

# *Proceedings of Congress*

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## **Joint Annual Scientific Meeting 2014**

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## Oral Presentation

### **Incidence of Deferasirox-associated Renal Tubular Dysfunction in Children and Young Adults with Beta-thalassaemia**

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Renal tubular dysfunction has been reported in patients on deferasirox therapy but no systematic study has been performed. This study aimed to determine the cumulative incidence of renal tubular dysfunction and serum electrolyte imbalances among children and young adults with transfusion-dependent beta-thalassaemia on deferasirox and study their intermediate term outcomes. Mean age was 15.89 years (range: 2.6-24.10 years). Nine (50.0%) patients were on deferasirox, three (16.7%) were on deferoxamine, two (11.1%) were on deferiprone, and four (22.2%) were on combination deferiprone and deferoxamine. Twelve (66.7%) patients had renal tubular dysfunction, defined by elevated urine  $\beta$ -2 microglobulin, of whom nine were on deferasirox. Eight patients (44.4%) had concomitant serum electrolyte imbalance, all of whom were on deferasirox. Compared to those on other chelation regimens, patients on deferasirox were more likely to develop renal tubular dysfunction (OR 35.3,  $p=0.009$ ), hypokalaemia (OR 23.2,  $p=0.015$ ), hypophosphataemia (OR 57.0,  $p=0.001$ ), hypocalcaemia (OR 23.2,  $p=0.015$ ), and Fanconi syndrome (OR 15.6,  $p=0.004$ ). Cumulative incidence of renal tubular dysfunction was estimated to be 89% at 6 years of deferasirox therapy. These effects were reversible with suspension or dose reduction of deferasirox. Vigilant monitoring of renal tubular dysfunction and serum electrolyte is recommended in patients who receive deferasirox.

### **Ventricular-arterial Coupling in Patients Late After Successful Intervention of Coarctation of the Aorta**

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**Background:** Despite surgical or catheter intervention of coarctation of the aorta (CoA), dysfunction of systemic arteries proximal to coarctation site may persist.

**Objective:** We aimed to test the hypothesis that structural and functional alterations of systemic arteries occur in adolescents and young adults late after successful intervention of CoA and may impact on left ventricular (LV) mechanics.

**Method:** Thirty-one (16 males) patients, aged  $23.4\pm 6.3$  years, at  $20.6\pm 5.2$  years after surgical ( $n=17$ ) or catheter ( $n=14$ ) intervention and 31 (15 males) age-matched healthy controls were studied. Patients with significant residual CoA and those associated complex cardiac lesions were excluded. Carotid arterial stiffness and carotid intima-media thickness (IMT) and brachial-ankle pulse wave velocity (PWV) were determined by radiofrequency-based echocardiography and oscillometry, respectively. Tissue Doppler and speckle tracking echocardiography were performed to assess LV myocardial tissue velocities and linear and torsional deformation.

**Results:** Compared with controls, patients had significantly greater carotid arterial stiffness ( $p=0.007$ ) and IMT ( $p=0.002$ ), but similar brachial-ankle PWV ( $p>0.05$ ). For LV mechanics, patients had significantly lower mitral annular systolic ( $p<0.001$ ), early ( $p<0.001$ ) and late ( $p=0.026$ ) diastolic velocities, global LV systolic longitudinal ( $p=0.001$ ) and radial ( $p=0.004$ ) strain, and early diastolic longitudinal ( $p<0.001$ ) and radial ( $p=0.022$ ) strain rates than controls. Peak torsion ( $p<0.001$ ) and peak diastolic untwisting velocity ( $p<0.001$ ) were also significantly reduced in patients. For the whole cohort, carotid stiffness correlated negatively with mitral annular early diastolic velocity ( $r=-0.403$ ,  $p=0.001$ ), global LV longitudinal ( $r=-0.489$ ,  $p<0.001$ ) and radial strain ( $r=-0.275$ ,  $p=0.004$ ), and LV longitudinal systolic ( $r=0.361$ ,  $p=0.004$ ) and early diastolic strain rate ( $r=-0.464$ ,  $p<0.001$ ), while carotid IMT correlated negatively with mitral annular systolic ( $r=-0.441$ ,  $p<0.001$ ), early ( $r=-0.459$ ,  $p<0.001$ ), and late ( $r=0.324$ ,  $p=0.010$ ) diastolic velocities, early diastolic longitudinal strain rate ( $r=0.274$ ,  $p=0.031$ ), and peak diastolic untwisting velocity ( $r=-0.317$ ,  $p=0.014$ ).

**Conclusion:** In adolescents and young adults after repair of CoA, structural and functional impairment of central arteries may have a negative impact on LV systolic and diastolic deformation.

## Central Nervous System Tumours in Children Under the Age of Three: A Population Study by the Hong Kong Paediatric Haematology/Oncology Study Group

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**Background:** Central nervous system (CNS) tumours in children below the age of three represents special challenge to pediatric oncologists. Population-based epidemiological data on this particular patient group is lacking in Hong Kong.

**Method:** Review of data from a population-based pediatric tumour registry of the Hong Kong Paediatric Haematology/Oncology Study Group between 1999 and 2011.

**Results:** Eighty-one children with primary CNS tumours from 0-3 years were identified (annual incidence: 4.16 cases per 100,000). Forty-one (50.6%) were male and mean duration of FU was 94 months ( $\pm 8.1$ ). Primary tumours were infratentorial in 43 (53.1%). The tumour types in decreasing frequency were astrocytoma (n=17), medulloblastoma (n=16), ependymoma (n=13), CPT (n=7), PNET (n=7), ATRT (n=6), GCT (n=5), craniopharyngioma (n=4) and ganglioglioma (n=3). Three patients presented antenatally. Treatment included surgery in 82.7%, chemotherapy in 50.6% and radiotherapy in 25.9%. There were 29 deaths (35.8%) and 19 relapses (23.5%) during the review period with the 1y-OS, 5y-OS, 1y-EFS and 5y-EFS being 79.4% ( $\pm 4.6$ ), 63.5% ( $\pm 5.9$ ), 68.9% ( $\pm 5.3$ ) and 52.5% ( $\pm 5.9$ ) respectively. Significantly better OS and EFS were observed in patients who received gross-total resection but those with high-grade tumours, antenatal diagnosis or ATRT/PNET had worse outcome. Survival did not differ with age. Comparison with statistics from other studies revealed higher rates of embryonal tumour, GCT and craniopharyngioma in Hong Kong Chinese. Disease outcome appeared to be better in our cohort comparing to previous reports probably due to the higher proportion of GCT locally.

**Conclusion:** We described the epidemiology and treatment outcome of CNS tumours in young children locally; ethnical differences in the frequency of particular tumour types might exist.

## Application of High-resolution Anorectal Manometry in Children with Intractable Constipation

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**Aim of the study:** Chronic constipation can be due to slow colonic transit or sphincter problem or both. The role of sphincter pathophysiology in constipation is still unclear. This study aims to review the application of high-resolution anorectal manometry (HARM) in children with intractable constipation.

**Methods:** From March 2013 to January 2014, a retrospective review was conducted to study the children aged above 5 years who underwent HARM at our institution. They had either (i) chronic constipation according to the Rome III criteria, or (ii) complications due to fecal retention needing hospitalisation, followed by poor response to a 6-month period of conservative treatment. These patients were compared with the historical control, from September 2011 to February 2013, who received conventional manometry (CARM) by station pullthrough technique.

**Main results:** Nineteen children (16 boys, median age 9 years) received HARM and 18 children (10 boys, median age 8 years) received CARM.

|  | HARM  | CARM  | p-value |
|--|-------|-------|---------|
| Resting anal pressure $\geq 70$ mmHg             | 73.7% | 55.6% | 0.248   |
| Relaxation of anal sphincter $< 10\%$ on pushing | 47.1% | 12.5% | 0.031   |

Patients with resting anal pressure  $\geq 70$ mmHg were considered to have anal sphincter hypertonicity. Patients who had anal pressure relaxation of  $< 10\%$  or paradoxical contraction on pushing were regarded to have anal sphincter dysynergia.

In HARM patients with anal sphincter hypertonicity, 66.6% also had sphincter dysynergia, comparing to 0% for those with resting sphincter pressure  $< 70$  mmHg (p=0.012).

**Conclusion:** With HARM, 73.7% patients were demonstrated to have sphincter hypertonicity, which has no significant difference from CARM. HARM had significantly increased sensitivity in showing sphincter dysynergia than CARM. Anal sphincter dysynergia is more commonly seen in those with sphincter hypertonicity, which may imply that both external and internal anal sphincters contribute to the pathophysiology of intractable constipation.

### **A Meta-Analytic Review of Studies on the Mental Health of Chinese Victims with History of Childhood Physical Abuse**

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**Background:** Childhood physical abuse (CPA) is an international phenomenon. Rapid social changes in Chinese societies over the recent decades have raised public concern and research into CPA. The relationship between CPA and psychiatric conditions is well established in western countries. However, the questions of whether this link exists in the Chinese context and, if exists, how strong the association is remain uncertain.

**Objectives:** To examine the impact of CPA on mental health in the Chinese context, we performed a meta-analytic review of relevant studies published up to April 2013.

**Methods:** Literature search strategies included a comprehensive online search of three English databases and one Chinese database. Studies were included if all participants were Chinese; the study used an observational design to study the link between CPA and mental health; participants' mental well-being was assessed with objective measures and/or diagnostic interviews. We extracted data on publication details and methodological characteristics including the participants' mental health outcomes and then conducted a meta-analytic examination of the data.

**Results:** Total 24 effect size measures from 22 studies were included in this review. All studies were retrospective: 5 studies on antisocial personality disorder (APD; 4 on its tendency and 1 on its diagnosis), 5 on borderline personality disorder (BPD; 4 on its tendency and 1 on its diagnosis), 6 on depression (5 on its symptom and 1 on its diagnosis), 2 on anxious symptoms, 2 on cluster-B personality disorders, 2 on post-traumatic stress disorder (PTSD), 1 on conduct disorder (CD) and 1 on obsessive-compulsive

disorder (OCD). Our results revealed significant association between CPA and mental health (OR, 2.16; 95% CI, 1.87-2.49). According to Cohen's calculations, this corresponds to a small-to-medium effect. Specifically, CPA in the Chinese context was associated with APD, BPD, cluster-B personality disorders, conduct disorder, depression, anxious symptoms, and PTSD. Methodological factors and sample characteristics appear to affect the findings of the included studies. We found stronger CPA-mental health association in studies with high-risk sample (criminals) than those with low-risk sample (students). Moreover, the statistical significance of the association appears to partly depend on the use of standardized assessment tool.

**Conclusions:** This meta-analytic review is the first to examine the impact of CPA on Chinese mental health. Our findings replicate the western evidence that childhood abuse in the form of severe physical discipline can contribute to later adverse mental health and highlight the need for more effective policies and interventions to address the hidden costs of CPA for Chinese societies.

### **Impact of Sleep Deprivation on Neurocognitive and Clinical Performance of Paediatric Residents in Hong Kong – A Prospective Study**

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**Objective:** Sleep deprivation adversely affects neurocognitive ability. We aimed to study the effects of acute sleep deprivation on neurocognitive and clinical performance of paediatric residents in Hong Kong.

**Method:** A prospective within-subject study was carried out to compare the performances of residents on days with normal sleep duration with days after night duty (i.e. after sleep deprivation). Assessments include standard neuropsychological tests (Conner's Continuous Performance Test and Beery's Visual-Motor Integration) and simulated clinical scenario (emergency drug dosage calculation).

**Results:** 26 residents working in the Department of Paediatrics of the Prince of Wales Hospital, Hong Kong were recruited from January 2013 to January 2014. Median age of subjects was 30 years (range 23-46 years). Sixteen were female (61.5%). The residents have been working in the field of Paediatrics for a mean of 5.5 years (range 1-17 years). Significant shorter sleep duration was documented on post-call day (Call day median sleep duration: 387.5

minutes; Post-call day median sleep duration: 229 minutes; p-value 0.001). Lower standard global score in Beery's Visual-Motor Integration (VMI) test was noted on post-call day (p-value 0.025) with a consistent association of poorer performance with increasing age on post-call day. No difference in simulated clinical scenario performance was detected despite the differences in neurocognitive outcomes.

**Conclusion:** Acute sleep deprivation adversely affects neurocognitive outcomes including visual perception and motor coordination in paediatric residents. This adverse effect is more prominent with increasing age.

### **Molecular Diagnosis for Paediatric Genetic Disorders Using Whole Exome Sequencing of the Next Generation Sequencing Technology**

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Molecular diagnosis for paediatric genetic diseases is important for targeted or tailored treatment, more informative genetic counselling and guiding the families for prenatal or pre-implantation diagnosis. Traditionally, linkage analysis using large multiplex families or multiple families with the same molecular cause is essential and the process could take years before a diagnosis can be reached. Candidate gene screening is usually the only method available for clinical laboratories for genetic diseases in Hong Kong.

Next generation sequencing technology has virtually revolutionised the way genetic studies are conducted and provides opportunities for molecular diagnosis for genetic disorders that were never available before. With the possibility of sequencing the whole genome or almost all the coding exons of the genome, the method does not require the availability of large, multiple affected families and prior

knowledge of candidate causal genes. It can be applied to a single patient or, as a usual practice, whole genome or whole exome sequencing for the patient plus parents. For whole exome sequencing (WES), it usually produces up to 100 million short sequencing reads of usually 100bp long. These short reads were firstly compared with sequences of a reference human genome and mapped to genomic regions from which they were generated. Each position (base pair) of a coding exon is usually covered with dozens to hundreds of sequencing reads. Analysing the sequences of these reads allows for identification of mutations that are different from the reference sequences.

For WES for a single individual, up to 100,000 variants can be identified, with some of which are common variants in a population and some of which rare or private. The population frequencies of these variants are looked up in public databases such as those from the 1000 Genome Project or ESP6500, a project that sequenced 6500 individuals in the US. An internal database is also established with WES data from 200 samples from the local population. For rare, severe genetic disorders that are likely to be caused by mutations from a single gene, we can safely rule out the common (>1% in a population) variants and only focus on the rare or private variants. The nature of the mutations, such as with or without amino acid changes, changes in the open reading frame of the protein, the nature of the amino acid changes (similarity of the amino acid changed to), the conservation of this amino acid in different species, and the function of the gene in relationship to the disease phenotype, are considered to help pinpoint the causal mutations.

We will present examples on using WES for molecular diagnosis for paediatric genetic disorders in our Department. These include detection of de novo mutations (mutations that are not detected in parents), somatic mutations and compound heterozygous mutations, and mutations missed by traditional laboratory testing, which demonstrated the power of this new technology in providing accurate molecular diagnosis.

## Poster Presentation

### Feasibility of Using Cell Phone Reminders to Motivate Behaviour Change in Obese Adolescents in Hong Kong

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**Background:** Obesity is an increasing public health problem affecting young people. Behavioural treatment is the mainstay of weight control programs for obese teens. Traditional behavioural-based programs are usually done face-to-face by health professionals and include strategies to increase physical activity and modify dietary habits. However, seeing medical professionals for individual consultation to discuss weight management on a regular basis can be expensive and time-consuming. In addition, many Hong Kong adolescents rely heavily on the internet to acquire updated knowledge and cell phone texting for communication.

**Research purpose:** We undertook a pilot study investigated the feasibility of using both an adapted internet-based curriculum with cell phone reminders and a simplified lifestyle modification programme (sLMP), consisting of four nutritional counselling sessions, as adjuncts to usual care for obese adolescents and their parents. Here we report on the feasibility of using cell phone reminders for weight management.

**Methods:** Forty-eight obese Chinese teens aged 12 to 18 years were randomised into three groups (control group, internet intervention (IT) group, sLMP group). The study was conducted over 24 weeks. IT group subjects were asked to view a 12-week internet-based curriculum weekly and to set specific goals related to (a) diet and (b) physical activity at baseline and then every month. A weekly semi-personalised SMS (incorporating subjects' diet and exercise goals) was sent to participants and they were asked to reply with an emotion icon to represent whether they had achieved their targets during the week. Participants were also asked in these SMSs about factors which were incentives or barriers to meeting dietary and exercise goals.

**Results:** The most preferred dietary and physical activity goals were eating at least two servings of fruit (62.5%) and increasing frequency of exercise by doing 30 minutes of moderate to vigorous activity three to five times per week

(75%), respectively. A total of 400 messages were sent to the 16 subjects. Fifteen subjects opted to receive the message by Whatsapp and one preferred email. The research assistant spent an average of 2 hours per week to send out the personalised cell phone reminders to the 16 participants. Participants' response rate to meeting dietary and exercise goals were 78.3% and 77.5%, respectively. However, response rate for stating personal incentives and barriers were 51.4% and 47%, respectively. All subjects and nearly 93.8% of parents found the weekly SMS and setting dietary and exercise goals useful or very useful in terms of managing their child's obesity.

**Conclusions:** The findings suggest that the use of cell phone reminders is feasible and well accepted by participants. Given that mobile phones are a common device among adolescents, setting specific goals related to dietary and physical activity along with personalised cell phone reminders may be an innovative adjunct to usual care of obesity. Further study is required to determine whether this intervention is a cost-effective approach to changing weight and promoting a healthy lifestyle in obese youth.

### With Others and for Others: Accounting for Decisions about Genetic Testing in the Clinic

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While there are various factors influencing clients' decisions about genetic testing; testing for the sake of others is not uncommon. This paper focuses on decisions about testing (DOT) when a genetic mutation is identified in a Sudden Arrhythmia Death Syndromes (SADS) patient and it is unclear whether the mutation is the cause of the disease. Family members are then asked to consider genetic testing to ascertain the client's genetic status and future risk. The paper examines, at the interactional level, how genetic counselors, clients and family members negotiate decision-making involving others.

The data consists of 23 video-recorded consultations obtained from a Hong Kong hospital. Episodes of decision-making about testing are identified and extracted from the transcribed data. By using theme-oriented discourse

analysis, the analysis focuses on the discourse strategies that participants employ to foreground the possible benefits when other family members undergo the genetic test.

Preliminary findings show a disjuncture of perspectives between genetic counselors and family members in terms of the benefits of testing. While genetic counselors see testing as a means of confirming the diagnosis and managing risk, family members voice concerns about the usefulness of the test for a client's treatment. To mitigate these different perspectives on DOT, participants use a range of discourse strategies, such as contrast, foregrounding, self-and-other construction as a way of emphasizing future scenarios. This study, in sum, elucidates how other-oriented decisions are made in the clinical setting.

### **Oral Intake of $\beta$ -glucans Containing Products Does Not Affect the Serum $\beta$ -glucans Level by Using Chromogenic Limulus Ameobocyte Lysate Assay (Fungitell Test)**

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**Background:** *Ganoderma Lucidum* (*G.lucidum* or Lingzhi, 靈芝) is a form of fungus that has been used as medication for a long time. *G.lucidum* has been advocated to enhance health and longevity. It has been applied to a wide spectrum of diseases including hypertension and cancer. One of the major bioactive components in *G.lucidum* is  $\beta$ -glucan.  $\beta$ -glucans are glucose polymers joined together by  $\beta$ -1-3 / 1-6 glycosidic linkages. Human does not have the enzymes to digest the  $\beta$ -1-3 / 1-6 glycosidic bonds and therefore the glucose polymers form 3-dimensional helical structures after ingestion and activate immune cells via dectin-1 receptor. However, most of the fungi also have  $\beta$ -glucans in their cell wall and therefore  $\beta$ -glucans found in the blood can be used a marker for the diagnosis of fungal infection. Chromogenic Limulus Ameobocyte Lysate (LAL) assay (Fungitell, Pyrochrome, Associates of Cape Cod, Inc, East Falmouth, USA) is the commercial product designed for the detection and quantitation of  $\beta$ -glucans, especially the  $\beta$ -(1, 3)-glucans.

Fungitell is now approved by FDA for clinical diagnosis of fungal infection. However, whether the intake of the  $\beta$ -glucans containing products such as Lingzhi may affect the serum  $\beta$ -glucans level remains unknown.

**Methods:** The commercially available *G.lucidum* products are provided by YorkBest International, Hong Kong. The  $\beta$ -glucans level has been confirmed previously by our group. Oral dosage in this study followed the manufacturing instructions (2 to 4 capsules). The serum samples from patients with confirmed fungal infection were retrieved from the department of Microbiology in order to assess the specificity and sensitivity of the Fungitell kit. Pre/Pro blood samples were collected from the volunteers before and 1hr after the intake of *G.lucidum*. The serum  $\beta$ -glucans level was then measured by Fungitell kit according to the manufacturer's instruction. The  $\beta$ -glucans level <60pg/mL is considered as negative, whereas, the values >80pg/mL is considered as positive. The values between 60 to 79pg/mL are considered as indeterminate. The data was analysed by GraphPad Prism version 5.0 software (GraphPad Software Inc, CA, USA). Comparisons between means were based on paired student's t test.

**Results:** Fungitell test results of patients with confirmed fungal infection shown that 5 out of 11 were detected as positive whereas another 5 were detected as negative. One out of 11 was detected as equivocal. The data showed no correlation between the fungal agents and the serum  $\beta$ -glucans level. The 11 confirmed fungal infection patient samples were considered as positive population while the 11 healthy donors were considered as negative population. True positive was 5 but the false negative was also 5. Since all the healthy donors were negative, the true negative cases were considered as 11 and false positive was considered as 0. In this study, all the samples from pre/pro blood collection after the intake of the  $\beta$ -glucans containing products fell into negative range. No significant statistical difference was observed when comparing the results from pre/pro blood samples.

**Conclusion:** Fungitell test has a sensitivity of 50% and specificity of 100% in detecting various fungal infections. The positive predictive value is 100% while the negative predictive value is 68.75%. The accuracy is 72.72%. It is non-specific for various fungi. The oral intake of  $\beta$ -glucans containing products did not affect the serum  $\beta$ -glucans level by using Fungitell assay.

### Iron Overload Induced TNF- $\alpha$ Bearing Monocyte Microparticles Enhance Osteogenesis of MSC

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**Introduction:** Heterotopic ossification (HO) occurs at a high frequency in severe trauma induced injuries. Whether haemorrhage induced local iron overload after trauma related to enhanced osteogenesis is virtually unclear. In this study, our first objective was to determine the in vitro effect of iron on microparticles (MPs) generation by monocytes cell line. And to find out what possible mechanisms underlying iron-induced monocyte MP generation, this including iron overload induced apoptosis and oxidative stress in relation with MPs generation. Furthermore, to examine effect of excessive iron induced monocyte MPS on mesenchymal stromal cell osteogenesis.

**Methods and results:** By using monocyte cell line, we showed that excessive iron induced monocyte MPs generation in dose dependent manner, iron increased reactive oxygen species (ROS) production (37.5-300  $\mu$ M). Apoptotic cells were found to be significantly increased under iron treatment (75  $\mu$ M-300  $\mu$ M, 48 hours) as showed by AnnexinV/PI assay. Secondly, we found that MSC engulfed iron induced monocyte MPs and enhance the osteogenesis as showed by mineralisation assay and alkaline phosphatase assay. By using western blotting method, we found excessive iron upregulated TNF- $\alpha$  gene and protein expression of monocyte. In order to confirm it is TNF- $\alpha$  caused the enhanced osteogenesis effect, we using monocyte TNF- $\alpha$  knock down model. Our data showed that iron induced monocyte MPs from TNF- $\alpha$  knock down cells had a lower stimulation ability on osteogenic differentiation of MSCs. To further confirm this data, we using antibody neutralisation assay, anti-TNF- $\alpha$  had anti osteogenic effect on TNF- $\alpha$  induced osteogenic differentiation of MSCs.

**Conclusions:** Our findings suggest that iron-overload induces monocyte MPs which may caused by ROS induced cells apoptosis. Furthermore iron induced monocyte MPs which contain TNF- $\alpha$  may contribute to etiotopic bone formation in HO.

### Serious Pandemic Influenza A/H1N1 Infection in Paediatric Intensive Care Unit From 2009-2014

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**Background:** Influenza is usually an acute and self-limiting febrile illness. In April 2009, a new variant, the Pandemic Influenza A/H1N1 virus (pH1N1) was identified causing human infection, leading to a pandemic alert issued by WHO. Most cases had mild or subclinical disease, but some cases experienced serious complications with high mortality.

**Objective:** To review the clinical course and outcome of serious pH1N1 infection that required intensive care since its outbreak in 2009 at a local hospital in Hong Kong

**Method:** This is a retrospective case cohort review. Patients with the diagnosis of influenza infection with admission to paediatric intensive care unit from June 2009 to March 2014 were traced from CDAR system of Hospital Authority. Patients with nasopharyngeal aspiration or swab confirmed with positive pH1N1 were retrieved from the microbiology laboratory database. Demographic data, reasons for admission to intensive care, severity of organ involvement, use of ventilator support, inotropic support, treatment and outcome were recorded and reviewed.

**Results:** During this period, 1657 specimens were tested on 1541 hospitalised paediatric patients for pH1N1 in our hospital, among them 104 specimens were positive. Twenty patients with 22 specimens (21%) positive for pH1N1 were admitted to PICU. All twenty patients were positive for PCR of M gene and pH1N1 specific haemagglutinin (HA) gene, but only 11 patients showed positive immunofluorescence (IF) rapid test. Eighteen patients also had positive viral cultures. Among these 20 patients, 9 were male and 11 were female, with median age of 5 years (range: one month to 20 years old). The median duration of PICU stay was 12 days (range: 2 days to 37 days). All patients survived and were discharged from PICU. Six patients (30%) had pre-existing comorbidities like Down syndrome, Prader Willi syndrome, craniopharyngioma, depression and Turner syndrome. Five patients (25%) had co-infection. Eleven patients (55%) were admitted for respiratory distress and failure, 4 patients (20%) had neurological complications and 5 patients (25%) had other clinical indications. Among the eleven patients with severe respiratory complication, 6 patients had severe pneumonia requiring ventilator support. One received non-invasive ventilation and 5 patients were

intubated with median ventilator-days of 9.5 (range: 2-17 days). The median of maximum oxygen required was 0.74 (range: 0.5-1). Two patients required iNO and the support of one to three inotropes. Among the 4 patients with serious CNS complications, one had status epilepticus and one had repeated seizures. One patient had Acute Disseminated Encephalomyelitis (ADEM) and one patient had necrotising encephalitis. Both patients were treated with intravenous immunoglobulin and pulse methylprednisolone. Because of the poor neurological state, plasmapheresis was performed in both patients with improvement in neurological outcome. All patients were treated with oral oseltamivir with doubling of the usual dose and the duration of therapy was 5-10 days. Two patients required intravenous peramivir with satisfactory response.

**Conclusion:** Most of the serious pH1N1 cases were reported in 2009, yet there continues to be sporadic serious pH1N1 infection. Co-infection and negative rapid IF test for pH1N1 are not uncommon, hence a high index of suspicious is important to identify and combat this potential serious infection.

### Short Inversion Detection From Next Generation Sequencing Data

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Inversions are known to be related to human diseases and are mostly under positive selection in evolutionary courses. However, unlike other types of genetic variations, inversions remain poorly studied due to the fact that they're balanced variants and can hardly be mapped to the genome accurately. Investigation of the most updated public databases for structural variation shows that few inversions shorter than 500 bp have been detected so far. Here we introduce SRInversion: a new method specific for detection of inversions shorter than 1kb. The method would split and re-map next generation sequencing reads back to reference genome to locate inversion breakpoints.

Both simulated and real data were used for comparison of the performance by SRInversion with that of the published structural variation detection software. Results on simulated data showed that when inversions are shorter than 100bp, SRInversion has both higher specificity and sensitivity than other software, and SRInversion is the only

software that can detect inversions smaller than 20bp. A set of high-coverage whole genome child-parents trio data (NA12878, NA12891, and NA12892) from the 1000 genomes project was also used for further validation of the performance of SRInversion. After filtering, de novo assembly was performed around detected inversion regions, and these assembly contigs were aligned back to reference genome using web-based BLAST. Regions within which assembly and alignment results suggest inverted sequences were marked as assembly validated. Ten assembly validated inversion regions were selected from each of the trio data, for which PCR will be performed for experimental validation.

### The Antitumour Activity Evaluation of DpC & Dp44mT on Orthotopic Xenograft Neuroblastoma Mouse Model

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**Background:** Neuroblastoma is a childhood high-incidence disease. The side-effects of methemoglobin formation and hypoxia in patients unfortunately postpone its further clinical application. The recently synthesized thiosemicarbazones, including di-2-pyridylketone-4,4-dimethyl-3-thiosemicarbazone (Dp44mT) and di-2-pyridylketone-4-cyclohexyl-4-methyl-3-thiosemicarbazone (DpC) have gained better performance through in vitro experiment.

**Methods:** The apoptosis of neuroblastoma and normal cell lines induced by DpC/Dp44mT/L1 was evaluated through Flow cytometry and XTT test. Based on the orthotopic SK-N-LP/Luciferase nude mouse models, the decrease of tumour burden post-DpC treatment was measured. Hematoxylin-eosin staining was used to detect tissue toxicity. The neuroglobin and cytoglobin expression were tested in normal /neuroblastoma cell lines and mouse tissues.

**Results:** The viability of human neuroblastoma cell lines (SK-N-LP/SK-N-AS/Be(2)C/SH-SY5Y), rat cardiomyoblast cell line H9C2, human hepatocyte cell line MIHA, human kidney cell line HK2 and human mesenchymal stem cell line MSC normal cell post-DpC treated in 24 hour were lower than Dp44mT/L1 treatment. Tumour burden decreased in DpC-treated group compared

with control group ( $p < 0.05$ ). Body weight changes compared with control groups were significant difference ( $p < 0.05$ ). Tissue necrosis could be found in tumour, spleen and lung via haematoxylin-eosin staining. The neuroglobin and cytoglobin expression were increased post-DpC than Dp44mT both in neuroblastoma cell lines and mouse tumour tissue.

**Conclusion:** The new generation thiosemicarbazone DpC could be chosen as a better candidate for neuroblastoma treatment strategy.

### **Congenital Pyloric Web: Common Cause for an Uncommon Neonatal Problem?**

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**Background/purpose:** Pyloric or antral webs are a rare surgical entity causing gastric outlet obstruction in children. In this paper we report three cases that were seen in our Paediatric surgical centre within a 15 months period.

**Methods:** Consecutive patients diagnosed with pyloric web at our centre between July 2012 and October 2013 were retrospectively reviewed. Their demographics, diagnosis and treatment were summarised and reported.

**Results:** Two out of three patients were full term neonates. All (two female and one male) presented with intolerance of feeds during the first week of life. Radiological findings include dilated stomach bubble but presence of small bowel gas. All were diagnosed with upper GI contrast study and with subsequent laparotomy and Heineke- Mikulicz (HM) pyloroplasty performed. At follow up all three patients recovered well.

**Conclusions:** We have described three isolated cases of pyloric web that were seen at our centre over a short period of time. No causal relationship has been found between cases. Web excision followed by HM pyloroplasty in the neonatal period yielded good outcome. Neonatal endoscopic treatment may obviate open surgery in selected cases but warrants further study.

### **Bio-active Constituents from Yinqiaosan Has Anti-Influenza Effect**

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Influenza epidemics have become a major public health concern worldwide. Recurring emergence of new influenza viruses and viruses that are resistant to currently approved antiviral medications pose a critical need to explore new or alternative medications. A classical traditional Chinese medicine decoction, Yinqiaosan (YQS), has a long history for treating respiratory diseases in China. However, the efficacy of YQS has not been investigated mechanistically. In the present study, the effectiveness of YQS in treating influenza virus infection, as well as the potential mechanism of action of an active antiviral compound present in the decoction were investigated.

Our results showed that YQS increased the survival rate of the mice in an *in vivo* influenza virus infection model with significant reduction in lung viral titers. In order to further delineate the mechanisms of action of YQS, a panel of herbal extracts and compounds were prepared from YQS and were screened for the anti-influenza virus effect. One of the compounds, named as forsythoside A, suppressed the viral titers of a wide range of influenza virus subtypes including the oseltamivir-resistant and the 2009 pandemic H1N1 viruses *in vitro*. It also showed protective effects in the mouse infection model. Through electron microscopy, slow or abnormal viral budding events were observed upon forsythoside A treatment during influenza virus infection. Furthermore, Western blot analysis revealed a reduced influenza virus M1 protein expression. As a previous report showed that assembly of viral components into an infectious particle required a threshold level of M1 protein, reduced M1 expression in the cells treated with forsythoside A may contribute to the virus replication suppression.

To conclude, traditional Chinese medicine has been recognised as an important part in complementary and alternative medicine and it is a plentiful source of antimicrobial drugs. Our study not only supports the efficacy of YQS, but also identified an active antiviral compound from the decoction. This may provide a lead compound for future drug development.

## The Role of Oncogene in Mycobacteria-induced Autophagy in Human Macrophages

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Macrophages are the major immunocytes to initiate both innate and adaptive immune responses against *Mycobacterium tuberculosis* (Mtb), a causative agent of tuberculosis. Upon mycobacteria infection, macrophages could eliminate the intracellular bacteria through different cell death pathways, including apoptosis and autophagy.

c-Myc is a transcription factor that regulates a variety of target genes and control different cellular functions such as proliferation and immune response. Recently, our group revealed that c-Myc has a potential role in regulating the antimicrobial responses in macrophages.

Here we use BCG, a live attenuated strain of *Mycobacterium bovis*, which is similar to Mtb in antigenic composition, as a model to study the role of c-Myc in regulating mycobacteria-induced autophagy. We first investigated the role of c-Myc in BCG-induced LC3BII levels. Knocking down c-Myc by siRNA could decrease BCG-induced LC3BII levels. We found that BCG-induced autophagy is dependent on JNK and p38 and independent on PI3K or ERK pathways. And knocking down of c-Myc could significantly inhibit phosphorylation of p38. In conclusion, c-Myc may play a positive role in mycobacteria-induced autophagy in human macrophages.

## Right Ventricular Mechanics in Adolescents and Young Adults Long-term After Repair of Coarctation of the Aorta

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**Background:** Alteration of right ventricular (RV) function has been found in patients with pressure-loaded left ventricles due to systemic hypertension and aortic stenosis. We tested the hypothesis that RV function may be altered in adolescents and adults with repaired coarctation of the aorta (CoA) and related to left ventricular (LV) mass.

**Methods:** Twenty-eight (15 males) patients with CoA, aged  $23.7 \pm 6.5$  years, at  $20.6 \pm 5.4$  years after surgical or

catheter interventions and 28 (14 males) aged matched healthy controls were studied. Patients with significant residual CoA were excluded. M-mode, tissue Doppler imaging, and speckle tracking echocardiography were performed to assess LV mass and shortening fraction, anterior RV wall thickness, and RV myocardial tissue velocities and deformation.

**Results:** Systolic ( $p=0.14$ ) and diastolic ( $p=0.32$ ) blood pressure was similar between patients and controls. Compared with controls, patients had significantly greater LV shortening fraction ( $p=0.028$ ), indexed LV mass ( $p=0.016$ ), and indexed RV anterior wall thickness ( $p=0.012$ ). With regard to RV function, patients had significantly lower tricuspid annular systolic ( $p<0.001$ ) and early diastolic ( $p<0.001$ ) velocities, isovolumic acceleration ( $p=0.004$ ), global RV systolic longitudinal strain ( $p=0.03$ ), systolic strain rate ( $p=0.012$ ), and early ( $p=0.021$ ) and late ( $p=0.012$ ) diastolic strain rates than controls. Patients with an associated ventricular septal defect ( $n=6$ ) requiring closure compared to those without had even lower tricuspid annular systolic ( $p=0.01$ ) and early diastolic ( $p=0.041$ ) velocities. For the whole cohort, LV mass correlated negatively with RV systolic strain rate ( $r=-0.27$ ,  $p=0.045$ ) and tricuspid annular early diastolic velocity ( $r=-0.40$ ,  $p=0.002$ ), while RV anterior wall thickness correlated negatively with tricuspid annular systolic ( $r=-0.42$ ,  $p=0.002$ ) and late diastolic ( $r=-0.40$ ,  $p=0.003$ ) velocities, and positively with e/a ratio ( $r=0.31$ ,  $p=0.024$ ).

**Conclusion:** RV systolic and diastolic function is impaired in patients late after repair of CoA and related to increased LV mass and RV thickness, even in the absence of residual CoA and systemic hypertension.

## Investigating the Role of Interleukin-17A on Cytokines Production by Macrophages in Response to Bacterial Infections

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Interleukin-17A (IL-17A) has been shown to associate with a variety of infection diseases. In this study, we investigate whether IL-17A affects cytokines production of human peripheral blood-derived macrophages during *Mycobacterium bovis* BCG or *Klebsiella pneumoniae* infection. We observed that IL-17A-treated macrophages exhibited suppressed productions of TNF- $\alpha$  and IL-6 in

response to BCG infection. The reduction of cytokines production was not associated with cell death. On the other hand, IL-17A promoted TNF- $\alpha$  and IL-6 production by macrophages during *K. pneumoniae* infection. Furthermore, IL-17A did not affect TNF- $\alpha$  production induced by LPS and Pam<sub>3</sub>Cys, which are TLR4 and TLR2 agonists, respectively. The data suggest that the differential regulation of cytokines production by IL-17A requires whole bacterium infection.

### **HIV-1 Tat Dysregulation of KSHV Induced Immune Response Through the Production of IL-8**

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Human immunodeficiency virus (HIV) causes acquired immunodeficiency syndrome (AIDS) and is a major health issue around the world. HIV is known to induce a number of pathological problems in AIDS patients via the transactivator (Tat) protein that is expressed and released by infected cells. One of the most important function of Tat is the dysregulation of the immune response. IL-8 is a chemokine known to be highly expressed in AIDS patients and Tat plays a major role in its production. IL-8 increases the HIV transmission and replication rate; and plays a role in Kaposi's sarcoma associated herpesvirus (KSHV) infection, which is a major opportunistic pathogen that AIDS patients are at risk to. KSHV is also known to induce the expression of IL-8 in patients, and IL-8 is known to assist tumour development by increasing angiogenesis. In our study, we investigated the role that Tat may have in manipulating the expression of IL-8 induced by KSHV in primary blood monocyte derived macrophages (PBMac). The results showed that pretreatment of PBMac with Tat inhibited the expression of IL-8 induced by KSHV by approximately 40%. We also found that Tat was able to inhibit the phosphorylation of STAT-1 induced by KSHV, and the inhibition of STAT-1 phosphorylation was related to the expression of IL-8 induced by KSHV. In conclusion, we found that Tat was able to manipulate the expression of IL-8 induced by KSHV in macrophages, and this inhibition of IL-8 expression was regulated through the STAT-1 related pathways.

### **Coping When a Child has Special Needs: Exploring the Function of Information from Community and Online Sources**

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**Background:** Managing children with special needs can be physically and mentally demanding at times. Having adequate healthcare information can aid parents in their decision making.

**Objective:** This study recruited family members of children with special needs and professionals in relevant fields in Hong Kong. It aimed to explore the role and function of information from community and online sources with a specific focus on special parenting.

**Methods:** Qualitative focus group interviews were conducted in a semi-structured format. After obtaining informed consent, forty-nine participants were interviewed on issues of both general and online information seeking experiences. Two themes (information needs and sources of information supplementary to healthcare professional advice) were identified from the interviews.

**Results:** Results showed that caregivers need health- and service-related information to effectively manage the daily life of children with special needs. Having adequate information related to caregiving can foster parents' mental health. The Internet emerges as a new source for today's parents to seek information and identify "similar others" for support.

**Conclusions:** Lack of information and emotional support can harm the mental health and parenting skills of the parents especially those having children with special needs. Despite an increasing amount of health-related information in the Internet, parents' Health literacy is critical for proper use and interpretation of online information. Conventional sources of information such as community groups are particularly important for parents with low eHealth literacy.

## Longitudinal Study of Viral Loads and Polyfunctional T cell Responses Towards Epstein-Barr Virus in Paediatric Liver Transplant Recipients Who Developed Post-transplant Lymphoproliferative Disorder

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This study aims to investigate the long-term viral control and T-cell immunity towards Epstein-Barr virus (EBV) in paediatric liver transplant patients who developed post-transplant lymphoproliferative disorder (PTLD). We performed a longitudinal study on viral loads and T cell functions in six PTLT patients (two boys and four girls) up to 5 years after diagnosis. Median age at diagnosis was 2.4 years (range: 0.8-5.2 years). Primary disease was congenital biliary atresia with a median age of transplantation at 0.6 years (range: 0.5-1.3 years). Median time from date of transplantation to diagnosis of PTLT was 1.3 years (range: 0.3-4.7 years). All patients were seronegative towards EBV prior to transplantation and were maintained on a range of tacrolimus doses for immunosuppression. Three patients entered continual clinical remission after one course of rituximab while two patients had relapsed disease and one had persistent lymphocytosis requiring additional treatment with second course of rituximab or chemotherapy to enter clinical remission. Plasma EBV loads were determined by qPCR. Despite entering clinical remission, persistently elevated plasma loads were found in 4 of 6 patients indicating suboptimal long-term viral control. Two patients were selected for longitudinal profiling of EBV latent cycle and lytic cycle antigen-specific polyfunctional T-cell (PFC) responses from 1-4 years after PTLT diagnosis. Upon stimulation by overlapping peptides of EBNA1, EBNA3a/3b/3c and BZLF1, the production of three cytokines (IFN- $\gamma$ , TNF- $\alpha$ , IL-2) and expression of two cytotoxic markers (perforin, CD107a) were examined. Although CD4+ and CD8+ PFC responses could be detected at 3 years after diagnosis, the PFCs were predominantly perforin-negative and the proportion of PFCs with three-five functions was low ranging from 0-3 percent of total antigen-responsive T-cells. Long-term viral control is suboptimal concurrent with slow development of EBV-specific PFC responses signifying the continual risk of re-emergence of PTLT in this cohort of young PTLT patients.

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## Suspected Malrotation in Infants: The Diagnostic Accuracy of Upper Gastrointestinal Contrast Study

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**Aim:** To determine the accuracy of upper gastrointestinal (UGI) contrast study in the diagnosis of malrotation in infants.

**Methods:** A total of 24 UGI contrast study radiographs of infants with suspected malrotation were collected in a period of 3 years. Of those, 12 cases had confirmed diagnosis of malrotation through positive operative findings, and 12 cases had malrotation excluded clinically. The radiographs were reviewed independently by 3 paediatric radiologists without knowing the diagnosis. Radiological signs of malrotation, including the position of duodenal-jejunal junction (DJJ) not located to the left of left vertebral body, low position of the DJJ, duodenal distension and jejunum on the right of abdomen, were commented by the radiologists. Moreover, they need to commit the overall impression for or against the radiological diagnosis of malrotation.

**Results:** Regarding the position of the DJJ not located to the left of left pedicle, the sensitivity and specificity of the sign were 75.7% and 71.0% respectively. For the low position of the DJJ, the sensitivity and specificity were 77.1% and 67.7%. Reported sensitivity and specificity of duodenal distension were 30.6% and 94.4% respectively. Jejunum being on the right of abdomen had a sensitivity and specificity of 30.3% and 90.6%. As for the overall impression of radiological diagnosis of malrotation, the false positive and false negative rates were both 23.3% respectively. Among the 3 radiologists, concordant and correct radiological diagnosis occurred in 10 out of 24 cases (41.7%). Equivocal diagnoses of malrotation were made in 12 out of 72 observations (16.7%).

**Conclusion:** Diagnosis of malrotation should not be based on individual radiological signs as there is significant discordance among different radiologists. Real time interpretation of UGI contrast studies by paediatric surgeons and radiologists is recommended as diagnosis should be based on both clinical and radiological findings.

### Upper Gastrointestinal Contrast Study in the Diagnosis of Malrotation in Infants: A 7-year Experience

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**Aim:** To review the role of upper gastrointestinal (UGI) contrast study in the diagnosis of malrotation in infants.

**Methods:** Hospital notes of paediatric patients with the diagnosis of malrotation with laparotomy done in the past 7 years (2006-2013) were retrieved. Children were excluded if malrotation was found intra-operatively as an incidental finding, UGI contrast study was not performed, or congenital abdominal wall defects were present.

**Results:** Of 29 patients identified, 17 (88.2% male, median age 10 days) were included. Excluded were 7 with malrotation discovered incidentally, 4 without a UGI series, and 1 with cloacal extrophy. Of the 17 patients, all of them had operation performed during their neonatal period, and 76.5% presented with bilious vomiting and 52.9% presented with sepsis. Fourteen patients had a positive UGI contrast examination with a sensitivity of 82.4%. Corkscrew appearance was present in 1 patient and was later found to have volvulus intra-operatively. There were no patients with false positive UGI study and 3 patients with false negative examination. On retrospective review of the 3 UGI studies, the duodenal-jejunal junction (DJJ) were located to the left of the vertebral body and were at level of the duodenal bulb. However, there were contrast holdup in the duodenum with delayed duodenal contrast emptying. All 3 patients were later found to have volvulus and 2 of them had gangrenous bowel requiring resection and stoma.

**Conclusion:** Diagnosis of malrotation should not be based on individual radiological signs such as location of the DJJ alone. Subtler signs such as contrast holdup and delayed duodenal contrast emptying should also be considered. Real time interpretation of UGI contrast studies by paediatric surgeons and radiologists is recommended as diagnosis should be based on both clinical and radiological findings.

### Combination of HDAC and Proteasome Inhibitors Up-regulates p16<sup>INK4A</sup> and p21<sup>WAF1</sup> and Induces Apoptosis of EBV-transformed Lymphoblastoid Cell Lines

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Epstein-Barr virus (EBV) transforms B cells through its unique set of latent genes into continually proliferating lymphoblastoid cell lines (LCLs). Amongst the latent genes, EBV nuclear antigen (EBNA)-3A and -3C exert anti-apoptotic effects on the LCLs through down-regulation of p16<sup>INK4A</sup> and p21<sup>WAF1</sup>. Histone deacetylase (HDAC) inhibitors induce apoptosis of various types of cancer cells through up-regulation of p16<sup>INK4A</sup> and p21<sup>WAF1</sup> and proteasome inhibitors can enhance the anti-tumour effect of HDAC inhibitors through the generation of reactive oxygen species (ROS). Here, we hypothesise that combination of HDAC and proteasome inhibitors can counteract the anti-apoptotic effects of EBNA-3A and -3C in LCLs. We found that combination of HDAC (SAHA or romidepsin) and proteasome inhibitors (bortezomib or carfilzomib) could synergistically inhibit cell proliferation and mediate apoptosis, as evidenced by the annexin V-positive and sub-G1 populations as well as proteolytic cleavage of PARP and caspase-3, in three LCLs. The drug combinations did not affect the expression level of EBNA-3A and -3C whereas they directly induced the expression of p16<sup>INK4A</sup> and p21<sup>WAF1</sup>. Both up-regulation of p16<sup>INK4A</sup> and p21<sup>WAF1</sup> and induction of apoptosis were dependent on the generation of ROS. We conclude that combination of HDAC and proteasome inhibitors may counteract the anti-apoptotic effects of EBNA-3A and -3C by the up-regulation of p16<sup>INK4A</sup> and p21<sup>WAF1</sup>, thereby inducing apoptosis of EBV-transformed LCLs.

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## **Homozygous Missense Mutation in *ABR* Causes Cerebellar Hypoplasia with Early Lethality – A New Condition Identified by Exome Sequencing?**

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We performed whole exome sequencing (WES) in a consanguineous Pakistani family with a recurrent pattern of cerebellar hypoplasia, intra-uterine growth restriction, and various CNS/non-CNS malformations, resulting in early lethality (1 perinatal death and 1 intrauterine death). Karyotype (in the first pregnancy) and oligonucleotide array (in the 2nd affected pregnancy) were normal. Parents declined post-mortem examination. By WES, a novel homozygous missense mutation was identified in the *ABR* gene (*ABR*: NM\_021962.4:c.G2455T: p.A819S) in both affected pregnancies. Both parents were identified to be heterozygous of the same mutation while the healthy child did not carry any mutation. The mutation is located in a highly conserved region and is predicted to be highly

damaging by all the commonly used *in silico* mutation prediction tools. The protein encoded by *ABR* gene contains a GTPase-activating protein domain, a domain found in members of the Rho family of GTP-binding proteins. Previous reports showed that *OPHNI*, mutations in which cause X-linked mental retardation with cerebellar hypoplasia (OMIM300486), also encodes for a regulator of GTPase-activating protein. Both *OPHNI* and *ABR* are highly expressed in the human brain especially in the cerebellum, and both contain a GTPase-activating domain. Rho proteins are important mediators of intracellular signal transduction, which affects cell migration and cell morphogenesis. Other studies have demonstrated a regulatory role of Rho GTPase in differentiation of cerebellar neurons, and that ethanol-associated impairment of Rho GTPase might contribute to brain defects in fetal alcohol syndrome. Further functional studies, including zebrafish morpholino studies, are currently ongoing. WES can be helpful in individual families with undiagnosed lethal MCA syndromes to identify potentially responsible autosomal recessive mutations and may lead to a better understanding of the role of various developmental pathways in human embryogenesis.