

Cuban Research in Current International Journals

The following selection—alphabetical by title—reflects Cuban publishing in international medical and population health journals over the last quarter on an array of topics. Links to these journal articles may be found at www.medicc.org/mediccreview.

AFCo1 as nasal adjuvant of capsular polysaccharide from *Neisseria meningitidis* serogroup C induces systemic and mucosal immune responses. Romeu B, González E, Zayas C, Campo JD, Acevedo R, Cuello M, et al. *Scand J Infect Dis.* 2011 Jun 15.

Increasing emphasis is being placed on the mucosal administration of vaccines in order to stimulate mucosal as well as systemic responses. Findings from our group suggest that proteoliposome-derived cochleate (AFCo1) acts as a potent mucosal adjuvant. As an alternative to chemical conjugation, the current study aimed to determine the benefit of using AFCo1 to improve the mucosal and systemic immune responses to capsular polysaccharide of *Neisseria meningitidis* serogroup C (PsC), a model of a thymus-independent (TI) antigen. Therefore, intranasal (i.n.) immunization of 3 doses, 1 week apart with AFCo1 plus PsC in mice was conducted. Highly specific anti-PsC IgA responses and an anti-PsC IgG response were obtained. The subclass pattern induced against PsC was similar to that induced with the meningococcal vaccine. In summary, AFCo1 as nasal adjuvant was demonstrated to be capable of eliciting mucosal and systemic specific responses against a TI antigen.

A methodology based on molecular interactions and pathways to find candidate genes associated to diseases: its application to schizophrenia and Alzheimer's disease. Ochagavía ME, Miranda J, Nazábal M, Martín A, Novoa LI, Bringas R, et al. *J Bioinform Comput Biol.* 2011 Aug;9(4):541–57.

Experimental techniques for the identification of genes associated with diseases are expensive and have certain limitations. In this scenario, computational methods are useful tools to identify lists of promising genes for further experimental verification. This paper describes a flexible methodology for the *in silico* prediction of genes associated with diseases combining the use of available tools for gene enrichment analysis, gene network generation and gene prioritization. A set of reference genes, with a known association to a disease, is used as bait to extract candidate genes from molecular interaction networks and enriched pathways. In a second step, prioritization methods are applied to evaluate the similarities between previously selected candidates and the set of reference genes. The top genes obtained by these programs are grouped into a single list sorted by the number of methods that have selected each gene. As a proof of concept, top genes reported a few years ago in SzGene and AlzGene data-

bases were used as references to predict genes associated with schizophrenia and Alzheimer's disease, respectively. In both cases, we were able to predict a statistically significant amount of genes belonging to the updated lists.

A new oral vaccine candidate based on the microencapsulation by spray-drying of inactivated *Vibrio cholerae*. Año G, Esquisabel A, Pastor M, Talavera A, Cedré B, Fernández S, et al. *Vaccine.* 2011 Jun 15.

The aim of this work was to evaluate the microencapsulation by spray-drying of inactivated *Vibrio cholerae*, using methacrylic copolymers Eudragit® L30D-55 and FS30D. The microparticles obtained presented a particle size around 3.0µm. The preparation temperature affected the morphology and the antigenicity of microparticles, but it did not affect the *V. cholerae* content. *In vitro* release studies showed that in acid medium less than 5% of bacteria was released, and in neutral medium, Eudragit® L30D-55 microparticles released 86% after 24h, whereas FS30D released less than 30%. Rats inoculated with microparticles exhibited vibriocidal antibody titres. Microencapsulation by spray-drying of inactivated *V. cholerae* could be proposed as a method to obtain an oral vaccine which provides controlled release of the bacteria.

Association of MICA and MICB alleles with symptomatic dengue infection. García G, Puerto FD, Pérez AB, Sierra B, Aguirre E, Kikuchi M, et al. *Hum Immunol.* 2011 Jul 1. [Epub ahead of print]

Dengue viruses (DV) are one of the most important arthropod-borne viral diseases in the developing world. DV can cause syndromes that are either self-limiting or severe. Allelic variants of human leukocyte antigen (HLA) genes have been demonstrated to be associated with disease susceptibility. Here we report the association of nonclassical HLA class I MICA-MICB genes with disease outcome during DV infection. A sequencing-based typing method and genotyping of MICA and MICB in a well-characterized group of Cuban individuals with dengue hemorrhagic fever (DHF), dengue fever (DF), or asymptomatic dengue infection (ADI) was performed. Statistical analysis revealed a tendency for MICA*008 and MICB*008 to associate with susceptibility to illness when symptomatic versus asymptomatic cases (odds ratio [OR] = 2.1, $p(v) = 0.03$, and OR = 10.4, $p = 0.0096$, respectively) were compared. Surprisingly, a stronger association of both allelic forms was observed for the DF patients compared with the ADI group (MICA*008, OR

= 5.2, $p = 0.0001$; and MICB*008, OR = 13.2, $p = 0.0025$) rather than the severe cases. Major histocompatibility class I-related gene-related natural killer cells and/or $\gamma\delta$ and $\alpha\beta$ T-cell activation might regulate the development of symptomatic DF and DHF.

Biological fitness and action opportunity of natural selection in an urban population of Cuba: Plaza de la Revolución, Havana. Vázquez V, Alonso V, Luna F. *J Biosoc Sci.* 2011 Jul 15.

This paper describes the biological fitness of an urban population of Havana city, Plaza de la Revolución, which has the lowest fertility and the highest demographic ageing in Cuba. The aim is to assess the biological fitness of this community through the indexes of action opportunity of natural selection, to determine its evolutionary pattern and the influence of its socio-cultural peculiarity. Demographic data were obtained from the reproductive histories of 1200 women between the ages of 55 and 64. Data concerning mortality and surviving offspring from the first embryonic stages until age of reproduction were also collected. In order to measure the level of biological fitness two indexes were used: the Crow index of action opportunity of natural selection and the corrected index proposed by Johnston and Kensinger, which takes into account prenatal mortality. This corrected index was calculated including and excluding induced abortions in order to evaluate the contribution of these to biological fitness. When only postnatal mortality was considered, the results showed an evolutionary pattern similar to that of developed countries, based on low mortality and fertility. However, when prenatal mortality was taken into account, biological fitness decreased and the corrected index of natural selection was 4.5 times higher than when miscarriages and fetal deaths were not considered. Moreover, this corrected index was 2.65 times higher when induced miscarriages were considered, indicating the large decrease in biological fitness as a result of the current reproductive behaviour of frequent induced abortion.

Cancer survival in Cuba, 1994–1995. Garrote LF, Álvarez YG, Babie PT, Yi MG, Álvarez MG, Cicili ML. *IARC Sci Publ.* 2011;(162):89–95.

The population-based cancer registry in Cuba is a national cancer registry established in 1964; cancer registration is entirely done by passive methods. Data on survival from 13 cancer sites or types registered during 1994–1995 are reported. Follow-up has been carried out predomi-

nantly by passive methods, with median follow-up ranging from 13–54 months. The proportion with histologically verified diagnosis for various cancers ranged between 34–100%; death certificates only (DCOs) comprised 8–50%; 50–89% of total registered cases were included for the survival analysis. The 5-year age-standardized relative survival for selected cancers were breast (69%), colon (41%), cervix (56%), urinary bladder (64%), rectum (48%) and non-Hodgkin lymphoma (49%). The 5-year relative survival by age group showed no distinct pattern or trend, and was fluctuating. A decreasing survival with increasing clinical extent of disease was noted for all cancers studied. The data on survival trend revealed that the 5-year relative survival of most cancers diagnosed in 1994–1995 was greater than that in 1988–1989.

Caregiver perspectives for the prevention, diagnosis and treatment of childhood giardiasis in Havana City, Cuba. A qualitative study. Escobedo AA, Almirall P, Alfonso M, Avila I, Cimeman S, Salazar Y, et al. *Acta Trop.* 2011 Aug;119(2-3):99–106.

Although long considered a non-pathogenic protozoan, *Giardia lamblia* is now a well recognized cause of abdominal discomfort, diarrhoea and failure-to-thrive in children. The overall prevalence of this infection in Cuban population is about 7.2%; however, higher prevalences have been found among young children attending day-care centres and primary school in the country. Anecdotally, clinical giardiasis is generally considered to place a large burden on both diagnostic and treatment services in Cuba. In order to gain insight into caregivers' perspectives with respect to this infection in children, a qualitative study was carried out in a paediatric hospital in Cuba. Focus group discussions were conducted to gather information about the awareness of the giardiasis, their mode of transmission and symptoms, diagnosis process, treatment seeking behaviour, possible ways of prevention, barriers for not adopting preventive behaviours, and the source and channels of information about this disease. Caregivers have knowledge of giardiasis, although there were myths and misconceptions regarding giardiasis. Manifestations like diarrhoea, abdominal pain and nausea were cited; however, asymptomatic forms of these infections are hardly accepted. Boiling water and washing hands before eating and after defecation and washing vegetables were mentioned among the principal ways of preventing this infection. The most commonly mentioned reasons for not adopting preventive behaviours included lack of time due to outdoor activities and limitation of combustible distribution. Treatment-seeking behaviour when giardiasis suspected mainly included visiting the nearby family doctor. The findings of this study reveal the need for a health education intervention in areas of misperceptions and confusion.

CIGB-300, a synthetic peptide-based drug that targets the CK2 phosphoacceptor do-

main. Translational and clinical research. Perea SE, Baladron I, García Y, Perera Y, López A, Soriano JL, et al. *Mol Cell Biochem.* 2011 Jul 7.

CK2 represents an oncology target scientifically validated. However, clinical research with inhibitors of the CK2-mediated phosphorylation event is still insufficient to recognize it as a clinically validated target. CIGB-300, an investigational peptide-based drug that targets the phosphoacceptor site, binds to a CK2 substrate array in vitro but mainly to B23/nucleophosmin in vivo. The CIGB-300 pro-apoptotic effect is preceded by its nucleolar localization, inhibition of the CK2-mediated phosphorylation on B23/nucleophosmin and nucleolar disassembly. Importantly, CIGB-300 shifted a protein array linked to apoptosis, ribosome biogenesis, cell proliferation, glycolysis, and cell motility in proteomic studies which helped to understand its mechanism of action. In the clinical setting, CIGB-300 has proved to be safe and well tolerated in a First-in-Human trial in women with cervical malignancies who also experienced signs of clinical benefit. In a second Phase 1 clinical trial in women with cervical cancer stage IB2/II, the MTD and DLT have been also identified in the clinical setting. Interestingly, in cervical tumors the B23/nucleophosmin protein levels were significantly reduced after CIGB-300 treatment at the nucleus compartment. In addition, expanded use of CIGB-300 in case studies has evidenced antitumor activity when administered as compassionate option. Collectively, our data outline important clues on translational and clinical research from this novel peptide-based drug reinforcing its perspectives to treat cancer and paving the way to validate CK2 as a promising target in oncology.

Effects of D-003 (10 mg/day) on Bone Mineral Density of the Lumbar Spine and Femoral Neck in Postmenopausal Women: A Randomized, Double-Blinded Study. Ceballos A, Castaño G, Mendoza S, González J, Mas R, Fernández L, et al. *Korean J Intern Med.* 2011 Jun;26(2):168–78.

Background/Aims Increased osteoclast activity is a pivotal finding in osteoporosis. This increase is mediated via the mevalonate-to-cholesterol pathway, which is involved in producing the intermediates required for osteoclast activity. D-003, a mixture of high molecular weight sugarcane wax acids, has been shown to inhibit cholesterol synthesis prior to mevalonate production, resulting in a reduction of bone loss and resorption in ovariectomized rats. Moreover, previous studies have demonstrated that short-term D-003 treatment reduces urinary excretion of deoxypridinoline/creatinine in postmenopausal women. **Methods** We performed a double-blinded, placebo-controlled study to investigate the effects of D-003 (10 mg/day) treatment for 3 years on bone mineral density (BMD) in 83 postmenopausal women with low BMD. **Results** Over 3 years, D-003 treatment increased lumbar spine BMD (5.1%, $p < 0.01$) and improved

osteoporosis-related quality of life scores as compared with placebo-treated controls. D-003 was also well tolerated; the frequency of adverse events in the bone, joints, or muscle with D-003 treatment ($p < 0.05$) was lower than in the placebo group. **Conclusions** D-003 treatment (10 mg/day) for 3 years increased lumbar spine BMD and produced clinical improvements in postmenopausal women with low BMD. Further studies, however, will be required to confirm these results.

Ethical dilemmas in genetic testing: examples from the Cuban program for predictive diagnosis of hereditary ataxias. Mariño TC, Armiñán RR, Cedeño HJ, Mesa JM, Zaldivar YG, Rodríguez RA, et al. *J Genet Couns.* 2011 Jun;20(3):241–8.

Predictive testing protocols are intended to help patients affected with hereditary conditions understand their condition and make informed reproductive choices. However, predictive protocols may expose clinicians and patients to ethical dilemmas that interfere with genetic counseling and the decision making process. This paper describes ethical dilemmas in a series of five cases involving predictive testing for hereditary ataxias in Cuba. The examples herein present evidence of the deeply controversial situations faced by both individuals at risk and professionals in charge of these predictive studies, suggesting a need for expanded guidelines to address such complexities.

Evidence on impact of community-based environmental management on dengue transmission in Santiago de Cuba. Toledo ME, Rodríguez A, Valdés L, Carrión R, Cabrera G, Banderas D, et al. *Trop Med Int Health.* 2011 Jun;16(6):744–7.

During the dengue outbreak that struck Santiago de Cuba in 2006–2007, we conducted an observational study in the Mariana Grajales district, the former setting of a community trial for *Aedes aegypti* control. In the trial, community working groups (CWG) had been created in 29 randomly selected intervention house blocks, and routine vector control activities alone were conducted in the remaining 30 control blocks. The CWG elaborated and implemented with the population plans and activities to reduce *Aedes* infestation. They were still functional in 2006 and continued organizing community-based environmental management activities. The attack rate of dengue fever during the outbreak was 8.5 per 1000 inhabitants in the former intervention blocks and 38.1 per 1000 inhabitants in the control blocks, which corresponds to a relative risk of 4.5 (95% CI 3.1–6.5). There was a significantly higher proportion of unaffected intervention blocks, and affected blocks had on average substantially less cases than affected control blocks. This study indicates that community-based environmental management inserted in the routine *A. aegypti* control programme can not only sustainably curb vector infestation but also have an impact on dengue transmission.

Generation of HIV-1 and internal control transcripts as standards for an in-house quantitative competitive RT-PCR assay to determine HIV-1 viral load. Armas Cayarga A, Perea Hernández Y, González González YJ, Dueñas Carrera S, González Pérez I, Robaina Álvarez R. *Biotechnol Res Int.* 2011;2011:964831. Epub 2011 Jun 23.

Human immunodeficiency virus type-1 (HIV-1) viral load is useful for monitoring disease progression in HIV-infected individuals. We generated RNA standards of HIV-1 and internal control (IC) by *in vitro* transcription and evaluated its performance in a quantitative reverse transcription polymerase chain reaction (qRT-PCR) assay. HIV-1 and IC standards were obtained at high RNA concentrations, without DNA contamination. When these transcripts were included as standards in a qRT-PCR assay, it was obtained a good accuracy ($\pm 0.5 \log(10)$ unit of the expected results) in the quantification of the HIV-1 RNA international standard and controls. The lower limit detection achieved using these standards was 511.0 IU/mL. A high correlation ($r = 0.925$) was obtained between the in-house qRT-PCR assay and the NucliSens easyQ HIV-1 test (bioMérieux) for HIV-1 RNA quantitation with clinical samples ($N = 14$). HIV-1 and IC RNA transcripts, generated in this study, proved to be useful as standards in an in-house qRT-PCR assay for determination of HIV-1 viral load.

Intersectoral action for health at a municipal level in Cuba. Spiegel J, Alegret M, Clair V, Pagliccia N, Martínez B, Bonet M, et al. *Int J Public Health.* 2011 Aug 16.

Objective To consider how Cuba's acknowledged achievement of excellent health outcomes may relate to how health determinants are addressed intersectorally. **Methods** Our team of Canadian and Cuban researchers and health policy practitioners undertook a study to consider the organization and practices involved in addressing health determinants in two municipalities (one urban and one rural). The study included a questionnaire of municipal Health Council members and others involved in health and non-health sectors, key informant interviews of policy makers, focus groups in each municipality and examination of three common case scenarios. **Results** Regular engagement of different sectors and other agencies in addressing health determinants was quite systematic and comparable in both municipalities. Specific policies and organizational structures in support of intersectoral actions were frequently cited and illustrated in case scenarios that demonstrate how maintenance of regular linkages facilitates regular pursuit of intersectoral approaches. **Conclusions** The study demonstrates the feasibility of examining processes of intersectoral action for health processes and suggests that further examination in evaluating factors such as training, particular practices, etc., can be a fruitful direction to pursue comparatively and with analytical designs.

Low Prevalence of Helicobacter pylori Among Symptomatic Children from a Hospital in Havana, Cuba. Llanes R, Millán LM, Escobar MP, Gala A, Capó V, Feliciano O, et al. *J Trop Pediatr.* 2011 Jul 13. [Epub ahead of print]

The aims of this study were to assess the prevalence of *Helicobacter pylori* infection and to introduce a new algorithm to improve its diagnosis in Cuban symptomatic children. One hundred and thirty-three consecutive children with upper gastrointestinal symptoms were studied. Patients were endoscoped and antral biopsies were obtained for rapid urease test (RUT), culture and histology. Prevalence of *H. pylori* infection was 30.8%. No statistical differences were found concerning demographic, socio-economic factors or chief clinical complaints, between *H. pylori*-positive and negative children, except for haematemesis, which was significantly higher in infected children ($p = 0.003$). Histologically, there was statistical association between moderate chronic gastritis in infected children ($p = 0.04$). Culture and RUT had the highest specificity and sensitivity, respectively. The prevalence of *H. pylori* infection in Cuban symptomatic children is similar to the one observed in developed countries. Culture and RUT is a useful combination to diagnose *H. pylori* infection in paediatric patients.

Phase I clinical trial of the (131)I-labeled anticarcinoembryonic antigen CIGB-M3 multivalent antibody fragment. González GP, García IG, González JG, Sánchez LP, Mirabal MV, Marín CC, et al. *Cancer Biother Radiopharm.* 2011 Jul 5;26(3):353-63.

Center for Genetic Engineering and Biotechnology (CIGB)-M3 is a trivalent recombinant single-chain Fv antibody fragment specific for carcinoembryonic antigen (CEA). Preclinical studies with radiolabeled CIGB-M3 have showed that the antibody fragment accumulates in human colon tumor xenografts growing in nude mice. A Phase I clinical trial was carried out to determine safety, biodistribution, and pharmacokinetics of the radiolabeled CIGB-M3 in two groups of patients with CEA+ colorectal cancers. Group I (10 patients) received a single intravenous injection of 0.3 mg of (131)I-CIGB-M3 (16.7-23.3 mCi/mg). Group II (7 patients) received 1 mg (5-7 mCi/mg). No adverse events related to the injected product were recorded, and no immunology response was detected up to 6 months after the injection. Tumors were detected in 15 of the 17 studied cases. The pharmacokinetic profile showed beta half-times of 14.1 and 6.3 hours for Groups I and II, respectively. Seventy-two hours after the administration of the product, 85% of the total injected activity was excreted in urine in the form of free (131)I. The kidneys were identified as the organs that can limit the maximum tolerated dose. The (131)I-CIGB-M3 was safe in patients with colorectal cancer. The biodistribution and pharmacokinetic data suggest that the product can be further tested for molecular radiotherapy of CEA+ tumors.

Saccadic latency is prolonged in Spinocerebellar Ataxia type 2 and correlates with the frontal-executive dysfunctions. Rodríguez-Labrada R, Velázquez L, Seigfried C, Canales N, Auburger G, Medrano J, et al. *J Neurol Sci.* 2011 Jul 15;306(1-2):103-7.

Data on saccadic latency in patients with Spinocerebellar Ataxia 2 (SCA2) are sparse and contradictory. In order to determine whether saccadic latency is definitely prolonged, identify its possible determinants and evaluate it as disease biomarker we assessed the saccadic latency by electronystagmography in 110 SCA2 patients and their paired controls. Mean saccadic latencies were significantly longer in patients when compared to controls for all tested target displacements. Forty-six percent of SCA2 patients had saccadic latencies above the normal range. Reciprobit plots of saccadic latency demonstrated a skewed distribution in the direction of longer latencies for the patients compared to controls. As saccadic latency increased, the velocity and amplitude of saccades significantly decreased in SCA2 subjects but not in controls. Saccadic latency was not influenced by any demographical, clinical or molecular SCA2 variables, but it showed a significant correlation with the performance of the Stroop test, the verbal fluency test and the Wisconsin Card Sorting Test in SCA2 patients. This paper demonstrated that saccadic latency is prolonged in SCA2 patients and it significantly correlates with the performance of frontal-executive functions, thus this parameter could be a useful biomarker to evaluate the efficiency of future therapeutical options on these dysfunctions.

Therapeutic vaccination with an EGF-based vaccine in lung cancer: a step in the transition to a chronic disease. Rodríguez G, González G, Crombet T, Lage A. *Expert Rev Respir Med.* 2011 Jun;5(3):337-42.

The trend of increased survival in advanced tumors suggests the possibility of the transformation of cancer into a chronic disease. That goal will require therapeutic weapons with low toxicity that can be used chronically. Here we summarize the development of a therapeutic vaccine consisting in recombinant EGF chemically linked to a protein from *Neisseria meningitidis*. In mice, the vaccine elicited antibodies to self-EGF and had anti-tumor activity. Clinical trials have shown that the vaccine is also immunogenic and well tolerated in humans. The vaccination produced a decrease in plasma EGF concentration. Advanced lung cancer patients eliciting high antibody titers of EGF had better survival. The vaccine can be used long term and integrated with other treatment modalities.