Cuban Research in Current International Journals

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

Admixture estimates for the population in Havana City. Cintado A, Companioni O, Nazabal M, Camacho H, Ferrer A, Fernández De Cossio ME, et al. Ann Hum Biol. 2009 May;36(3):350–60.

Background The Cuban population is essentially a result of the admixture between Spanish, West African and, to a lesser degree, Amerindian tribes that inhabited the island. **Aim** The study analyzed the genetic structure of the three principal ethnic groups from Havana City, and the contribution of parental populations to its genetic pool. **Subjects and methods** According to genealogical information and anthropological traits, 206 subjects were classified as Mulatto, of Spanish decent or of African descent. Seventeen Ancestry Informative Markers, with high difference in frequency between parental populations, were selected to estimate individual and group admixture proportions. The statistical analyses were performed using the ADMIX, ADMIX95 and STRUCTURE 2.1 packages. **Results** The results demonstrate a high level of European and African admixture in Mulattos (57–59% European; 41–43% West African). The European contribution was higher in those of Spanish descent (85%) while in those of African descent, the West African contribution ranged between 74% and 76%. Genetic structure was only detected in Mulattos and those of African descent. An Amerindian contribution was not detectable in the studied sample. **Conclusion** Our findings indicate the existence of admixture and genetic structure in the population of Havana City. This study represents one of the first steps towards understanding Cuban population admixture in order to produce successful experimental designs for admixture mapping.

An inter-sector participatory strategy in Cuba using an ecosystem approach to prevent dengue transmission at the local level. Díaz C, Torres Y, de la Cruz AM, Álvarez AM, Piquero ME, Valero A, et al. Cad Saude Publica. 2009;25 Suppl 1.

Cuba is located among a group of countries with high dengue incidence. Following several epidemics in the last 10 years, the country designed, implemented, and evaluated a participatory strategy based on the Ecohealth approach. The aim was to promote inter-sector ecosystem management to decrease *Aedes aegypti* infestation and prevent dengue transmission in the municipality of Cotorro, in Havana city. The study adopted a participatory research methodology. The strategy ensured active participation by the community, diverse sectors, and government in the production of healthy ecosystems. Timely and integrated measures for prevention and control were developed, thereby decreasing the risk of vector proliferation and local dengue transmission. The approach allowed holistic problem analysis, priority setting, and administration of solutions. The strategy has been sustained two years after concluding the process.

Antioxidant effects of D-004, a lipid extract from the *Roystonea regia* fruit, on the plasma of healthy men. López E, Molina V, Illnait J, Oyarzábal A, Fernández LC, Más R, et al. Asian J Androl. 2009 May;11(3):385-92.

The aim of this study was to conduct a randomized, double-blind and placebo-controlled study to investigate the effects of D-004, a lipid extract of the *Roystonea regia* fruit that prevents testosterone- and phenylepinephrine-induced prostate hyperplasia in rodents, on plasma oxidative markers in healthy men. We enrolled male volunteers (20–55 years) in good health and without lower urinary tract symptoms. Thirty-four eligible participants were randomized to placebo or D-004 (320 mg) capsules administered daily for 6 weeks. An interim check-up and a final visit were conducted after 3 and 6 weeks of therapy, respectively. Physical examinations were performed at each visit, and laboratory tests were performed at baseline and at treatment completion. Oxidative variables included plasma malondialdehyde (MDA), total hydroxyperoxides (TOH), sulphydryl (SH) groups and total antioxidant status (TAS). We assessed treatment compliance and addressed adverse experiences (AEs) at weeks 3 and 6. At 6 weeks, with D-004, the mean reductions of plasma MDA (26.7%), TOH (18.8%) and SH groups (31.6%), and the mean increase of TAS (35.3%) were significantly different from those of placebo ($P < 0.001$ for plasma TAS, $P < 0.0001$ for all other comparisons). D-004 did not differ from the placebo in safety indicators. There were two withdrawals (both in the D-004 group), with one due to dyspepsia (the only AE during the trial). In conclusion, D-004 displayed antioxidant effects on plasma oxidative markers in healthy men, which was consistent with findings from laboratory experimental studies.

Association between the expression of IL-10 and T cell activation proteins loss in early breast cancer patients. Llanes Fernández L, Arango Prado MC, Alcocer González JM, Guerra Yi ME, Franco Odio S, Camacho Rodríguez R, et al. J Cancer Res Clin Oncol. 2009 Feb;135(2):255-64. Epub 2008 Jul 24.

Breast cancer patients may express abnormal cellular immune responses affecting their immunological competence. The analysis of immunological parameters may be useful as indicators of T cell function. To determine the expression of lymphocyte activation proteins and cytokines in tumor and non-metastatic axillary lymph nodes, 30 breast cancer patients were monitored. CD3 polypeptides, PTKs (protein tyrosine kinases) and phosphorylated tyrosines were studied by Western Blot and cytokines mRNA expression was determined by RT-PCR (reverse transcription-polymerase chain reaction). This group of patients had shown high immunohistochemistry expression of IL-10 in tumors. Activation proteins were mainly expressed in involved lymph nodes comparing with their expression in tumors. The differences in expression of CD3 polypeptides and $p56^{lck}$ between both locations were significant. There was no statistical association between PTKs and IL-10 in the tumor but more than 50% of cases who express IL-10 lost $p56^{lck}$, $p59^{fyn}$. A direct association between IL-10 and CD3 polypeptides was observed, however 52.2% of patients who express IL-10 did not express 41 kDa CD3-ζ form. IL-10 mRNA was detected in more than 50% of tumors contrary to the prevalence of type 1 cytokines in regional nodes (40%). The lack of expression of lymphocyte activations proteins and the high expression of IL-10 suggest a down-regulation on T cells function in the tumors. These results are useful in order to understand the local immune response that would be key in the control of the tumor progression.

Cuban neonatal screening of phenylketonuria using an ultramicro-fluorometric test. González EC, Frómeta A, del Río L, Castells E, Robaina MS, García SM, et al. Clin Chim Acta. 2009 Apr;402(1-2):129-32. Epub 2009 Jan 11.

Background Guthrie's bacterial inhibition assay has been used in Cuba since 1983. A decentralized program for the newborn screening of hyperphenylalaninemias started in the year 2000 using an ultramicro-fluorometric test (UMTEST PKU). **Methods** A simple and rapid ultramicro-fluorometric test based on McCaman and Robin's method has been designed, developed and applied for the measurement of Phe in dried blood spots on filter paper. **Results** The UMTEST PKU exhibited an acceptable precision and accuracy. Samples of 27,528 newborns on filter paper Schleicher & Schuell 903 (S&S 903) from the national neonatal screening program were collected and analyzed, and the mean Phe concentration was 66.5 µmol/l. Our assay showed high Pearson and concor-

dance correlations with 2 commercially available kits. A total of 521,923 Cuban newborns were studied from the year 2000 to 2007 using the UMTEST PKU. Elevated blood phenylalanine levels were found in 1,764 infants (0.34%) and no false negative were noted. Ten cases were diagnosed with phenylketonuria, all of them with an initial phenylalanine concentration over 360 µmol/l. **Conclusions** The analytical performance characteristics of our assay and its use in the national program have demonstrated its suitability for the neonatal screening of PKU.

***Helicobacter pylori* is not associated with anaemia in Latin America: results from Argentina, Bolivia, Brazil, Cuba, Mexico and Venezuela.** Santos IS, Boccio J, Davidsson L, Hernández Triana M, Huanca Sardinas E, Janjetic M, et al. Public Health Nutr. Epub 2009 Mar 4.

Objective To investigate the association between *Helicobacter pylori* infection and anaemia. **Design** Six cross-sectional studies. *H. pylori* infection was assessed by the [^{13}C]urea breath test using MS or IR analysis. Hb was measured for all countries. Ferritin and transferrin receptors were measured for Argentina, Bolivia, Mexico, and Venezuela. **Setting** Health services in Argentina, Brazil and Mexico or public schools in Bolivia, Cuba and Venezuela. **Subjects** In Argentina, 307 children aged 4–17 years referred to a gastroenterology unit; in Bolivia, 424 randomly selected schoolchildren aged 5–8 years; in Brazil, 1,007 adults (157 men, 850 women) aged 18–45 years attending 31 primary health-care units; in Cuba, 996 randomly selected schoolchildren aged 6–14 years; in Mexico, 71 pregnant women in their first trimester attending public health clinics; in Venezuela, 418 children aged 4–13 years attending public schools. **Results** The lowest prevalence of *H. pylori* found was among children in Argentina (25.1%) and the highest in Bolivia (74.0%). In Bolivia, Cuba and Venezuela children showed similar prevalence of *H. pylori* infection as in Brazilian and Mexican adults (range 47.5% to 81.8%). Overall anaemia prevalence was 11.3% in Argentina, 15.4% in Bolivia, 20.6% in Brazil, 10.5% in Cuba and 8.9% in Venezuela. Adjusted analyses allowing for confounding variables showed no association between *H. pylori* colonization and anemia in any study. Hb, ferritin and transferrin receptor levels were also not associated with *H. pylori* infection in any country. **Conclusions** The present study showed no evidence to support the hypothesis that *H. pylori* contributes to anemia in children, adolescents, adults or pregnant women in six Latin American countries.

Immunogenicity of CIGB-230, a therapeutic DNA preparation, in HCV-chronically infected individuals in a Phase I clinical trial. Álvarez Lajonchere L, Shoukry NH, Grá B, Amador Cañizares Y, Helle F, Bédard N, et al. J Viral Hepat. 2009 Mar;16(3):156-67. Epub 2008 Oct 31.

Summary Hepatitis C virus (HCV) is a worldwide health problem. No vaccine is available against this pathogen and therapeutic treatments currently in use are of limited efficacy. In the present study, the immunogenicity of the therapeutic vaccine candidate CIGB-230, based on the mixture of pIDKE2, a plasmid expressing HCV structural antigens, with a recombinant HCV core protein, Co.120, was evaluated. CIGB-230 was administered by intramuscular injection on weeks 0, 4, 8, 12, 16 and 20 to 15 HCV-chronically infected individuals, non-responders to previous treatment with interferon (IFN) plus ribavirin. Interestingly, following the final immunization, neutralizing antibody responses against heterologous viral pseudoparticles were modified in eight individuals, including six de novo responders. In addition, 73% of vaccinees exhibited specific T cell proliferative response and T cell IFN-gamma secretory response 24 weeks after primary immunization with CIGB-230. Furthermore, 33.3% of individuals developed de novo cellular immune response against HCV core and the number of patients (46.7% at the end of treatment) with cellular immune response against more than one HCV structural antigen increased during vaccination (P = 0.046). In addition, despite persistent detection of HCV RNA, more than 40% percent of vaccinated individuals improved or stabilized liver histology, particularly reducing fibrosis, which correlated with cellular immune response against more than one HCV antigen (P = 0.0053). In conclusion, CIGB-230 is a promising candidate for effective therapeutic interventions based on its ability for enhancing the immune response in HCV chronically infected individuals.

Intralesional administration of epidermal growth factor-based formulation (Heberprot-P) in chronic diabetic foot ulcer: treatment up to complete wound closure. Fernández-Montequín JI, Betancourt BY, Leyva-González G, Mola EL, Galán-Naranjo K, Ramírez-Navas M, et al. Int Wound J. 2009 Feb;6(1):67-72.

Previous studies have shown that an epidermal growth factor-based formulation (Heberprot-P) can enhance granulation of high-grade diabetic foot ulcers (DFU). The aim of this study was to explore the clinical effects of this administration up to complete wound closure. A pilot study in 20 diabetic patients with full-thickness lower extremity ulcers of more than 4 weeks of evolution was performed. Mean ulcer size was 16.3 ± 21.3 cm^2. Intralesional injections of 75 µg of Heberprot-P three times per week were given up to complete wound healing. Full granulation response was achieved in all 20 patients in 23.6 ± 3.8 days. Complete wound closure was obtained in 17 (85%) cases in 44.3 ± 8.9 days. Amputation was not necessary in any case and only one relapse was notified. The most frequent adverse events were tremors, chills, pain and ardor at site of administration and local infection. The therapeutic scheme of intralesional Heberprot-P administration up to complete closure can be safe and suitable to improve the therapeutic goal in terms of healing of chronic DFU.

Molecular diagnosis of Toxoplasma gondii infection in cerebrospinal fluid from AIDS patients. Alfonso Y, Fraga J, Fonseca C, Jiménez N, Pinillos T, Dorta Contreras AJ, et al. Cerebrospinal Fluid Res. 2009 Mar 6;6:2.

Background Toxoplasmic encephalitis (TE) is one of the most common opportunistic infections in immunocompromised patients. In Cuba, despite the highly active antiretroviral therapy, TE is still the most important cause of cerebral mass lesions in patients infected with the human immunodeficiency virus (HIV). The detection of *Toxoplasma gondii* by PCR may facilitate the diagnosis and follow-up of TE in acquired immunodeficiency syndrome (AIDS) patients by direct identification of parasite DNA in clinical samples. The aim of the present study was to evaluate a rapid PCR method using the B1 gene to detect *T. gondii* in cerebrospinal fluid (CSF) samples from patients with suspected TE. **Methods** CSF samples from AIDS and HIV-negative patients were analyzed. Patients were divided into two groups according to the Centers for Disease Control and Prevention (CDC) criteria for AIDS-related TE: AIDS patients with suspected neurotoxoplasmosis and AIDS and HIV-negative patients with other confirmed neurological diseases but no suspicions of TE. Predictive values, diagnostic accuracy, sensitivity and specificity of the PCR B1 method were calculated. **Results** The results obtained from 190 patients showed that this assay has a good sensitivity and specificity (83.3% and 95.7%, respectively) for the diagnosis of TE in AIDS patients. **Conclusion** PCR using the B1 gene and B22/B23 set of primers is a single, rapid and reliable method that may be valuable for discrimination between toxoplasmosis and other central nervous system (CNS) diseases.

Molecular epidemiology of spinocerebellar ataxias in Cuba: Insights into SCA2 founder effect in Holguín. Velázquez Pérez L, Sánchez Cruz G, Santos Falcón N, Almaguer Mederos LE, Escalona Batallan K, Rodríguez Labrada R, et al. Neurosci Lett. 2009 Apr 24;454(2):157-60.

The objective of this study was to determine the prevalence of hereditary ataxias in Cuba, with a special focus on the clinical and molecular features of SCA2. Clinical assessments were performed by neurological examinations and application of the SARA scale. Molecular analyses of genes SCA1–3, SCA6, SCA17 and DRPLA identified 753 patients with SCA and 7,173 asymptomatic relatives, belonging to 200 unrelated families. 86.79% of all SCA patients were affected with SCA2. In the Hol-

guin province, the average population prevalence of SCA2 is 40.18 × 10^5 inhabitants, with the remarkable figure of 141.66 × 10^5 in the Baguanos municipality. The high prevalence of the SCA2 mutation in Holguin reflects most likely a founder effect. The stabilization of the prevalence along time suggests the existence of premutated chromosomes with pure CAG, acting as reservoir for further expansions. CAG repeat length correlated inversely with age at onset, accounting for 80% of the variability. Genetic anticipation was observed in the 80% of transmissions. Repeat instability was greater in paternal transmissions whereas CAG expansions without anticipation was observed in 10.97% suggesting the effect of CAA interruptions in the CAG segment, which decrease the toxicity of the abnormal ataxin-2, and/or other protective factors.

Motor Decline in Clinically Presymptomatic Spinocerebellar Ataxia Type 2 Gene Carriers. Velázquez Pérez L, Díaz R, Pérez González R, Canales N, Rodríguez Labrada R, Medrano J, et al. PLoS ONE. 2009;4(4):e5398. Epub 2009 Apr 29.

Background Motor deficits are a critical component of the clinical characteristics of patients with spinocerebellar ataxia type 2. However, there is no current information on the preclinical manifestation of those motor deficits in presymptomatic gene carriers. To further understand and characterize the onset of the clinical manifestation in this disease, we tested presymptomatic spinocerebellar ataxia type 2 gene carriers, and volunteers, in a task that evaluates their motor performance and their motor learning capabilities. **Methods and Findings** 28 presymptomatic spinocerebellar ataxia type 2 gene carriers and an equal number of control volunteers matched for age and gender participated in the study. Both groups were tested in a prism adaptation task known to be sensible to both motor performance and visuomotor learning deficits. Our results clearly show that although motor learning capabilities are intact, motor performance deficits are present even years before the clinical manifestation of the disease start. **Conclusions** The results show a clear deficit in motor performance that can be detected years before the clinical onset of the disease. This motor performance deficit appears before any motor learning or clinical manifestations of the disease. These observations identify the performance coefficient as an objective and quantitative physiological biomarker that could be useful to assess the efficiency of different therapeutic agents.

Pulmonary tuberculosis case detection through fortuitous cough screening during home visits. González-Ochoa E, Brooks JL, Matthys F, Calisté P, Armas L, Van der Stuyft P. Trop Med Int Health. 2009 Feb;14(2):131-5.

Objective To compare the yield of active tuberculosis (TB) case detection among risk groups during home visits with passive detection among patients at health services. **Methods** In April 2004, in a first phase, we introduced, active screening for coughing among all family members of patients that were visited at home by their family doctor or nurse for other reasons. Subsequently, from October 2004 onwards, active screening was restricted to family members belonging to groups at risk of TB. **Results** The overall detection rate of TB increased from 6.7 per 100,000 during passive detection at health services before the intervention to 26.2 per 100,000 inhabitants when passive detection was complemented by active case finding. Active screening among risk groups yielded 35 TB cases per 1000 persons screened compared to 20 TB cases per 1000 persons passively screened at health services. Active case finding was particularly efficient in those coughing for 3 weeks or more (107/1000 screened). **Conclusion** This study demonstrates that active case finding in groups at risk during home visits increases the case detection rate in the population and permits the identification of cases that may not be detected through passive case finding at health facility level.

Reduced frequency of ALS in an ethnically mixed population: a population-based mortality study. Zaldivar T, Gutiérrez J, Lara G, Carbonara M, Logroscino G, Hardiman O. Neurology. 2009 May 12;72(19):1640-5.

Objective To describe ALS mortality rates in the well-characterized ethnically mixed Cuban population over a 6-year period. **Background** There have been few population-based epidemiologic studies of ALS in non-Europeans. Preliminary data from the United States suggest a lower frequency of ALS in Hispanic and African groups compared with those of European descent. The Cuban population of 11 million comprises three main ancestral groups classified by skin color as white (65%), mixed (24%), and (black 10%). Medical care is of a high standard and is free. Cuba is ideally placed to establish the frequency of ALS in an admixed population of diverse ethnic origin. **Methods** Multiple-cause mortality files from the Central Statistics office in Cuba for the years 2001 through to 2006 were searched for codes corresponding to ALS. Age-adjusted mortality rates were calculated by sex, race/ethnicity, age, and geographic region at time of death. **Results** Four hundred thirty-two patients with a diagnosis of ALS were identified. The mean age at death was 63.7 years. There was a slight male predominance (1.1:1). The adjusted death rate from ALS for the total population older than 15 years was 0.83 per 100,000. The adjusted mortality rate per 100,000 was considerably lower in the mixed population (0.55; confidence interval [CI] 0.4–0.72) than in whites (0.93; CI 0.83–1.03) and blacks (0.87; CI 0.62–1.17). There was no correlation between the number of neurologists in each region and the mortality rate from ALS (r = 0.268, p = 0.335). **Conclusions** The overall mortality rate from ALS in Cuba is similar to that described in Hispanic populations in the United States and is lower than in Northern European populations. Mortality from ALS is lowest in a population of mixed ancestry. Ancestral origin is likely to play a role in ALS susceptibility.

Safety and preliminary efficacy data of a novel Casein Kinase 2 (CK2) peptide inhibitor administered intralesionally at four dose levels in patients with cervical malignancies. Solares AM, Santana A, Baladron I, Valenzuela C, González CA, Díaz A, et al. BMC Cancer. 2009 May 13;9(1):146. [Epub ahead of print]

Background Cervical cancer is now considered the second leading cause of death among women worldwide, and its incidence has reached alarming levels, especially in developing countries. Similarly, high grade squamous intraepithelial lesion (HSIL), the precursor stage for cervical cancer, represents a growing health problem among younger women as the HSIL management regimes that have been developed are not fully effective. From the etiological point of view, the presence of Human Papillomavirus (HPV) has been demonstrated to play a crucial role for developing cervical malignancies, and viral DNA has been detected in 99.7% of cervical tumors at the later stages. CIGB-300 is a novel cyclic synthetic peptide that induces apoptosis in malignant cells and elicits antitumor activity in cancer animal models. CIGB-300 impairs the Casein Kinase (CK2) phosphorylation, by targeting the substrate's phosphoaceptor domain. Based on the perspectives of CIGB-300 to treat cancer, this "first-in-human" study investigated its safety and tolerability in patients with cervical malignancies. **Methods** Thirty-one women with colposcopically and histologically diagnosed microinvasive or pre-invasive cervical cancer were enrolled in a dose escalating study. CIGB-300 was administered sequentially at 14, 70, 245 and 490 mg by intralesional injections during 5 consecutive days to groups of 7–10 patients. Toxicity was monitored daily until 15 days after the end of treatment, when patients underwent conization. Digital colposcopy, histology, and HPV status were also evaluated. **Results** No maximum-tolerated dose or dose-limiting toxicity was achieved. The most frequent local events were pain, bleeding, hematoma and erythema at the injection site. The systemic adverse events were rash, facial edema, itching, hot flashes, and localized cramps. 75% of the patients experienced a significant lesion reduction at colposcopy and 19% exhibited full histological regression. HPV DNA was negative in 48% of the previously positive patients. Long term follow-up did not reveal recurrences or adverse events. **Conclusions** CIGB-300 was safe and well tolerated. This is the first clinical trial where a drug has been used to target the CK2 phosphoaceptor domain providing an early proof-of-principle of a possible clinical benefit.