Introduction and Project Objectives: Autism Spectrum Disorder (ASD) is known for deficits in social communication and repetitive behaviors. To reveal the neural mechanism of these two major symptom domains, the current study investigated the difference in the frontotemporal brain networks between ASD and normal control in their pre-attentive detection of emotional (angry and happy speech) versus non-emotional change (neutral speech and pure tone). By using an optical brain imaging method known as the event-related optical signal (EROS), the frontotemporal networks can be localized in spatial and temporal dimensions simultaneously.

Methods: Children aged 6-12 diagnosed with ASD, and their age and gender matched normal controls watched a silent subtitled movie and were instructed to ignore the auditory stimuli presented at the background. Four types of speech tones, including angry, happy, and neutral speeches as well as pure tone, were presented, while EROS mismatch responses were recorded from the frontal and temporal cortices.

Results: Control group showed temporal-followed-by-frontal pattern to both the angry speech and pure tone changes, and early frontal-temporal-late frontal patterns to the happy and neutral speech changes in both hemispheres, which is consistent with the ambiguity level of each type of change. The ASD group demonstrated an early frontal-temporal-late frontal pattern for happy speech, neutral speech, and pure tone changes in the right hemisphere, while no mismatch response was observed for the angry speech change.

Conclusion: ASD subjects were able to detect non-emotional and happy speech changes with the same frontotemporal brain network disregarding ambiguity levels of the changes. They showed deficits in detecting angry speech change. The neural mechanism underlying the pre-attentive change detection process in ASD is different from that of the normal control.

Project No.: 02130386

AMR-22

Risk of Intracerebral Haemorrhage in Chinese Patients Taking Oral Anticoagulants for Atrial Fibrillation with Cerebral Microbleeds. A Prospective Study in Warfarin. The IPAAC Warfarin Study

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Introduction and Project Objectives: Patients with atrial fibrillation (AF) have 5-fold increase in risk of ischaemic stroke. Anticoagulants can prevent stroke effectively, but its use is limited by the risk of devastating intracerebral hemorrhage (ICH), which has higher prevalence in Chinese compared with Caucasians. Tiny haemosiderin deposits, appeared as cerebral microbleeds (CMBs) in the brain, can now be detected using advanced Magnetic Resonance Imaging (MRI) techniques. Presence of CMBs indicates previous asymptomatic mild vascular leakage, which predicts future clinical ICH. Studies are underway to explore how CMBs can guide anticoagulation decisions in AF. We aimed to evaluate the risk of warfarin-related ICH in Chinese AF patients with CMBs. We hypothesized that patients with CMBs may have higher risk of ICH.

Methods: In this multicenter prospective observational study, we recruited Chinese AF patients anticoagulated with warfarin for evaluation of CMBs with 3T MRI brain. Patients were followed-up clinically for 2 years. Primary outcome was clinical ICH. Secondary outcomes included ischaemic stroke, systemic embolism and mortality of all causes.

Results: Total 237 patients were included. CMBs were observed in 83 (35.0%) patients. Compared with patients without CMBs, patients with CMBs had a trend towards higher rate of ICH (3.6% vs 0.6%, p=0.091) and systemic embolism (2.4 vs 0%, p=0.053) at follow-up. The rate of ICH in the CMBs group (18.5 per 1000 patient years) was much higher than that observed than similar studies in Caucasians (9.8 per 1000 patient years in CROMIS-2 study). For patients with ≥ 5 CMBs, there were more patients who developed ICH than ischaemic stroke during follow-up. After adjustment for age in multiple logistic regression, ≥ 5 CMBs (OR 18.53, p =0.023) and ischaemic heart disease (OR 14.23, p =0.025) were predictive for ICH. Furthermore, CMB count (C-index 0.82) was more sensitive than conventional HAS-BLED (C-index 0.55) and CHA2DS2-VASc (C-index 0.63) scores in predicting ICH.

Conclusion: To our knowledge, this is the first prospective study evaluating risk of warfarin-associated ICH in Chinese AF patients with CMBs. The results suggest that Chinese AF patients with multiple CMBs may be associated with higher risk of warfarin-associated ICH who may benefit from stroke preventive therapies with better safety profile. Adding CMBs evaluation to conventional clinical scores can help improve risk stratification for ICH. Further individual patient data meta-analyses are needed for more precise evaluation of risk-benefit ratio of anticoagulants in AF patients with CMBs of different ethnic origins.

Project No.: 01120136

AMR-23

Using a Novel and Noninvasive Cerebral Augmentation Index as Evaluated by External Counterpulsation to Predict Long-term Clinical Outcome after Acute Ischemic Stroke

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Introduction and Project Objectives: External counterpulsation (ECP) is a non-invasive method used to augment cerebral blood flow of patients with ischaemic stroke via induced hypertension. We aimed to whether the cerebral augmentation index (CAI) evaluated by ECP can predict clinical outcomes after acute ischemic stroke, and whether the hemodynamic effect of ECP assessed by CAI is associated with dynamic cerebral autoregulation to autoregulation index (ARI).

Methods: We enrolled acute ischemic stroke patients within 7 days after stroke onset. Spontaneous arterial blood pressure and bilateral

middle cerebral arteries of patients were monitored using transcranial Doppler (TCD). Flow velocity changes before, during and after ECP were, respectively, recorded for 3 min. The cerebral augmentation index (CAI) was the increase in percentage of the middle cerebral artery mean flow velocity during ECP compared with baseline. TCD data were analysed based on the side ipsilateral or contralateral to the infarct. Transfer function analysis was applied to obtain autoregulatory parameters, ARI, phase difference (PD), and gain. The modified Rankin Scale (mRS) (good outcome: mRS 0~2; poor outcome: mRS 3~6) was evaluated 3 months after the index stroke.

Results: 200 patients were included (mean age, 64.5±8.9 years; 86.5% males). At month 3 after stroke onset, univariate analysis showed that the National Institutes of Health Stroke Scale at recruitment was significantly higher in the poor outcome group, while the bilateral CAIs were significantly lower in the good outcome group than that in the poor outcome group (ipsilateral 3.72±2.94 vs 7.92±6.25, p=0.032; contralateral 4.04±3.82 vs 6.98±6.05, p=0.048). Multivariate logistic regression showed that bilateral CAIs were independently correlated with an unfavourable functional outcome after adjusting for confounding factors. In the total cohort, 46 patients were included for analysing the correlations between CAI and ARI. However, there were no significant correlations between them.

Conclusion: The higher degree of cerebral blood flow velocity augmentation on the both sides ipsilateral and contralateral to the infarct induced by ECP is independently correlated with an unfavourable functional outcome after acute ischemic stroke. Besides, the hemodynamic effect of external counterpulsation evaluated by CAI is a different measure of impaired cerebral autoregulation from the assessment using spontaneous blood pressure fluctuation.

Project No.: 02130836

AMR-24
Investigating Oxyresveratrol as Potential Neuroprotective Drug in Experimental Models of Parkinson's Disease

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Introduction and Project Objectives: Oxyresveratrol is a stillbenoid extracted from mulberry. It has one more hydroxyl group on its structural analog, resveratrol. We have demonstrated that oxyresveratrol elicits neuroprotective effects on cultured neurons exposed to parkinsonism mimetic 6-hydroxydopamine (6-OHDA). However, its molecular mechanisms are unclear. Furthermore, it is not demonstrated yet of its neuroprotective effects on animal model of Parkinson's disease (PD). The role of endoplasmic reticulum (ER) stress and the unfolded protein response (UPR) has been greatly demonstrated as a pathological phenomenon in PD. These signaling mechanisms are activated when there is an increased burden of misfolded proteins in neurons, primarily alpha synuclein, in the case of PD. In this study we aim to elucidate the effects of oxyresveratrol, a potent antioxidant, on the UPR in PD. Furthermore, the neuro-protective potential of oxyresveratrol in an animal model was examined.

Methods: The murine dopaminergic Mes 23.5 cell line was treated with the parkinsonian mimetic 6-OHDA. Effects of oxyresveratrol on the downstream effectors mediating the UPR were investigated by immunoblotting and quantitative PCR. For animal model, male Sprague-Dawley rats were injected stereotactically with 6-OHDA. Subsequently, immunohistochemistry to study midbrain pathology and behaviour tests to assess motor debilitation were performed.

Results: Oxyresveratrol reduced the expression of ATF4 and CHOP, following induction of ER stress in the Mes 23.5 cells by 6-OHDA. The activation of this branch of the UPR, downstream of PERK, propels the cell towards apoptosis. Motor dysfunction was also alleviated in the oxyresveratrol-treated rats, along with a partial preservation of dopaminergic cells in the substantia nigra pars compacta.

Conclusion: This study suggests that attenuation of apoptosis driven by the UPR may be an important mechanism of action of oxyresveratrol. Moreover, a protection of motor function and cell loss found in experimental PD rat model suggests that oxyresveratrol may be a useful therapeutic candidate for PD.

Acknowledgement: The study is supported by Health Medical Research Fund (02131496) from Food and Health Bureau of Hong Kong SAR Government to RCCC.

Project No.: 02131496

AMR-25
Investigation on Ataxia Disorder by Using a Novel Genetically-modified Mouse Model

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Introduction and Project Objectives: In the cerebellar cortex, Purkinje cells (PCs) receive signals from different inputs through their extensively branched dendrites and serve as an integration centre. Defects in the dendritic development of PCs thus disrupts cerebellar circuitry and causes ataxia. Here we report that specific inactivation of both Lhx1 and Lhx5 in postnatal PCs results in ataxic mutant mice with abnormal dendritic development. The PCs in the mutants have reduced expression of Espin, a novel F-actin cytoskeleton regulator. Objectives: 1) To investigate the roles of Espin and different actin regulatory proteins in causing the Purkinje cell dendritic spine defects and ataxic phenotypes in Lhx1/5 DKO mutants. 2) To examine the distribution of neurotransmitter receptors in the dendritic spines of Purkinje cells in Lhx1/5 DKO mutants. 3) To rescue the defects of dendritic development in Lhx1/5 DKO mutants by overexpression of Espin in Purkinje cells of the DKO mutants. 4) To testify whether the activation of other actin regulatory proteins can functionally compensate the loss of Espin and induce normal dendritic development in Purkinje cells of the DKO mutants.

Methods: By using our novel genetically-modified Lhx1/5 double conditional knockout ataxic mouse model, we are able to identify some of the underlying cellular and molecular mechanisms of ataxic

disorder due to cerebellar defect. We carried out mice breeding, quantitative RT-PCR, organotypic culture of cerebellar slices, biolistic transfection, histological analysis, transmission electron microscopy for analysis of the Lhx1/5 double conditional knockout ataxic mouse model.

Results: Conditional knockout of Lhx1/5 in postnatal Purkinje cells leads to downregulation of Espin, a novel F-actin cytoskeleton regulator, resulting in F-actin mislocalization in the proximal dendrites of Purkinje cells. This thereby impairs the dendrite and spine development of Purkinje cell in Lhx1/5 mutants. The mutant PCs therefore fail to form proper synapses and show aberrant electrophysiological properties. By over-expressing Espin, we can successfully rescue the defects in the mutant PCs.

Conclusion: Our findings suggest that Lhx1/5, through regulating Espin expression, control dendritogenesis and spine morphogenesis in postnatal PCs. Our novel genetically-modified ataxic mouse model can serve as an entry point for testing of gene-based or biochemical-based treatment for the ataxic disorder.

Project No.: 02133326

AMR-26

The Role of platelet-T cell interactions in pathogenesis of Multiple Sclerosis (MS) and Neuromyelitis Optica (NMO)

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Introduction and Project Objectives: Platelets are known to participate in vascular pathologies; however, their role in neuroinflammatory diseases, such as multiple sclerosis (MS), is unknown. Autoimmune CD4 T cells have been the main focus of studies of MS, although the factors that regulate T-cell differentiation toward pathogenic T helper-1/T helper-17 phenotypes are not completely understood. The main objective of the study was to understand the role of platelets in modulation of functions of pathogenic CD4 T cells in MS.

Methods: We investigated the role of platelets in the modulation of CD4 T-cell functions in patients with MS and in mice with experimental autoimmune encephalitis (EAE), an animal model for MS.

Results: We found that early in MS and experimental autoimmune encephalitis, platelets degranulated and produced soluble factors serotonin (5-hydroxytryptamine), platelet factor 4, and platelet-activating factor, which specifically stimulated differentiation of T cells toward pathogenic T helper-1, T helper-17, and interferon-γ/interleukin-17-producing CD4 T cells. At the later stages of MS and experimental autoimmune encephalitis, platelets became exhausted in their ability to produce proinflammatory factors and stimulate CD4 T cells but substantially increased their ability to form aggregates with CD4 T cells. Formation of platelet-CD4 T-cell aggregates involved the interaction of CD62P on activated platelets with adhesion molecule CD166 on activated CD4 T cells, contributing to downmodulation of CD4 T-cell activation, proliferation, and production of interferon-γ. Blocking of formation of platelet-CD4 T-cell aggregates during progression of experimental autoimmune encephalitis substantially enhanced proliferation of CD4 T cells in the central nervous system and the periphery leading to exacerbation of the disease.

Conclusion: Our study indicates differential roles for platelets in the regulation of functions of pathogenic CD4 T cells during initiation and progression of central nervous system autoimmune inflammation.

Project No.: 02130636

AMR-27

Rewiring the deafferented thalamic and cortical neurons to the remaining auditory inputs: A model for treatment of tinnitus patients

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Introduction and Project Objectives: Tinnitus is an auditory phantom sensation when no external sound is present. Our working theory is that tinnitus is caused by the parasitic positive feedback oscillation in the thalamocortical circuit, neurons of which become hypersensitive due to lack of cochlear input and cause tinnitus after hearing loss. Cholecystokinin (CCK) has plasticity enabling properties towards cortical neurons. In the present study, we aim to rewire weak connections from the remaining inputs towards the deafferented ones. We hypothesize that this will suppress the hypersensitivity and therefore silence tinnitus permanently.

Methods: To accomplish this study, a reliable tinnitus induction and assessment models had to be established first. Gap-prepulse inhibition of the acoustic startle reflex is used for the assessment of tinnitus, whereas unilateral noise induced hearing loss was chosen as a model to induce tinnitus.

Results: After various applications of CCK4 paired together with sound treatment, we were able to obtain long term observations of GPIAS performance that show improvement in tinnitus behavioural evaluation.

Conclusion: This animal study shows a promising future way how patients with chronic tinnitus could be treated with the help of plasticity-enabling properties of Cholecystokinin system in the brain.

Project No.: 01121906

AMR-28
Effects of Early Life Adversity on the Development of Enteric Neurons

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Introduction and Project Objectives: Irritable bowel syndrome (IBS) is one of the commonest digestive diseases characterized by recurrent abdominal pain and disturbances in bowel habits, but its cause is not fully understood. Studies have implicated that adverse early life experiences predispose individuals to IBS. The objectives of the present study are (1) to determine the effects of neonatal maternal separation as one type of adverse early life experiences on the early postnatal development of the enteric nervous system (ENS) in neonatal rats, (2) to assess the phenotypic changes of enteric neurons of the ENS in adult rats after neonatal maternal separation, and (3) to identify similar phenotypic changes in human biopsies with IBS and correlate these changes with stresses in early life.

Methods: This study involved animal experiments and human tissues. Sprague-Dawley rats underwent neonatal maternal separation from postnatal days 2 to 21, and changes in the developing ENS and/or gut contractility during the early postnatal period, after weaning and in adulthood were analyzed and correlated with IBS. Parallel changes of neuronal projections in human colonic biopsies from IBS patients as compared with those in from healthy individuals were analyzed and correlated with early stress experiences of the patients.

Results: Neonatal maternal separation in neonatal rats induced colonic hypersensitivity and increased colonic motility later in adulthood. This separation did not affect the morphological development and the neurochemical phenotype of the early ENS before weaning. However, when these maternally separated rats grew up to adulthood, a lower percentage of nNOS immunoreactive neurons and higher percentages of both calbindin and calretinin immunoreactive neurons were found in the myenteric plexus, implicating that subtle alterations in the myenteric neurons might have already been induced by neonatal maternal separation. In humans, a positive correlation between IBS symptoms (stool forms) and early trauma history scores was found in patients with IBS, but however in the mucosal biopsies of the distal colon, similar morphologies of neurons and neuronal projections were found in both IBS patients and normal subjects, and the expression of neuronal markers Tuj1 and neuropeptide Y was also found normal in IBS patients.

Conclusion: Early life adversity such as neonatal maternal separation was not able to induce significant morphological and neurochemical changes in the developing enteric nervous system, but however, it predisposed individuals to irritable bowel syndrome developed later in adulthood. The cellular and molecular processes leading to irritable bowel syndrome following early life adversity await further investigations.

Project No.: 02130116

AMR-29
Can Modulator of Mitochondrial Dynamics be Translated into Neuroprotection?

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Introduction and Project Objectives: Mitochondria are dynamic organelles undergoing continuous membrane remodeling. Proper balance of mitochondrial dynamics is crucial for maintaining cellular energy status. Excessive fission causes mitochondrial fragmentation, which predisposes intrinsic apoptosis. Abnormal activation of mitochondria fission or inhibition of fusion have been suggested as early events during the pathogenesis of many diseases. Neurons have especially high demand for mitochondrial metabolism, thus they are particularly sensitive to mitochondrial dysfunction. Abnormalities in mitochondrial dynamics are associated with many neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease. This study investigates whether modulation of dynamic balance, especially for targeting at dynamic shift to fusion side, could be a therapeutic target for neurodegenerative diseases.

Methods: Mitochondrial division inhibitor-1 (mdivi-1) and mitochondrial fusion promoter M1 hydrazone are modulators of mitochondrial dynamics, which inhibits fission and promotes fusion, respectively. The neuroprotective effects of mdivi-1 and M1 hydrazone were assessed by using primary cultured cortical neurons, which were subjected to treatment of free radical generator 6-hydroxydopamine (6-OHDA). Lactate dehydrogenase assay, DAPI (4´ ,6-diamidino-2-phenylindole) staining and Western-blot analysis of apoptotic markers (e.g. cleaved caspase-3) were performed to assess neuronal death. Mitochondria functional features including oxidative stress and membrane potential were further examined for their neuroprotective effects.

Results: Mdivi-1 and M1 hydrazone restored 6-OHDA-induced neuronal apoptosis. Both drugs restored 6-OHDA-induced mitochondrial abnormalities including oxidative stress and outer membrane permeabilization.

Conclusion: These findings suggested the neuroprotective effects of mdivi-1 and M1 hydrazone against 6-OHDA induced toxicity in primary cultures of cortical neurons. The current study paves the road of whether mitochondria dynamics can be served as a platform for evaluating any potential modulators of mitochondrial dynamics to elicit neuroprotection .

Acknowledgement: The study is supported by Health and Medical Research Fund (02131956).

Project No.: 02131956

AMR-30

A small molecule targeting the interaction between PTEN PDZ-binding motif and PSD-95 rescues cognitive defects in 5XFAD mice

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Introduction and Project Objectives: Alzheimer's disease (AD) is a major public health issue affecting the aging population. Effective drugs that can curtail disease progression are lacking. Phosphatase and tensin homolog deleted from chromosome 10 (PTEN) is a tumor suppressor that possesses both lipid and protein phosphatase activities. It hydrolyzes the 3'-phosphate of phosphatidylinositol [3,4,5]-triphosphate (PIP3) to suppress growth and proliferative signaling. PTEN has been reported to mediate amyloid beta (A β)-induced long-term depression in hippocampal brain slices, which requires an interaction with the postsynaptic scaffold protein PSD-95 through its carboxyl-terminal PDZ-binding motif (PDZ-BM).PSD-95 is one of the most abundant scaffolding proteins in the PSD and is involved in synaptic strength and the maintenance of the PSD architecture. PSD-95 has been implicated in the negative regulation of the N-methyl-d-aspartate receptor (NMDAR)-dependent long-term potentiation (LTP). The fact that the PTEN PDZ-BM could mediate A β-induced synaptic dysregulation suggests that pharmacological tools that can specifically target the interaction between PTEN and PDZ domain-containing proteins may have therapeutic effects in AD patients. The major objective of the project aims to define the role of PTEN PDZ-BM in Aβ-induced synaptic dysregulation in animal system and to search for small molecules that can attenuate cognitive impairment by targeting this PTEN protein-protein interaction domain.

Methods:

1. To define the role of PTEN PDZ-BM in Aβ-induced synaptic dysregulation, we crossed the AD mouse strain 5XFAD (APPSwFlLon, PSEN1*M146L*L286V) with PTEN PDZ-BM knockout (Pdz-/-) mice. 5XFAD mice lacking the PTEN PDZ-BM (5XFAD; Pdz-/-) were subjected to behavior tests.
2. To search for small molecules that can attenuate cognitive impairment, a small molecule chemical library was screened by using a fluorescence polarization (FP) method that monitors the interaction between the PDZ2 domain of PSD-95 and a Fluorescein isothiocyanate (FITC)-conjugated PTEN PDZ-BM peptide.

Results: Using 5XFAD and Pten knockin mouse models, we provide the first in vivo evidence linking memory deficits to the PDZ-binding motif of the mouse Pten gene. We further identified a small molecule, ZPDZBMi to block PTEN from binding to PSD-95. Infusion of ZPDZBMi into 5XFAD mice alleviated memory deficits and was associated with a reduction of the PTEN levels in the postsynaptic density.

Conclusion: Our results suggest a new therapeutic approach against β-amyloid-induced memory deficits, with ZPDZBMi as a potential treatment for cognitive impairment in AD.

Project No.: 02130026

AMR-31

A study of the role of CDK5RAP3 in neuronal cell differentiation

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Introduction: Cyclin-dependent kinase 5 (CDK5) is proline-directed serine/threonine kinase important for the regulation of diverse biological processes, including neuronal migration and differentiation, neurite extension, axonal growth, synaptic plasticity, neurogenesis, dopamine signaling, stress response, and senescence. Dysregulation of CDK5 activity has been implicated in a number of neurodegenerative diseases, including Alzheimer's and Parkinson's Diseases. The activity of CDK5 is mainly regulated by the interaction of CDK5 activator protein, called p35 and p39. Using a yeast two-hybrid screening with p35 as bait, the CDK5 regulatory subunit associated protein 3 (CDK5RAP3, or C53/LZAP) was identified as a novel CDK5 activator interacting protein. Studies have shown that CDK5RAP3 promotes apoptosis induced by genotoxic stress via G2/M arrest. Furthermore, CDK5RAP3 is shown to be a putative tumor suppressor by suppressing the NF-κB cell survival pathway in head and neck squamous cell carcinomas. More recently, we demonstrated that CDK5RAP3 is frequently overexpressed in human liver cancer and contributes to its metastasis by activating p21-activated protein kinase 4 (PAK4). While evidence has shown that CDK5RAP3 is involved in cancer formation, little information is available for the roles of CDK5RAP3 in neuronal function.

Objective: To characterise the role of CDK5 regulatory binding protein 3 (CDK5RAP3) in neuronal differentiation

Methods: Two neuroblastoma cell lines, i.e. human SK-N-BE(2)-C and mouse N1E-115, and primary neurons was used to study the effect of CDK5RAP3 in neuronal differentiation and neurite outgrowth. In vitro kinase assay was used to identify the potential phosphorylation site on CDK5RAP3 and ubiquitination degradation assay was performed to evaluate the effect of CDK5RAP3 on p35 protein stability.

Results: We showed that CDK5RAP3 were downregulated at both mRNA and protein levels during neuronal differentiation. Ectopic overexpression of CDK5RAP3 inhibits neurite outgrowth in primary cortical neurons, and stable knockdown of CDK5RAP3 confers a more differentiated phenotype of the BE(2)C cells, indicating that CDK5RAP3 is a suppressor of neuronal cell differentiation.. We mapped the CDK5/p35 phosphorylation site of CDK5RAP3 to serine 218 residue and found that p21-activated protein kinase 4 (PAK4) are potential downstream targets of CDK5RAP3 in differentiation.

Conclusion: Our data suggest that CDK5RAP3 is an inhibitor of neuronal differentiation and a substrate regulated by CDK5/p35. Finding from our study provide a better understanding of how CDK5 regulates neuronal differentiation and may develop CDK5RAP3 as a marker for neuronal differentiation.

Project No.: 02131756

AMR-32

Adipocyte Fatty Acid Binding Protein (A-FABP) Is a Potential Therapeutic Target of Both Alcoholic and Non-alcoholic Steatohepatitis

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Introduction and Project Objectives: Steatosis and liver fibrosis, the first and last reversible stages before progression to irreversible cirrhosis and hepatocellular carcinoma, are considered as the potential treatment window for chronic liver diseases. Adipocyte fatty acid binding protein (A-FABP) is implicated in different stages of chronic liver diseases. In the present study, we investigated the roles of A-FABP in alcoholic- and obesity-related steatohepatitis and the underlying mechanisms whereby A-FABP contributes to the disease pathogenesis.

Methods: Male A-FABP-KO mice and their wild-type (WT) littermates were fed with high fat high cholesterol diet (HFHC) or standard chow (STC) for 16 weeks to induce non-alcoholic steatohepatitis (NASH) or fed with liquid-ethanol diet or iso-caloric maltose dextrin for 6 weeks to induce alcoholic steatohepatitis (ASH). Histological analysis of liver morphology, various body parameters, markers of liver injury, hepatic expression of fibrotic genes, inflammatory markers and A-FABP were examined. The effect of circulating A-FABP on the disease progression was evaluated by infusion of recombinant A-FABP into the A-FABP KO mice. Selective A-FABP inhibitor BMS309403 was administrated to mice for disease treatment. The underlying mechanism whereby A-FABP regulates steatosis and fibrosis was elucidated using primary hepatocytes and hepatic stellate cells (HSCs).

Results: A-FABP is increased in the circulation and/or liver under obese condition or alcohol consumption. Genetic ablation of A-FABP attenuates ASH in mice associating with decreased lipid accumulation and liver fibrosis. On the other hand, genetic ablation and pharmacological inhibition of A-FABP significantly attenuates HFHC-diet induced steatohepatitis and liver fibrosis. Mechanistically, A-FABP acts as lipid chaperone to promote fatty acid uptake into hepatocytes causing steatosis and enhances the expression of transforming growth factor β1 (TGF-β1) through JNK-cJun signaling in hepatic stellate cells (HSCs) and promotes the efflux of fatty acid from HSCs contributing to liver fibrosis. The present findings suggest that A-FABP is a therapeutic target and the selective A-FABP inhibitor BMS309403, which acts by competing FA binding pocket of A-FABP and impairs JNK-AP-1 signaling, is a potential drug for the treatment of alcoholic- and obesity-related liver diseases at different stages.

Conclusion: In conclusion, A-FABP is a key mediator of alcoholic- and obesity-related liver diseases. Selective A-FABP inhibitor BMS309403 is a potential drug for the treatment of alcoholic- and obesity-related liver diseases at different stages.

Project No.: 02131906

AMR-33

FGF21 Resistance as a Potential Mediator of Systemic Insulin Resistance and Type 2 Diabetes

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Introduction and Project Objectives: Fibroblast growth factor 21 (FGF21) is an endocrine factor that has pleiotropic effects on glucose and lipid homeostasis. However, the clinical relevance and underlying mechanism of FGF21 resistance in obesity remain elusive. MicroRNA-34a (miR-34a) is one of the most highly elevated miRs in obesity and contributes to metabolic dysfunctions. The aim of this study is to characterize the dynamic alteration of FGF21 sensitivity during the progression of obesity and explore the possible role of adipose miR-34a in FGF21 resistance.

Methods: miR-34a expression and its association with FGF21 receptor complex were assessed in visceral and subcutaneous adipose tissue from both humans and mice with obesity. The pathophysiological roles of adipose miR-34a in FGF21 resistance, adipose inflammation and obesity-related metabolic complications were evaluated by using adipose-selective miR-34a knockout mice.

Results: Elevated adipose miR-34a expression was negatively correlated with FGF21 receptor complex and adiponectin expressions in human subjects. Blockage of miR-34a actions with either lentivirus-mediated miR-34a sponge or adipose tissue specific genetic depletion counteracted obesity-induced reduction of FGFR1 and β-klotho expressions and restored the capacity of FGF21 to induce adiponectin secretion in adipose tissue. Furthermore, adipose tissue miR-34a deficiency alleviated obesity-induced metabolic dysfunction and chronic inflammatory responses in mice.

Conclusion: During the development of obesity, a progressive increase of miR-34a in visceral adipose tissue is accompanied by a gradual decline of FGF21 sensitivity, thereby leading to hypoadiponectinemia, metabolic inflammation and insulin resistance. Inhibition of miR-34a selectively in adipose tissue may represent a novel therapeutic approach for obesity-related metabolic complications.

Project No.: 02132836

AMR-34

A randomized controlled trial of a laser-aided orthodontic treatment for patients with periodontitis

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Introduction and Project Objectives: To investigate the analgesic and inflammation-modulatory effects of low-level laser therapy among orthodontic patients with compromised periodontium. Low-level laser therapy (LLLT) shows effects in orthodontic pain relief

and periodontal inflammation control. However, no evidence is available regarding its application in tooth movement with compromised periodontium.

Methods: A randomised controlled trial was conducted in 27 adults with treated and controlled chronic periodontitis over 6 months. One side of the dental arch underwent repeated treatment with a 940-nm diode laser, whilst the other side received pseudolaser treatment. Subjective pain, periodontal status, cytokines in gingival crevicular fluid and periodontopathic bacteria in supragingival plaque were assessed.

Results: The intensity of pain was lower on the laser-irradiated side at multiple follow-up visits ($P<0.05$). The pain subsided 1 day earlier on the laser side, with a lower peak value during the first week after initial archwire placement ($P<0.05$). The laser side exhibited a smaller reduction in bite force during the first month ($P<0.05$). A smaller increase was observed in the plaque index scores on the laser side at 1-month and in the gingival index scores at the 3-month follow-up visit ($P<0.05$). Laser irradiation inhibited the elevation of interleukin-1 β, prostaglandin E2 and substance P levels during the first month ($P<0.05$). However, no intergroup difference was detected in the bacteria levels.

Conclusion: Low-level laser therapy exhibits benefits in pain relief and inflammation control during the early stage of adjunctive orthodontic treatment in periodontally compromised individuals.

Project No.: 01121056

AMR-35

Inflammation-related impairment of left ventricular functional reserve in patients with systemic lupus erythematosus: A speckle-tracking exercise echocardiographic study

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Introduction and Project Objectives: Cardiovascular disease is a leading cause of death in patients with systemic lupus erythematosus (SLE). An overactive inflammatory response in SLE may lead to myocardial injury and impaired left ventricular (LV) functional reserve. Such abnormalities may not be detectable at rest.

Purpose: To assess (i) LV systolic functional reserve in SLE using exercise speckle-tracking echocardiography; (ii) relation of systolic functional reserve with inflammatory burden.

Methods: 44 patients with SLE and 31 age/sex-matched healthy controls (46±12 years vs. 46±10 years; female/male=43/1 vs. 29/2, p=NS) were studied with bicycle exercise echocardiography. LV ejection fraction and global longitudinal strain (GLS) were measured at rest and low-level exercise. The average level of erythrocyte sedimentation rate (ESR) in the past 12 months was measured.

Results: Compared with normal controls, ΔGLS from rest to exercise (-5.8% vs. -4.3%, p=0.005) of SLE patients were significantly impaired despite preserved LV ejection fraction (61% vs. 59%, p=0.10) and GLS (-20.5% vs. -19.7%, p=0.10) at rest. There was a significant inverse correlation of average ESR with GLS at peak stress (r=-0.32, p=0.005).

Conclusion: LV systolic functional reserve is impaired in patients with SLE and is related to inflammatory disease burden. Low-level exercise speckle-tracking echocardiography can detect subclinical diminishment of LV functional reserve during SLE progression.

Project No.: 02130686

AMR-36

Can achieving Minimal Disease Activity (MDA) prevent progression of subclinical atherosclerosis and arterial stiffness? A two-year prospective cohort study in Psoriatic Arthritis

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Introduction and Project Objectives: While MDA was associated with articular benefits, its effect on disease-related elevated CVD risk remained uncertain. This study aimed to investigate the effect of achieving MDA on subclinical atherosclerosis(SCA) and arterial stiffness(AS).

Methods: 101 PsA patients without overt CVD received protocolized treatment aiming at MDA for 2-years. High-resolution ultrasound(for SCA) and AS were assessed annually. The primary objective was to investigate the effect of achieving MDA(MDA group) at 12-months on the progression of SCA(carotid intima-media thickness[IMT]&plaque) over 24-months. Secondary objectives were to compare 1)the changes in AS(branchial-ankle pulse wave velocity[baPWV] and augmentation index[AIX]) over 24-months between the MDA and non-MDA groups;2)changes in SCA and AS markers in patients who achieved sustained MDA(sMDA: defined as achieving MDA from month 12to24) compared to those who didn't(non-sMDA group). Carotid plaque progression was defined as increase in number or region harboring plaque.

Results: 90 PsA patients [male:52;age:50±11] completed 24-months follow-up were included in this analysis. Significantly increased proportion of patient achieved MDA(baseline:16.7%;12-months: 63.3%;24-months:68.9%)after intensive treatment. SCA outcomes were similar between MDA and non-MDA group(Figure1). 41(45.6%) patients achieved sMDA. At baseline, higher prevalence of subjects in the non-sMDA group were smokers, were treated with NSAIDS and csDMARDs; fewer subjects were on bDMARDs, and had higher disease activity compared with the sMDA group. 34/90(37.8%) patient had plaque progression. The prevalence of plaque progression was numerically higher in the non-sMDA group [22(44.9%)vs12(29.3%),p=0.128]. Using multivariate analysis, achieving sMDA had protective effect on plaque progression[OR=0.273,95%CI:0.088-0.846,p=0.024]after adjusting baseline difference(Table 1). Achieving sMDA was also related to less progression of total plaque area(TPA), mean&maximum IMT, baPWV&AIX(Table 1).

Conclusion: Effective suppression of inflammation by achieving sustained MDA may prevent progression of subclinical atherosclerosis and arterial stiffness in PsA patients.

Project No.: 01120496

AMR-37

Immunotherapy and Modulatory Mechanisms of Hypoallergen DNA Vaccines for Shellfish Allergy

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Introduction and Project Objectives: Shellfish is the most common allergen causing food allergy in the United States, Hong Kong and most Asia-Pacific regions. Tropomyosin is identified as the major cross-reactive allergen in shellfish. However, no allergen-specific immunotherapy is currently clinically available. This study thus aims at (1) examining the therapeutic efficacies of two hypoallergen-encoding DNA vaccines in a BALB/c mouse model of shrimp tropomyosin-induced hypersensitivity; and (2) evaluating the immuno-modulatory mechanisms of hypoallergen vaccine-based immunotherapy.

Methods: Hypoallergen DNA vaccines, namely pMEM49 and pMED171, were constructed by cloning the corresponding nucleotide sequences in the mammalian expression plasmid pCI-Neo. BALB/c mice (n = 8 per group) were sensitized and challenged with the recombinant shrimp tropomyosin rMet e 1, followed by intradermal treatment with either pMEM49 or pMED171 for three times at weekly interval. Animals receiving PBS throughout experiment or sham treated with PBS or empty pCI-Neo PBS were included as controls. Upon a second rMet e 1 challenge, blood, spleen and small intestine were collected for humoral and cellular immunological analysis. To assess the modulatory roles of the pMED171-induced regulatory T (Treg cells), CD4+CD25+ Treg cells and CD4+CD25- T cells were isolated from PBS- and pMED171-treated mice and transferred to rMet e 1-sensitized mice 24 h prior to allergen challenge.

Results: Treatment of BALB/c mice with pMEM49 or pMED171 led to the remarkable reduction in tropomyosin-challenge-induced allergic symptoms, serum tropomyosin-specific IgE levels, as well as Th2 cytokine expression and inflammatory cell infiltration in the intestine when compared with controls. Treatment with either pMEM49 or pMED171 increased the number and activity of T regulatory cells, but to a greater extent for pMED171. Adoptive transfer of CD25+ Treg cells from pMED171immunized mice to rMet e 1-sensitized mice led to limited generation of specific IgE, Th2 activity and intestinal inflammation in the recipient mice.

Conclusion: This study is the first to demonstrate the potency of hypoallergen-encoding DNA vaccines as a therapeutic strategy for shellfish allergy and illustrate with robust experiments the induction of functional Treg cells via this treatment modality.

Project No.: 02130206

AMR-38

PLGA/β-TCP composite scaffold incorporating salvianolic acid B promotes bone fusion by angiogenesis and osteogenesis in a rat spinal fusion model

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Introduction and Project Objectives: Spinal disorders often require surgical treatment called spinal fusion to restore a stabilized spine where bone grafts are implanted for the fusion of adjacent vertebras. In this study, we developed a bioactive composite scaffold incorporated with salvianolic acid B (SB), an active component extracted from Danshen. This study aimed to evaluate the effects of SB-incorporated porous scaffold on spinal fusion models.

Methods: The composite scaffolds composed of poly (lactic-co-glycolic acid) and tricalcium phosphate (PLGA/β-TCP) were fabricated with low-temperature rapid prototyping technique, which incorporated SB at low (SB-L), middle (SB-M), high (SB-H) doses, and pure PLGA/β-TCP as blank control (Con). The release profile of SB from the scaffolds was determined by high performance liquid chromatography. Osteoconductive and osteoinductive properties of the scaffolds were reflected by the osteogenic differentiation ability of rat primary mesenchymal stem cells. The angiogenesis was determined by the forming of tube-like structures resembling capillaries using endothelial cell line (EA hy9.26). A well-established spinal fusion model was used to evaluate the in vivo bony fusion. Animals were transplanted with scaffolds, or autografts from iliac crest as positive controls. Micro-computed tomography (CT) analysis, CT-based angiography, manual palpation test, histomorphometry, and histology were performed after 8 weeks of transplantation.

Results: Results revealed that incorporated SB was steadily released from the scaffolds. The aliquot of released SB promoted osteogenesis and angiogenesis in vitro in a dose-dependent manner. In animal study, a dose-dependent effect of SB on new bone formation, mineral apposition rate, and vessel density within the scaffold were demonstrated. Manual palpation test showed little numerical improvement in fusion rate when compared with the blank controls.

Conclusion: In summary, our results suggested that SB-incorporated PLGA/β-TCP composite scaffold could enhance bony fusion through the promotion of osteogenesis and angiogenesis.

Project No.: 02130436

ID-1

Genetic susceptibility to Tuberculosis: latest findings suggests a role of gene-environment interaction

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Introduction and Project Objectives: Genetic predisposition for tuberculosis is well established by both human and animal studies. Recently, several GWAS had reported some predisposition genes. However, few of them have been replicated by other studies (1,2). Such difficulties of replication are in sharp contrast with many other diseases in which GWAS results were readily replicated, like body anthropometry traits and even scoliosis (3,4).

Methods: We carried out a replication study to examine some of the recently GWAS reported predisposition genes for TB with a large local patient cohort of 1150 Chinese TB patients and 1280 population controls. None of the SNPs in ASAP1, chromosome 18q, DUSP14 and HLA-DQA1 showed significant association in whole group analysis. On the other hand, subgroup analysis with stratification by age showed that a SNP rs9272785 in HLA-DQA1 were associated with young-onset TB (onset at 20-40 years old, N=396).

Results: The results lend support to the hypothesis of gene-environmental interaction in TB susceptibility. It suggests that different pathogenesis operates in TB patients of different age group. Young patients are more likely to have a genetic predisposition in the type II HLA gene. We would like to see if the results could be confirmed in future cohorts in other populations.

Conclusion: Young patients are more likely to have a genetic predisposition in the type II HLA gene. We would like to see if the results could be confirmed in future cohorts in other populations.
Part of the results have been published (5)
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Project No.: 14130282

ID-2

Modelling the Impact of Control Strategies for Tuberculosis Transmission in Hong Kong

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Introduction and Project Objectives: To assess the effectiveness of latent tuberculosis infection (LTBI) treatment in older people.

Methods: We developed a mathematical model to evaluate the impact of treating LTBI in the elderly in addition to current TB control strategies. The model was calibrated using the annual age-stratified TB notifications from 1965-2013. We assessed the scenario that LTBIs in older people (≥60 years) were screened and treated annually, and examined the impact of varying proportions of LTBI patients who were willing to be screened (acceptability) and who completed treatment (adherence).

Results: Our results showed that at present, approximately 75% of annual new notifications were from reactivations. Even if only a low to moderate proportion (approximately 20% to 40% annually) of elderly people were willing to be screened and treated for LTBI, the overall TB incidence could be reduced by almost 50%, to reach the 2025 milestone of the global End TB Strategy. Nevertheless, due to a high risk of hepatotoxicity in elderly population, benefit-risk ratios were mostly below unity.

Conclusion: Hong Kong is a high-income city with intermediate tuberculosis (TB) burden primarily driven by endogenous reactivations. A high proportion of remote latently infected people, particularly elderly, hinders the effectiveness of current strategies focusing on passive TB detection. According to our results, intervention programs of TB control should be carefully formulated, including LTBI treatment for elderly homes residents with a close monitoring of possible adverse side effects. Our findings also underscore the need for new anti-TB drugs/drug regimens with greater efficacy and safety for treating LTBI.

Project No.: CU-16-C11

ID-3

How can we prevent and control tuberculosis in older people of Hong Kong: a logic model toward TB elimination goal

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Introduction and Project Objectives: Tuberculosis (TB) in older people increasingly becomes a major public health challenge in Hong Kong. With the ageing of population and TB epidemic, the current strategy has limited impacts on achieving the End TB targets

by 2035. This study aimed at identifying targeted and prioritized TB interventions in older people of Hong Kong. The evidence was synthesized in a strategic framework to assist decision-making in improving the local TB program.

Methods: A scoping review was conducted to map potentially effective TB interventions for older people; while another literature review was performed to identify the TB epidemiology, high-risk factors, and policy gaps in older people of Hong Kong. Accordingly, a questionnaire was developed for a two-round Delphi survey in a panel of knowledgeable and experienced TB experts. Interventions identified from the review were rated for importance and feasibility on a 5-point Likert-scale under a consensus level of 70%. After the second-round survey, the interventions that reached consensus as being very important and very feasible were recommended to be prioritized. This study used a logic model to synthesize evidence for a strategic framework of TB prevention and control in older people of Hong Kong.

Results: Nineteen articles met the inclusion criteria of scoping review. Interventions were mapped according to the purpose of preventing infection, early detection, appropriate treatment, and program management. In the literature review, high disease burden, reactivation from latent TB infection (LTBI), and high-risk groups of TB were identified in older people of Hong Kong. However, few targeted and proactive actions were developed. In the two-round Delphi survey, 15 TB experts represented an accredited degree of authority from public health, clinical, and laboratory perspectives. Among the 67 interventions, 48% (32/67) reached the consensus to be prioritized and provided evidence for activities in the logic model. In addition to optimizing the current case-finding strategy, infection control, a high index of TB suspicion, adopting innovative diagnostic tests, screening for LTBI and TB on admission to residential care homes for the elderly, appropriate treatment with pre-treatment assessment and follow-up tests, monitoring, and evaluation, and enhanced surveillance, evaluation, and training program were the main prioritized processes under the current resources. Further modelling analysis and evidence-based pilots were the expected outputs to determine effectiveness and cost-effectiveness in the local health system.

Conclusion: This study provides a case analysis and reference framework of preventing and controlling TB in older people. By capturing casual relationships, the logic model makes the evidence and assumptions explicit toward the long-term outcome and goal of achieving the End TB targets. The findings are useful for policy-makers to improve the TB program in an area with intermediate TB burden such as Hong Kong, and for researchers to concentrate on the prioritized knowledge gaps.

Project No.: CU-15-C12

ID-4

Genomic Characteristics of Hospital-associated and Community-associated Methicillin-resistant Staphylococcus aureus

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Introduction and Project Objectives: The epidemics of community- (CA-) and healthcare-associated (HA-) methicillin-resistant Staphylococcus aureus (MRSA) have been driven by a small number of lineages/clonal complexes through recombination of mobile genetic elements (MGEs). The dynamic evolution processes and the population structure of Staphylococcus aureus are under the influence of multiple important factors, such as point mutation events, recombination and horizontal gene transfer (HGT). The HA-MRSA clone belonging to ST239 lineage has descended from ST8 and ST30 parents through large scale insertion of at least 635 kb sequence of ST30 chromosome to the ST8 parent and become a successful epidemic clone that spread globally. We hypothesize that new emerging MRSA clones in Hong Kong underwent evolutionary changes that involved recombination and HGT of MGEs, to become predominant representative clones in Hong Kong.
We sought to characterize MRSA strains from patients hospitalized with MRSA infections to determine the prevalent clones of HA- and CA-MRSAs and to examine the role of large scale recombination events and HGT via MGEs in the evolutionary changes and establishment of new MRSA clones in our locality.

Methods: Non-duplicate isolates obtained from blood (235), pus/tissue specimens (234) of hospitalized patients in 3 out of 7 hospital clusters of the Hospital Authority were characterized on their sequence types using multiplex PCR (mPCR), spa typing and MLST. Whole genome sequencing were performed on representative isolates of each ST type.

Results: The predominant MRSA clones identified in hospitalized patients belonged to ST45 by mPCR (34.5%), followed by ST22 (mean 31.1%), ST1048 and ST1774. The recombination analyses of single nucleotide polymorphism and mobile genetic elements from representative ST strains confirmed two major events: of ST1774 which was a hybrid of ST45 and ST188 and ST1048 being derived from ST45 and ST7. The prevalence of ST45, ST1774 and ST1048 have predominantly replaced the ST239 clonal dominant in hospitals of Hong Kong. Large scale recombination events of MRSA led to new ST types becoming predominant in Hong Kong.

Conclusion: Recombination events played an important role to the development of new MRSA ST types in Hong Kong. The study unravelled insights to the dynamic evolution processes and selective advantages that shape the landscape of MRSA associated with healthcare and community-associated infections in Hong Kong.

Project No.: CU-15-B3

ID-5

A feasibility study on the inhibition of Staphylococcus aureus infection using AdsA specific antibodies

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Introduction and Project Objectives: Staphylococcus aureus (S. aureus) is a common pathogen in hospitals and in the community. Development of immunotherapeutic approaches against S. aureus, either active or passive, has seen a resurgence in recent years due to

the emergence of multiple drug-resistant strains. Adenosine synthase A (AdsA), a cell wall–anchored enzyme that catalyzes AMP to adenosine, is a critical virulence factor necessary for S. aureus to escape phagocytic clearance by polymorphonuclear leukocytes. Our work provided the first evidence for the potential use of recombinant AdsA as an antibody target to prevent invasive S. aureus infection in a mouse model. We showed that an anti-AdsA serum had a therapeutic effect against S. aureus infection. To prepare monoclonal antibodies (MAbs) targeting AdsA to prevent S. aureus from escaping phagocytic clearance in vitro and in vivo.

Methods: In order to produce monoclonal antibodies (MAbs) targeting rAdsA by hybridoma technology, recombinant AdsA protein was used to immunize mice to generate specific antibody-secreting B-lymphocytes. Cell fusion was carried out between the splenocytes and NS-1 myelomas to obtain hybridoma cell lines correspondingly secreting anti-rAdsA antibodies, which was confirmed by antibody-capture assay.

Results: To produce monoclonal antibodies targeting rAdsA by hybridoma technology, recombinant AdsA protein was used to immunize mice to generate specific antibody-secreting B-lymphocytes. Cell fusion was carried out between the splenocytes and NS-1 myelomas to obtain hybridoma cell lines correspondingly secreting anti-rAdsA antibodies, which was confirmed by antibody-capture assay. These cell lines underwent mouse ascites fluid expansion to massively produce the monoclonal antibodies. All MAbs demonstrated a high titer, showing that a high concentration of specific antibodies was produced. In the lethal challenge experiment, however, the eight monoclonal antibodies exhibited no function in extricating the mice from S. aureus infection and their survival rate reached zero within three days after the inoculation of Newman or USA300 strain S. aureus at lethal dosage.

Conclusion: Unfortunately, these antibodies cannot block AdsA activity, thereby preventing S. aureus infection.

Project No.: 14130742

ID-6
Photodynamic Therapy Mediated by Natural Products on Methicillin-Resistant Staphylococcus Aureus (MRSA)-Infected Wound

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Introduction and Project Objectives: Owing to the nature of multiply-resistance associated with methicillin-resistant Staphylococcus aureus (MRSA), the options for treatment of MRSA infections are limited and remains a clinical challenge. The main objective is to find an effective therapeutic agent/method, other than antibiotics, to tackle MRSA infections. Photodynamic therapy (PDT) mediated by natural products is effective in treating MRSA-infected wound.

Methods: In comparison with methylene blue (MB), the effects and underlying action mechanisms of photodynamic therapy mediated by pheophorbide a (Pa) and hypericin (Hy) on MRSA-infected wound have been investigated. This project is mainly divided into three parts. Firstly, the in vitro efficacies of Pa-PDT, Hy-PDT and MB-PDT in killing MRSA were evaluated. Secondly, their wound healing and immunomodulatory activities in a mouse model of MRSA-infected wound were studied. Lastly, their effects on human neutrophils were examined.

Results: Pa-PDT, Hy-PDT and MB-PDT were all capable of killing MRSA in vitro and Hy-PDT showed the highest efficacy against different MRSA strains. In the murine model, both Hy-PDT and Pa-PDT showed better efficacy than MB-PDT in decreasing bacterial load and stimulating wound healing of the MRSA-infected wounds. The efficacy of Hy-PDT was the best among all treatments tested. On the other hand, Pa-PDT, Hy-PDT and MB-PDT were cytotoxic to human neutrophils in vitro. However, their damage could be minimized by topical application of photosensitizers and local illumination of light.

Conclusion: Pa-PDT and Hy-PDT could inhibit the growth of MRSA. They could promote wound healing of MRSA-infected wound by direct bactericidal effect. This study was supported by the Health and Health Services Research Fund, Food and Health Bureau, Hong Kong SAR government (13120302).

Project No.: 13120302

ID-7
Characterization the role of OqxAB in Salmonella virulence and antimicrobial resistance

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Introduction and Project Objectives: The plasmid-mediated quinolone resistance gene oqxAB is known to encode a resistance-nodulation-division efflux pump and confer reduced susceptibility towards fluoroquinolones in various bacterial hosts. The aims of this study are to determine the role of oqxAB in contributing to Salmonella virulence and antimicrobial resistance, as well as stress tolerance. Regulation of this plasmid-mediated efflux pump by local and global regulators in Salmonella is also elucidated.

Methods: Clinical and environmental Salmonella Typhimurium, S. Enteritidis and S. Indiana strains were included and the role of oqxAB in expression of antimicrobial resistance, virulence and stress response was evaluated by antimicrobial susceptibility testing, macrophage and mouse infection assays and tolerance assays. Whole plasmid sequencing was performed to determine the key vectors responsible for dissemination of this mobile element. Regulatory mechanisms were elucidated by knock-out and cloning, followed by qRT-PCR and Western Blotting.

Results: Our results showed that oqxAB conferred reduced ciprofloxacin susceptibility and enhanced mutation development within the QRDR region in S. Typhimurium. Although no direct linkage between the presence of oqxAB and elevated virulence was

observed, this mobile efflux element was demonstrated to enhance the survival of Salmonella under unfavourable conditions. Plasmid sequencing and PCR screening showed that IncHI2 plasmids are the major vector mediating dissemination of oqxAB in S. Typhimurium clinical strains. Gene knockout and western blotting revealed that this mobile efflux pump is also subjected to regulation by the global regulator RamA.

Conclusion: The mobile efflux pump oqxAB enhanced survival of Salmonella under antimicrobial and environmental stresses. The deliverables from this study further our understanding of the role of this and other similar mobile resistance genes, and the mode of regulation of such genetic elements.

Project No.: 14130402

ID-8

Molecular Epidemiology of Transferrable Multidrug-Resistant Pump OqxAB in Escherichia coli from Human and Animal Sources

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Background: OqxAB is the most prevanet plasmid-mediated quinolone resistance determinant in Hong Kong and China.

Aims and Objectives: To investigate the clonal structure of multidrug-resistant E. coli populations originating from food animals and human sources; and the molecular epidemiology of plasmids carrying oqxAB originating from E. coli isolates of food animal and human sources.

Methods: This is a retrospective study. Escherichia coli isolates originating from patients with UTI (n = 205, collected in 2004-2013) and food-producing animals (n = 136, collected in 2012-2013) in Hong Kong were investigated by molecular methods. Forty-three oqxAB positive isolates were further investigated by genome sequencing.

Results: The oqxAB gene was highly prevalent among NIT-intermediate (11.5%-45.5%) and -resistant (39.2%-65.5%) isolates but rare (0%-1.7%) among NIT-susceptible isolates. In our isolates, the oqxAB gene was associated with IS26 and was carried by plasmids of diverse replicon types. Nonetheless, the most prevent plasmid replicon type carrying oqxAB is repFII. Multilocus sequence typing revealed that the clones of the oqxAB-positive E. coli were diverse. Multilocus sequence typing showed that 10 STCs were shared by isolates originating from both human and animal sources. Plasmid sequencing and genomics analysis provide evidence that highly similar oqxAB plasmids of IncF type are carried by genetically diverse isolates from different host sources. Of note, these oqxAB plasmid often co-carried multiple resistance and virulence-associated genes.

Conclusion: Plasmid-mediated oqxAB is an important nitrofurantoin resistance mechanism. Clones and plasmids are involved in the dissemination of this resistance mechanism in isolates of diverse host origins.

Project No.: 14130722

ID-9

Ecological Relationship Between Legionella Pneumophila And Biofilm Communities In Potable Water Distribution System

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Introduction and Project Objectives: The project aimed to study the trends of culturable and viable but not culturable (VBNC) L. pneumophila in free water and biofilm samples collected from simulated water distribution system (SWDS) with different flow rates; to characterise the microbial communities in the SWDS; to analyse the relationship between abundance of bacterial species and the amount of culturable and VBNC L. pneumophila.

Methods: Culturable L. pneumophila was quantified using culture-based methods. Microbiome profiles in the water and biofilm were characterised using 16S and 18S rDNA-targeted sequencing.

Results: Trends of culturable and VBNC L. pneumophila in free water and biofilm were similar under different flow rates. Similarly, microbial communities were similar under different flow rates. Novosphingobium, Bradyrhizobium and Hartmannella were most abundant in biofilm. Novosphingobium had negative correlation with L. pneumophila.

Conclusion: Flow rate did not significantly affect the trends of culturable and VBNC L. pneumophila and microbial communities. Hartmannella and Novosphingobium were most predominant in biofilm, the former supports Legionella multiplication while the latter had negative correlation with Legionella survival. Further studies are needed to address the interaction between these organisms.

Project No.: 14130462

ID-10

Epidemiology of Scarlet Fever in Hong Kong

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Introduction and Project Objectives: Scarlet fever was a common infectious disease mainly affecting children before the 20th century. In Hong Kong, scarlet fever is a notifiable disease with a relatively stable incidence in the previous decades. However, an upsurge of scarlet fever cases, with about 10-fold of previous average incidence, was reported in 2011. Similar upsurge was reported in other parts of China and the United Kingdom. Improved understanding in the epidemiological characteristics may facilitate public health intervention against scarlet fever in Hong Kong and other places. This project describes the epidemiology of scarlet fever in Hong Kong and identifies potential differences before and after the upsurge in 2011.

Methods: We used individual data of scarlet fever cases between 2005 and 2015 notified to the Department of Health. Data on age, sex, dates of illness onset, and travel history from 7,756 scarlet fever cases were retrieved. Of these, 7,266 local cases were under 15 years and were analyzed. A multivariable negative binomial regression

model was fitted to examine the effect of various epidemiological and meteorological variables on weekly scarlet fever incidence rate.

Results: In the final regression model, variables such as age, sex, school holidays in the preceding week, temperature, relative humidity, rainfall, long-term and bimodal seasonal trend were included. The model showed that scarlet fever incidence was stable before 2011, but decreased mild at about 8% annually after the upsurge in 2011. Our results show that scarlet fever usually peaked in December to January, with a milder peak in May to June.
Children aged 3-5 years (kindergarten students) were most affected throughout the study period, followed by children aged 6-11 years (primary school students). Boys aged 3-5 years had the highest risk (adjusted incidence rate ratio (IRR)=1.5, 95% CI=1.3-1.7), comparing to girls aged 0-2 years. School holidays (adjusted IRR=0.6, 95% CI=0.5-0.7) and higher temperature (adjusted IRR=0.96, 95% CI=0.94-0.99) were found to be negatively associated with scarlet fever incidence after the upsurge.

Conclusion: Our study showed elevated scarlet fever activity for 5 years after the 2011 upsurge. Younger children had a higher risk of scarlet fever, especially during school days. School-based control strategy is likely to be effective for controlling outbreaks. Monitoring of the high risk group, or potential change, is needed for effective control of the disease. The re-emergence of scarlet fever is still poorly understood and further community-based studies are needed.

Project No.: HKS-16-E09

ID-11
Development of Smart Theranostic Agents for Imaging and Photoinactivation of beta-Lactamase-Producing Bacteria

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Introduction and Project Objectives: The increasing worldwide occurrence of infectious diseases caused by antibiotic-resistant pathogens has become a great threat to humans. It has been known that bacteria can develop various means to enhance their resistance to antibiotics. As a result, conventional antibiotics are becoming ineffective against certain bacteria and the drug-resistant problem has been threatening public health worldwide. Having a remarkably different mode of action, antimicrobial photodynamic therapy (PDT) has been extensively studied as an alternative treatment modality for conditions caused by bacterial infection. It utilizes the combined action of three individually non-toxic components, namely a photosensitizer, molecular oxygen, and light with appropriate wavelength to generate cytotoxic reactive oxygen species. Different classes of photosensitizers have been examined for their antimicrobial photodynamic activities. Their photocytotoxicities are generally higher toward the Gram-positive bacteria compared with the Gram-negative counterparts as a result of the highly organized outer membrane of the latter that imparts a strong permeability barrier. In this project, we aimed to prepare a series of beta-lactamase-activated phthalocyanine-based photosensitizers and study their photophysical properties and antimicrobial activities against beta-lactamase-producing bacteria.

Methods: A series of beta-lactamase-activated phthalocyanine-based photosensitizers were synthesized, in which a zinc(II) phthalocyanine was conjugated to either a black hole quencher BHQ3 or another phthalocyanine unit through a cleavable beta-lactam ring linker. We then studied their electronic absorption and basic photophysical properties in different solvents. The effects of beta-lactamase on their fluorescence emission and singlet oxygen generation efficiency were also examined. The dark- and photocytotoxicity of these compounds were finally investigated against the Escherichia.

Results: Upon interaction with beta-lactamase secreted by Klebsiella pneumoniae, the fluorescence emission and singlet oxygen generation by these compounds could be restored in solution. For the tetralysine-substituted dimer, it was photocytotoxic against both bacterial strains Gram-negative Escherichia coli ATCC 35218 (beta-lactamase positive) and ATCC 25922 (beta-lactamase negative), showing that it could not be selectively activated by beta-lactamase-producing bacteria.

Conclusion: Phthalocyanines conjugated with a black-hole quencher via a beta-lactam ring exhibit a higher quenching efficiency than the beta-lactam-ring-linked dimeric phthalocyanines. The photoactivities of these conjugates can be restored upon cleavage of the beta-lactam ring by beta-lactamases. It has been found that the three photosensitizers prepared in this study are either non-cytotoxic against the Gram-negative Escherichia coli ATCC 35218 and ATCC 25922 or show no selectivity toward the beta-lactamase positive bacterial strain. Further chemical modification of the photosensitizers and optimization of the experimental conditions are needed to improve their in vitro responses.

Project No.: 15140322

ID-12
RNomics Study of the Emerging Incompatibility Group X3 Plasmid Carrying blaNDM-1 in Hong Kong

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Introduction and Project Objectives: The emerging of New Delhi metallo-beta-lactamase (NDM-1) is a major health threats to the clinical management of gram-negative bacteria infections. In Enterobacteriaeae, NDM-1 have been found on plasmids with narrow or board host range and rarely on the chromosome. A novel incompatibility group X3 plasmid carrying blaNDM-1 (pNDM-HN380) has recently found to epidemiologically link to multiple geographical areas in China. However, the mechanism of plasmid dissemination and their control of the resistance genes remains unclear.
The objective of this project is to identify and characterize the novel chromosomal and plasmid-encoded sRNA which involve in the dissemination of the IncX3 plasmid and regulation of antibiotics resistance, and to study the plasmid stability and fitness to the host by determining the global changes of the RNA repertoire of E. coli upon introduction of pNDM-HN380.

Methods: We performed The Next Generation Sequencing to profile the RNA repertoires of transconjugants E. coli J53 in the presence and absence of pNDM-HN380 plasmid at log and stationary phases. Novel sRNAs were predicted and validated by Northern blot analysis. Putative sRNA targets were validated using qRT-PCR. Bacterial growth, swimming motility test, and plasmid stability were studied.

Results: Transcriptomes profiling of transconjugants revealed the downregulation of genes involved in motility and chemotaxis. The low motility of bacteria carrying plasmid was further confirmed by swimming motility test. We also identified three novel intragenic region sRNAs (IGR plas1, IGR plas2 and IGR gen2) and validated by Northern blot analysis. Small RNA plas2 was further characterized by studying its influence in fucose metabolism, and we found that it acts as a master regulator to control the fucose metabolic pathways, which facilitates the enhancement of virulence and modulation of pathogenesis of gut bacteria.

Conclusion: This study provided comprehensive pictures and relationship between sRNAs and transcriptomes of bacteria carrying pNDM-HN380 plasmid, a mobile element widely spreads NDM-1 genes. The understanding of sRNA-regulated fucose metabolism which facilitates the pathogenesis of bacteria may help us to develop therapeutic to tackle the virulence of pathogen.

Project No.: 14130532

ID-13

Development of novel tridenate inhibitors targeting to active site residues and Zinc ions of New Delhi Metallo β-lactamases-1 (NDM-1)

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Introduction and Project Objectives: Carbapenemases-mediated resistance in Gram-negative bacteria is the most clinically important mechanism, as carbapenemases possess versatile hydrolytic capacities that can break down nearly all β-lactam antibiotics, in particular, class B1 metallo-β-lactamases NDM-1. Currently, inhibitors of NDM-1 are not available in the clinic. The rapid worldwide transmission of NDM-1 producing "superbug" further emphasizes the urgent need for the development of effective NDM-1 inhibitors.

Methods: Based on our previous results of site-directed mutagenesis on NDM-1 and the structure of a well-known NDM-1 inhibitor L-captopril, we aim to construct a compound library of novel 3,4-disubstituted pyrrolidine derivatives by using a 3+2 cycloaddition reaction and to characterize their synergistic activities in combination with meropenem against NDM-1 producing bacteria.
After construction of the compound library, the synergistic activities of all newly synthesized compounds were tested by combination with meropenem against E. coli BL21 (NDM-1). The most promising compound was selected to test against a panel of clinically isolated strains and confirm its inhibition on NDM-1 by the standard enzymatic assay and the ESI-MS analysis using a purified NDM-1 enzyme.

Results: Most of the pyrrolidine derivatives exhibited low cytotoxicity (MIC > 200 μM) but weak synergistic activity (RF ranged 2 to 16) against the screening strain. Interestingly, we have identified a promising compound Chen45, which demonstrated very potent synergistic activity (RF = 256) against E. coli BL21 (NDM-1) and clinically isolated strains. Enzymatic assay and ESI-MS revealed that it acts by extracting the zinc ions in the active site of NDM-1.

Conclusion: We have efficiently constructed a novel 3,4-disubstituted pyrrolidine derivatives by using a 3+2 cycloaddition approach. The synthetic routes to the target compound library are straightforward and concise with high yield. Through the MIC screening, compound Chen45 with promising synergistic activity was identified. Structure-activity relationship revealed that the bis (pyridin-2-ylmethyl)amine group on R1 of Chen45 is crucial for the NDM-1 inhibition. Compound Chen45 provide a novel scaffold for further development of pyrrolidine-type NDM-1 inhibitors.

Project No.: 14130432

ID-14

Mechanism of substrate recognition and specificity of B2 and B1 subclasses of metallo-β-lactamases: insights into their different spectrums of substrate specificity

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Introduction and Project Objectives: Carbapenems are one of the last line defense agents for Gram-negative bacterial infections, such as those caused by Enterobacteriaceae. Despite the fact that most carbapenems are resistant to extended-spectrum β-lactamase (ESBL), they may be degraded by emerging metallo-β-lactamases (MBLs) such as B1 (VIM-1, NDM-1) and B2 (Sfh-I) MBLs. Crystallographic study sheds light on the mode of antibiotic binding to MBLs, yet mechanisms governing substrate recognition and specificity are largely unclear. This study provides a connection between crystallographic study and functional significance of MBLs, with an emphasis on substrate specificity and catalysis of various β-lactams.

Methods: Structural and mutational analysis were performed on different carbapenemases to understand the exact mechanisms of action of these enzymes.

Results: In NDM-1, L1 loop residues, L59, V67 and W87 were found to be important for the activity of NDM-1, most likely through maintaining the partial folding of the L1 loop or active site conformation through hydrophobic interaction with the R groups of β-lactams. Substitution of L59 by alanine resulted in significant reduction in minimum inhibition concentrations (MICs) to ampicillin and selected for resistance to cephalosporins, whereas substitutions of V67 by alanine exhibited impact on the MICs of carbapenems. K224 and N233 on the L3 loop played an important role in recognition and hydrolysis of substrate. In VIM-1, residues F61, Y67 and W87 in L1 loop were important for the activity of VIM-1, most likely through maintaining the partial folding of the L1 loop or active site conformation via hydrophobic interaction with the R groups of β-lactams or the β-lactam ring. N233 in the L3 loop of VIM-1 also played an important role in both substrate recognition and hydrolysis. In Sfh-I, K224 of L3 loop may interact with the carboxylate oxygen of β-lactam fused ring through hydrogen bonding. Residue, Y244 of Sfh-I also played an important role possibly in stabilization of the conformation of the active site. Structure comparison of B1 and

B2 subclasses of MBLs and biochemical characterization revealed that the broad substrate spectrum of B1 enzymes such as NDM-1 and VIM-1 could be due to the wide active site cavity that accommodates a wide range of β-lactams. A helical structure was identified in the active site of Sfh-I, blocking accommodation of ß-lactams other than carbapenems.

Conclusion: This study provides insight into the development of effective inhibitors of carbapenemases and offers an efficient tactic to investigate the molecular basis of substrate specificity of β-lactamases.

Project No.: 14130422

ID-15
The Consumption of Antimicrobials in Hospitalized Patients in Hong Kong

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Introduction and Project Objectives: Antibiotics have been widely used to treat infections for over decades. It also leads to a pressing global concern about the development of antibiotic resistance in bacteria potentially induced by selective pressure from exposure to antibiotics and other mechanisms. Understanding the prescribing patterns not only provides valuable data to study the relationship between antibiotic use and resistance but also help evaluate adherence to guidelines and policies in the clinical setting. We therefore would like to make use of publicly available data to investigate the prescription of antibiotics for all inpatients admitted into Hong Kong public hospitals from 2000 to 2015.

Methods: Inpatient antibiotic prescribing data in public hospitals from 2000 to 2015 were acquired from the Hong Kong Hospital Authority. Aggregated data on the overall number of hospital admissions stratified by age were also acquired. We obtained the mid-year population data from the Hong Kong Census and Statistical Department as the denominator of the incidences. Interrupted time series analysis was conducted to assess the effect of change in prescribing guideline on the actual prescribing.

Results: A total of 35,535,506 antibiotic prescriptions were dispensed among 2,161,360 patients from 2000 to 2015. The most frequently dispensed antibiotics are amoxicillin–clavulanate, cefuroxime, metronidazole, piperacillin-tazobactam, levofloxacin, cloxacillin, ampicillin, cefoperazone, ceftriaxone, and ciprofloxacin, which accounted for 68% of the total number of antibiotics dispensed. There were 27.6% of admissions to the public hospitals that had at least one antibiotic dispensed in 2000. The trend gradually increased to 31.5% in 2015. The incidence of overall antibiotics dispensing remained stable over the study period. However, there was an increasing trend among drug classes including benzylpenicillin and phenoxymethylpenicillin, broad-spectrum penicillins, carbapenems, and tetracyclines among patient aged 85 or above. For the interrupted time series analysis, the change in tetracyclines prescribing coincided with the change in the prescribing guideline in November 2012.

Conclusion: The trend of overall antibiotic use in Hong Kong inpatients admitted to public hospitals is increasing over the study period. Increasing trends were observed among all patients especially in patients aged 85 years and above. A worrying increase in prescriptions was observed in carbapenems and polymyxins which are the "last resort" drug classes. Our study also implied that the change in local antibiotic prescribing guidelines seemed to have an immediate effect on the practice of prescription.

Project No.: HKS-16-E10

ID-16
Intra-season waning of influenza vaccination effectiveness in children

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Introduction: The protection conferred by influenza vaccination is generally thought to last less than a year, necessitating annual re-vaccination. However, the speed with which influenza vaccine effectiveness (VE) might decline during a year is unknown. This is of particular importance for locations with year-round influenza activity. In this test-negative study, we aimed to assess how influenza VE changes by time intervals between vaccination and admission to hospital, taking advantage of almost year-round circulation of influenza in Hong Kong.

Methods: We analyzed influenza VE in children aged 6 months to 17 years for over 5 consecutive years from September 2012 through August 2017 who were admitted to general wards in 4 public hospitals in Hong Kong with a fever (≥38°C) and any respiratory symptom, such as runny nose, cough, or sore throat. We compared characteristics of positive influenza viruses cases and negative controls examined how VE changed by time between vaccination and hospitalisation.

Results: We analysed data on 15,695 hospitalized children. Among them, the majority of vaccinations occurred in the final quarter of each year. Influenza admissions occurred year-round with peaks in January through March in most years and a large summer peak in 2016. We estimated that VE against influenza A or B decreased from 79% (95% CI: 64%-88%) for children vaccinated within 0.5 to 2 months, to 60% (95% CI: 46%-71%) within 2 to 4 months, to 57% (95% CI: 39%-70%) within 4-6 months of vaccination, and to 45% (95% CI: 22%-61%) within 6-9 months of vaccination. In separate analyses by type/subtype, we estimated that VE declined by 2-5 percentage points per month.

Conclusion: We observed clear evidence of reductions in VE during the 9 months after vaccination in children. Our findings confirm the importance of annual vaccination in children. Influenza vaccines that provide broader and longer-lasting protection are needed to provide year-round protection in regions with irregular influenza seasonality or prolonged periods of influenza activity.

Project No.: HKS-18-E18

ID-17

Development of system using routine surveillance data to estimate influenza vaccine effectiveness for hospitalised Hong Kong children

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Introduction and Project Objectives: Influenza is a leading cause of paediatric hospitalisation in Hong Kong. Discharge diagnoses and laboratory results for patients admitted to public hospitals are being collected and saved in the Clinical Management System. However, the influenza vaccination status is not routinely collected. This study aimed to determine if data on influenza vaccination status could be routinely collected and used with laboratory data to monitor influenza vaccine effectiveness in hospitalised Hong Kong children aged from 6 months to below 6 years.

Methods: This was a 'test-negative' case-control study conducted over two summer and one winter influenza seasons in five public hospitals during 2015 and 2016. Case-patients were respiratory-associated admissions with nasopharyngeal aspirate or nasopharyngeal swab specimens obtained during the first 48 hours of hospitalisation that tested positive for influenza A or B. Control-patients were those with specimens that tested negative for both influenza A and B. Vaccine effectiveness for administration of full or partial series of influenza vaccination was calculated as 1 minus the odds ratio for influenza vaccination history for case-patients versus control-patients. An influenza immunisation status form was designed and tested for reliability to obtain current influenza vaccination status of hospitalised children by ward staff.

Results: 2900 eligible subjects with influenza vaccination status available were included in the vaccine effectiveness analysis. During the period June 2015 to November 2016, influenza vaccine effectiveness for preventing influenza A or B hospitalisation in Hong Kong children aged from 6 months to below 6 years was 68% (96% confidence interval [CI]: 55%, 77%) from unconditional analyses and 64% (95% CI: 46%, 75%) from conditional analyses. 1239 influenza immunisation status forms were filled in by ward staff, of which 95% agreed with the combined vaccination status of immunisation records and guardians' self-report to research staff at interview, and had a Cohen's Kappa coefficient of 0.70 (moderate agreement).

Conclusion: Influenza vaccine is effective in preventing influenza A or B associated hospitalisation in Hong Kong children aged from 6 months to below 6 years. Implementation of the influenza immunisation status form in all paediatric wards of public hospitals with centralising the data in the Clinical Management System could monitor influenza vaccine effectiveness routinely.

Project No.: 14131442

ID-18

Chinese adults' preference for influenza vaccination: a discrete choice experiment

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Introduction and Project Objectives: To measure the relative effects of altering different factors (attributes) in determining influenza vaccination choice in Hong Kong adults and examine whether manipulating risk priming changes the relative influence of influenza or vaccine risk on vaccination choice.

Methods: A tablet-assisted discrete choice experiment (DCE) survey was developed and adults (aged ≥18 years) were recruited from the public venues and non-government organizations distributed in the 18 districts of Hong Kong using convenience sampling. Subjects were randomly allocated to either the control condition in which they viewed a neutral video, or one of the three health risk-priming conditions: viewing a video about influenza risk, vaccine risk or air pollution risk, prior to the DCE. Mixed logit models were conducted to evaluate relative preference weights for seven pre-determined attributes (i.e. infection probability, case-fatality ratio (CFR) of influenza, vaccine safety, vaccine efficacy, vaccination cost, community vaccination coverage rate (CVCR) and doctors' advice) for vaccination choice under each condition.

Results: A total of 800 participants completed the DCE, with ~200 being allocated to each of the four conditions. Across all four conditions, influenza CFR compared with infection probability, Vaccine Efficacy compared with external cues (CVCR and doctors' advice) and external cues compared with vaccine safety had a greater effect on vaccination choice. Vaccine-relevant attributes (vaccine safety and efficacy) were more important for a mild pandemic but less important for a severe pandemic than disease-relevant attributes (infection probability and CFR)in determining vaccination choice regardless of priming condition. In addition, regardless of priming condition, vaccination preference increased when a CVCR changed incrementally from 5% to 60% but declined thereafter when the CVCR reached 80%. Compared with Control participants, a CVCR increased by 80% had a smaller and insignificant effect for participants primed by intervention risk. The importance of disease risk increased relative to vaccine risk increased following influenza risk priming.

Conclusion: It is important to emphasize information about disease risk particularly that about consequences of infection during a severe pandemic but information about vaccine efficacy during a relatively mild pandemic, for promoting vaccination uptake. Doctors' recommendation and social cues indicating an increasing uptake rate in the community can compensate the negative effect of perceived uncertainty vaccine safety in influencing vaccination choice. However, free riding likely occur as people notice a sufficient high vaccination coverage in the community.

Project No.: 14130942

ID-19

Vaccination Scheme Development to Stimulate Both B and T Cell Dependent Heterosubtypic Protection Against Influenza A Virus in Mice

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Introduction and Project Objectives: The limited protection of current commercial vaccines necessitates the investigation of novel vaccine strategies for unpredictable outbreaks. To investigate the feasibility of using vaccines derived from Group 1 influenza A virus to induce broadly cross-reactive immune responses against multiple influenza subtypes, we tested a panel of sequential 4-dose immunization regimens in mice.

Methods: Mice were treated with inactivated (seasonal H1N1/A/Brisbane/59/07, pandemic H1N1/A/California/09/2009, and highly pathogenic H5N1/A/VN/1203/04) and vaccinia virus-based H5N1 live-attenuated (Wyeth/IL-15/5Flu) vaccines in different combinations. Wyeth/IL-15/5Flu is a novel pentavalent vaccine, expressing HA, NA and NP proteins from H5N1/A/Vietnam/1203/2004, M1 and M2 proteins from H5N1/A/CK/Indonesia/PA/2003 virus, and human IL-15 as a molecular adjuvant. Mice were then challenged by a lethal dose of either Group 1 (H1N1/A/Puerto Rico/8/1934) or Group 2 (mouse-adapted H3N2 / A / Hong Kong / 1 / 1968, HAPI H7N7/A/Netherlands/219/2003) influenza virus.

Results: All studied sequential 4-dose vaccinations could induce some degrees of heterosubtypic protection in mice. Amongst all these regimens, the combined use of inactivated and live-attenuated vaccines could achieve the best heterologous protection.

Conclusion: The results of immune profiles from such regimens provide related information about synergistic effect of combining different vaccine platforms for universal vaccinne development and optimization. Further research will focus on developing alternative vaccine strategies, including the use of different kinds of viral immunogens or adjuvants, for universal protection against influenza.

Project No.: 14130962

ID-20

Association Between Basal Leukocyte Transcriptome Profile And Symptom Development & Disease Severity After Influenza Virus Infection In Humans

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Introduction and Project Objectives: The variability in individual susceptibility, e.g. host gene expression profile at the time of infection, could be critical in determining disease outcome at the time of the establishment of infection after exposure. The objective of this study was to investigate the association between host leukocyte basal transcriptome profile and influenza symptom development and disease severity.

Methods: We recruited patients with acute respiratory illnesses from outpatient clinics in Hong Kong between 2015-2017, and followed up their household members regardless of symptom presentation to identify secondary transmission. We collected whole blood samples in Tempus tubes from all household members at enrollment, which was typically prior to their illness onset; nasal and throat swabs at enrollment after 3 and 6 days, regardless of illness, for confirmation of influenza virus infection by PCR. Whole blood RNA from exposed symptomatic and asymptomatic contacts who had confirmed influenza virus infection were selected as cases, with matched influenza negative controls, were selected for microarray analysis using the Affymetrix GeneChip Human Gene 2.0 ST array. Differential expressed genes (DEGs) between groups were identified by ANOVA, and subjected to Gene Ontology (GO), pathway, transcription factor binding site (TFBS)over-representation analyses. Molecular interaction network of genes of interest was generated and visualized using Cytoscape software.

Results: We screened 553 index patients in the outpatient clinics, among which 67 (12%) were subsequently confirmed to have influenza virus infection at the time of recruitment by PCR. These 67 index patients had 120 exposed household contacts who had provided blood samples for microarray analysis. 19 (16%) of the exposed household contacts had confirmed influenza virus infection (cases) during follow-up but not at enrollment. From 12 cases and 24 matched controls, we identified DEGs in influenza-infected vs non-infected exposed contacts which clustered into pathways related to G protein coupled receptor (GPCR) signaling pathway and Cell cycle. Among those infected samples, genes such as SCARNA5, KIR2D2K and ZNFs were most differentially expressed, and innate immune response was identified as the most significant biological process in symptomatic vs asymptomatic infected contacts. Transcription factor binding site (TFBS) analysis suggested that POU3F2 and FOXJ2 are important transcription factors that regulate the gene expression in symptomatic vs asymptomatic infected contacts.

Conclusion: Our data revealed that genes related to innate immune response were downregulated in the basal gene expression profile of symptomatic vsasymptomatic infected contacts, highlighting the importance of innate immunity in controlling the establishment of influenza virus infection and development of illness after infection.

Project No.: 14130662

ID-21

Genetic Susceptibility of Hong Kong Ethnic Chinese to Severe Influenza and RSV Infections

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Introduction and Project Objectives: Influenza virus and respiratory syncytial virus (RSV) are major causes of severe respiratory infections worldwide. Accumulating data have suggested that genetic variation in host immunity related genes may play an

important role in determining susceptibility to severe influenza or RSV diseases. The objective of this study was to investigate relationship of host genetic susceptibility to severe influenza and RSV infections among Hong Kong ethnic Chinese.

Methods: This is a two-year prospective study and laboratory investigation. Adults (aged ≥18 years) hospitalized for influenza A or RSV infections in two acute-care, general public hospitals in Hong Kong were recruited. Single nucleotide polymorphisms (SNPs) of 4 genes related to innate immunity were determined by Sanger sequencing on host DNA isolated from respiratory samples: IFITM3 (rs12252), CD55 (rs2564978), TLR3 (rs5743313), and TLR4 (rs4986790 and rs4986791). Univariate and multivariate analyses were performed to study potential correlation of SNPs with clinical characteristics.

Results: A total of 563 in-patients laboratory-confirmed with either influenza A virus or RSV infections between December 2014 and February 2017 were studied. Fatal cases (<3%) and risky SNP genotypes were less frequent than in our earlier study. In influenza A, there was a tendency of enrichment of IFITM3 genotype CC among those who developed pneumonia. Extensive effort was made to analyze potential correction of SNP genotype with an array of clinical parameters, including duration of hospitalization, requirement for oxygen or mechanical ventilation, ICU admission, and fatal outcome. However, no significant findings were observed.

Conclusion: We found no association of SNPs in IFITM3, CD55, and TLR3 with severe influenza A and RSV infections during mild seasons in ethnic Hong Kong Chinese. Host genetic factors may have strongest effect during initial phase of new virus emergence in a relatively immune naïve population. This should be considered in designing study to investigate genetic host factors with influenza severity.

Project No.: 14130202

ID-22
Host Inflammatory Responses in Adults with Severe Respiratory Syncytial Virus Lower Respiratory Tract Infections

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Introduction and Project Objectives: We aim to study the host inflammatory responses in adults hospitalized for respiratory syncytial virus (RSV) infections.

Methods: We performed a prospective, observational study in an acute-care general public hospital in Hong Kong. Adults hospitalized for laboratory-confirmed RSV infections are studied. Clinical data and peripheral blood samples (for cytokine/chemokine assays) are prospectively collected for study. Peripheral blood samples (15 ml each) are collected at acute phase for cytokine/chemokine studies, including interleukin (IL)-1b, IL-6, IL-10, IL-12p70, TNF-α, CXCL8/IL-8, CXCL9/MIG, CXCL10/IP-10, CCL2/MCP-1, and CCL5/RANTES using cytometric bead array (CBA), and sTNFR-1, MIP-1α, interferon(IFN)-γ and IFN-α, interleukin(IL)-17A and IL-18, and Thymic Stromal Lymphopoietin (TSLP) using ELISA. Correlations between baseline cytokine levels and disease severity and outcomes were studied.

Results: Fifty-seven patients with documented RSV infection were recruited. 34 (59.6%) were male, median (interquartile range) age was 75 (64.5-83) years, 56 (98.2%) were Chinese, and 11 (19.3%) were nursing home residents. 47 (82.5%) patients had one or more major comorbidities (including chronic pulmonary disease, cardiovascular disease, chronic kidney disease, diabetes, and malignancy). Fifty (87.7%) patients had one or more complications, including pneumonia (47.7%) and cardiovascular (15.8%) complications. 31 (54.4%) required supplementary oxygen. Their median length of stay in acute hospital was 7 (IQR 4.5-13) days, with 38 (66.7%) staying for 5 or more days in hospital. Two (3.5%) required intensive care, and 6 (10.5%) patients died.
Higher IL-6, IL-8 and CCL2/MCP-1 were associated with in-hospital death on univariate analysis, and IL-6 was associated with death, after adjusting for age and comorbidities. Higher IL-6, CCL2/MCP-1 and IL-18 were associated with death/intensive care on univariate analysis, and IL-18 was associated with death/intensive care after adjusting for age and comorbidities, while IL-6 and CCL2/MCP-1 were associated with a trend towards death/intensive care. Higher IL-8 and lower IFN-α predicted length of stay in hospital greater than 5 days, after adjustment by age and comorbidities.

Conclusion: Our data show that inflammatory cytokines may play a role in immunopathogenesis and determine disease severity and outcomes in adults hospitalized with RSV infection.

Project No.: CU-16-A2

ID-23
A systematic review and meta-analysis of estimates of excess mortality associated with influenza

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Introduction and Project Objectives: Influenza viruses are associated with substantial global burden of morbidity and mortality every year. It is important to assess the mortality impact of influenza to support planning and resource allocation. However estimates of influenza-associated mortality often vary between studies due to differences in study settings, methods and measurement of outcomes. We aim to identify published population-based studies of the impact of influenza virus infections on mortality in defined populations, to report the estimates of the influenza-associated mortality and to examine methodological factors and their influence on reported estimates.

Methods: We searched PubMed and Embase for published journal articles using pre-defined searching terms. Eligible studies were selected for an extraction of population-based estimates of the influenza-associated mortality from either of the following four causes of death: pneumonia and influenza, respiratory diseases, respiratory and cardiovascular diseases and all causes. Methodological details were also retrieved from all the selected studies. Estimates of the influenza-associated excess mortality were

reported by age group and cause of death. Statistical heterogeneity in estimates reported by different studies was assessed using Cochran's Q test and the I2 statistic. A meta-regression was also carried out to identify factors potentially associated with variations observed in the reported age-specific estimates of the influenza-associated mortality.

Results: Based on the estimates reported in 103 studies, we identified that the influenza-associated excess mortality increased with age and ranged widely from -0.3-1.3 and 0.6-8.3 respiratory deaths per 100,000 population for children and adults to 4-119 respiratory deaths per 100,000 population for older adults. Meta-regression analysis identified that the observed variation in estimates was associated with the study approaches. The multiplier methods tended to produce lower estimates, while Serfling-type models were associated with higher estimates compared with other methods.

Conclusion: Substantial variation in the published estimates of the influenza-associated excess mortality could be partially attributable to methodological differences in the studies. Standardization of the methods may allow effective comparisons of estimates of the influenza-associated disease burden across studies in the future.

Project No.: HKS-16-E08

ID-24
Real-time estimation of the impact of influenza viruses

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Introduction and Project Objectives: Statistical models are very useful in estimation of the influenza-associated excess mortality that is often underestimated by laboratory-confirmed influenza deaths reported from healthcare facilities due to various reasons. Timely and reliable estimates of the impact of influenza virus infection are important for assessment and implementation of control measures during an ongoing influenza epidemic. However, methodology for real-time estimation of impact remains limited because of the delay in mortality data. The objectives of our proposal study are: (1) to estimate the influenza-associated excess mortality risk by virus type and subtype in each influenza season in Hong Kong in 2001-2016; (2) to validate the model performance of real-time estimation of excess mortality risk on data from influenza seasons in Hong Kong in 2011-16; and (3) to estimate minimum number of cases required for reliable real-time estimation of the influenza-associated excess mortality risk.

Methods: We compared different approaches for real-time estimation of the influenza-associated excess mortality between 2001 and 2016 in Hong Kong, including linear regression model (Method 1), Poisson regression model with (2) log links (Method 2) and (3) identity links (Method 3), and time series models (Method 4), fitted to historical mortality and influenza surveillance data. The excess mortality was estimated by subtracting the predicted mortality estimated from the fitted model setting influenza activity to zero from the predicted mortality from the model based on the observed influenza activity.

Results: We calibrated the alternative approaches to the estimation of the influenza-associated excess mortality by types/subtypes in each influenza epidemics between 2001 and 2016 in Hong Kong, and validated a new approach for the real-time estimation of the influenza-associated excess mortality. With the new approach, we could predict that there were approximately 351 (95% confidence interval: 120, 606) influenza-associated excess all-cause deaths and 192 (95% confidence interval: 64, 311) influenza-associated excess respiratory deaths in Hong Kong, compared to 261 reported laboratory-confirmed deaths, during the winter of 2017/2018.

Conclusion: We explored new approaches for real-time estimation of the mortality impact of influenza virus infections. These appear to provide robust preliminary estimates of the impact of influenza, and complement surveillance data on laboratory-confirmed deaths. These results improve our understanding of the impact of influenza virus infections and provide a practical approach to reliably estimate the impact of influenza in time during an on-going epidemic.

Project No.: 16150782

ID-25
Understanding Alternate Immune Correlates of Protection in Household Transmission of Influenza

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Introduction and Project Objectives: Defining immune functions which limit influenza infection will enable the design of next generation influenza vaccines. HAI antibodies are widely accepted as the standard immune correlate of protection from influenza infection, yet individuals with high HAI titers can become infected. Therefore we postulate that antibody function contributes to protection from influenza infection. Cross reactive antibodies which engage NK cells, to mediate Antibody Dependent Cellular cytotoxicity (ADCC), have been shown to be increased in older adults accounting for reduced H1N1 pandemic infection and risk of infection in a human challenge study, whilst also showing evidence for cross reactivity to avian viruses. Furthermore, human experimental challenge studies have shown a correlation with higher baseline ADCC titers and reduced risk of infection. Data on the protective role these cross reactive responses play in acquisition of infection and the severity of infection from baseline levels prior to infection is scarce.

Methods: Our study reports on the context of household acquired infection in the Hong Kong community, and baseline PBMC and serum samples were collected from households reporting an index case of infection. Contacts of infected subjects were recruited and monitored for acquisition of infection. Antibody function from index and contacts who became infected during the study from baseline was assessed by activation of NK cells, isotype and avidity by ELISA assays.

Results: The baseline ADCC influenza-specific responses of uninfected contacts was found to be higher in magnitude and avidity to multiple influenza proteins than infected contacts, indicating a protective role of ADCC antibodies in the acquisition of influenza infection. Higher baseline HA-stem and NP specific IgG antibodies

also correlated with reduced infection irrespective of ADCC function. Recent infection boosted specific ADCC responses, but not HA-stem or cross-reactive H7 responses.

Conclusion: This study provides rare data on the context of community acquired influenza infection and the protective threshold of baseline immune responses for ADCC antibodies.

Project No.: 14130672

ID-26

Role of influenza virus surface glycoproteins in virus-induced cell stress and inflammation

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Introduction and Project Objectives: Human infections by avian influenza A (H5N1) viruses are characterized by respiratory failure and high mortality. Fatal outcomes were associated with prolonged hyper-cytokinemia accompanied with high viral load, suggesting induction of aberrant and yet ineffective innate responses after H5N1 infections. We investigated the role of surface glycoproteins derived from H5N1 and H1N1 influenza viruses in inducing NLRP3 inflammasome activation and cellular distress.

Methods: Murine bone marrow derived dendritic cells (BMDC) derived from wild-type and NLRP3 knockout mice were infected with recombinant A/PR/8/34 virus carrying the HA and NA surface glycoproteins derived from the H1N1 A/HK/54/98 (HK5498) or H5N1A/VN/1203/04 (VN1203) viruses to monitor ER stress response and pro-inflammatory cytokine induction. ER stress inhibitors TUDCA and 4-PBA were applied to reduce ER stress associated inflammatory response in BMDC and in mice upon infection with VN1203 virus.

Results: We observed differential NLRP3 inflammasome activation triggered by HK5498 and VN1203 viruses in BMDC, with a significantly higher IL-1β induced by the VN1203 virus. Multiple host damage signals, including TNF-α signaling, mitochondrial ROS, and ER stress may serve as Signal 2 and trigger NLRP3 inflammasome activation. Using recombinant HK5498 and VN1203 viruses that only differed by the surface glycoproteins, we observed that the VN1203 virus specifically activated the ATF6 branch of the ER stress response when compared to the HK5498 virus. Over-expression of H5N1 surface glycoproteins alone was sufficient to promote NLPR3 inflammasome activation in HEK293T cells, leading to increased release of IL-1β. The use of ER stress inhibitors TUDCA and 4-PBA showed promising effect in reducing ER stress, NLPR3 inflammasome activation, influenza virus replication, and pro-inflammatory cytokine induction in vitro. However, protective effect was not observed in mice when 4-PBA was administered alone prior to lethal challenge with VN1203 virus.

Conclusion: Our results suggest that H5N1 surface glycoprotein-induced ER stress may trigger NLRP3 inflammasome activation and act as an additional mechanism for H5N1 immunopathogenesis. The ER stress inhibitors with immunomodulatory effect may be considered as an adjunctive therapy in combination with influenza antivirals.

Project No.: 14131072

ID-27

Characterization of Influenza A Viruses with Polymorphism in PB2 Residues 701 and 702

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Introduction and Project Objectives: The 701 and 702 positions of influenza PB2 polymerase subunit are previously shown to have roles in host range. However, limited polymorphisms at these two residues are identified in natural isolates, thereby limiting the study of their role in the polymerase. In this study, we aim to investigate the potential genetic polymorphism of the PB2-701 and 702 residues and their roles in viral properties.

Methods: Site-directed random mutagenesis at residues 701 and 702 of the PB2 gene of the influenza virus in mammalian and avian cells were performed. Mutant viruses were rescued by reverse genetics. The polymerase activity, viral replication and pathogenicity of the mutant viruses generated were characterized.

Results: Thirty-one viable viruses were generated by random mutagenesis and reverse genetics, indicating that the PB2-701 and 702 positions can tolerate a wide range of amino acids. These mutants demonstrated varying polymerase activities and viral replication rates in mammalian and avian cells. Notably, some mutants displayed enhanced polymerase activity, yet their replication kinetics were comparable to the wild-type virus. Surface electrostatic charge predication on the PB2 structural model revealed that the viral polymerase activity in mammalian cells generally increases as this region becomes more positively charged. One of the mutants (701A/702E) showed much reduced pathogenicity in mice while others had a pathogenicity similar to the wild-type level. Distinct tissue tropisms of the PB2-701 and 702 mutants were observed in infected chicken embryos.

Conclusion: Overall, this study demonstrates that the PB2-701 and 702 region has a high degree of sequence plasticity and sequence changes in this region can alter virus phenotypes in vitro and in vivo.

Project No.: 14130622

ID-28

The pathogenic role of the adaptation in the polymerase basic 2 protein of the new identified duck isolated H7N9 lineage in mammalian hosts

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Introduction and Project Objectives: We have recently identified a cluster of H7N9 viruses in duck which were circulated in China before the outbreak and have distinct genetic background to the human H7N9 lineage. We hypothesize that the duck isolated H7N9 lineage may cause pathogenesis in mammalian hosts if acquire specific adaptive mutation at PB2 (Q591, E627K or D701N).

Methods: To investigate the role of the amino acid substitutions Q591K, E627K or D701N in the PB2 gene segment of the duck isolated H7N9 lineage in relation to the 1a) polymerase activity 1b) virus

replication in human cells 2) the pathogenicity of these recombinant viruses in mice infection model.

Results: We found that the polymerase activities were significantly enhanced in the background of duck H7N9 after acquiring Q591K, E627K or D701N at the PB2 gene. The Q591K and E627K can increase the replication compared to the wild type virus at 37oC. However, the duck H7N9 virus from this cluster only caused significant pathogenesis in mice after it acquired PB2 mutation with K at 627 but not K at 591 or N at 701. We also found that the inductions of pro-inflammatory cytokines including TNF-α, IP-10, MCP-1 and MIP-1α were associated to the higher disease severity during the infection.

Conclusion: We concluded that the mammalian adaptation occurs at the PB2 gene of the duck H7N9 viruses can also enhance the pathogenicity in mammalian host when compared to the human H7N9 isolates. Our results thus showed that the genetic background of the H7N9 virus does not determine the virulence caused by the PB2 mutation.

Project No.: 14131042

ID-29
Molecular determinants for the interspecies transmission of an avian H6N1 virus that caused the human infection in Taiwan

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Introduction and Project Objectives: Interspecies transmission of avian influenza viruses (AIVs) to mammals poses a continuous threat to agriculture and public health. As one of the most frequently detected subtypes of AIVs, H6 was mostly detected in aquatic birds. The H6N1 lineage that established in chickens in Taiwan since 1990s has caused the first human case of H6 subtype in 2013, followed by another virus isolated from a dog in 2014. How did this virus develop the mammalian infectivity remains a question.

Objectives: 1) To evaluate the virus infectivity and transmissibility of the H6N1 virus that caused the first human case of H6 subtype; 2) To identify the molecular signatures that may be associated with the adaptation of the Taiwan H6N1 virus in chickens; and 3) To test whether these signatures contribute to the increased virus infectivity or transmissibility in humans (using ferrets as a proxy for humans).

Methods: Based on genomic sequences of all available Taiwan H6 viruses, three strains, A/Chicken/Taiwan/CF19/2009 (Ck/09), A/Chicken/Taiwan/2267/2012 (Ck/12) and A/Taiwan/2/2013 (Hu/13), were selected to represent viruses from the different evolutionary stages. Reverse genetic technique was used to reconstruct a series of recombinant viruses and infection was conducted to test how the different gene segments, mutations, or combinations of mutations affect the virus infectivity and transmissibility in the ferret model.

Results: Inoculation of each of the three viruses showed that Hu/13 exhibited the best virus replication and transmission in ferrets, followed by Ck/12. Ck/09 could not be transmitted from ferret to ferret, while both Ck/12 and Hu/13 were transmissible via physical contact. Hu/13 even showed limited airborne transmissibility in the ferrets. Introduction of the polymerase basic 1 (PB1) or 2 (PB2) genes or the Ribonucleoprotein (RNP) complex from Hu/13 virus significantly increased viral infectivity and transmissibility in the genetic context of Ck/09 virus. PB2-E156A and I491T mutations increased the viral RNP activity, virus replication and transmissibility of Ck/09, while PB2-A156E/T491I reduced these in Hu/13. Mutations at positions 251, 265, 292, 293 or 648 on PB2 could enhance the phenotypic changes caused by the E156A and I491T mutations.

Conclusion: The findings generated here provided insights into the genetic basis for the interspecies transmission of Taiwan H6N1 avian influenza virus to mammals, and thus will help to suggest appropriate strategies for influenza surveillance, disease control and pandemic preparedness. The molecular determinants for the viral virulence and transmissibility may be used as potential targets for drug development.

Project No.: 14131402

ID-30
Kinetics of Hemagglutinin Stalk Binding Neutralizing Antibody Responses During Natural Influenza Infection

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Introduction and Project Objectives: Influenza is a major public health concern in Hong Kong. Influenza hemagglutinin(HA) stalk binding antibodies provide broad protection against a range of genetically diverse influenza strains. During the 2009 pandemic, a novel H1N1 influenza subtype with an antigenic shift emerged as the predominantly circulating virus and our previous data indicated that the breath of protection increased in individuals who acquired 2009 pandemic infection. One possibility for this observation is the boosting of broadly reactive stalk antibody responses by the 2009 pandemic virus. Therefore, here we utilized this opportunity to investigate the kinetics of HA stalk antibody during 2009 pandemic infection in Hong Kong.

Methods: We implemented the novel stalk antibody detection assay platforms for the first time in Hong Kong. These responses were measured in a well-characterized cohort of ethnic Chinese individuals who had pandemic and seasonal influenza infections during 2009.

Results: We observed a significant increase in the stalk antibody responses among those who had 2009 pandemic seroconversion (33.7%), as opposed to those who had seasonal H1N1(10.7%) or H3N2(5.5%) seroconversions and followed a similar trend to the stalk responses of PCR confirmed individuals. When compared to HA head binding responses the stalk responses appeared to be significantly lower. The stalk antibody responses were significantly higher in the 61-70 age decade ($p<0.001$) compared to all the other age groups. The longitudinal stalk titers albeit at low levels appear to be relatively more stable compared to HA head responses.

Conclusion: The prevalence and magnitude of stalk antibody titers increased significantly after the 2009 pandemic infection. However, the naturally induced stalk antibody titers remained relatively low compared to HAI titers. Therefore, novel vaccination strategies are required to boost these highly cross-protective stalk antibody responses in order to increase the heterosubtypic protection in the population. Additionally, the stalk antibody detection assays we implemented here could be utilized to measure broadly protective antibody responses in the Hong Kong population during natural infection or after vaccination.

Project No.: 15141212

ID-31
Targeting Deubiquitylases at the Intersection of Host Immunity and Viral Pathogenesis

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Introduction and Project Objectives: Deubiquitylases (DUBs) regulate critical signaling pathways at the intersection of host innate immunity and viral pathogenesis. Unlike some of the larger viruses, influenza virus itself does not come equipped with its own genome encoded DUB; however, total cellular ubiquitylation profile is altered during IAV infection depending on its pathogenicity. Using a ubiquitin C-terminal electrophile we profiled DUBs that function during influenza A virus (IAV) infections, and isolated several that regulate antiviral responses and viral restriction. The specific objectives of this proposal was to characterise the role of these DUBs by genetic manipulation and pharmacological inhibition, and determine outcomes in viral infection and host immune responses.

Methods: We employed a ubiquitin modified with a vinylmethylester group at its C-terminus (Ub-vme) to isolate DUBs from IAV-infected cells. Mock and H1N1 pandemic IAV-infected primary lung epithelial or A549 cells were permeabilised using perfringolysin O , and DUBs activated during infection were isolated with Ub-vme, either carrying a TAMRA or HA-tag. Ub-vme reactive material from control and infected cells were resolved by SDS-PAGE and visualised by fluorescent scanning or enriched on anti-HA beads. Potential candidates were identified by trypsin digestion, mass spectrometry and spectral counting on immunoprecipitated material. Further validation of hits was performed on lysates from mock and virus-infected or IFN-I treated samples, followed by CRISPR/Cas9 mediated genome editing to generate knock-out cells, and finally define their mechanism by in vitro reconstitution.

Results: We identified OTUB1 as a key regulator of RIG-I dependent antiviral immunity. OTUB1 was interferon-inducible, and interacted with RIG-I, viral PB2 and NS1. Upon infection, OTUB1 relocalised from the nucleus to mitochondrial membranes, and stabilised the RIG-I signaling complex in a coordinated manner via hydrolysis of K48 polyubiquitin chains and by forming a repressive complex with UBCH5c. Using a reconstituted system composed of in vitro translated [35S]IRF3, purified RIG-I, mitochondrial membranes and cytosol expressing OTUB1 variants, we recapitulated the mechanism of OTUB1-dependent RIG-I activation. This layer of regulation in the RIG-I signaling cascade was antagonized by proteasomal degradation of OTUB1, triggered by a wide range of IAV NS1 proteins.

Conclusion: This study underscores the intricate and complex dynamic that deubiquitylases afford in the host-pathogen arms race. Characterisation of DUBs that are induced upon infection with significant consequences on virus replication and immune responses offer promising targets for developing therapeutics against infection and spread.

Project No.: 14131102

ID-32
Tropism of the novel human betacoronavirus lineage C virus in human ex vivo and in vitro cultures, as an assessment of its potential transmissibility and pathogenesis in humans

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Introduction and Project Objectives: Middle East respiratory syndrome coronavirus (MERS-CoV) is a zoonotic infection causing severe viral pneumonia, with index cases having resided in or recently travelled to the Arabian Peninsula, and is a global concern for public health. Although MERSCoV infection is ubiquitous in dromedaries across Africa and in the Arabian Peninsula, zoonotic disease appears confined to the Arabian Peninsula. Phenotypic characterization of camel MERS-CoVs is limited. In this study we aimed to compare MERS-CoV isolates from dromedaries in Saudi Arabia, Africa (Egypt) and Africa with a prototype human MERS-CoV to assess virus replication competence and cell tropism in the human respiratory tract.

Methods: We characterized MERS-CoVs from dromedaries in Saudi Arabia and Egypt and compared them with a human MERS-CoV reference strain. We assessed viral replication kinetics and competence in Vero-E6 cells, tissue tropism in cultures of exvivo human bronchial and lung tissues, and cytokine and chemokine induction, gene expression, and quantification of viral RNA in Calu-3 cells. We used mock-infected tissue as negative controls for ex-vivo experiments and influenza A H5N1 as a positive control for cytokine and chemokine induction experiments in Calu-3 cells.

Results: We isolated five dromedary strains, two from Saudi Arabia (AH13) and (AH19D), one from Egypt (NRCE-HKU270), one from Burkina Faso (BF785) and one from Nigeria (Nig1657). Both BF785 and Nig1657 have deletions in the accessory gene ORF4b. The human and dromedary MERS-CoV strains from Saudi Arabia and Egypt had similar viral replication competence in Vero-E6 cells and respiratory tropism in ex-vivo cultures of the human respiratory tract, and had similar ability to evade interferon responses in the human-respiratory-tract-derived cell line Calu-3. In contrast, the two isolates from West Africa (BF785 and Nig1657) had lower virus replication competence in ex-vivo cultures of human bronchus and lung.

Conclusion: The similarity of virus tropism and replication competence of human and dromedary MERS-CoV from the Arabian Peninsula, and genetically diverse dromedary viruses from Egypt, in ex-vivo cultures of the human respiratory tract suggests that dromedary viruses from Saudi Arabia and Egypt are probably infectious to human beings. The genetic and phenotypic differences in African viruses may be relevant to zoonotic potential. There is an urgent need to investigate the MERS-CoV at the animal-human interface.

Project No.: 13121132

ID-33
Suppression of PACT-dependent antiviral response by measles virus V protein

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Introduction and Project Objectives: Measles virus is a highly transmissible respiratory pathogen and also associated with the post-infectious encephalitis and subacute sclerosing panencephalitis. During early measles virus infection, host cells elicit innate immunity to combat virus infection and viruses employ different strategies to antagonize the host antiviral response. We previously report that a cellular PACT protein functions as a potent activator of RIG-I, which is a key viral RNA sensor against several RNA viruses. In this proposal, we demonstrated that Measles virus encoded V protein could bind and abolish PACT-mediated RIG-I activation. In addition we identified a Measles virus defective interfering RNA as the PACT/RIG-I agonist. Our study of host-virus interaction could enhance our current knowledge of innate immunity and generate new knowledge for future vaccine improvement.

Methods: We fully characterized the PACT-dependent antiviral immunity in measles virus infection. We utilized wild-type and PACT knockout mouse embryonic fibroblast to examine the innate antiviral immunity in response to wild-type and mutant measles virus. We applied biochemical approach to dissect the molecular mechanism of the inhibition of PACT-dependent antiviral response by V protein. Finally, we investigated the strain-to-strain difference of V protein in the ability of inhibiting PACT-mediated antiviral response.

Results: The exact role of PACT against measles virus infection were defined. The molecular mechanism of the inhibition of PACT-dependent antiviral response by V protein reveals a new layer of knowledge on virus-host interaction, which provides important implications in vaccine development.

Conclusion: Measles virus derived defective interfering RNA is the agonist of PACT/RIG-I, while measles virus encoded V protein antagonizes PACT/RIG-I mediated interferon signaling.

Project No.: 14130862

ID-34
The Transmissibility of Hand Foot and Mouth Disease in Hong Kong

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Introduction and Project Objectives: Hand-foot-and-mouth disease (HFMD) causes a substantial disease burden in Asian regions including Hong Kong, mainly in children below 5 years of age. HFMD has a clear seasonal pattern in temperate regions with annual peaks in the summer, while in tropical and subtropical regions including Hong Kong, multiple peaks may occur within a year. In the literature, there were mixed results on the associations between HFMD incidence and driving factors, and limited studies examined their associations with transmissibility.

Methods: In this study, we estimated the transmissibility of HFMD over time and examined potential associated factors in Hong Kong. Specfically, we used a likelihood-based procedure to estimate time-dependent effective reproduction number (Rt), based on weekly number of HFMD-associated hospitalizations from 2010 to 2014. The associations between the estimated Rt versus between-year effects, depletion of susceptibles, absolute humidity and school holidays were examined using linear regression.

Results: We observed a main summer peak in HFMD-associated hospitalizations from May to August every year, followed by a milder autumn wave in around September to October. The HFMD epidemics usually peaked before the start of the summer holidays. Absolute humidity was high and stable during summer, from May and August. The estimated effective reproduction number (Rt) usually surpassed 1 during the period around early January to late March. Rt usually started increasing between early spring and summer and peaked in around April to May at about 1.2–1.3. Rt mostly remained above 1 for about 4 to 5 months from spring to mid-summer. Rt usually rebounded between August and October in autumn, then fell below 1 for most of the time during winter.
We assessed the effect of depletion of susceptible, between-year effects, absolute humidity and school holidays on Rt. We found a negative association between Rt and depletion of susceptibles, with the estimated coefficients ranging from -0.14 to -0.03 in 2010-2014 in the linear regression model. The variation in transmissibility was found to be mainly affected by depletion of susceptibles (explained 19% of the variation), followed by between-year effects (13%). The estimated effects for absolute humidity and school holidays were found to be insignificant.

Conclusion: Overall, the transmissibility of HFMD was moderate in Hong Kong. The main driver of the HFMD epidemics is the depletion of susceptibles, while meteorological factors, including humidity, and school vacations appeared to have limited impact on the transmission of HFMD in Hong Kong.

Project No.: HKS-16-E07

ID-35
The Clinical Severity Profile and Subclinical Infections of Enterovirus 71 in Children

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Introduction and Project Objectives: Hand-foot-and-mouth disease (HFMD) causes a substantial disease burden in Asian regions including Hong Kong, mainly in children below 5 years of age. Enterovirus 71 (EV-A71), coxsackievirus A16 (CV-A16) and the newly emerging coxsackievirus A6 (CV-A6) are the most common enterovirus serotypes causing HFMD in Hong Kong. However, EV-A71 is of particular interest as it is more likely to lead to severe outcomes including neurological complications.
Only a small proportion of EV-A71 infections lead to severe disease. A larger proportion was mild or even subclinical cases. For EV-A71, the clinical severity pyramid has not been characterized. The proposed objective of this study is to characterize the risks of subclinical and clinical infections, and associated severe outcomes based on published evidence.

Methods: We obtained data on EV-A71 associated events and the prevalence of antibody titers against EV-A71 among healthy children aged 6-35 months from the published results of unvaccinated children reported by phase III clinical trials of EV71 vaccine candidates. Titer distribution and geometric mean titer (GMT) for unvaccinated children were also obtained from the trial reports. Case-severity risks of HFMD cases caused by EV-A71 were extracted from a large scale epidemiological study in China. Serological correlates of protection against EV-A71 associated disease were also extracted from the literature.
We applied a hierarchical Bayesian model, which synthesized published evidence to reconstruct the severity profile of EV-A71 infections, including medically attended symptomatic disease, hospitalization, severe complications and death.

Results: We estimated that on average, 15.1% of children were infected by EV-A71 in a year. Most EV-A71 infections were mild, with about 10% symptomatic and seeking medical attention and 2.2% hospitalized. The model also suggested that 70% of children had ≥ 4-fold rises in antibody titers after infection.

Conclusion: The hierarchical Bayesian model provided a unified framework to synthesize evidence from multiple sources. Our model provided good estimates, consistent with other published studies and supported by simulation studies. Aggregated data on the serological correlates of protection against infection and the immune response were used, which are routinely reported by vaccine clinical trials. The approach can be applied to other diseases, allowing characterization of the severity profile which is important to the understanding of disease burden at the population level, transmission dynamics and guiding public health measures.

Project No.: HKS-18-E16

ID-36
Development of Pin1 Inhibitors for the Treatment of Hepatitis C

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Introduction and Project Objectives: Prolyl-isomerase 1 (Pin1) is a characteristic enzyme that is responsible for various cell processes such as transcriptional regulation, cell cycle progression, and apoptosis. Pin1 mRNA and protein expression levels were significantly elevated in hepatitis C virus (HCV) replicon cells and HCV-infected cells, and treatment of those cells with a Pin1 inhibitor decreased HCV propagation. Moreover, overexpression of Pin1 is correlated with a higher probability of prostate tumor recurrence. These observations render Pin1 as a promising target for the treatment of HCV and has stimulated the development of Pin1 inhibitors for the potential treatment of cancer.

Methods: A database of natural product and natural product-like compounds was established, and a molecular docking strategy was utilized to identify new Pin1 inhibitors. The action of the hit compounds against Pin1 activity was studied using multiple methods, including cell thermal shift, western blotting, fluorometric enzymatic assay and other techniques.

Results: A structure-based virtual screening identified compound 1 as a lead natural-product-like inhibitor of Pin1 activity. Compound 1 selectively targeted Pin1 and disrupted the interaction between Pin1 and the p65 subunit of NF-kB in cellular systems. Moreover, compound 1 induced apoptosis in prostate cancer cells.

Conclusion: Compound 1 represents a natural product-like Pin1 inhibitor that acts via targeting the Pin1–p65 interaction. We anticipate that compound 1 may serve as a useful scaffold for the development of highly potent inhibitors of Pin1, as potential agents for treatment of HCV and prostate tumor.

Project No.: 14130522

ID-37
Prevalence, Perceptions and Intention related to Various Modes of HIV Testing among Men Who Have Sex With Men in Hong Kong

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Introduction and Project Objectives: We investigated among men who have sex with men (MSM) in Hong Kong: 1) prevalence of uptake and acceptability various modes of HIV testing (existing modes, free routine opt-out testing if offered at primary care settings, self-testing (HIVST) and free home-sampling testing) and 2) factors associated with acceptance of HIVST and opt-out testing at primary care settings.

Methods: A cross-sectional survey was conducted. Participants were: 1) Chinese males of 18-60 years old, 2) holding a Hong Kong ID card, and 3) had had anal intercourse with at least one man in the last six

months. Those self-reported as being HIV positive were excluded from the study. A total of 336 participants (response rate: 67.6%) were recruited from multiple sources.

Results: The prevalence of taking up any mode of HIV testing was 73.2% in lifetime and 44.0% in the last six months. Among all participants, 48.5% intended to take up any existing mode of HIV testing in the next year. If real-time counselling was made available, 11.0% and 59.2% intended to take up HIVST at market rate (300 HKD/episode) and free HIVST. If free routine opt-out HIV testing became available in primary care setting, 37.2% would like to use such service. The prevalence of behavioral intention to use home-sampling HIV testing was 22.9% (free of charge) and 10.1% (300 HKD/episode). Adjusted for significant background variables, positive attitudes toward HIVST, perceived significant others would support use of HIVST, perceived higher behavioural control, and perceived counselling supporting HIVST to be important were associated with higher intention to use free HIVST with online real-time counselling, while a negative association was found for fear toward HIV positive results.
Four variables were positively associated with behavioral intention to take up free routine opt-out testing in the next year in the adjusted analysis. They were: 1) previous HIV testing experience, 2) positive attitudes toward such mode of testing, 3) perceived over 60% of the participants' significant MSM friends would take up such testing, and 4) perceived high/very high level of trust in health care professionals in primary care setting.

Conclusion: This was the first study to investigate and compare a full range of HIV testing modes among MSM as well as the facilitators and barriers. The results may assist policy making and service planning. HIVST with real-time online/telephone counselling services is of good potential to increase testing rate among local MSM.

Project No.: CU-15-C9

ID-38
Role of Δ42PD-1 in HIV Infection

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Introduction and Project Objectives: Human immunodeficiency virus (HIV) is the causative agent of acquired immunodeficiency syndrome (AIDS). HIV continues to spread, leading to 36.9 million people living with the virus and about 40 million deaths worldwide. In Hong Kong, despite active prevention and timely introduction of combination antiretroviral therapy (cART), the number of cumulative infections has increased from 776 in 1996 to 9715 in 2018. Although program death one (PD-1) protein is associated critically with functional exhaustion of HIV-specific T cells, the role of its isoform namely Δ42PD-1 remains elusive in HIV infection. In this study, we hypothesize that host protein Δ42PD-1 may initiate intestinal inflammation, leading to mucosal damage and subsequent bacterial translocation.

Methods: Δ42PD-1 specific monoclonal antibodies were generated by the conventional hybridoma technology after mouse immunizations. Peripheral blood mononuclear cells (PBMCs) were isolated from healthy donors and HIV-infected patients. Human γδ-T cell and other immune cell populations were purified from PBMCs using magnetic beads. Δ42PD-1 protein expression was measured by flow cytometry. The ligand-receptor interaction was determined by several methods. HIV-induced Vδ2 T cells with Δ42PD-1 high expression were adoptively transferred into humanized mice to investigate the in vivo role of Δ42PD-1 on γδ-T cells.

Results: We found that during HIV infection Δ42PD-1 is primarily expressed on the γδ subset of T cells, which are important innate cell types for fast immune response. Characterization of Δ42PD-1+ γδ-T cells showed that Δ42PD-1 expression is induced by HIV-1 infection likely through cytokines IL-12/IL-15. We found that Δ42PD-1+ γδ-T cells are markedly increased in acute HIV-1 patients compared to chronic patients or healthy individuals, and acquired a gut-homing phenotype. By using the humanized mice model, we further found that Δ42PD-1+ γδ-T cells can migrate to the small intestines and cause inflammatory damage. The function of Δ42PD-1+ γδ-T cells is mediated through TLR4 as its newly discovered receptor. Moreover, we generated a Δ42PD-1-specific antibody that can block the function of the Δ42PD-1/TLR4 pathway and alleviate intestinal damage in humanized mice.

Conclusion: Our study demonstrated the function of Δ42PD-1 in terms of a human T cell subset and its role during acute HIV infection. Future work to develop the antibody as a therapeutic is warranted. Results in this study have resulted in two peer-reviewed publications.

Project No.: 14130582

ID-39
Molecular Epidemiology of Human Papillomavirus (HPV) Infection in Hong Kong Women with Cervical Cytological Abnormalities

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Introduction and Project Objectives: The study aimed to update the molecular epidemiology of HPV infection among cytological abnormalities after the approval of HPV vaccines in HK. The objectives included 1) evaluate the prevalence of genital HPV genotypes among cervical cytological abnormalities and its association between HPV genotypes and various stages of cervical pre-cancerous lesions; 2) to explore epidemiological risk factors associated to HPV genotypes; 3) to examine the willingness of taking HPV vaccination; and 4) to assess utilisation of health service related to cervical infection.

Methods: A mixed study design with a cross-sectional survey, and medical record retrieval was used in the study. Women aged 25 years or older were recruited. Behavioural and clinical information, cervical specimens for HPV detection were obtained. Prevalence of HPV infection among cytological abnormalities and its association with behavioural risk factors were analysed by univariate and multivariate logistic regressions.

Results: A total of 342 eligible respondents were recruited, with a mean age of 46.0 ± 10.9. Of the 342 respondents, 73.1% had cervical cytological abnormalities. HPV infection was detected among women with cytological abnormalities is 79.2% in which 80.8% had high-risk HPV and 38 (19.2%) had only low-risk HPV infection. Age is an independent risk factor of HPV infection and women aged 25-44 years were more likely detecting high-risk HPV genotypes. Other potential risk factors associated to high-risk HPV infection include use of condom (protective factor) and history of Sexually Transmitted Diseases (STD). The uptake rate of HPV vaccine among the recruited subjects was 10.5% which was relatively low, however, the non-vaccinated subjects were unlikely to receiving HPV vaccination in future. In addition, majority of the respondents (81.9%) used cervical screening services in public sectors.

Conclusion: This study shows that HPV infection is highly related to cervical inflammatory as well as pre-cancerous lesions. Condom use was found as a protective factor for any HPV infection. Furthermore, condom use and sexual transmitted disease were strongly associated with high risk HPV infection. However, the relationship between other epidemiological factors including smoking and alcohol related to HPV genotypes differ from previous studies which requires further investigation in larger sample size. This study provides a better understanding on the evolution of HPV infection in cervical cancer with an update on its epidemiological profile among women with cytological abnormalities, which enables the policy marker, educator and service provider to implement appropriate education and preventive measures to cervical HPV infection in Hong Kong.

Project No.: CU-15-C7

ID-40
Parental Acceptability of HPV Vaccination for Chinese Boys and Girls aged 9-13 years in Hong Kong --- A Population-based study

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Introduction and Project Objectives: Human papillomavirus (HPV) vaccination for children prior to the initiation of sexual activities is the most efficacious. This study was to investigate parental acceptability of HPV vaccination for their sons and daughters aged 9-13 years under different cost scenarios, and factors associated with parental acceptability of free vaccination.

Methods: Participants were: 1) Chinese speaking parents aged 18-60 years with a Hong Kong ID card; 2) had a son or a daughter aged 9-13 years at the date of the survey; 3) the child had the right to abode in Hong Kong. Random telephone numbers were selected from up-to-date telephone directories of Hong Kong. A total of 300 eligible parents (boys' parents: 162; girls' parents: 138, response rate: 68.9% & 69%) provided verbal informed consent and completed the anonymous telephone interview during March to October, 2016. Using parental acceptability of free HPV vaccination as the dependent variable, univariate and multiple logistic regression models were fitted.

Results: The prevalence of HPV vaccination was very low among boys and girls (0.6% vs. 2.2%, p=0.242). Among those whose children had not taken up HPV vaccination, the prevalence of parental acceptability of HPV vaccination for the index daughter was significantly higher than the index son at market rate (27.4% versus 14.9%, p=0.008), 50% discount of market rate (48.9% versus 29/2%, p=0.001), and free vaccination (63.0% versus 51.6%, p=0.048).
Among boys' parents, adjusted for significant sociodemographic variables, potential facilitators of parental acceptability of free HPV vaccination for their sons included perceived benefit of HPV vaccination and anticipated regret if not having the son vaccinated against HPV. Embarrassment of discussing HPV-related topics, and perceived difficulties of discussing HPV vaccination were associated with lower parental acceptability.
Among girls' parent and in adjusted analysis, in addition to anticipated regret if not having the daughter vaccinated against HPV, some attitudinal variables based on the Health Belief Model were also associated with higher parental acceptability of free HPV vaccination for their daughters (perceived susceptibility and perceived severity of HPV infection among females, perceived benefit of HPV vaccination and perceived self-efficacy)

Conclusion: Coverage of HPV vaccination among children aged 9-13 years was very low. Majority of the parents would likely to vaccinate their children if free vaccination is made available. Free universal vaccination for both boys and girls should be considered. Different strategies promoting HPV vaccination should be applied to boys' and girls' parents.

Project No.: CU-15-C16

ID-41
Distribution and current infection status of Biomphalaria straminea in Hong Kong

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Introduction and Project Objectives: Schistosomiasis, also generally known as snail fever, is a parasitic disease caused by trematode flatworms of the genus Schistosoma. In Hong Kong and mainland China, the freshwater snail Biomphalaria straminea has been introduced and has the potential to transmit intestinal schistosomiasis caused by S. mansoni, a parasite of man which has a wide distribution in Africa and parts of the New World, especially Brazil. The first identification of B. straminea in Hong Kong dates back to the 1970s, and its geographical distribution, phylogenetic relationships, and infection status have not been updated for more than 30 years. Thus, this study aims to reveal the distribution and current infection status of B. straminea in contemporary Hong Kong.

Methods: Snails were collected from different parts of Hong Kong from July 2016 to January 2017. Both anatomical and molecular methods were applied to identify B. straminea. Cytochrome c oxidase subunit 1 (cox1), internal transcribed spacer 1 (ITS1), 5.8S rDNA, internal transcribed spacer 2 (ITS2), and 16S ribosomal DNA (rDNA)

were sequenced from individual snails and analyzed. To detect the presence of S. mansoni, both biopsy and PCR analyses were carried out.

Results: Using both anatomical and molecular analyses, this study demonstrated the existence of black- and red-coloured shell B. straminea in different districts in the New Territories in Hong Kong, including places close to the mainland China border. None of the B. straminea (n = 87) investigated were found to be infected with S. mansoni when tested by biopsy and PCR. The Hong Kong B. straminea are genetically indistinguishable, based on the chosen molecular markers (cox1, ITS1-5.8S-ITS2, and 16S rDNA), and are similar to those obtained in mainland China and South America.

Conclusion: Biomphalaria straminea is now well established in freshwater habitats in Hong Kong. No evidence of infection with S. mansoni has been found. Surveillance should be continued to monitor and better understand this schistosomiasis intermediate host in mainland China and Hong Kong.

Project No.: 15140012

ID-42
Commissioned study on conducting tests for Zika virus among asymptomatic people returning from the 2016 Olympic Games and Paralympic Games

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Introduction and Project Objectives: Zika virus (ZIKV) infection may be associated with severe complications, such as congenital microcephaly and Guillain-Barré syndrome. In addition to mosquito-borne transmission, ZIKV may be transmitted by sexual and other non-vector-borne routes. However, most patients with ZIKV infection may be asymptomatic. The risk of travel-related ZIKV transmission from asymptomatic persons returning from the 2016 Rio Olympic and Paralympic Games in Brazil to Hong Kong was unknown. In this study, we investigated the prevalence rate of asymptomatic ZIKV infection and persistence of viral shedding in bodily fluids among these returned travellers.

Methods: The subjects of this study included asymptomatic travellers who returned from the Rio 2016 Olympic or Paralympic Games to Hong Kong and/or their sexual partners, starting on 15 days to 6 months after their return. Serum samples were collected from each subject for anti-ZIKV antibody detection ELISA, immunofluorescence test, and viral load study by real-time qRT-PCR. Additionally, end-stream urine and semen (males) were collected from each consenting subject for detection of ZIKV RNA by real-time qRT-PCR assays.

Results: Between 22-August-2016 and 28-February-2017, a total of 214 subjects (114 males and 100 females) joined the study. Their median age was 34 years. Seven (3.3%) were pregnant. Their median duration of stay in Brazil was 16 days. Eighty-seven (40.7%) recalled having been bitten by mosquitoes during their stay. Two hundred and eight (97.2%) participants reported using mosquito repellents consistently during their travel. A total of 209 serum, 201 urine, and 42 semen samples were collected from the subjects. ZIKV RNA was not detected in any of the serum, urine, and semen samples. Sixteen of 209 (7.7%) serum samples were weakly positive anti-ZIKV antibody by immunofluorescent test, none was positive for anti-ZIKV IgM or IgG by ELISA. All 16 of these individuals had received yellow fever vaccination prior to serum collection, which was likely the reason for the weakly positive antibody response detected by immunofluorescence test.

Conclusion: The findings in this study suggested that the risk of acquiring ZIKV infection was low if appropriate preventive measures were implemented. Similar studies with more subjects should be considered for other mass gathering events in ZIKV-endemic regions to better characterize the risk of acquiring ZIKV infection during these events and the effects of these preventive measures. Public education of Hong Kong residents travelling to ZIKV-endemic areas on these measures continues to be an important means of preventing importation and local dissemination of ZIKV.

Project No.: ZIKA-HKU