nausea, vomiting, internuclear ophthalmoplegia, facial weakness, dysphagia, aspiration pneumonia, autonomic dysfunction, facial sensory loss and long-tract sign (7, 70%), (ii) hemispheric syndrome with hemiparesis, homonymous hemianopia (2, 20%), (iii) cortical signs including aphasia, acalculia, agnosia, agraphia, neglect, and cognitive impairment (1, 10%), (iv) hyperphagia, weight gain from hypothalamic involvement (1, 10%), (v) trigeminal neuralgia in 1 (10%).

Conclusion: Cerebral involvement is common in our NMOSD patients; brainstem is the most frequently affected site.

Post-stroke orthostatic hypotension, its pattern of recovery

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Introduction: Orthostatic hypotension (OH) is common in stroke patients due to damage to central autonomic centres or pathways. Its pattern of recovery is not well defined.

Methods: First-ever stroke patients admitted to Queen Mary Hospital were recruited, excluded those with known cardiac illness or disorders that can affect ANS. 60° tilt table was performed in Tung Wah Hospital at 14 and 90 days post-stroke. NIHSS and BI were measured at 6-month follow-up.

Result: A total of 75 patients were recruited; 72 underwent initial assessment (3 patients died before assessment), 69 patients completed second assessment 3 months later (4 died and 2 dropped out), and 67 completed 6-month follow-up assessment. Of those 25 patients (34.7%) with OH, only 23 underwent reassessment. OH resolved in 13 (57%) of these 23 patients, 4 (17%) have milder degree of OH and 6 (26%) have similar degree of OH. There is a 10% relative reduction of prevalence of OH at 3 months. Patients with OH resolved is demonstrated to have made better recovery at 6 months with mean NIHSS 1.83 versus 6.78 compared to those with persistent OH (P=0.003). No patients developed new-onset OH at 3-month assessment.

Conclusion: Over 50% of patients with OH recovered at 3 months post-stroke. Patients with OH resolved at 3 months' assessment were expected to have better recovery at 6 months compared to those with persistent OH.

Impact of stroke on autonomic nervous system

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Introduction: Disturbances of the autonomic nervous systems (ANS) are not uncommon in stroke patients, attributed to damage of the central autonomic networks. The resultant alternation in autonomic dynamics caused an imbalance between sympathetic and parasympathetic tone. The most common clinical problems include abnormalities in heart rate and blood pressure control. Vasomotor and sudomotor disturbances post stroke are less well defined.

Methods: First-ever stroke patients admitted to Queen Mary Hospital were recruited excluded those with known cardiac illness or disorders that can affect ANS. Autonomic function test and sympathetic skin response (SSR) were performed in Tung Wah Hospital within 14 days of admission.

Result: A total of 75 patients were recruited, only 72 underwent full assessment (3 patients died before assessment). A total of 25 patients (34.7%) have orthostatic hypotension (OH), majority asymptomatic, found to be more common in patients with severe stroke (NIHSS) and poor functional level (BI). There was no difference in number of antihypertensive used between those with and without OH. Forty-five patients (62.5%) have impaired SSR where 60% has bilateral involvement. SSR impairment was more common in patients with low BI score or insular involvement.

Conclusion: Sympathetic impairment is common post stroke, ranged from 34.7% for OH to 62.5% for sudomotor impairment. Graded vasomotor training should be advocated in patients with severe stroke with monitoring of BP during training as majority of patient with significant OH were asymptomatic. SSR is found to associate with post-stroke functional status.

Predictors common to cardiovascular and cancer outcomes in a population-based 13-year prospective study in Hong Kong

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Introduction: Cardiovascular diseases (CVD) and cancer are the two leading causes of death in Hong Kong. Obesity is becoming increasingly prevalent in the local population and has been reported to be associated with CVD and some forms of cancer in the western world. In this study, we attempted to identify the aetiological factors linking obesity to both CVD and cancer among Hong Kong Chinese.

Methods: Subjects were recruited from the Hong Kong Cardiovascular Risk Factor Prevalence Study (CRISPS), a population-based prospective study commenced in 1995-6. CVD and cancer outcomes were identified and confirmed by physician-entered diagnosis codes in the Hospital Authority Computer Database.

Results: A total of 2091 subjects from CRISPS-1 had full anthropometric and biochemical data and outcome data for analysis; 113 subjects had confirmed incident CVD. Male sex, age, baseline body mass index, waist circumference (WC), fasting glucose, 2-hour glucose after oral glucose tolerance test, insulin resistance index HOMA-IR, systolic and diastolic blood pressure, triglycerides, high-density lipoprotein (HDL), diabetes, hypertension, dyslipidaemia and metabolic syndrome were all predictive of incident CVD over 12.9 years even after controlling for age ($P < 0.01$). On multivariate analysis, baseline age, WC, HOMA-IR, hypertension and diabetes were independent risk factors for incident CVD. A total of 176 subjects had incident cancer. Age and female sex were significantly associated with cancer. In addition, baseline HDL, HOMA-IR and diabetes were significantly associated with cancer after adjustment for age. On multivariate analysis, age, female sex, low HDL and diabetes were the independent risk factors for the development of cancer.

Conclusion: Age and diabetes mellitus are risk factors common to CVD and cancers in our population. The data from this prospective study suggest that diabetes plays an important role in the development of CVD and cancer, the top killers in Hong Kong. Primary care health policies should focus on community-wide education and strategies aiming to reduce insulin resistance and diabetes development, and hence future CVD and cancer events in our ageing population.

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N-acetylcysteine promotes post-transplantation survival of cardiomyocytes

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Introduction: Previous studies have fully demonstrated a considerable prospect in clinical application of the indefinitely proliferating human embryonic stem cells (hESCs), which in turn makes the hESC-derived cardiomyocytes one of the most promising targets for treating irreversible cardiac events, for example, chronic heart failure (CHF). However, numerous hurdles stand in the way of an effective clinical approach, among which, limited post-transplantation survival ratio of cardiomyocytes still need to be conquered. This study investigated the self-repair potential of hESCs and hESC-derived cardiomyocytes. N-acetylcysteine (NAC), a widely used clinical antioxidant, is also studied for its cardiac protective effect against ischaemia/reperfusion injury-induced cellular DNA damage.

Methods: hESCs and hESC-derived cardiomyocytes were parallel cultured. H₂O₂ was used as the DNA damage agent, mimicking Ischemia/Reperfusion injury throughout heart transplantation. The formation and resolution of gamma-H2AX foci can reflect DNA damage and self-repair capacities of both hESCs and hESC-CMs upon H₂O₂ damage. N-acetylcysteine was then added into hESC-CMs culture medium 12 hour prior to H₂O₂ damage. Comparison between NAC pre-conditioned and control groups was carried out by quantifying formation and resolution of gamma-H2AX foci of each.

Results: hESCs and hESC-derived cardiomyocytes showed similar gamma-H2AX foci formation upon H₂O₂ treatment, while foci resolution of hESCs occurred much faster than that of hESC-CMs. When comparing NAC-preconditioned hESC-CMs with non-NAC group, less gamma-H2AX foci formation was observed in NAC-preconditioned group. Meanwhile, the foci resolution rate was also faster in NAC-preconditioned group.

Conclusion: We conclude that N-acetylcysteine, a widely used antioxidant, is able to protect hESC-CMs from ischaemia/reperfusion injury, thus, may promote the post-transplantation survival of cardiomyocytes in the real clinical application.

Insulin induces vasoconstriction through both endothelin-1 and cyclooxygenase-2 in mesenteric arteries from *db/db* obese mice

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Introduction: Obesity is associated with increased expression of endothelium-derived constricting factors and augmented insulin-mediated vasoconstriction, yet the underlying mechanism remains unknown. The major objective of this study was to elucidate how insulin induces vasoconstriction in arteries using a genetically inherited obese mouse model with type 2 diabetes.

Methods: Mesenteric arteries isolated from *db/db* mice were subjected to isometric force measurement of vasoreactivity using wire myograph. The expression levels of cyclooxygenase 1/2 and putative constrictor prostanoids in the arteries were determined by quantitative real-time PCR analysis and ELISA, respectively.

Results: Insulin elicits vasoconstriction in small mesenteric arteries from *db/db* obese mice, but induces vasodilatation in arteries isolated from lean littermates. Inhibition of ERK or blockade of both the endothelin receptor A and the TP receptor can alleviate insulin-induced vasoconstriction in *db/db* mice, indicating that this insulin action is mediated by endothelin-1 and the constricting prostanoids through ERK activation. Further study showed that the expression of cyclooxygenase-2 but not cyclooxygenase-1 is up-regulated in the arteries of *db/db* mice compared to lean littermates. Inhibition of cyclooxygenase-2 by its specific inhibitor NS398 can alleviate insulin-evoked vasoconstriction. Insulin enhances the production of prostaglandin F₂alpha in arteries of *db/db* mice through ERK and cyclooxygenase-2 signalling pathways. Treatment with recombinant endothelin-1 or prostaglandin F₂alpha induces vasoconstriction in a dose-dependent manner, whereas treatment with both endothelin-1 and prostaglandin F₂alpha further enhance their vasoconstriction effects.

Conclusion: Taken together, these results suggest that insulin-induced vasoconstriction is mediated by both endothelin-1 and cyclooxygenase-2 derived prostaglandin F₂alpha through ERK signalling cascades in obese mice.

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Assessing self-efficacy behaviour of type 2 diabetes mellitus in primary care

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Introduction: Self-efficacy is a process that helps patients in self-management to take care of their quality of life and achieve satisfactory disease control. The study aimed to find out whether higher degree of self-efficacy was associated with better health-related quality of life (HRQOL) and glycaemic control in patients with diabetes mellitus in Hong Kong primary care.

Methods: A total of 488 type 2 diabetic patients (T2DM) attending a government GOPC in Hong Kong were interviewed face-to-face using a structured questionnaire containing the Chinese version of the 26-item diabetes self-efficacy scale (C-DSES) and the Chinese (Hong Kong) SF-12 Health Survey. Each C-DSES total score was correlated with glycaemic control measured by HbA1c, and the SF-12 Physical Component Summary (PCS) and Mental Component Summary (MCS) scores.

Results: 54.3% of subjects had HbA1c >7% (mean, 7.38±1.27%) and 51.2% were elderly (mean age, 65.3±11.0 years). C-DSES total score was correlated with SF-12 PCS ($r=0.26$, $P<0.001$) and MCS ($r=0.12$, $P=0.007$) scores. There was no correlation between C-DSES and HbA1c level, and no significant difference on C-DSES between good and bad glycaemic control. No correlation between SF-12 scores and HbA1c was found. Multivariate regression analyses showed that C-DSES total score was an independent predictor of PCS and MCS after adjustment for age, gender, BMI, duration of diabetes.

Conclusion: Our study has demonstrated a relationship between self-efficacy and HRQOL among diabetic patients. Improving self-efficacy may help improve HRQOL probably by improving illness coping even if there is no change to the disease severity. Further prospective studies should evaluate interventions for the improvement of self-efficacy of patients with diabetes mellitus.

Acknowledgement: This study was supported by the Seed Funding Programme for Basic Research grant (project no. 200711159119) from the University of Hong Kong.

Confirmatory factor analysis on traditional Chinese version of 26-item Diabetes Self-Efficacy Scale (C-DSES) in Chinese patients with type 2 diabetes mellitus

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Introduction: Diabetes Self-efficacy Scale (C-DSES), translated to traditional Chinese version from the West, assesses the strength of patients' beliefs in their own abilities to respond to diabetes mellitus. The aim of study was to validate the factorial structure of traditional Chinese version of C-DSES by confirmatory factor analysis (CFA) for a use in a Hong Kong population with diabetes mellitus.

Methods: Traditional Chinese version of the 26-item C-DSES was administered by interviewers to 488 subjects with type 2 diabetes mellitus (T2DM). The C-DSES has six subscales including dietary management, regular exercise, medication taking, regular self-monitoring, foot care, and hyper- or hypoglycaemia prevention/treatment. The six-factor CFA model was examined by goodness-of-fit indices according to threshold criteria suggested by Joreskog & Sorbom.

Results: The fit of CFA model was good when Root Mean Square Error of Approximation (RMSEA) was close or less than 0.08; the ratio of Chi-square statistic to degree of freedom was between two and five; the goodness-of-fit index (GFI) was close to or greater than 0.9. Because RMSEA was 0.065 (90% CI, 0.060-0.070) and model Chi-square statistic of 869 with 284 degrees of freedom (ratio, 3.06), the CFA model provided a good fit even when the GFI with 0.88 was a bit lower than 0.9.

Conclusion: The six-factor model of C-DSES was revealed good factorial structure, supporting the construct validity of C-DSES in Chinese subjects with T2DM. This scale is an applicable and valid instrument to measure the effectiveness of interventions to control the blood glucose and prevent diabetic complications.

Acknowledgement: This study was supported by the Seed Funding Programme for Basic Research grant (project no. 200711159119) from the University of Hong Kong.

Practical limitations of convalescent plasma collection: a case scenario in pandemic preparation for influenza A (H1N1) infection*

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Background: To ensure a good preparedness for pandemic influenza A (H1N1), a study was conducted to investigate clinical effectiveness of hyperimmune intravenous immunoglobulin (H-IVIG) prepared from convalescent plasma donated by recovered patients. This article reports on the outcome of the collection phase of the study.

Methods: Starting on 26 August 2009, all confirmed patients aged between 18 and 55 years were invited for participation into the study and screened for plasma donation eligibility. Effective on 17 September 2009, those who were unwilling to consider screening for plasma were asked to donate whole blood. Plasma collected or separated from whole blood had to demonstrate sufficient neutralisation antibodies titers of 40 or more before being channelled for H-IVIG production.

Results: By 31 October 2009, a total of 9101 persons were successfully contacted. A total of 1309 screening and 619 whole blood donation appointments were made. In the former, 786 (60.0%) attended screening but only 301 could donate plasma by apheresis because of failure to meet blood donation eligibility criteria, failed laboratory tests, insufficient neutralisation antibody titers, and inability to make the apheresis appointment. For those who opted for whole blood donation, 379 (61.2%) had attended and donated. A total of 276 L of convalescent plasma with sufficient neutralisation antibodies titers was collected for H-IVIG production.

Conclusion: The study highlighted a number of practical limitations in convalescent plasma collection programs and plasmapheresis is always the preferred mode of collection. It provided valuable learning experience for the blood transfusion service in future planning when large-scale collection is required.

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Case fatality of acute myocardial infarction in Queen Mary Hospital in 2009

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Introduction: Acute myocardial infarction (AMI) is one of the major causes of death in Hong Kong. With the advance in management, mortality can be reduced with timely revascularisation utilising thrombolytic therapy or percutaneous coronary intervention. However it is difficult to compare mortality among different centres or its trend over past years if a standardised methodology in calculation of mortality is not adopted. Therefore in the present study we applied the method of case fatality in calculating the mortality rate of AMI in Queen Mary Hospital in the calendar year 2009 so that the result may be compared with internationally published figures and can be used as a reference to assess the trend of mortality in future.

Methods: All hospital admissions in Queen Mary Hospital in the calendar year 2009 with the diagnosis of AMI (ICD code 410) and acute coronary syndrome (ACS) [ICD code 411] documented as the principal, second and third admission diagnosis were retrieved and reviewed. Those who had AMI, including ST-elevation myocardial infarction and non-ST-elevation myocardial infarction, as the principal diagnosis after review were included for analysis. All in-hospital deaths were retrieved. In-hospital case fatality was defined as total number of in-hospital death divided by total number of AMI admissions respectively.

Results: Preliminary results of the analysis revealed that a total of 747 hospital admission episodes were retrieved and reviewed in which 227 episodes of ST-elevation myocardial infarction, 370 episodes of non-ST-elevation myocardial infarction and 72 episodes of ACS were confirmed as the principal diagnosis while the remaining 78 admissions had principal diagnosis other than AMI/ACS. In-hospital case fatality was 11.9%. 30-Day fatality figures were also in the process of computation. Important outcome indicators, like whether the patients received timely revascularisation, were being analysed.

Conclusion: The Queen Mary Hospital AMI in-hospital case fatality was 11.9%. This was compared with the internationally published figures, 14.5% in-hospital mortality reported by Australian Institute of Health and Welfare and the previous published WHO MONICA Studies and the 1995-6 Hong Kong Atrial Fibrillation Registry (hospital mortality 22.9%). Direct comparison is not possible due to demographic variables.

Long-term follow-up for EBUS-TBNA negative mediastinal lesions

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Introduction: Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TNBA) is recognised as an accurate diagnostic and staging technique in patients having abnormal mediastinal lesions. Negative results of EBUS-TBNA could be resulted from true negative lymph node involvement in patients having malignancy, or from other benign conditions. Prevalence of tuberculosis was 80 per 100 000 population in Hong Kong during the study period. This study aimed to evaluate the results of these negative lymph nodes upon follow-up for at least 1 year.

Methods: A prospective study on patients having EBUS-TBNA for mediastinal lymphadenopathy by thoracic CT scan from July 2007 to April 2009 was conducted in a tertiary referral centre in Hong Kong where tuberculosis is endemic. Rapid on-site pathological examination was not available. Demographic data and occupational dust exposure were recorded. Negative results were defined as cases without specific diagnoses from the EBUS-TBNA results. Patients having negative results of EBUS-TBNA would pursue surgery, further investigation or clinical follow-up for at least 1 year as necessary.

Results: A total of 122 patients (malignancy in 84.4%) underwent EBUS-TBNA. The sensitivity was 90.4%, with a negative predictive value of 70%. Thirty-seven patients (30%) had negative EBUS-TBNA results. The mean size of lymph nodes was 1.21 ± 0.56 cm and mean number of needle pass per lymph node was 2.80 ± 1.05 . One patient defaulted follow-up and 36 patients were analysed. Seventeen patients (47%) had subsequent surgery and same number of patients decided for clinical follow-up. Repeat EBUS-TBNA or CT-FNA was performed in the remaining two patients and both confirmed non-small-cell lung cancer (NSCLC). Among 24 patients confirmed malignancy (23 NSCLC, 1 nasopharyngeal carcinoma), 14 patients confirmed true negative lymph node involvement, seven had false-negative lymph node (19% of all negative results) and three refused surgery. Three patients (8%) were confirmed tuberculosis. Seven patients (19%) had no definitive diagnosis likely due to inflammatory changes including three patients with background of silicosis. After 1 year of follow-up, five patients were clinically and radiologically stable, while one patient had mediastinal lesions resolved spontaneously.

Conclusion: Negative results from EBUS-TBNA lymph nodes should be pursued for further investigations, as up to 19% patients had false-negative lymph nodes and 8% had tuberculosis.

Use-dependent block of hKv1.5 channels and the molecular determinant by the natural flavone acacetin

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Introduction: We have recently demonstrated that the natural flavone acacetin is an atrial-selective compound that inhibits ultra-rapid delayed rectifier potassium current (IKur) and transient outward potassium current (Ito) in human atrial myocytes, and also acetylcholine-activated potassium current (IK.ACh). It increased atrial effective refractory period and effectively prevented atrial fibrillation (AF) in anaesthetised dogs without prolonging QT interval of ECG. The present study was designed to determine whether the IKur block of acacetin is rate- and/or use-dependent, and the molecular determinant of the channel block in HEK 293 cells expressing hKv1.5 channels (coding IKur in human atrial myocytes).

Method and Results: It was found acacetin was an open channel blocker of hKv1.5 channels and inhibited hKv1.5 current in use- and frequency-dependent manner. The IC50 of acacetin for inhibiting hKv1.5 was reduced from 3.7 μ M at 0.2 Hz to 3.1 μ M at 0.5Hz, 2.9 μ M at 1Hz, 2.1 μ M at 3 Hz, and 1.7 μ M at 4 Hz. The mutagenesis study showed that the hKv1.5 mutant I508A in the S6-segment exhibited a significant reduction of the channel block by acacetin (IC50, 19.4 μ M, 5.2-fold of WT). MCAb BI was significantly higher for SLE patients compared.

Conclusion: These results demonstrate that acacetin is an open channel blocker by binding to the S6 domain of hKv1.5 channels. The use- and rate-dependent blocking property of hKv1.5 by acacetin indicates that this natural compound could exert a strong suppressive effect on atrial fibrillation in man.

Effects of increased cholesterol level on BK channels

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Background: The large conductance Ca^{2+} -activated K^+ (BK, or slo) channels are ubiquitously expressed in different tissues and play an important role in regulating various physiological processes such as cell excitability, hormone secretion, vascular activity, etc. The present study was designed to investigate how/whether BK channels are regulated by increased cholesterol level.

Methods: Whole-cell BK current and BK single channel current were recorded in whole-cell patch clamp mode and cell-attached single channel recording, respectively, in HEK 293 cells stably expressing Maxi-K with beta1-subunit.

Results: Whole-cell BK current was significantly suppressed in cells expressing both with α and $\beta 1$ subunits with cholesterol-enrichment by cholesterol-saturated methyl-beta-cyclodextrin (M β CD), whereas cholesterol depletion by M β CD had no effect on the current amplitude. Low-density lipoprotein (LDL) also decreased BK current. Single channel recording showed that cholesterol enrichment significantly reduced the open probability of BK channels. However, in cells expressing only α -subunit of BK channels (without $\beta 1$ -subunit), cholesterol-saturated M β CD had no significant effect on the current amplitude of BK channels.

Conclusion: Our results demonstrate the important evidence that BK channels exhibit $\beta 1$ -subunit-dependent response to cholesterol. The enriched-cholesterol and LDL reduce the activity of BK channels in cells co-expressed with both α and $\beta 1$ subunits, which may at least in part accounts for the occurrence of hypertension in patients with high plasma cholesterol level, since both of α and $\beta 1$ subunit transcripts are abundant in vascular smooth muscle.

Association of *JAG1* gene and its variant sequence with bone mineral density and osteoporotic fractures: a genome-wide association study and follow-up replication studies

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Introduction: Bone mineral density (BMD), a diagnostic parameter for osteoporosis and a clinical predictor of fracture, is a polygenic trait with high heritability. The identified variants so far demonstrate only a small effect size on BMD variation. Many additional associated loci remain to be identified. The identification of additional genes underlying BMD variation would therefore provide insight into the pathogenetic mechanisms of osteoporosis and offer strategies for therapeutic development.

Methods: We performed a genome-wide association study (GWAS) on 800 unrelated Southern Chinese women with extreme BMD and follow-up replication studies in six independent European descent and Asian populations including 18 098 subjects. The potential biological function of the identified variant was further validated via electrophoretic mobility shift assay (EMSA) and gene expression study in human bone-derived cells (HBDCs) and peripheral blood mononuclear cells (PBMCs).

Results: In the meta-analysis, rs2273061 of the Jagged 1 (*JAG1*) gene was associated with high BMD ($P=5.27 \times 10^{-8}$ for lumbar spine [LS] and $P=4.15 \times 10^{-5}$ for femoral neck [FN], $n=18\ 898$). This SNP was also associated with the low risk of osteoporotic fracture ($P=0.009$, OR=0.7, 95% CI 0.57-0.93, $n=1881$). Furthermore, we performed an EMSA which demonstrated the binding of c-Myc to the 'G' but not 'A' allele of rs2273061. An mRNA expression study in both HBDCs and PBMCs confirmed the high BMD-related allele 'G' of rs2273061 was associated with higher *JAG1* expression.

Conclusion: Our results support the *JAG1* gene as a novel candidate for BMD regulation and it is a potential key factor for fracture pathogenesis.

Acknowledgement: This project was supported by Hong Kong Research Grant Council; The KC Wong Education Foundation; The Bone Health Fund of HKU Foundation; Matching Grant, CRCG Grant and The Osteoporosis Research Fund of The University of Hong Kong.

Association of Cdx1 binding site of periostin gene with bone mineral density and vertebral fracture risk

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Introduction: The genetic determination of osteoporosis is complex and ill-defined. Periostin, an extracellular matrix secreted by osteoblasts and a regulator of osteoblast differentiation and bone formation, may affect susceptibility to osteoporosis.

Methods: We adopted a gene-wide association method followed by imputation-based verification and identification of causal variant(s). Bone mineral density (BMD) was measured by dual X-ray absorptiometry at the lumbar spine and femoral neck. Morphometric vertebral fractures were identified by assessing vertebral height from X-rays of the thoracolumbar spine. Single marker and haplotype association analyses were performed with PLINK and imputation analyses with MACH. The putative transcription factor binding with target sequence was confirmed by electrophoretic mobility shift assay (EMSA).

Results: SNPs of *POSTN* gene were associated with BMD or vertebral fractures. The most significant polymorphism was rs9547970, located -2,327bp upstream ($P=0.0007$, $OR=1.41$). It was further determined to be the variant that could best explain the association. A specific binding of Cdx1 to the wild-type site centering the rs9547970 major allele A of *POSTN* but not the variant G allele was confirmed by EMSA.

Conclusions: Our results suggest *POSTN* as a novel candidate gene for osteoporosis, and further studies are required to confirm its influence on osteoporosis risk.

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Relationship between ventricular dyssynchrony and T-wave alternans in patients with coronary artery disease

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Introduction: Coronary artery disease (CAD) is associated with increased dispersion of repolarisation and sudden cardiac death. We sought to investigate whether ventricular dyssynchrony is associated with proarrhythmic repolarisation dispersion as measured by T-wave alternans (TWA) in patients with CAD.

Method: We studied 154 patients (67 ± 9 years, 123 men) with documented CAD who underwent exercise treadmill testing and echocardiographic examination. TWA was analysed continuously during treadmill testing in all standard precordial leads by time-domain method. Tissue Doppler imaging (TDI) was performed to measure inter- and intra-ventricular dyssynchrony.

Results: As defined by ≥ 75 percentile of TWA, 39 patients had increased TWA ≥ 62 μV . There were higher prevalences of female (33 vs 16%; $P=0.022$) and body mass index (25.7 ± 2.7 vs 24.6 ± 3.0 kg/m^2 ; $P=0.04$) in TWA ≥ 62 μV group than TWA < 62 μV group. TDI showed significantly increase in index of interventricular dyssynchrony, Ts-RL, time difference between lateral and right free wall (77.0 ± 39.4 vs 61.6 ± 36.0 ms, $P=0.04$), but not intraventricular dyssynchrony (all $P>0.05$) in patients with TWA ≥ 62 μV compared with TWA < 62 μV . Furthermore, a modest but significant positive correlation was observed between TWA and Ts-RL ($r=0.23$, $P=0.009$). Multivariate analysis revealed that body mass index (odds ratio [OR]=1.16, 95% confidence interval [CI] 1.01-1.11, $P=0.032$) and Ts-RL (OR=1.01, 95% CI 1.00-1.02, $P=0.031$) were independent predictors for increased TWA.

Conclusion: Our results demonstrated that interventricular dyssynchrony in patients with CAD was associated with increased TWA, suggesting that interventricular dyssynchrony may contribute to proarrhythmic repolarisation dispersion.

Over-expression of miR-106b promotes metastasis in hepatocellular carcinoma by activating epithelial-mesenchymal transition process

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Introduction: Hepatocellular carcinoma (HCC) is the third most common cause of cancer death in Hong Kong. Patients with metastatic HCC have a relatively poor prognosis than those patients without metastasis development. Molecular pathological studies demonstrated that microRNA (miRNA) is involved in development of cancer metastasis. Our aim was to study the role of miRNA in HCC and study the functional role of miRNA in metastasis development.

Methods: HCC cell lines PLC and MHCC97H were orthotopically implanted onto liver of the SCID mice. Primary tumour and metastatic lung nodules were isolated for primary culture. Both primary and metastatic cell lines were established. MicroRNA microarray analysis was used to compare the miRNA expression profile in both primary and metastatic cell lines. Clinical relevance of the identified miRNAs was analysed by QPCR. The functional role of this miRNA was also demonstrated by both in-vitro and in-vivo experiments. Epithelial-mesenchymal transition (EMT) markers were also analysed by Western blotting.

Results: The metastatic cell lines show higher aggressiveness than the primary tumour cell lines in vitro. MicroRNA microarray study demonstrated that 15 human miRNAs were involved in the metastatic development in vivo. miR-106b was identified and clinical relevance was studied by QPCR in 99 pairs of HCC sample. QPCR study demonstrated miR-106b was significantly over-expressed in HCC tumour when compared with the non-tumour counterpart which is correlated with the tumour grade. In-vitro and in-vivo functional studies were carried out and miR-106b over-expressing cells show higher migration ability in vitro and promote metastasis in vivo. Rho GTPase, RhoA and RhoC are essential for cell motility and were over-expressed in miR-106b over-expressing cells which provide the molecular evidence that miR-106b expression promote the cell migration in vitro. EMT is an essential process activated during cancer progression. EMT markers were studied in miR-106b LNA knockdown cells by Western blotting. EMT process was found to be activated in miR-106b over-expressing cells which indicated that miR-106b may be involved in promoting development of metastasis by activating the EMT process.

Conclusion: miR-106b was found to be involved in metastatic HCC development. miR-106b, which is over-expressed in HCC tumour, can promote cell migration and metastasis development by activating EMT.

Inactivation of Toll-like receptor 4 alleviates non-alcoholic steatohepatitis through inhibiting endoplasmic reticulum stress and production of reactive oxygen species in ApoE-deficient mice

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Background and Aims: Hepatic inflammation is the major feature of non-alcoholic steatohepatitis (NASH). Toll-like receptor 4 (TLR4) is one of the important mediators of the systemic inflammation. Yet, the molecular link between hepatic inflammation and the pathogenesis of NASH remains elusive. The present study aimed to investigate the role of TLR4 in pathogenesis of obesity-induced NASH and to further elucidate the underlying mechanisms involved.

Methods: ApoE^{-/-}/TLR4^{-/-} mice were generated by cross-breeding the ApoE deficient (ApoE^{-/-}) mice with TLR4-deficient mice. ApoE^{-/-}/TLR4^{-/-} mice and their TLR4 wild-type littermates were fed with high-fat high-cholesterol (HFHC) diet for 12 weeks to induce NASH. Hepatic reactive oxygen species (ROS) were determined by lucigenin-enhanced chemiluminescence. Kupffer cells were isolated by collagenase perfusion and isopycnic sedimentation in Percoll. The splicing of the transcription factor X-box binding protein-1 (XBP1) was detected by PCR amplification.

Results: HFHC diet induced a typical symptom of NASH in ApoE^{-/-}/TLR4-WT mice. TLR4 expression was significantly enhanced in Kupffer cells in obesity-induced fatty liver. TLR4 inactivation prevented HFHC diet-induced liver injury and reduced hepatic triglyceride and cholesterol contents in ApoE-deficient mice. In addition, inactivation of TLR4 reversed HFHC diet-induced hyperglycaemia and improved insulin sensitivity. Inactivation of TLR4 also resulted in a significant reduction in hepatic recruitment of macrophages and prevented HFHC diet-induced production of ROS and proinflammatory cytokines tumour necrosis factor and macrophage chemoattractant protein-1 in ApoE-deficient mice. Furthermore, in ApoE^{-/-}/TLR4^{-/-} mice on HFHC diet, phosphorylation of eukaryotic translational initiation factor 2 α was enhanced in liver tissues, whereas XBP1 splicing and I κ B α phosphorylation was partially repressed when compared with those in ApoE^{-/-}/TLR4-WT mice.

Conclusions: TLR4 inactivation protects against dietary obesity-induced NASH through inhibiting both ER stress and ROS production in liver tissue, suggesting a central role of TLR4 in linking innate immunity to obesity-induced NASH and other inflammatory conditions.

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Evaluation of the combined treatment with fractional laser and fractional radiofrequency for acne scars in Asians

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Background: The fractionated radiofrequency (RF) induces deep dermal heating while leaving the epidermis less affected. Thus it may reduce downtime and lower the risk of post-inflammatory hyperpigmentation. Combining fractional bi-polar RF and diode laser is intended to improve acne scars by enhancement of collagen production in the scar indentation and by causing ablation and resurfacing of the scar edges. The objective of this study was to determine the safety and efficacy of the combined treatment on acne scars in Asians.

Methods: Twenty-one Asians with skin types IV-V and acne scars were recruited. Each received three treatments with fractional 915-nm laser using Matrix IR (Syneron, Irvine CA) with fluence at 70 J/cm², RF at 100 J/cm³, double passes followed by fractional RF using Matrix RF at energy of 62 mJ/pin, at 4-week intervals. Serial standardised photographs were assessed by two independent observers. Subjective evaluation was also obtained.

Results: Nineteen patients completed third treatment. There was significant improvement of acne scarring with mean score decreased from 7.5 to 5.9 out of 10 (P<0.001) and 67% were rated at least moderate objective global improvement. There was also significant objective improvement of mean scores for skin texture, pore size and pigmentary irregularities (P<0.001). 57.1% of subjects rated moderate-to-significant improvement and 81.9% were satisfied with the procedure. One case developed blisters near the jaw area and 7.5% developed transient post-inflammatory hyperpigmentation.

Conclusions: Combining fractional laser and RF appears to be safe and effective for acne scars in Asians.

Prognostic role of coronary calcification in patients with rheumatoid arthritis and systemic lupus erythematosus

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Introduction: Coronary calcification score (CCS) detected by multi-detector computed tomography (MDCT) is predictive for future cardiovascular (CVS) events in the general population. However, its prognostic role in patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) remains unknown. The aim of the study was to evaluate the predictive value of CCS for future CVS events detected by MDCT in patients with RA and SLE.

Methods: A total of 152 patients with RA and SLE, and 106 healthy controls underwent MDCT to measure CCS. All patients were prospectively followed up for major CVS events.

Results: Compared with controls, patients with RA and SLE had a significantly higher mean CCS (42.2±154.3 vs 1.4±13.0, P<0.01) and the prevalence of CCS 1-10, CCS 11-100 and CCS >100 (all P<0.05). After a mean period of 4.3±0.6 years, major CVS events (stroke and myocardial infarction) occurred in 10 patients with RA and SLE. In patients with RA and SLE, a higher major CVS event rate occurred in patients with CCS 1-10 (5.0%), CCS 11-100 (14.3%) and CCS >100 (40.0%) than those with CCS=0 (1.0%, P<0.01). Multivariate Cox regression analysis revealed that hypercholesterolemia (hazard ratio [HR]=11.2, confidence interval [CI] 1.4-89.3, P=0.02) and CCS >100 (HR=11.1, CI 1.31-95.0, P=0.03) were independent predictors of CVS events.

Conclusion: Coronary calcification detected by MDCT independently predicts CVS events in patients with RA and SLE. Accordingly, risk stratification by assessment of CCS may have an important prognostic role in patients with RA and SLE.

Hyperthyroidism-induced left ventricular diastolic dysfunction: implication in hyperthyroidism-related heart failure

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Background: Heart failure occurs in 6% of hyperthyroid patients. Nonetheless only half of those with hyperthyroidism-related heart failure have impaired left ventricular (LV) systolic function. Thus diastolic dysfunction may play an important role in the pathogenesis.

Methods and Results: We performed serial echocardiographic examinations in 70 consecutive patients with hyperthyroidism (39 ± 2 years, 47 women) to determine their diastolic function and repeated the examinations 6 months after achieving a euthyroid state. All patients had normal LV systolic function, but diastolic dysfunction was detected in 22 cases (mild: 3, moderate: 15, and severe: 4). The prevalence of diastolic dysfunction increased with age from 17.9% in patients <40 years to 100% in those >60 years. Increasing age was the only independent predictor for diastolic dysfunction in hyperthyroid patients. After achievement of a euthyroid state, most patients (16/22, 72%) had completely normalised diastolic function: 100% of patients <40 years, 33.3% of those ≥ 60 years. Further analyses revealed significant age-related differences in the cardiovascular response to hyperthyroidism. Among patients <40 years, hyperthyroidism resulted in a marked reduction in total peripheral vascular resistance, increased cardiac output, and enhanced diastolic function as determined by E' . No such significant change in total peripheral vascular resistance or cardiac output was observed in hyperthyroid patients ≥ 40 years. In addition, hyperthyroidism was associated with reduced E' , signifying diastolic dysfunction in older hyperthyroid patients.

Conclusion: Hyperthyroidism is associated with diastolic dysfunction, particularly in older patients. It is partly reversible following achievement of a euthyroid state.

Clinical features of a cohort of patients with palindromic rheumatism

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Introduction: Palindromic rheumatism (PR) is an idiopathic, recurrent, intermittent and transient episode of afebrile attacks of mono- or oligo-arthritis and/or para-arthritis without residual joint damage. Patients feel well in between attacks. The condition may preclude the onset of other rheumatic conditions, especially rheumatoid arthritis (RA).

Methods: We conducted a retrospective study to analyse the demographic characteristics, clinical features, treatment and outcome of a cohort of PR patients.

Results: Thirty-one PR patients were identified. Male-to-female ratio was 1:1.4. Three patients (9.7%) have a positive family history of autoimmune disease. The mean age of onset was 29 years and the mean age at diagnosis was 35 years. Attacks occurred suddenly, irregularly and uncommonly accompanied by constitutional symptoms. Attacks were very short in duration, in the majorities less than 48 to 72 hours and were always shorter than a week. The frequency of attacks was very variable. Any joint may be affected, although attacks were more predominantly over the joints of the upper limbs (joints of upper limb-to-lower limb ratio was 2:1) and over the small joints (small joints-to-large joints ratio was 2:1). During the attacks, there could be a mild-to-moderate elevation in the inflammatory markers. Rheumatoid factor (RF) was found to be positive in five patients (16.1%), among these two with positive anti-cyclic citrullinated peptide (anti-CCP) antibodies. Antinuclear antibodies (ANA) usually were absent or only with low titre. Negative anti-dsDNA and anti-ENA were noted. Normal radiological findings with absence of bone and cartilage destruction even after extended periods of time were encountered. Acute attacks respond favourably to non-steroidal anti-inflammatory drugs (NSAIDs) and NSAIDs were prescribed in 25 patients (80.6%). Among these 31 PR patients, five patients (4 female and 1 male) eventually developed into rheumatoid arthritis (RA) with a latency period range from 1 to 27 years since the onset of their PR. None of them developed into rheumatic diseases other than RA otherwise.

Conclusion: Whether PR is a distinct rheumatological entity or is a variant of RA remains controversial. As this may be the initial manifestation of RA or other connective tissue diseases, it is worthwhile to follow-up these patients on a long-term basis. The availability of anti-CCP may help identify a subgroup of patients who may have higher chance of developing RA.

Detection of subclinical synovitis in patients with rheumatoid arthritis in clinical remission

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Objectives: To detect the prevalence of subclinical synovitis in patients with rheumatoid arthritis (RA) in clinical remission by musculoskeletal ultrasonography (USG) and to define possible predictors for the presence of subclinical synovitis.

Methods: A total of 37 RA patients receiving disease-modifying anti-rheumatic drugs (DMARDs) with disease in clinical remission were recruited. They were subject to clinical, laboratory, functional status or quality-of-life and radiographic evaluation at baseline. Disease Activity Score 28-joint assessment (DAS-28) was calculated. Musculoskeletal USG including both grey-scale and power Doppler techniques to the dorsal aspect of both wrists and all metacarpophalangeal joints was performed on each subject.

Results: Of 37 RA patients with clinical remission, nine were found to have increased power Doppler signal by USG, signifying the presence of subclinical synovitis, the prevalence rate being 24.3%. The continuous DAS-28 with three variables version using C-reactive protein (DAS-28 CRP v3) was the only independent predictor for the presence of USG-detected subclinical synovitis in the multivariate analysis with the odds ratio (OR) of 8.158, $P=0.052$. The cut-off value of DAS-28 CRP v3 was found to be 2.32 with the sensitivity of 66.7% and specificity of 78.6% for the presence of USG-detected subclinical synovitis.

Conclusion: Musculoskeletal USG is more sensitive than clinical assessment to detect subclinical synovitis. USG with grey-scale and power Doppler in combination with clinical assessment allows more accurate evaluation of the disease status, especially for the definition of true remission. DAS-28 CRP v3 may be used as a guide to stratify those relatively higher-risk stable RA patients for proceeding to musculoskeletal USG examination to delineate the true disease status and to optimise maintenance therapy.

High-sensitivity C-reactive protein and other inflammatory markers in predicting cardiovascular risk in Hong Kong Chinese

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Introduction: Inflammation is increasingly recognised as a key player in atherosclerosis, and C-reactive protein measured using high-sensitivity assay (hsCRP) is the most promising inflammatory marker in predicting the risk of cardiovascular diseases (CVD). In this prospective cohort study, we examined the predictive value of hsCRP for CVD in Hong Kong Chinese and determined if other biomarkers would enhance the predictive value of hsCRP.

Methods: Subjects were recruited from the Hong Kong Cardiovascular Risk Factors Prevalence Study 2 (CRISPS-2) cohort. Those with known cardiovascular disease(s) were excluded. Baseline serum levels of adiponectin, leptin, soluble tumour necrosis factor alpha receptor 2 (sTNFR2) and hsCRP were determined and subjects were followed prospectively for 6 years.

Results: A total of 1785 subjects were included in the final analysis. The cumulative incidence of CVD was 3.4%. At baseline, subjects with incident CVD were older, and had higher body mass index (BMI), waist circumference (WC), systolic blood pressure (BP), HOMA-IR, and fasting glucose levels (all $P<0.001$), compared to those who did not develop CVD (non-CVD). They also had higher baseline levels of leptin and sTNFR2 (both $P<0.001$) and hsCRP (median [interquartile range], 1.76 [0.87-2.55] mg/L vs 0.69 [0.32-1.49] mg/dL in non-CVD; $P<0.001$), but similar adiponectin levels, compared to non-CVD subjects. Logistic regression showed that baseline hsCRP was an independent predictor of CVD ($P=0.003$) after controlling for the conventional CVD risk factors, and for leptin and sTNFR2. The predictive value of leptin or sTNFR2 was not significant after adjustment for conventional CVD risk factors, hsCRP and each other. Serum hsCRP levels tertile analysis (<0.45 mg/L, 0.45-1.2 mg/L and >1.2 mg/L) showed that compared to subjects in the lowest tertile, those in the highest tertile had an odds ratio of 3.362 for incident CVD (95% CI, 1.249-9.052; $P=0.016$). Receiver operating characteristics curve analysis found that an hsCRP level of ≥ 1 mg/L had the most optimal sensitivity and specificity for CVD prediction.

Conclusion: In this 6-year prospective study, hsCRP was an independent predictive factor of CVD in Hong Kong Chinese, in addition to conventional CVD risk factors. Measurement of adiponectin and other inflammatory biomarkers tested in this study did not provide any adjunctive predictive value.

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The effect of rapamycin on anti-DNA antibody binding to human mesangial cells and subsequent changes to inflammatory processes

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Introduction: Rapamycin is an effective immunosuppressive agent that is used in the prevention of organ transplantation rejection. Its effect on the pathogenesis of lupus nephritis has not been defined. We investigated the effect of rapamycin on anti-DNA antibody binding to human mesangial cells (HMC) and the functional consequences thereafter.

Methods: Polyclonal anti-DNA antibodies were isolated from the sera of 20 patients with lupus nephritis using affinity chromatography. Binding of anti-DNA antibodies to HMC was assessed by cellular ELISA. HMC were incubated with control IgG or anti-DNA antibodies (10 µg IgG/mL) for periods up to 72 hours. The effect of anti-DNA antibodies on the activation of MAPK and phosphatidylinositol-3-kinase (PI3K), and cytokine secretion was assessed by Western blot and ELISAs.

Results: Anti-DNA antibodies bound significantly to HMC compared to control IgG, and induced activation of ERK, mTOR and PI3K, and secretion of MCP-1 and IL-6 ($P < 0.05$, for all after 48 hours). Incubation of HMC with rapamycin prior to the addition of anti-DNA antibodies decreased antibody binding by 40.03% ($P < 0.01$). Rapamycin decreased anti-DNA antibody-induced activation of mTOR and ERK by 36.7% and 34.7% respectively after 48 hours ($P < 0.01$, for both), but had no effect on PI3K activation. Inhibition of mTOR and ERK activation resulted in a subsequent reduction of anti-DNA antibody-induced MCP-1 (17.8 ± 2.9 vs 14.5 ± 3.4 ng/µg cellular protein), and IL-6 (250.7 ± 59.9 vs 181.6 ± 73.2 pg/µg cellular protein) secretion ($P < 0.05$, for both).

Conclusion: Our data demonstrate that rapamycin can act directly on non-immune cells and partially inhibit anti-DNA antibody binding to mesangial cells and down-regulate inflammatory processes. Further studies are warranted to determine whether rapamycin may be beneficial in the treatment of lupus nephritis.

Characterisation of multiple ion channels in human-induced pluripotent stem cells-derived mesenchymal stem cells

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Introduction: Our recent studies demonstrated that functional mesenchymal stem cells (MSCs) can be derived from human-induced pluripotent stem cells (iPS) which can be used as an alternative source of stem cells for cardiac repair.¹ However, transplantation of iPS-MSC with undesirable electrical ionic profile into human myocardium can be potentially lethal. Here, we characterised the electrophysiological properties of iPS-MSCs in comparison with that of bone marrow (BM)-derived MSCs,² since human iPS-MSCs showed similar phenotype and expression of surface markers for MSCs as BM-MSCs.

Methods and Results: The expression of various common ion channels for sodium (Na^+), potassium (K^+), calcium (Ca^{2+}) and chloride (Cl^-) in human iPS-MSCs were examined by reverse transcription-polymerase chain reaction (RT-PCR). Those functional ion channels identified by RT-PCR were confirmed by whole-cell patch clamp technique. RT-PCR revealed the molecular identities (mRNAs) of some ion channels and their possible existence in human iPS-MSCs, including KCa.1.1 (responsible for the big conductance Ca^{2+} -activated K^+ current, BK_{Ca}), Kv10.1 (for the delayed rectifier K^+ current, IK_{DR}), Kir2.1 and Kir2.3 (for the inwardly-rectifying K^+ current, I_{Kir}), KCa3.1 (for the intermediate conductance Ca^{2+} -activated K^+ current, IK_{Ca}), Clcn3 (for the chloride current, I_{Cl}), SCN9A (for the tetrodotoxin-sensitive sodium current, $\text{I}_{\text{Na,TTX}}$), CACNA1C (for the nifedipine-sensitive L-type Ca^{2+} current, $\text{I}_{\text{Ca,L}}$) and Kv4.3 (for the transient outward potassium current, I_{to}), but not Kv1.4 and Kv4.2 (for I_{to}). Patch clamp experiments were conducted to verify the existence of functional ion channels, five types of currents (BK_{Ca} , IK_{DR} , I_{Kir} , IK_{Ca} and I_{Cl}) were found in human iPS-MSCs, but not the three ($\text{I}_{\text{Na,TTX}}$, $\text{I}_{\text{Ca,L}}$ and I_{to}) reported in BM-MSCs.²

Conclusion: Although human iPS-MSC and BM-MSC showed similar phenotype, they have different ionic channel profile. The functional implication for these differences in the ionic profile merits further investigation.

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Identification of transient receptor potential channels in human atrial myocytes

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Introduction: Generation of cardiac arrhythmias, especially human atrial fibrillation underlying mechanisms, is not fully understood. Recent studies have demonstrated that transient receptor potential (TRP) channels play important roles in the regulation of physiological and pathological cellular function. Little information is documented about TRP channels in human hearts.

Methods: The present study was designed to investigate the expression of TRP channels in human atrial myocytes using whole-patch voltage clamp and molecular biological approaches.

Results: It was found that the previously reported background non-selective cation current was inhibited by the TRPC channel blocker La^{3+} in a concentration-dependent manner ($\text{IC}_{50}=46 \mu\text{M}$), suggesting the contribution of TRPC channels. In addition, we recorded a current that is sensitive to inhibition by divalent cations, eg Mg^{2+} , Ni^{2+} , Ba^{2+} , etc. The current was enhanced by removing intracellular Mg^{2+} or extracellular Mg^{2+} ion, but blocked by Ni^{2+} or Ba^{2+} . This divalent cation-sensitive current was inhibited by 2-aminoethoxydiphenyl borate (2-APB, $\text{IC}_{50}=32 \mu\text{M}$). The current increased when the bath medium pH was reduced from 7.3 to 4.0. These properties were similar to those of TRPM7 channels. RT-PCR and Western blot analysis, and immunocytochemistry revealed that mRNAs and proteins of TRPC1, TRPC3, and TRPM7 were significant in human atrial myocytes.

Conclusion: These results demonstrate the novel information TRPC1, TRPC3, and TRPM7 channels are present in human atrial myocytes. Activation of TRP channels likely contributes to the genesis of human atrial fibrillation, and therefore TRP channel may be a target for the development of anti-atrial fibrillation approach.

Characterisation of ion channels in human cardiac progenitor cells

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Background: Cardiac progenitor cells play an important role in cardiac repair and regeneration; however, cellular biology and electrophysiology are not understood. The present study was designed to investigate ion channels in human cardiac c-kit⁺ progenitor cells.

Method: Cardiac c-kit⁺ progenitor cells were isolated and expended from human atrial specimens from patients who had undergone coronary artery bypass surgery on the basis of written consent. Ion channel currents and their phenotypes were determined with whole-cell patch voltage-clamp and molecular biological approaches.

Results: Multiple ion channel currents were recorded in human cardiac c-kit⁺ progenitor cells. These include a large conductance Ca^{2+} -activated K^+ current (BK_{Ca}) in most (93%) cells, an inwardly-rectifying K^+ current (I_{Kir}) in 87% of cells, a transient outward K^+ current (I_{to}) in 39% of cells, a voltage-gated tetrodotoxin-sensitive Na^+ currents ($\text{I}_{\text{Na,TTX}}$) in 76% of cells. RT-PCR and Western-blot analysis revealed the molecular identities (mRNAs and protein) of these ion channel currents, including KCa.1.1 (responsible for BK_{Ca}), Kir2.1 , Kir2.2 (for I_{Kir}), Kv4.3 (responsible for I_{to}), $\text{Na}_v1.2$, $\text{Na}_v1.3$, $\text{Na}_v1.6$, $\text{Na}_v1.7$ (for $\text{I}_{\text{Na,TTX}}$).

Conclusion: Our results demonstrate for the first time that multiple ion channels are heterogeneously present in human cardiac c-kit⁺ progenitor cells. The potential physiological roles of these ion channels are being studied.

Plasma amyloid beta peptides and oligomers levels in Alzheimer's disease

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Introduction: Amyloid beta (A β) exists in different forms including A β peptides, oligomers, protofibrils, and fibrils. It has been believed that A β fibrils in Alzheimer's disease (AD) brain contribute to AD pathogenesis, but recent evidences suggest that A β oligomers have stronger relationship with AD pathogenesis.

Aims: To study the plasma A β_{40} , A β_{42} , and A β oligomers levels in AD patients and non-demented age-matched controls, and the correlations between plasma A β_{40} , A β_{42} , and A β oligomers levels and cognitive function.

Methods: We studied 44 AD patients and 22 controls. Cognitive functions were assessed by cognitive assessment tools: Chinese version of mini-mental state examination (MMSE), Abbreviated Metal Test (AMT), Alzheimer's Disease Assessment Scale Cognitive Subscale (ADAS-cog), and Delayed 10-Word Recall Test (DWRT). Plasma A β_{40} and A β_{42} levels were measured by ELISA kits (Invitrogen). Plasma A β oligomers level was detected by ELISA, using A β oligomeric antibody as detecting antibody and A β N-terminal antibody (against residues 1-14) as capturing antibody.

Results: There was no difference in plasma A β_{40} and A β_{42} levels between AD patients (median A β_{40} level 145.93 pg/mL, median A β_{42} level 9.94 pg/mL) and controls (median A β_{40} level 130.34 pg/mL, median A β_{42} level 8.42 pg/mL; P=0.196 and P=0.187, respectively). In women with AD, increased plasma A β_{42} level was associated with increased MMSE (P=0.043) and decreased ADAS-cog (P=0.034) suggestive of positive correlation between plasma A β_{42} level with cognitive function. Plasma A β oligomers level was higher in AD patients (median 642.54 ng/mL, range 103.33-2676.93 ng/mL) than controls (median 444.18 ng/mL, range 150.19-1311.18 ng/mL; P=0.047), and was negatively correlated with cognitive function evidenced by increased plasma A β oligomers level associated with decreased MMSE, AMT, DWRT scores and increased ADAS-cog scores (P=0.037, P=0.043, P=0.025, P=0.036, respectively).

Conclusion: Plasma A β_{42} and A β_{40} levels are not suitable biomarkers for AD diagnosis; but plasma A β_{42} level may reflect severity of cognitive impairment in women with AD. Plasma A β oligomers level may help diagnose AD patients, but the range is wide among AD patients, making it not an ideal biomarker for AD diagnosis.

Plasma amyloid beta peptides and oligomers antibodies in Alzheimer's disease

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Introduction: Various forms of amyloid beta (A β) including A β peptides, oligomers, protofibrils and fibrils are thought to be pathogenic in Alzheimer's disease (AD). The exact pathophysiological role of endogenous A β autoantibodies (Ab) in healthy subjects and AD patients are uncertain. Potential protective role of A β Ab has been suggested.

Aims: To study the serum A β monomers and A β oligomers Ab levels in AD patients and non-demented age-matched controls, and the relationship between A β monomers and A β oligomers Ab levels and cognitive function.

Methods: A total of 44 AD patients and 22 controls were recruited. Cognitive functions were assessed by cognitive assessment tools: Chinese version of mini-mental state examination (MMSE), Abbreviated Metal Test (AMT), Alzheimer's Disease Assessment Scale Cognitive Subscale (ADAS-cog). A β Ab levels were assayed by ELISA. A β_{42} monomer and A β_{42} oligomers were coated on 96-well plates for measuring A β monomer Ab and A β oligomers Ab levels respectively. The secondary antibody was rabbit-anti-human antibody (detecting antibody). Calibration curves were made by 2C8 (against A β residues 1-16) and 7A1a (A β oligomers antibody) for A β monomer and A β oligomers Ab respectively. Negative control was incubating with secondary antibody only without incubation with patients/controls' serum.

Results: There was no difference in serum A β monomer Ab level between AD patients (median 177.43 μ g/mL) and controls (median 190.88 μ g/mL, P=0.55). In AD patients, A β monomer Ab level was negatively correlated with cognitive function evidenced by increased A β monomer Ab level associated with decreased MMSE, AMT and increased ADAS-cog scores (P=0.004, P=0.013, P=0.005, respectively). Serum A β oligomers Ab level was higher in AD patients (median 42.81 μ g/mL, range 11.91-241.62 μ g/mL) than controls (median 24.15 μ g/mL, range 2.32-329.93 μ g/mL; P=0.014).

Conclusion: Serum A β monomer Ab is not a suitable biomarker for AD diagnosis, but may reflect the severity of AD. Serum A β oligomers Ab level may help in AD diagnosis, but wide range of titers among AD patients makes it not an ideal biomarker for AD diagnosis.

The adaptor protein APPL2 inhibits insulin-stimulated glucose uptake through both Akt and its downstream target TBC1D1 in skeletal muscle

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Introduction: APPL1 and APPL2 are the two intracellular adaptor proteins containing a PH domain, a PTB domain, and a Leucine zipper motif. Mounting evidence demonstrates that APPL1 is a positive regulator of insulin sensitivity by acting as a common relay of both adiponectin and insulin signalling pathways. However, the cellular functions of APPL2 and its relationships with APPL1 remain poorly understood. The objective of this study was to investigate the molecular relationships of APPL1 and APPL2 in insulin-mediated glucose uptake in skeletal muscle cells.

Methods: Proteins physically associated with APPL1 or APPL2 are retained by affinity purification and co-immunoprecipitation, followed by mass spectrometry-based proteomic identification. The effects of APPL1 and APPL2 in regulating insulin signaling are measured by Akt phosphorylation and in-vitro or ex-vivo glucose uptake assay.

Results: APPL1 potentiates, but the Bar domain of APPL2 inhibits insulin-stimulated phosphorylation of the protein kinase Akt and subsequent glucose uptake in both skeletal muscles isolated from the transgenic mice and cultured myotubes. Proteomic analysis demonstrates that APPL2 but not APPL1 interacts with TBC1D1, which is a key player for contraction and insulin-stimulated glucose transport in skeletal muscle. Moreover, insulin stimulates the disassociation of APPL1 and APPL2 heterodimers, but facilitates the interaction of TBC1D1 with APPL2 through Akt activation. Furthermore, insulin-evoked binding of APPL2 with TBC1D1 on Serine 229 suppresses phosphorylation of TBC1D1 on Threonine 590, leading to further suppression of glucose uptake.

Conclusion: APPL1 and APPL2 act as a pair of 'Yin-and-Yang' regulators of insulin signalling and glucose uptake in skeletal muscle. APPL2 inhibits insulin-stimulated glucose uptake through its dual effects via the Bar domain on both APPL1 and TBC1D1. These findings shed new light on our understanding of the molecular mechanisms for the metabolic actions of insulin in skeletal muscle.

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