

“III International Symposium of Hereditary Ataxias”

Guardalavaca Beach, Holguín, Cuba

1st-3rd October 2008

Ataxia UK contribution: £1000

The III International Symposium on Hereditary Ataxia was held from 1st to 3rd October in Cuba, which has the highest concentration of people with hereditary ataxias in the world. SCA2 is most prevalent form.

The aim of the meeting was to bring together researchers from around the world to facilitate interaction and collaborative research into hereditary ataxias. Unfortunately, hurricane Ike had hit the country in September and caused much damage and not all delegates attended the meeting in October. However, 26 Cuban researchers and 24 researchers from other countries (Germany, India, Canada, Guatemala, Mexico, Argentina and Colombia) were present. Over the course of the three days, 60 presentations took place (lectures, oral presentations and poster presentations).

A variety of topics were covered in the meeting, by both junior and senior researchers. Hot topics that were presented included the study of sleep in SCA2 patients (Roberto Rodríguez Labrada PhD, Cuba), genetic and epigenetic modifiers (José Laffita PhD, Cuba), apoptotic pathways triggering cell death in transgenic SCA2 mice (Dany Cuello PhD, Cuba), models of brain recovery in damaged rats with Fe⁺⁺ (Antonio Bueno Navas PhD, Mexico), epidemiology of SCA2 in Cuba (Prof Luís Velázquez Pérez PhD), metabolic therapies useful in ataxias (Patrick McLeod PhD, Canada), basis of neurotransmissions in SCAs (Prof Henry Stokes Brown PhD, Guatemala) and SCA12 in India (Achal Shrivastava PhD, India). An award aimed at young scientists working in neurodegenerative conditions, particularly SCAs, was presented to José Miguel Laffita PhD and to Luís Almaguer Mederos PhD for their work on SCA2.

As well as researchers, patients were also present at the meeting, and were particularly involved in the neurorehabilitation activities and workshops.

A list of abstracts from the conference is below. We are grateful to Prof Luís Velázquez Pérez and Dr José Miguel Laffita Mesa for providing the abstracts and the information in this summary.

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Lectures

1st October 2008
ROOM A

C-01: Biotechnology in Cuba: The CIGB experience

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The existence in Cuba of a well developed, sophisticated and productive biotechnology industry is well recognized worldwide. Some typical traits characterize the biotechnological sector in Cuba: The investor is the Cuban government-Biotechnology is part of the health system- The biotechnology success is essentially supported by Cuban scientists and professionals-Operability in a “closed cycle” way from research to commercialization by fully integrated institutions -National collaboration instead of individual competition -“Spin off” companies derived from scientific or production institutions In the period from 1990 to 1996 the Cuban government invested around 1 billion dollars to give rise to what is currently known as “The Western Havana Bio-Cluster” (the first and more important), comprising by 52 institutions related to the biotechnology field. They carry on more than 100 research projects, which have generated a product pipeline with more than 60 new products. The Cuban biotechnology sector has registered 24 medical products among biopharmaceuticals and vaccines. Several of them Cuban biotech products are unique, such as the meningitis B vaccine, Haemophilus influenzae type b synthetic vaccine, the PPG anti-cholesterol pill, and several monoclonal antibodies and vaccines for the treatment of cancer, together with agro-biotech products such as the tick vaccine and a bionematicide. The available production capacities have been able to supply for Cuban needs and for exports, and to achieve regulatory standards according to current Good Manufacturing Practices. The Centre for Genetic Engineering and Biotechnology (CIGB) has become in one of the most important research-production facilities at the “West Havana Bio-Cluster”. The CIGB works in the fields of healthcare-biotechnology and agro-animal biotechnology. The CIGB has more than 20 years experience in the production of several recombinant biopharmaceutical molecules such as interferon, Hepatitis B vaccine, streptokinase, epidermal growth factor, and many other products, which are already producing a positive impact on public health in Cuba and a cash flow sales to more than 30 countries in the world. To date, 131 new drug applications for nine CIGB products have been approved in 57 countries. CIGB with under 300 scientists and engineers work in more than 50 ongoing research and development projects which involve new vaccines, therapeutics recombinant proteins and peptides, molecular vaccines, therapeutic monoclonal antibody and diagnostic systems.

C-02: Spinocerebellar Ataxia Type 2: The Most Frequent Mutation in Cuba. From the Prevalence to the Endophenotypical Markers for Therapeutic Evaluation.

Velázquez Pérez Luis, Sánchez Cruz Gilberto, Velázquez Mercedes, Almaguer Luis Enrique, Rodríguez Labrada Roberto, Escalona Karel, Almira Yosvanis, Reynaldo Rubén, Prieto Lisandra, Aguilera Rodríguez Raúl, Laffita Mesa José M. (Cuba)
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The hereditary ataxias in Cuba make up the highest concentration of these patients in the world. *Methods:* We made an epidemiological and descriptive study of 757 patients and 7 173 members of families at risk from this disorder in Cuba. Genetic analyses were done for various SCA subtypes *Results:* 86.79% of patients with autosomal dominant inheritance segregated the SCA2. In Cuba, the general prevalence for cerebellar ataxia is 9.12 cases per 100 000 inhabitants. However in the province of Holguin the prevalence reaches up values up to 42 cases per 100 000 inhabitants with remarkable figure of 503 cases per 100 000 inhabitants in Potrerillo, an area of the municipality of Báguano. The age group that was most affected was that of 30-39 years, with a prevalence of 63.97 cases per 100,000 inhabitants. The risk of members of affected families showing the disorder was 159.33 cases per 100,000 inhabitants in this province. The highest incidence was 18.08 cases per 100,000 inhabitants in Cacocum. The age onset was inversely correlated with the CAG repeat expansion. Sixty seven percent of the patients with (CAG)₄₁ and 99% of the patients with (CAG)₄₂₋₇₉ presented clinical symptoms at 30 years old or younger. Clinical manifestations of SCA2 were cerebellar syndrome, slowing of eye movements and autonomic disorders. Repeat length correlated inversely with age at onset, accounting for 80% of the variability. Genetic anticipation was observed in the 80% of transmissions. Paternal transmissions lead to greater repeat instability. The proportion of SCA2 mutation in Cuba was highest at worldwide scale, reflecting a founder effect. *Conclusions.* The prevalence and incidence are the highest in the world. This together with the dominant pattern of inheritance, the effect of anticipation and inexorably progressive course of the disorder shows the serious health problem that affects the Eastern region of Cuba. This population is an special resource for genetic and non genetic studies focused in the developed of therapeutic alternatives

C-03: Hereditary Ataxias in the Indian population: Phenotype to Genotype correlations

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A vast majority of hereditary ataxias are associated with expansion of repetitive sequences. Till date 17 loci have been reported with expansion out of which 12 of them are associated with Spinocerebellar Ataxias (SCAs). Understanding Genotype-Phenotype correlations in Spinocerebellar Ataxia would be integral in clinical diagnosis, assessing prognosis and thereby planning optimal treatment/disease management strategies. We have created one of the largest locus specific variation databases for Spinocerebellar Ataxias (SCA-LSVD) which houses detailed information on the clinical features and genotype information pertaining to the various SCA loci of patients from more than 450 families across India. This would enable us to address pertinent questions with respect to Genotype-Phenotype correlations in SCAs. Of the total number of patients screened, 43% of the patients could be characterised to 6 known SCA types based on the genotypes at their respective genomic loci. Prevalence of ataxia varied with SCA2 having the highest frequency (33%) followed by SCA12 (26%),

SCA1 (19%), FRDA (11%), SCA3 (9%), SCA7 (2%) among genetically characterised cases. DRPLA, SCA6 and SCA8 were not observed in any of the families. Genotype-Phenotype correlations of the ataxias also revealed subtle features that distinguish SCA subtypes with overlapping cerebellar features. For example, individuals of SCA12 had a characteristic tremor not observed in other ataxias and this clinical feature was specific to an ethnic population. Once a close link with tremor, ethnicity and SCA12 was established, it became much easier to classify SCA12 and also identify uniqueness of the clinical features not overlapping with other ataxias. Similarly, reduced saccadic velocity in all SCA2 cases differentiates it from SCA1 and SCA3. This would be a very useful starting point for understanding the molecular correlates of phenotypes in ataxia which is a multi locus disease where related molecular mechanisms converge to overlapping phenotypes. SCA-LSVD would also be an important resource to identify novel candidate loci for SCAs.

C-05: Targeting Energy Metabolism in Polyglutamine Repeat Diseases?.

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With the completion of the Human Genome Project, attention is now focusing on the proteome, nutrigenomics, and the study of the bidirectional interactions between genes and diet, and metabolomics, the integrated study of the many small molecules produced by metabolism. Some nutrients, after interacting with a receptor, behave as transcription factors that can bind to DNA and acutely induce gene expression; while nutrient can induce epigenetic interactions that can alter the structure of histone proteins in chromatin so that gene expression is chronically altered; and genetic common genetic variations [single-nucleotide polymorphisms (SNPs)] can alter the expression or functionality of genes.

This presentation will review the current progress in using a combined nutrigenomic approach to the treatment of neurological disorders caused by dynamic mutations. This approach offers an opportunity to provide a neuroprotective strategy for the treatment of presymptomatic individuals with a known mutation such as we see in Spinocerebellar Ataxia 2.

C-06: Supplement of Zn in the diet protects transgenic mouse of the Ataxia type SCA-2. One year of study.

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CQF Havana, Cuba; CIGB Havana, Cuba.

The objective of this work was the evaluation of supplement of zinc during one year in the food in a transgenic animal model of the SCA2 disease, as well as its role in the neuropathological mechanisms that underline this human disorder. The worked line of mice was a Homozygote FO66 Line Transgenic that has as characteristic 75 repetitions of the chain of DNA of CAG.s The experiment to be carried out with three experimental groups, Ataxic mouse with normal diet (G1, n=11); Ataxic mice with diet with supplement of Zinc (G2, n=11) and healthy (G3, n=11). Frequently monthly was study, in each experimental group the corporal weight (CW), the Rotarod performance, Footprinting, clasp test, the electric conduction (EC) was studied in the later extremities of the animals and the survival (S) and histologist study (HS) was done. The data was carried out a descriptive statistical analysis using a parametric Factorial ANOVA and a contrast of ranges of Kruskal Wallis for not parametric. The results in the animals on: CW; Rotarod performance, Footprinting, clasp test, EC, S and HS were significantly better in the G2 when was compared with the animals of the G1 and similar during more time to the animals of the G3, The beneficial effect of the Zn⁺⁺ in the diet is absolute scientific novelty for this illness. Their action could be the result of rescuing the modulator effect of the Zn⁺⁺, for which gets lost the homeostasis gradually with the course of the illness. The reached results endorse our hypothesis about of a progressive lost of Zn⁺⁺ in this illness, given its biggest use, in the constant synthesis and degradation of the protein mutata Ataxina 2.

C-07: Pharmacological studies of *mangifera indica l.* Extract and mangiferin in spinocerebellar ataxia type 2 transgenic mice related with their possible neuroprotective actions.

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Center of Investigations and Rehabilitation of Hereditary Ataxias (CIRAH), Holguín, Cuba.

The Spinocerebellar Ataxia type 2 (SCA-2) is a molecular based disease that has a prevalence of 43 per 100,000 inhabitants in Holguin province, Cuba, which is the highest one reported world-wide. SCA2 is characterized by a high variability in its clinical and electrophysiological presentation, even intrafamiliarily and it is due to an intergenerational CAG repeat expansion contained in the first exon of disease-causing gene This neurodegenerative disease has been associated with neurotoxicity, whereas the primary specific degeneration target are the cerebellum Purkinge neurons. The identification of factors that contribute to explain the phenotypic variability of patients, which in some way are related with a delay in the onset of the disease, are basic points in the studies that we have been conducting in our lab. The efforts are directed towards clarifying the ethiology (genetic and non genetic factors), as well as towards obtaining a therapy for SCA2 aimed at delaying the onset of the disease and improving the patient's quality of life.

An aqueous stem bark extract of *Mangifera indica L* (MiE) has been used in pharmaceutical formulation in Cuba under the brand name of Vimang®. Scientific evidence indicates that MiE and its major constituent, mangiferin, have demonstrated antioxidant, anti-inflammatory

antiallergic, analgesic, and neuroprotective actions. Given the several biological actions of MiE and mangiferin and its potential uses as a therapeutic agent against brain disorders, we investigated the effect of oral supplementation of MiE or mangiferin on behavioral outcomes of neurological cognitive functions and several motor coordination-related parameters in SCA2 transgenic mice. Adults animals (the SCA2 transgenics and the progenitors healthy wild-type) provided by CIGB, Cuba, were treated daily, with 10, 50 and 100 mg/kg of MiE or 10 mg/kg of mangiferin dissolved in water given during 12 months (p.o.). Different experiments orientated to measure the mice behaviour were performed: A digitalized rotating rod apparatus, together with footprinting and cord tests were used to investigate the influence of antioxidant treatment on mice motor coordination. At the same time three neurobehavioral tests were performed: inhibitory avoidance, object recognition and open field. Analysis of SCA2 transgenic mice revealed significant differences in motor coordination compared with its progenitors. Daily oral administration of 100 mg/kg of MiE during one year markedly improved motor performance of SCA2 mice. At the same dose MiE recovered from the lack of weight of these animals. In all test developed, there were differences between male and female animals. Finally, the analysis of the behaviour of the animal when walking (footprinting test) corroborated that MiE (10-100) mg/kg) improve the lack of motor coordination of SCA2 transgenic mice. On the other hand, the highest doses of MiE (50 and 100 mg/kg) also improved selectively the aversive memory (inhibitory avoidance task) in the transgenic female mice. However, the treatments with MiE and mangiferin neither affected the locomotion and habituation (open field test) nor the declarative memory (object recognition task). The results provide important evidences that may conduced to considered the beneficial effects of MiE and mangiferin in behavioural parameters related to essential nervous system functions associated to neurological disorders; motor coordination, balance and some cognitive events associated to memory process. Further experimental and clinical studies will be needed to clarify the effects and mechanisms involved in the pharmacological actions of MiE and its main component mangiferina in SCA2.

2nd October 2008
ROOM B

SESSION
NEW ADVANCES IN NEUROPHYSIOLOGY AND IMAGENOLOGY

C-08: Cuban Human Brain Mapping Project

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As a contribution to International Human Brain Mapping Project, Cuba has initiated the construction of a comprehensive data base from a random sample of the population comprising medical, psychological and neuroimaging data. From a total sampling universe of 1939 more than 560 Normal subjects have been recruited for the project, screened for normality and provided high density EEG recording as well as T₁, T₂, PD, DWI images. Processing pipelines have been developed and applied for quantitative EEG tomography (qEEGt), volumetric measures. Currently available are estimates of population values for these measures for ages 18-69 which are in agreement with previous results. New insights are provided by the analysis of relations between anatomical and physiological information, examples of which are correlation between DWI, reaction time and alpha peak frequency. The normative values established have already provided information about brain abnormalities in subjects with dyscalculia and mild cognitive impairment. The project is currently projected to be extended to other countries in Latin America.

C-09: Saccadic pathology in SCA2 patients and presymptomatic carriers

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A distinctive clinical sign of Spinocerebellar ataxia type 2 (SCA2) is the early slowing of saccadic eye movements. We assessed maximal saccade velocity (MSV) in 82 patients and 80 controls and correlated it to disease duration, polyglutamine expansion size, age at onset, ataxia score, age, and sex. Little overlap with normal values was found even at earliest stages. Stepwise linear regression analysis showed that 60-degree MSV was strongly influenced by polyglutamine size and less by disease duration, whereas the reverse was found for ataxia score. In a second study, we assessed maximal saccade velocity (MSV) in 34 presymptomatic spinocerebellar ataxia type 2 (SCA2) mutation carriers and in 34 matched controls. MSV of saccades with 60-degree amplitudes was significantly reduced in mutation carriers, and again correlated to polyglutamine expansion size. Thus, saccade velocity is a sensitive, quite specific, and objective endophenotype, which reflects early pontine degeneration and precedes ataxia manifestation, and which is useful to search polyglutamine modifier genes.

C-10: PET imaging in parkinsonism and spinocerebellar ataxia

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Positron emission tomography (PET) and single photon emission computed tomography (SPECT) investigations of well-defined monogenic hereditary movement disorders allow visualizing the phenotypic sequels of a given genetic defect (phenotype-genotype correlation). Functional brain imaging enables the metabolic characterization of different types of spinocerebellar ataxia (SCA) in vivo. The most widely used radiotracers in SCA patients are 18-Fluorodeoxyglucose (FDG) as a measure of global and local neuronal energy metabolism and ligands for pre- and postsynaptic dopaminergic function (e.g. 18-Fluorodopa, ¹²³I-βCIT, ¹¹C-raclopride, ¹²³I-IBZM and others). The latter showed the involvement of nigrostriatal dopaminergic nerve terminals in the disease process of SCA2 corresponding to the clinical findings of limb slowness and dystonia in some affected individuals. Comparable findings were also demonstrated in SCA3, 6 and 17. Glucose hypometabolism of the

brainstem and cerebellum in FDG-PET is another common feature of SCA2 patients. Some studies showed slight FDG uptake diminutions in clinically asymptomatic carriers of CAG trinucleotide repeat expansions in the SCA2 gene. Therefore, FDG-PET might be useful in the early detection of subclinical disease activity in SCA2. A reduced FDG uptake also demonstrated the involvement of several cortical regions in SCA6 patients indicating a widespread neuronal dysfunction which is not restricted to the cerebellum. It has been shown that the severity of ataxia symptoms (measured with the International Cooperative Ataxia Rating Scale, ICARS) correlates best with frontal hypometabolism in FDG-PET suggesting that fronto-cerebellar projections play a major role for the development of ataxia in SCA.

C-11: Multimodal neuroimaging of unconscious-covert-recognition

Valdés Sosa Michel (Cuba)

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Models of information processing in Cognitive Neuropsychology (e.g. visual recognition) have primarily originated from single-case studies (relying heavily on the discovery of double dissociations). Although the behavioural consequences of focal lesions would seem to provide the ultimate test of brain structural-functional relations, these cognitive models are very poor in neurophysiological and neuroanatomical detail. Conversely, integrated analysis of brain images derived from different modalities (e.g. structural and functional magnetic resonance imaging, electrophysiological data, and tractography derived from diffusion tensor imaging), is leading to detailed proposals of the neural network involved in the recognition of visual objects (including faces) in typical subjects. Conscious face recognition is conceived to sequentially involve early visual areas, the “occipital face area (OFA)”, the “fusiform face area (FFA)”, and anterior-inferior-temporal cortex. Based on combined fMRI/tractography and source localization of the N_{170} , we suggest that the face recognition network has a highly parallel architecture. Recently application of multimodal imaging to single cases with informative neuropsychological syndromes, including prosopagnosia (a selective impairment of face recognition), has permitting stronger tests of the models developed from neuroimaging. Studies of prosopagnosia suggest separate networks underlying overt and covert (unconscious) face recognition, with several (possibility fast) routes connecting in parallel both OFA and FFA with orbitofrontal cortex enabling the latter process. These routes for unconscious face recognition could also operate in typical subjects.

C-12: Functional recovery after brain injury: the role of norepinephrine.

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It has been established that brain injury (BI) is followed by a spontaneous functional recovery (FR) in either, animals and man. Between the mechanisms involved, it has been suggested that, immediately after that motor cortical BI take place, a remote functional inhibition is performed in areas related with the site where the lesion occurred. There is also a neuronal basis for such a functional depression and the process is reversible. This fact is known as diaschisis [1]. It has been reported that motor cortical BI decreases the extracellular norepinephrine (NE) levels and that the use of NE agonist and antagonist enhance recovery, while a strong line of evidence demonstrates that dopamine and serotonin do not have any participation [2]. Moreover, intracerebellar infusion of NE also enhances recovery [3]. We

reported that pontine NE increased after 6 hours of a focal cortical ablation [4], while it decreased in rats with a chronic cortical lesion and it is related to an increase in lipid peroxidation. Such effects are reversible and are related with the FR. Thus, it is possible that the mechanisms involved in the recovery are dependent of the kind of the injury, and that the pons could regulate the effects observed in the cerebellum. We suppose that the NE depletion observed in the pons after BI is the cause of the cerebellar depletion observed by other authors and that this decrease could be due to a functional shift in the locus coeruleus, located in the pons, which changes its NE production to a protein synthesis in order to re-built the cortical-pontine damaged pathways. More research about these mechanisms is necessary. The knowledge on this field will contribute in the development of pharmacological strategies in order to enhance recovery and rehabilitation of patients with motor sequels of acute, chronic or degenerative cerebral lesions.

C-13: Mechanistic understanding and handling of movement disorders.

Alvarez González Lázaro (Cuba)

In this work the author will discuss about the knowledge and study of movement disorders, emphasizing in the handling and treatment of these diseases. Special attention will receive the Parkinson Disease and Hereditary Ataxias.

3rd October 2008

ROOM A

SESSION

EMERGING TRENDS IN MOLECULAR BIOLOGY IN PolyQ

C-16: Loss of purkinje cells linked with activation of apoptotic cell death in the SCA2 transgenic mice F066*

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Introduction: Spinocerebellar Ataxia type 2 is a neurodegenerative disease caused for an expansion of a CAG repeat in ATXN2 gene causing degeneration of cerebellum and related pathways. SCA2 show an autosomal dominant pattern. In healthy person CAG stretch ranged from 13-31 CAG meanwhile in carriers reach 32-79 CAG. At worldwide scale SCA2's

prevalence rates is $1\text{-}5 \times 10^5$ people, however in Cuba this values reach up to 43×10^5 being a serious health problem. Despite of efforts toward understanding the pathological mechanism underlying cell death in SCA2, remain challenging the searching for a therapy or a palliative treatment. Apoptosis is an endogenous mechanism of programmed cell death able to destroy tumorigenic and other pernicious factors in cell. Thus, is an attractive variant to modify acting factors causing cell death in order to treat SCA2. Moreover, in the last decade several animal models have been generated aimed to track mechanisms driving neurodegeneration. In SCA2, three important mouse models have been created however, despite its utility, lacks of theoretical validity hampering the real extrapolation to humans. In Cuba we generated the F066 founder mice with the physiological self-promoter from a SCA2 patient with 79 CAG. This model has shown ubiquitous expression of ATXN2. **Aims:** **1.** To analyze the validity of F066 model as tool in understanding SCA2 pathology. **2.** To asses the role of apoptotic cell death in the degeneration of Purkinje Cells. **3.** To gain insights in the apoptotic mechanism as possible therapeutic targets to treat SCA2. **4.** Also we are aimed to analyze the involvement of apoptotic cell death in SCA2 morphological changes in cerebellum. **Methods:** The study was performed in the SCA2 transgenic mice F066 which express ataxin-2 under the self promoter of humans. **Results:** By mean of morphological, immunohistochemical and electronical microscopy techniques was demonstrated in the F066 model a significant reduction of purkinje cells -pc-. The cell loss was associated with the activation of apoptotic cell death. Apoptosis was demonstrated by the immunodetection of the activated form of caspase-3 (Fig.1), Bax protein overexpression (Fig. 2) and morphological markers of apoptosis (Fig. 3). Analysis of transgenic mice revealed significant differences of motor coordination compared with the wild type littermates. **Discussion:** Our results point out to cell death as an early and progressive event in the degeneration of Purkinje cell. Apoptotic markers detected (Bax overexpression) might suggest a participation of intrinsic apoptotic pathway as driving mechanism in the cell death and in the progression of SCA2. Perhaps the nucleation of proteins to ATXN2 inclusion triggers the apoptotic cascades causing the marked disruption of ER. This early event can truncate the protein traffic of homing proteins in ER and finally cause the catastrophic cell death in cell expressing ATXN2. **Conclusions:** F066 is a usefull model to understand SCA2 pathology also. We demonstrated the marked loss of PC paralleling SCA2 in humans. This cell loss affects the motor coordination in F066 mice. The underlying mechanism of this cell loss is the apoptotic cell death. This mechanism is a potential therapeutic target in the future treatment of SCA2.

C-17: Genetics and Molecular investigations on SCA2: From genetic predisposition to genetic and epigenetic modifying mechanisms acting in a very frequent disease in Holguín*.

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5: Department of Gene Therapy, Division of Pharmaceutics Center for Genetic Engineering and Biotechnology (CIGB), Ave 31 % 158 and 190 Havana City, Cuba. .

Background: Spinocerebellar Ataxia 2 (SCA2) is an autosomal dominant neurodegenerative disorder caused by the expansion of a CAG repeat within the exon 1 of the ATXN2 gene. SCA2's worldwide prevalence ranges 1 to 5/100,000 people. However, Holguín province of Cuba, constitute a special pocket (43/100,000 people) of dwelling SCA2 subjects. Prevalence rates remain stable along time and the tremendous variability is an important pitfall to be cleared in order to improve the life quality of SCA2 patients. Many fundamental questions remain yet unanswered about the molecular mechanism causing ATXN2 locus instability. Unsolved remain which are the main modifiers of ATXN2 gene, and its contribution to phenotypic variance. It is observed that in SCA2, CAG repeats are interrupted by either CAA codons in the normal individuals. However, single point mutations of CAA to CAG can lead to repeat expansion and pathogenesis. Moreover, ATXN2 promoter is embedded in a CpG Island (CGI), being a unexplored target of methyl sensitive Transcription Factors (TF) and a rational mechanism to understand the variance of SCA2. Altogether, other important factor modulating the toxic load of polyQ stretch is the somatic mosaicism. **Methods:** Availability of large number of SCA2 patients with extended families allowed us to perform a Genetic, Haplotype, Sequence and Somatic Instability based approaches to trace and elucidate the mechanism of triplet repeat expansion and effect of SNP within the CAG repeat on instability. By other hand, we used *In Silico* approaches and designed novel Methyl Sensitive PCR -MSP- methods to characterize ATXN2 promoter and gain insights about the involvement of CpG Methylation as control of ATXN2 gene expression. Finally, we have also assessed the contribution of somatic mosaicism to phenotypic variance in a large cohort (500) of SCA2 patients. **Results:** Ancestral STR haplotypes were exclusively linked to SCA2 mutation and distinguished predisposed alleles associated ($p=0.0000$) with loss of the most proximal 5' CAA interruption, with an overrepresentation in the 29-31CAG range. STR haplotypes were very different to other SCA2 population. Extended analysis of the repeat interspersation pattern in 113 normal individuals revealed new alleles very different to other populations and that the majority of these normal alleles lacked the most proximal 5' CAA interruptions. Extended GeneScan analysis in our DNA bank revealed instability in the 38% of large alleles at somatic level, highlighting this group as predisposed. This finding was strengthened by the fact that a pedigree with SCA2 haplotype and pure large allele (30CAG), show that along with an intergenerational increase in repeat size there was a horizontal increase in repeat size with the birth order of the siblings indicating an important role for parental age in repeat instability during transmission. ATXN2 promoter shown a high concentration of CpG (100 CpG). Support Vector Machine (SVM) based tool predicted that 34% of CpG are methylatable (CpGm) with overrepresentation toward downstream 2nd ATG, perhaps reflecting a strong epigenetic control of putative more toxic ataxin-2 variants. CpG in dyads around the 1st in frame ATG, but none methylated, are potential targets for a more dynamic epigenetic control of the most common ataxin-2 isoform. Contrary to previous report (Aguilar et al., 1997) and to our SVM prediction, MSP results show that other CpG are methylated in control and patients. ATXN2 promoter shows high (80%) similarity with RBMPS a critical "hubs" from SCAs Subnetwork, connecting ATXN2 with ATXN3 and ATN1 proteins through ATXN1, reflecting a more global role of epigenetic control by

Methylation of critical genes causing, modifying or related with SCAs. These related promoters also show bidirectional alternative promoter, being a perfect target to self-silence gene expression by RNA interference (RNAi) machinery. Finally, we sought if the degree of mosaicism would be modifying factor of Age at Onset (AO). A clear segregation was found in each CAG group when mosaicism was used as stratifying factor despite identical CAG. **Conclusions:** Common haplotypes point out to unique origin of SCA2 mutation in Cuba. Our results demonstrate that large alleles are the principal source of SCA2 expansion. CAA loss is a key step toward the instability in SCA2 locus in Cuban kindreds. Somatic mosaicism in normal alleles is a valuable predictor to determine predisposition to genetic instabilities. The *de novo* methylation is an important mechanism to be explored in SCAs, the knowledge related will be useful in treating this disorders. **Relevance:** The present research is unique showing a broad number of results related with SCA2 performed in an unusual genetic resource.

C-18: Risk estimation and modifiers of clinical severity in spinocerebellar ataxia type 2 Cuban patients.*

Almaguer-Mederos Luis, Falcón Nieves, Zaldivar Yanetza, Almarales Dany, Almaguer Dennis, Laffita José, Cuello Almarales Dany, Jorge Humberto, Aguilera Raúl, Sánchez Gilberto, Auburger George, Gispert Susana, Velázquez Luis.
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Spinocerebellar ataxia type 2 (SCA2, OMIM 183090) is a neurodegenerative disorder with a markedly variable clinical severity and caused by the expansion of a CAG repeat tract in coding region of ATXN2 gene. *Objective:* Here we study a large sample ($n=924$) of SCA2 Cuban founder population in order to estimate a more objective age-dependent risk and the risk of developing SCA2 at a particular age being carrier of a specific CAG repeat size, and to identify modifying factors of clinical severity. *Methods:* The clinical assessment was carried out using the International Cooperative Ataxia Rating Scale and CAG repeat number was assessed by PCR. Age at onset was defined as the onset of motor impairment, and was based on a combination of information provided by the patient, a close relative or both. The study protocol was approved by the institutional review board and an informed consent was obtained from each study participant. *Results:* In our cohort, the mean (\pm SD) age at onset was 32.5 ± 13.8 years, and 30 years was the mode observed age at onset. There was no significant difference for age at onset between men and women ($F= 1.92$; $p = 0.166$). The calculation of the Bayesian risk for asymptomatic individuals with an *a priori* risk of 50% indicated the existence of a progressive decrease of the risk as the individual's age advances. By the use of Kaplan-Meier survival analysis, we obtained cumulative probability curves for disease manifestation at a particular age for each CAG repeat length in the 34-45 range. Expanded alleles were 75,4% unstable upon intergenerational transmission, and there was a mean anticipation of 16.3 years. Large normal alleles showed intergenerational instability and we identified a *de novo* mutation in one SCA2 family. Incomplete penetrance was observed for

CAG repeat sizes of 32-36 units. There was a double dose effect on the age at onset. The CAG repeat number at the expanded alleles was responsible for 74,8% of the variability of the age at onset. A 9,5% of the remaining variance was due to a combination of the CAG repeat number of the normal allele and the ratio of the length of the normal to the expanded CAG repeat length. The 48% of the remaining age at onset variance was due to genetic and shared environmental factors. Of the seven genes tested only SCA3, DRPLA and mitochondrial complex I gene polymorphism (10398G) were significantly associated with age at onset variation. There were significant differences for strongly positive antigliadin antibodies between SCA2 individuals and controls ($\chi^2 = 4.62$; $p= 0.032$); however, there were not significant differences between AGA positive and AGA negative patients in age at onset, disease duration, ataxia score, or CAG repeat number; neither in the prevalence of gastrointestinal symptoms. *Conclusion:* Here we present very valuable information for predictive-testing programs, for the planning of studies for the identification of other genetic and environmental factors as modifiers of age at onset, and for the design of clinical trials for people at enlarged risk for SCA2.

C-19: Cuban Program of Predictive Diagnosis of SCA2.*

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The existence in Cuba of between 8000 and 10,000 individuals at risk of suffering from SCA2 created the need for developing the Program for the Predictive Diagnosis as a means to prevent the intergenerational transmission of this disorder. This paper shows the results of the Cuban predictive program in a seven-year period, during which it was conducted by a multidisciplinary staff providing genetic counselling, psychological assessment and psychosocial support to the individuals under study before and after the molecular studies. Between February 2001 and May 2008 829 individuals have applied for gratuitous access to the Presymptomatic Diagnosis motivated by the uncertainty about their own risk and that of their offspring, and also to get ready to deal with the disease or aiming at family planning. Predominantly, the individuals were females (56,69%), aged 20 to 30 years, with a secondary school level or higher. 550 of them completed the protocol, out of which 136 resulted positive for the SCA2 gene mutation. In some families the diagnosis preceded the onset of the disease in three generations. 414 individuals were negative. Most of the individuals studied have 1 or up to 3 children, thus a group ranging from 800 to 1600 family members have profited from the certainty of not being at risk or being carriers and thus sparing their offspring from the risk of suffering the disease. The abandonment rate in the program was 22,91%, remarkably lower than that reported for other similar programs. Likewise 29 couples participated in the Prenatal Diagnosis program, with an average age of 25 years and pre-university school level, most of them having no children yet. In 12 of the cases the diagnosis was positive and 10 decided to interrupt the pregnancy. The lack of catastrophic occurrences (suicidal behaviours or psychiatric episodes), the decrease in the levels of anxiety and depression, as well as the growing number of applications to be included in the program show that the Predictive Diagnosis Program has had a favourable psychological impact in the studied individuals.

Oral Presentations/ Presentaciones Orales

**1st October 2008
ROOM A**

OP-01: Spinocerebellar Ataxia 12 found only in Agarwals in India

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Objective: To report the occurrence of SCA 12 in an endogamous population and also present the phenotype among the largest number of genotypically confirmed pedigrees. **Background:** Spinocerebellar ataxia 12 (SCA12) is autosomal dominant cerebellar ataxia (ADCA) associated with an expanded unstable CAG repeat on chromosome 5q31-33. **Methods:** Individuals from 468 Indian families clinically diagnosed as ADCA phenotype were tested for expansion of CAG/CTG repeats at SCA1, SCA2, SCA3, SCA6, SCA7, SCA8, SCA12, SCA 17 and DRPLA loci. **Results:** CAG repeat expansion associated with SCA 12 was identified in 50 affected and 32 asymptomatic individuals from 44 families. The expanded allele carried 47 to 69 CAG repeats (normal alleles 7 to 31 repeats). Age at onset of the disease was 26 to 65 years. All affecteds had dysarthria and mild or no gait ataxia. Majority presented with upper extremity tremor as initial symptom (37/50) and was found to have brisk reflexes (38/50). In 23 of 50 patients there was evidence of sub clinical sensory or sensory-motor neuropathy while imaging of brain (CT/MRI) showed cerebellar as well as cerebral cortical atrophy in 35 of 50 cases. All the subjects belonged to a particular Agarwal ethnic group in India. **Conclusions:** SCA 12 accounts for about 8% of ataxia families as 3rd/2nd common cause of ADCA in India. Patients presenting with tremor in hands and having minimal or no gait ataxia, brisk reflexes, evidence of sub-clinical peripheral neuropathy and cerebral cortical and cerebellar atrophy and belonging to the Agarwal ethnic group may be tested for SCA 12 mutation.

OP-02: Cuban Families with Spinocerebellar Ataxia Type 3/Machado-Joseph disease.

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Machado- Joseph disease (MJD)/Spinocerebellar ataxia type 3 (SCA3), belongs to a novel class of inherited neurodegenerative diseases caused by the expansion of CAG repeats in the respective genes. In general, longer expansions result in earlier onset and more severe clinical manifestations. SCA3/MJD is the most prevalent spinocerebellar ataxia worldwide, its frequency in Cuba is unknown. Between years 2001 and 2003 we carried out a nationwide genetic survey for spinocerebellar ataxia type 2, and found 43 no-SCA2 spinocerebellar ataxia families. In order to identify SCA3/MJD cases in this cohort, we searched 18 no-SCA2 families (30 affected individuals) for SCA3 mutation. We found 6 families (8 affected individuals) who were positive for this particular mutation (33,3%). Normal alleles varied between 15 and 37 CAG repeats, and the range for expanded alleles was 68-76 CAG repeat units. There was a significant correlation between age at onset and CAG repeat size ($r = -0,80$; $p < 0,01$). SCA3/MJD is present in Cuba, and represents about a third of no-SCA2 spinocerebellar ataxia families. Predictive diagnosis could be now provided for SCA3/MJD families.

OP-03: Are similar Somatic Mosaicism in SCA 3 and SCA2 patients?

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Background: Spinocerebellar ataxia type 2 and Machado-Joseph disease are two autosomal dominant cerebellar ataxias caused by expansions of unstable CAG repeats in the coding region of the causative genes. These SCAs remain as the more frequent in Cuba. Paralleled to other CAG diseases, the understanding of phenotypic variability is a goal to improve genetic counselling and the treatment of these entities. Somatic mosaicism is a rational modifying candidate. Previous works point out to important differences; however different technical conditions might hamper rational comparisons. **Aim:** To compare the somatic mosaicism in MJD and SCA2 Cuban patients and determine phenotypical association between somatic mosaicism in SCA2 and MJD. **Methods:** We determined the size of the (CAG) n in peripheral leukocytes from 15 MJD and 77 SCA2 unrelated kindreds. The degree of mosaicism was quantified by GeneScan. Genotype phenotype correlation was established and was compared the somatic instability profiles in affected subjects. **Results:** Somatic instability was more pronounced in SCA3 than in SCA2 patients. CAG size of ataxin 3 was not associated with somatic mosaicism measured as: Mosaicism Index (MI) or peak numbers (PN). Also, disease duration (DD) was none correlated neither MI nor PN. Contrary to SCA2 in which we found a strong correlation ($R^2=0.78$) with MI and PN with CAG and with the other phenotypic and genotypic markers. **Conclusions:** Differences in somatic mosaicism in similar substrates (CAG stretch) reflect different acting mechanisms generating instabilities. These molecular events are relocated perhaps out of CAG. Further studies in CNS structures are necessary to decipher the contribution of somatic mosaicism to the selectivity of cell death and the resulting neuropathological features in MJD and SCA2. CNS ataxin's mRNA expression

profiling is a demanding task to decipher the role somatic mosaicism in the toxic load of compromised structures in these two neurodegenerative disorders.

OP-04: Lipid Metabolism in Spinocerebellar Ataxia Type 2

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Lipids are a diverse group of compounds that have many key biological functions, such as acting as structural components of cell membranes, serving as energy storage sources and participating in signaling pathways. At proper levels, lipids perform important functions in the body, but can cause health problems if they are present in excess, such as the increase of risk of heart disease, stroke, and other vascular diseases. Latest studies, in an ataxin-2 deficient mouse (*Sca2^{-/-}*), show that the lack of ataxin-2 leads to obesity, diminished insulin receptor (*Insr*) protein in parallel to elevated *Insr* mRNA levels, hepatosteatosis, and dyslipidemia. These findings are compatible with a scenario where ataxin-2 directly regulates *Insr* activity and degradation at the endocytosis machinery. In our investigation levels of serum lipids of 56 Spinocerebellar Ataxia type 2 patients of mild stages were studied. Patients with history of Diabetes, Alcoholism, High Blood Pressure or others conditions that could alter the clinical parameters to study were excluded. High values of cholesterol were detected in 48.2 % of the sick persons and hypertriglyceridemia appeared in 33.9 % of patients. The group of ages of 30 to 39 years was the most affected, presenting hypercholesterolemia 71.4 % of them. Patients with smaller size of the mutation (between 32 and 40 repetitions of CAG) were those that more frequently presented high values of cholesterol. The means of the values of cholesterol (5.03 mmol/L) and triglycerides (1.84 mmol/L) are within normal limits, but very close to superior limits, 5.2 and 1.88 respectively. Prevalence of hypercholesterolemia in adult population reported in Cuba is 13.6 % in SCA2 patients we found 48.2%.

OP-05: Glutathione-s-transferases specific activity and spinocerebellar ataxia type 2.

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Spinocerebellar ataxia type 2 (SCA2), is a neurodegenerative and progressive disorder that reach its highest worldwide prevalence rate in Holguin province, Cuba. The understanding of the molecular mechanisms that trigger the neurodegenerative process is the most important issue in order to development therapeutic strategies. *Objective:* To evaluate specific activity of glutathione-s-transferases as a potential modulator of SCA2 phenotype. *Patients and Methods:* Glutathione-s-transferases catalytic activity was studied in serum samples of patients, presymptomatic individuals and matched controls. The clinical assessment was carried out using May Clinic standards for neurological examination, and CAG repeat number was assessed by PCR. *Results:* Glutathione-s-transferases catalytic activity was significantly higher in patients and presymptomatic individuals than controls ($F=14.16$; $p<0.001$), there was not significant correlation between glutathione-s-transferases catalytic activity and age at onset variability ($r=0.24$; $p=0.16$). Moreover, there was not significant correlation between glutathione-s-transferases catalytic activity and CAG repeat number ($r=$

0.21; $p=0.21$), or disease duration ($r=0.18$, $p=0.30$) *Conclusion:* Changes on glutathione-s-transferase catalytic activity could be considered a potential modulator of SCA2 phenotype.

2nd October 2008
ROOM B

OP-10: Involvement of cranial nerves in patients and presymptomatic relatives in SCA2: A neurophysiological follow-up study in 232 subjects.

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SCA2 is a degenerative disorder, caused by a dynamic mutation consisting in an intergenerational CAG repeat expansion in 12q.24.1 chromosome, and whose prevalence in our province, is considered the highest in the world. The aim of this study is to determine the neurophysiologic alterations of cerebral stem in 100 SCA2 patients, 36 presymptomatic relatives and 100 healthy subjects as control group by motor nerves conduction studies of hypoglossal, facial and accessory nerves, blink reflex, jaw jerk and PEATC. We also describe facial morphologic changes and evaluate the relation between neurophysiologic, clinics, morphologic and molecular variables. *Results:* Significant differences exist in: the latency and duration of the motor potential of the three studied nerves ($p \leq 0.05$); prolongation of the latency of bilateral R2 component of Blink Reflex and prolongation of the latency with fall of amplitude of T mentonianus reflex in the patients, appearing similar in the asymptomatic subjects. Quantitative alterations of the face morphology in both groups, given by atrophy of periorals, periorbitals and masseter muscles are described, in correspondence with the described electrophysiological alterations. *Conclusions:* Exist myelin damage of hypoglossy, facial and accessory nerves and axomyelinic injury of the trigeminal nerves and cerebral stem, becoming seriously at the year of made the first study; which suggests that degenerative changes of the cerebral stem happen with annual regularity.

OP-11: Progression patterns of peripheral and preclinical biomarkers in SCA2: A Twenty-Years Follow-up Study.

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Centro para la Investigación y Rehabilitación de las Ataxias Hereditarias.

Objective. To identified preclinical neurophysiological markers and evaluate the correlation between electrophysiological parameters and disease duration, polyglutamine expansion and ataxia score, and to judge its usefulness in assessing polyglutamine toxicity and clinical progression. **Methods:** Fifty five non- symptomatic first-degree relatives of SCA2 patients were studied 6 times over a period of 20 years by nerve conduction studies, multimodal evoked potentials, ataxia score and molecular genetic determination. **Results:** The most important findings were the progressive reduction of the sensitive potential amplitude and the

progressive increase of the latency of the N20, P40, central conduction time and poor replicability and unstable morphology with normal absolute and interpeak of the BSAEP. This electrophysiological characteristic continues evolving and it is more accentuated in the patients with the longer evolution time in the disease. The progressive velocity of the sensitive amplitude, N20 and P40 latencies were correlated with disease duration, polyglutamine expansion size and ataxia score. **Conclusions:** These observations identify that the progression of the preclinical sensitive amplitude and the central latency of the somatosensory evoked potentials as an objective and quantitative physiological markers that is under genetic control and disease duration. This is the first longitudinal study that is carried out in the world during 20 years in a large sample of presymptomatic relatives, with a defined molecular alteration from a degenerative disease. **Relevance:** Therefore, sensitive amplitude and the central latency of the somatosensory evoked potentials appears to be a promising surrogate marker for research projects into the duration of the diseases and the modulation of polyglutamine toxicity by modifier genes.

OP-12: Studies of visuomotor learning in healthy humans and patients with diferents neurodegenerative diseases.

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A fundamental ability for survival is the capacity to learn visuomotor skills. This capacity is possible because we can locate objects in space using visual coordinates and transform them into coordinates appropriate for movement, a construct known as the visuomotor map. This map should have mechanisms that allow its modification in case the original conditions change. We studied a basic visuomotor learning process in which the visual stimulus serves as the action target. For this purpose we used a prism adaptation task, which allows the quantification of motor performance, adaptation to an optical disturbance, and aftereffects shown after withdrawing the perturbation.

Initially we characterized the task in healthful young subjects to compare them with an aged population. The aged group showed slower adaptation and larger aftereffects, suggesting that the aging visuomotor system is less plastic, and has deficits in the aftereffect strategic control. Later we studied three populations of patients with neurodegenerative diseases. We began studying Parkinson's and Huntington's disease patients, which are diseases with different basal ganglia deficits. Our findings suggest that regardless of the differences, both populations show motor performance deficits but intact visuomotor learning. The third population we studied was the spinocerebellar type 2 ataxia. These patients did show significant visuomotor learning impairments, but with normal aftereffects. These results suggest that cerebellar, but not basal ganglia integrity, is needed for wedge prism visuomotor learning.

OP-13: Reversal of noradrenergic depletion and lipid peroxidation in the pons after brain injury correlates with motor function recovery in rats *

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Functional impairment after brain injury (BI) has been attributed to the inhibition of regions that are related to the injured site. Therefore, noradrenaline (NA) is thought to play a critical role in recovery from motor injury. However, the mechanism of this recovery process has not been completely elucidated. Moreover, the locus coeruleus (LC) projects from the pons through the rat sensorimotor cortex, and injury axotomizes LC fibers, depressing NA function. This was tested by measuring lipid peroxidation (LP) in the pons after sensorimotor cortex injury. Depression of function in the pons would be expected to alter areas receiving pontine efferents. Male Wistar rats were divided into three groups: control ($n = 16$), injured ($n = 10$) and recovering ($n = 16$), and they were evaluated using a beam-walking assay between 2 and 20 days after cortical injury. We performed measures of NA and LP in both sides of the pons and cerebellum. We found a decrease of NA in the pons and the cerebellum, and a concomitant increase in the motor deficit and LP in the pons of injured animals. Recovering rats had NA and LP levels that were very similar to those observed in control rats. These observations suggest that the mechanism of remote inhibition after BI involves lipid peroxidation, and that the NA decrease found in the cerebellum of injured animals is mediated by a noradrenergic depression in the pons, or in areas receiving NA projections from the pons.

OP-14: Changes of the excitability of the motoneurons in pos-poliomyelitis syndrome (PPS).

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INTRODUCTION. It has been described that PPS is a pathological entity that is presented in the patients, between 15 and 30 years after having suffered poliomyelitis. It consists of progressive fatigue, muscular weakness, muscular and joint pain, muscular atrophy, difficulty when breathing or in the swallowing, and cold intolerance. The PPS presents very controversial aspects and some authors refer some doubts about its existence like a specific pathology. The reports show a disagreement to the quantity of patients with PPS, for example Julien¹ indicated 25 to 28% prevalence and Ahlström² of 80%. There are not specific studies about PPS to differentiate the patients with symptomatic poliomyelitis sequels of the asymptomatic ones. **OBJECTIVE.** - The aim of the study is to compare the average values of latency and amplitude of H-reflex (HR) obtained in two different groups of patients, one with PPS diagnostic and another just clinical history of 20 year-old poliomyelitis or more but without PPS. With the purpose of finding possible differences in the excitability of the motoneurons that could be related to the PPS. **METHOD.** HR recording was obtained by means of the stimulation of the sciatic nerve and registering the provoked potential from gastronomic muscle of the patients. There were three groups: patients with PPS (G1) ($n = 5$), and patients without PPS (G2) ($n = 3$). And another group (G3) ($n = 6$) of patients with probably diagnostic of PPS. The parameter to study was the latency of HR (in msec), there were compared the values average and statistic difference of variances. The amplitude of the components will be measured in micro-volts and its values average will be analyzed. A Cadwell 5200-TO electromyography equipment was used. **RESULTS.** Mean latency of the HR were: G1 = 33.5; G2 = 32.41; G3 = 31.81. They didn't show significant differences

neither with the normal values reported in the literature (28 to 33 ms). However, as much in the G1 as in the G3 it was more difficult to obtain the HR (12 of 22 possible) that in the G2 (5 of 6 possible). The values average of nerve conduction velocity (NCV) of the tibial nerve were obtained in each one of the studied groups, there were not significant differences ($P > 0.05$) among the groups (G1 =, G2 =, G3 =), however, in the three groups the NCV values were smaller than the value that takes as normal in the clinic (48.7 ± 3.5 m / s). DISCUSSION. - The registrations of HR are more difficult of obtaining in these patients with polio sequels and mainly in those that show signs and symptoms of PPS. However, their latency remains in normal limits. In the other hand, the NCV of the tibial nerve was significantly diminished in all the patients compared to the normal values and the G1 had the value lower average. CONCLUSIONS. The results obtained by means of the electrophysiological studies indicated dysfunction of the nervous trunk, but there was not moto-neurons excitability alteration, there was an apparently decrease of the quick conduction nerve fibers and survival the slow fibers, that it could be related with the symptoms of the PPS.

3rd October 2008
ROOM A

OP-15: A Time Line from Rafael Estrada to the actual broad roads toward understanding SCA2.

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Rafael Estrada was the first who note the overrepresentation of dwelling subjects with OPCA (in he's time). Since these early years SCA2 has been studied for about 30 years. Several advances has been generated however many pitfalls shade the treatment. He was an assiduous scholar of pathology and he is considered as the neurology's father in Cuba. Here we present a time line from the first paper published by Estrada to the actuality. We analyzed the actual state of art in the study of SCA2 at worldwide scale. Also is discussed the emerging trends in the treatment and management of SCAs. Is highlighted the principal contributions of different research team, and is discussed other issues as collaborative researches necessary to reach the goal to improve the life quality of SCA2 patients.

OP-16: Allelic interaction at the SCA2 locus in the determination of the age at onset in spinocerebellar ataxia type 2 Cuban patients.

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Spinocerebellar ataxia type 2 is a neurodegenerative disorder with a markedly variable age at onset. It is caused by a CAG repeat expansion located in the first exon of the SCA2 gene, the

size of which is responsible for approximately seventy percent of the variability of the age at onset. In order to evaluate the influence of the CAG repeat number at the SCA2 locus on the age at onset in patients with spinocerebellar ataxia type 2, a study was carried out involving 534 Cuban patients -530 heterozygous and 4 homozygous-. The CAG repeat number at the SCA2 locus was assessed by PCR and polyacrilamide gel electrophoresis. We found no significant statistical differences for age at onset between men and women ($F = 0,17$; $p = 0,68$), nor among patients with paternal nor maternal transmission of the expanded allele ($F = 3,24$; $p = 0,07$). The CAG repeat number at the expanded alleles was responsible for 74,8% of the variability of the age at onset. A 9,5% of the remaining variance was due to a combination of the CAG repeat number of the normal allele and the ratio of the length of the normal to the expanded CAG repeat length. The 48% of the remaining age at onset variance was due to genetic and shared environmental factors. These results propose that there is an allelic interaction at the SCA2 locus and suggest that an increase in the CAG repeat number of the normal alleles may delay the age at onset.

OP-17: Molecular analysis of the Neurofibromatosis type 1 in Cuba: Detection of the four intragenic microsatellite markers*.

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Neurofibromatosis type 1 is a common autosomal dominant disorder caused by mutations of the NF1 gene on chromosome 17. The gene size and the heterogeneity of the mutations make molecular diagnosis quite complex. About 30–50% of NF1 patients represent new mutations. The molecular diagnosis can be performed using two different approaches: direct investigation of the disease-causing mutation or indirect diagnosis by linkage analysis, using linked DNA markers to identify the affected chromosome. Indirect diagnosis is performed using the linkage approach which, as in any dominant condition, needs at least one affected subject of different generations to determine the haplotype associated with the disease and the clinical diagnosis is crucial. In the present study we optimized the methods linkage analysis of NF1 gene. **Methods:** Genomic DNA was extracted from whole venous blood collected in EDTA using a salt precipitation method. The intragenic microsatellite markers: IVS27AC28.4, IVS27AAAT2.1, IVS38GT53.0 and Mfd15 were amplified in a thermal cycler (PCR). After addition of loading buffer the samples were analysed by non-denaturing polyacrilamide (12.5%) electrophoresis. The gels were silver stained. **Results and Discussion:** The microsatellites are highly polymorphic and are also useful for detecting de novo deletion in sporadic cases. Were standardized the intragenic microsatellite markers: IVS27AC28.4, IVS27AAAT2.1, IVS38GT53.0 and Mfd15. It was observed that the obtained alleles are in the range reported internationally. The intragenic microsatellite markers were informative for the families. In the future, could be offered this method in prenatal and preventive approaches to familial NF1. Our next goal is to detect mutations in our sporadic cases.

Posters/ Carteles

OP-18: First studies on the genotype-phenotype correlation and bioinformatics of Huntington's chorea in Cuba *

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Huntington's chorea is the highest prevalent pathology is due to the expansion of CAG triplets placed at exon 1 of the IT15 gene that presents a dominant hereditary autosomic pattern. In order to characterize our population in a molecular way and to know more about these issues that are not totally described in this pathology, it is pretended for the first time in our country to: Present a bioinformatics study about the stability of the mRNA conformed by different amount of CAG repeats and perform the study on the genotype-phenotype correlation of affected families. **Material and methods** To perform the bioinformatics study, Quickfold server from Michael Zucker's web page was used, and on the experimental job, three PCR techniques were conducted in order to detect CAG repeats in samples belonging to members of 10 affected families. **Results and discussion** With the Quickfold sever we were able to observer that molecules with 35 CAG repeats, shows many probable secondary structures, making this mRNA to be performing conformational changes that increase the probability of translation of mutated protein. This technique is used to demonstrate that when reaching 35 repeats, CAG alters the molecular functioning. In general, molecular-clinical results match with those internationally described, except in one of studied families, where a late debut takes place with respect to the number of CAG repeats, which supposes the presence of modifiers that alter the developing of this pathology. **Conclusions** This study increases the understanding of molecular events that take place within this pathology, as a start point to news therapeutics targets. Besides, this result – molecular diagnosis- allows the premature genetic sponsorship to those affected families; which is the only way of diminishing the presence of the pathology in the population.

2nd October 2008
14:30 pm

P-01: Total Antioxidant Status in spinocerebellar ataxia type 2 Cuban patients.

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Spinocerebellar ataxia type 2 (SCA2) is a hereditary neurodegenerative disease, as yet incurable. Has been suggested that oxidative stress contributes to pathologic process. *Objectives:* We intended to evaluate the presence of oxidative stress in SCA2 patients and presymptomatic individuals through the assessment of the total antioxidant status (TAS). *Subjects and Methods:* It was carried out a case-control study in SCA2 patients, presymptomatic individuals and healthy matched controls. It was determined the age at onset and disease duration, a neurological evaluation was carried out using the International Cooperative Ataxia Rating Scale for the Evaluation of the Cerebellar Syndrome (ICARS). The CAG repeat number was determined by PCR. *Results:* TAS was diminished in patients and presymptomatics with relation to controls ($p < 0,001$), and in the women patients in comparison with men ($p=0,04$); the age do not show any significant correlation with TAS in none of the studied groups. Neither we obtained significant association between TAC and the age at onset ($r=0,07$; $p=0,69$), the disease duration ($r=0,17$; $p=0,35$), the total ICARS score ($r=-0,28$; $p=0,21$), or the CAG repeat number ($r=0,24$; $p=0,19$). *Conclusions:* The presence of oxidative stress was confirmed in the patients and presymptomatic individuals. Further, the sex, and not the age neither the CAG repeat number, influence TAS in the patients, and TAS do not influence significantly upon the disease severity in the studied patients.

P-02: Superoxide dismutase and catalase enzymatic activities in spinocerebellar ataxia type 2 patients

Almaguer Gotay Dennis, Martínez Góngora Edilberto, Laffita José Miguel, Mejías Brito Sandor, González Zaldívar Yanetza, Cuello Almarales Dany, Velázquez Pérez Luis, Almaguer Mederos Luis E.

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Spinocerebellar ataxia type 2 (SCA2) is a neurodegenerative disorder that follows an autosomal dominant inheritance pattern and reaches the worldwide highest incidence and prevalence rates at Holguin province, Cuba. The understanding of disease mechanisms is vital for the development of therapeutic strategies. In order to evaluate the enzymatic activities for Cu/Zn-superoxide dismutase (Cu/Zn-SOD) and catalase (CAT) and its potential clinical consequences in SCA2 patients, we carried out a case-control study. We found no significant statistical difference for Cu/Zn-SOD activity between affected patients and control individuals ($p > 0,05$), but found a significant reduction for catalase activity in affected individuals ($p < 0,05$). However, catalase reduction was no significantly correlated with clinical variables nor with the CAG repeat size. These results reveal oxidative stress in SCA2 patients. Nonetheless, more work has to be done to clarify the clinical consequences of such an association.

P-03: Prevalence of antigliadin antibodies in spinocerebellar ataxia type 2 Cuban patients.

Almaguer Mederos Luis Enrique, Rodríguez Almira Yobanis, Martínez Góngora Edilberto, Almaguer Gotay Dennis, González Zaldívar Yanetza, Echevarría Pupo Ricardo, Sánchez Cruz Gilberto, Monte Brown Julio, Coello Almarales Dany, Velázquez Pérez Luis.

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The relevance of gluten sensitivity in hereditary ataxia pathogenesis is unclear. *Objective:* To evaluate the significance of anti gliadin antibodies levels for spinocerebellar ataxia type 2. *Methods:* We determined anti gliadin antibodies in 64 spinocerebellar ataxia type 2 patients and in 65 healthy matched controls. The clinical assessment was carried out using the International Cooperative Ataxia Rating Scale and CAG repeat number was assessed by PCR. *Results:* Antibodies were positive in 23,4% of the ataxia patients and 9,09% of the controls. Statistical comparison using (chi)² test with Yates's correction reveal significant differences between these two groups ($\chi^2 = 3.94$; $p= 0.047$). The same was obtained for strongly positive anti gliadin antibodies ($\chi^2 = 4.62$; $p= 0.032$). There were not significant differences between AGA positive and AGA negative patients in age at onset, disease duration, ataxia score, or CAG repeat number; neither in the prevalence of gastrointestinal symptoms, prevalence of wheat intolerance, or body weight. These results demonstrate an association between anti gliadin antibodies serum levels and SCA2. However, more work has to be done to clarify the clinical consequences of such an association.

P-04: Genetic anticipation in Spinocerebellar Ataxia type 2 Cuban families.

Almaguer-Mederos Luis, Falcón Nieves, Zaldívar Yanetza, Cuello Almarales Dany, Almaguer Dennis, Laffita José, Jorge Humberto, Aguilera Raúl, Sánchez Gilberto, Auburger George, Gispert Susana, Velázquez Luis.

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Genetic anticipation is phenomena typical of dynamic mutation disorders. *Objective:* To characterize genetic anticipation in Cuban families with spinocerebellar ataxia type 2. *Methods:* We assembled a data corresponding to 101 parent-to-child pairs belonging to 125 unique families from the largest worldwide SCA2 population in Holguín, Cuba. The CAG repeat number at the ATXN2 gene was assessed by PCR. Age at onset was defined as the onset of motor impairment, and was based on a combination of information provided by the patient, a close relative or both. The study protocol was approved by the institutional review board and an informed consent was obtained from each study participant. *Results:* Genetic anticipation was observed in 97% of parent-to-child pairs, with a range of 1-39 years. We found significant statistical differences for anticipation between patients with paternal or maternal transmission of the mutation. Genetic anticipation showed a significant correlation with the CAG repeat size at expanded alleles ($r=0,54$; $p<0,001$) and with conceptive age ($r=0,46$; $p<0,001$). There are an obvious "gender effect" operating on genetic anticipation in SCA2 Cuban families, and a significant influence of conceptive age suggesting a negative selection of ovocytes carrying larger mutant alleles.

P-05 Intergenerational CAG repeat instability in spinocerebellar ataxia type 2 Cuban patients.

Almaguer Mederos Luis Enrique, Rodríguez Almira Yobanis, Martínez Góngora Edilberto, Almaguer Gotay Dennis, González Zaldívar Yanetza, Echevarria Pupo Ricardo, Sánchez Cruz Gilberto, Monte Brown Julio, Cuello Almarales Dany, Velázquez Pérez Luis.

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Spinocerebellar ataxia type 2 is one among an increasing number of hereditary neurodegenerative diseases caused by unstable expansion of CAG repeat tracts. To characterize the intergenerational CAG repeat instability at the ATXN2 gene, we assembled a data corresponding to 202 parent-to-child pairs belonging to 125 unique families from the largest worldwide SCA2 population in Holguín, Cuba. Intergenerational instability varied between -8 and 17 units (mean=1,76±3,19), and occurred in 153 (75,7%) of the 202 transmissions studied. A total of 127 (83%) unstable transmissions corresponded to expansions and the remaining 26 (17%) to CAG repeat contractions. Expansions varied between 1 to 17 units (mean=3,36±2,77), whereas contractions varied between -8 to -1 units (mean= 2,69±1,91); this means shows not significant differences (F=1,38, p=0,24). Ninety seven (48,1%) of study parent-to-child pairs corresponds to paternal transmissions and 105 (51,9%), to maternal ones. Intergenerational instability was more frequent in paternal (85,6%) than in maternal (66,7%) transmissions of the SCA2 expanded alleles. We found that intergenerational CAG repeat instability at SCA2 locus is significantly influenced by paternal conceptive age in paternal transmissions. Although a similar trend was obtained for maternal transmissions this was not significant. A mean increase of CAG repeat instability was obtained for paternal transmissions (+0,15 per year), and a mean decrease for maternal transmissions (-0,03 per year). Large normal alleles showed intergenerational instability and we identified a *de novo* mutation in one SCA2 family.

P-06: Loss of CAA interruption in large SCA2 alleles is a risk factor to SCA2 gene instability: a haplotype and sequence based study in large Cuban kindreds.

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Spinocerebellar Ataxia 2 (SCA2) is an autosomal dominant neurodegenerative disorder caused by the expansion of a CAG repeat within the exon 1 of the SCA2 gene. In normal subjects CAG varies in length from 14-31 repeats and is interrupted by one or more CAA triplets, in patient's alleles contain a pure uninterrupted stretch of 32-200 CAG repeats. SCA2's worldwide prevalence ranges 1 to 5/100,000 people. However, in Holguín Province of Cuba, SCA2 reach the highest concentration (43/100,000 people). Aim: CAA loss linked to certain haplotypes in large alleles can be a predisposing factor for new expanded alleles in Cuba. Methods: We carried out haplotypes studies in 13 SCA2 pedigrees and 89 controls (n=132 chromosomes) using 6 microsatellite markers –STR- surrounding the mutant CAG. We found strong linkage disequilibrium (LD) between SCA2 mutation and some STR alleles. Haplotyping distinguished a common founder chromosome very different to other populations. CAG sequence analysis in 113 chromosomes revealed new large alleles 30-31 CAG. These alleles were either pure or lacked the most proximal 5' CAA interruption. Pure and interrupted 30 CAG allele was overrepresented respecting other alleles. CAA interspersed analysis in 37 chromosomes from our 13 pedigrees shows similar results. We found strong association (p=0.0070) between allele 4 at D12S1672 marker and 22 CAG allele lacking 5'CAA (configuration 13+8). Alleles with CAG different of 22 repeat, and lacked of

CAA interspersions were strongly associated ($p=0.0000$) with allele 4 of STR and the haplotype (3-3-4) ($p=0.0013$). Further tests narrowed this group to the 29-31 CAG range, showing association ($p=0.0063$) only with the haplotype 3-3-4 at D12S1332-D121672-D12S1333. We characterize the somatic instability in 500 SCA2 subjects. We found good fit between CAG and somatic mosaicism index ($R^2 = 0.28$, $p= 0.0000$). GeneScan analysis in alleles with 29-31 CAG shown that 38% of chromosomes are unstable at somatic level (No. peaks = 3 ± 0.18 SEM, range 3-8 peaks) contrasting to stable chromosomes (No. peaks = 1 ± 0.18 SEM, range 1-2 peaks) in our kindreds. Conclusions: Common haplotypes point out to unique origin of SCA2 mutation in Cuba. Our results demonstrate that large alleles are the principal source of SCA2 expansion. CAA loss is a key step toward the instability in SCA2 locus in Cuban kindreds. Somatic mosaicism in normal alleles is a valuable predictor to determine predisposition to genetic instabilities.

P-07: Methyl Specific PCR methods in SCA2: Preliminary results at a glance.

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Background and rationale: SCA2 is a neurodegenerative disease that affects $42 \cdot 10^5$ inhabitants in Cuba. The mutational status - CAG size - of SCA2 carriers is revealed by PCR. CAG size only explains 25–80% of the variability of phenotypical markers, hampering the genetic counselling, treatments and diagnosis. The searching of new molecular markers has a paramount importance. The Methyl Specific PCR (MSP) based methods are very sensitive and specific. **Aim:** Development of MSP methods for assessing the methylation status of SCA2 gene. **Results:** We designed and develop a battery of primers spanning regions from SCA2 5'UTR and was assessed by MSP in 25 SCA2 carriers and 15 and controls. Read-Outs inspection of signals reveals differences within patients and with controls. The involvement of the *de novo* Methylation in SCA2 is a new SCA2 face to be unravelled, toward gain insights in the treatment and management of SCA2. MSP methods can be used as prognostic method. The methyl profile will aid in the stratification of patient cohorts and will improve the genetic counselling. Also, palliative therapies approaches would be designed.

P-08: Serum and Cerebrospinal flows levels of cooper, iron and zinc in patients with type SCA-2 Ataxia of the Clinical Treal with Supplementation of zinc in the province of Holguin in Cuba.

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SCA-2 Ataxia constitutes to health problem for the province of Holguin and potentially for all Cuba. 757 persons in Cuba suffer this disease and almost 8000 descendent plows at risk. The present work investigated serum and cerebrospinal flows (CSF) copper (Cu), iron (Fe) and zinc (Zn) levels in 36 patients with SCA-2 Ataxia in two groups of 18 patients during the execution of the clinical trial Phase II, homocentric, randomized, controlled with placebo and to blind double with supplementation of Zn by oral way during 12 months (six months for each group of 18). Each group was divided at that time in two groups of nine patients each one. A group was administered the medication (Treatment A) and to the second group with placebo (Treatment B) until completing 6 months of the treatment each one. The quantification of the samples was carried out for atomic Absorption coupled with graphite oven in different samplings at different times: time zero in the study (basal state), at three months where only serum and finally at six months both serum and CSF. To significant increase of serum Zn, Cu and Fe levels were detected in both treatment groups. A significant increase of CSF Zn, Cu and Fe levels were detected in the Treatment A group to with regard to the Treatment B. This result indicates and impairment of homeostasis for these important microelements in type SCA-2 Ataxia and the possible role of Zn in the physiopathology of SCA2. These evidences support the application of new strategies for treating with Zn this disease.

P-09: Thalamic involvement in transgenic mice with spinocerebellar ataxia type 2.

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Introduction: In spite of the considerable progress in clinical and molecular research, knowledge regarding brain damage in spinocerebellar ataxia type 2 (SCA 2) still is limited and the extent to the thalamus is involved is uncertain. Have animal mice of the SCA2 disease for studies in brains can provide important insights into the pathogenesis of this polyglutamine disorder on the benefits of therapeutic molecules and neuropathological mechanisms that underline this human disorder. **Material and methods:** We performed a histological analysis using sections stained for lipofuscin granules and Nissl substance through the thalami of a 24 AGM. **Results:** We detected a moderate neuronal loss and synaptic failure in all nuclei of the thalamus. **Conclusions:** These findings indicate that

thalamic involvement can provide an adequate explanation for some of the disease related symptoms seen in the SCA 2 disease.

P-10: Neuroprotective effect of dietary zinc supplementation in a transgenic model of spinocerebellar ataxia SCA-2

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Spinocerebellar ataxia SCA-2, is the dominant autosomic molecular form more frequently in Cuba, especially in Holguín. Immunocytochemical stains of Purkinje cells generate esthetical appealing images, and, and atrophy of the idi dendritic tree in patients with hereditary ataxia has attracted much attention. Have a modify genetic animal (AGM) who carries the SCA-2 gen, is a main tool to localize new therapeutic targets to allow at least for improve the way of live and/or turn aside the course of this illness. Preliminary studies have been proving Zinc²⁺ deficiency in the serum and cerebrospinal fluid of these ataxic patients.

We established two experimental groups, One fed with normal diet (CENPALAB) and the other one fed with a normal diet enriched with sulphate of zinc. These animals were keeping with the maximum comfort under adequate conditions of temperature, humidity and light. As much the water as the food were given ad libitum. We establish the treatment with dietary Zinc²⁺ supplementation during at least a year and evaluated histological and morphometry aspects in the cerebellum as the target organ of this illness. We observed a moderated preservation of the Purkinje layer in the cerebellum, with a high number of synaptic terminals and in the molecular layer most of the stellate and basket cells persist. We discuss the role of the zinc as neuromodulator in the cerebellar neurons. Conclusions: This evidence will constitute a scientific novelty to help develop new zinc-based therapeutic strategies in front of the evolution of this disease and therefore to consider the emerging roles of zinc in the central nervous system in this illness.

P-11: An Electrophysiology and Pathologic Study of Peripheral Nerves in a Transgenic Model of Spinocerebellar Ataxia SCA-2.

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Introduction: Spinocerebellar ataxia type 2 (SCA-2) is an autosomal dominantly inherits, neurodegenerative disease. Characterized by cerebellar syndrome, that in his later stages involves mainly brainstem, spinal cord and thalamus. Electro physiologic and histological alterations of the peripheral nervous system have rarely been reported. Have an animal model

that carries the SCA-2 gen, is an important instrument for future studies on the benefits of therapeutic molecules and neuropathological mechanisms that underline this human disorder. One fed with normal diet (CENPALAB) and the other one fed with a normal diet enriched with sulphate of zinc with 10 animal by group. These animals were keeping with the maximum comfort under adequate conditions of temperature, humidity and light. As much the water as the food were given ad libitum. We establish the treatment with dietary Zinc²⁺ supplementation during at least a year and evaluated histological and morphometry aspects in the cerebellum as the target organ of this illness. The peripheral nerves from the both group (normal food and dietary Zinc²⁺ supplementation, were subjected to electrophysiological testing and histology study. Electro physiologic studies demonstrated a marked reduction of sensory action potential (p<0.05). Light microscopy of the sural nerves revealed marked loss of myelinated fibers, and morphometry studies showed a loss of large myelinated fibers. Conclusions: The peripheral nervous system was affected in this modify animal. These findings suggest a loss of sensory and motor fibers probably following a lesion of the dorsal root ganglion and the posterior horns in the spinal cord.

P-12: Effect of Zn enriched diet on the Motor Evoked Potentials of SCA2 transgenic mice.

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Spinocerebellar Ataxia type 2 (SCA2) is the triplet repeat neurodegenerative disorder with highest prevalence in eastern regions of Cuba. It is characterized by the progressive motor impairment as consequence of the degeneration of both Central and Peripheral Nervous System – including demyelination of spinal nerves. SCA2 patients also show a significant decrease of serum and cerebrospinal fluid zinc (Zn) concentrations. This disruption in the microelement homeostasis has remarkable influence in the function of nervous system. Therefore, we investigated the effect of a chronic Zn treatment on the amplitude of Motor Evoked Potentials (MEP) and the motor conduction velocity of SCA2 transgenic mice. Animals were feed with a Zn enriched diet containing twice the normal level of the microelement. Electrophysiological responses were obtained by bi-polar electrical stimulation of the motor components innervating the soleo muscle. Stimulus consisted in monophasic current pulses of 0.2 ms of duration. MEPs were differentially recorded with a bipolar needle electrode inserted in the muscle. Results show that the mean amplitude of the response of SCA2 animals was 4 times smaller compared with age-matched control animals – non-ataxic mice without treatment. Besides, Zn enriched diet increased the amplitude of MEPs 150 % regarding the values obtained in untreated SCA2 subjects. On the other hand, conduction velocity mean of the ataxic group was 3 times smaller than the control group whereas the mean of the Zn treated ataxic animals doubled the obtained for untreated SCA2 mice. Taken together, these results support the suggestion that Zn administration could be a useful therapeutically treatment to improve the live quality of SCA2 patients.

P-13: Effect of one year of dietary zinc supplementation in a transgenic model of spinocerebellar ataxia sca-2 on corporal condition.

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Introduction: Spinocerebellar ataxia SCA-2 is the dominant autosomic molecular form more frequently in Cuba, mainly in Holguín. This illness is characterized by a bad corporal condition and diminished motorboat capacity; it demands studies of the associate alimentary conduct. To have a modify genetic mouse, payee of the SCA-2 human gene, constitutes a significance tool for study of this illness and also to develop a new therapies that extend and/or improve the quality patient's life. In previous work we have demonstrated a substantial Zinc²⁺ deficiency in ataxic patient (serum and cerebrospinal fluid). **Methods:** Did prepare two experimental groups fed with diet based on pellet fodder (CENPALAB), a group fed with standard diet, another with a diet enriched with sulphate of zinc and a group control. It was given food and water *ad libitum*. Controlled maintenance conditions with required temperature, humidity and light. The treatment settled down with a dietary supplement of Zinc²⁺ during one year, minimum, and the alimentary conduct was analyzed keeping in mind the food and water consumption, corporal weight, average conversion, alimentary posture and physical aspect of the animals as considerable aspects of the corporal condition. **Results:** In the treated mice was perceived better corporal condition, evidenced by better physical aspect, correction of the alimentary conduct, bigger average conversion, alive weight and consummate of food and water. The zinc's action was discussed to level of the nervous system from the behavioral-alimentary point of view. **Conclusions:** The increment of the Zn in the diet improved the corporal condition of the mice significantly. This result agrees with our hypothesis that a relationship exists between the Zn and the physiopathological of this illness. Ulterior studies are in progress to clarify this hypothesis, guided to define the possible existent mechanisms between the Zn and the Ataxia SCA-2.

P-14: Effect of mangifera indica l. Extract and its main polyphenol mangiferin on the aversive and declaratives memories in spinocerebellar ataxia type 2 transgenic mice.

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The hereditary ataxias are progressive neurodegenerative disorders without specific therapy identified. The Spinocerebellar Ataxia Type 2 (SCA2) is related to the loss of function in the cerebellum, mitochondrial dysfunction, oxidative stress and neurotoxicity processes. An aqueous stem bark extract of the plant *Mangifera indica* L (MiE) has been used in pharmaceutical formulation in Cuba under the brand name of Vimang®. Scientific evidences indicate that MiE and its major constituent, mangiferin, display antioxidant, anti-inflammatory antiallergic, analgesic, and neuroprotective actions. Given the several biological actions of MiE and mangiferin and their potential as therapeutics agents, we investigated the effect of both antioxidants on behavioural outcomes of neurological function of SCA2 transgenic female mice. Adult animals were treated daily, with oral administration

of 10, 50 and 100 mg/kg of MiE or 10 mg/kg of mangiferin given during 12 months (p.o.), and the memory test were performed. The highest doses of MiE (50 and 100 mg/kg) improved selectively the aversive memory (inhibitory avoidance task) in SCA2 female mice. However, the antioxidant administrations neither affected the locomotion and habituation (open field test) nor the declarative memory (object recognition task). Mangiferin supplementation did not modify the behavioral parameters evaluated. The results indicate that MiE can affect behavioral parameters related to fear-related memory assessed in an inhibitory avoidance task. Further experiments will be needed to clarify the effect of MiE in SCA2.

P-15: Effect of *Mangifera indica* L. extract and mangiferin on motor coordination parameters in Spinocerebellar Ataxia type 2 transgenic mice.

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The hereditary ataxias are progressive neurodegenerative disorders for which no specific therapy has been identified. Cuba has 125 families that possess hereditary ataxias and the principal molecular form is the spinocerebellar ataxia type 2 (SCA2). A gene responsible for SCA2 has been mapped to human chromosome 12 and the mutation has been identified as an unstable and expanded (CAG)_n trinucleotide repetition. SCA2 is related to a loss of function in the cerebellum, mitochondrial dysfunction, oxidative stress and neurotoxicity process. *Mangifera indica* L. extract (MiE) has consistently shown anti-inflammatory, analgesic and antioxidant properties. These results have been obtained both in vitro and in vivo systems. It has also been demonstrated that the extract protects against neuronal cell death following transient ischemia/reperfusion injury. Given the several biological actions of MiE and mangiferin and its potential as a therapeutic agent, we investigated the effect of the aqueous MiE on some conductual parameters in SCA2 transgenic mice. A Rota rod apparatus together with a footprinting and cord tests were used to measure motor coordination of mice. The animals were weighed every week. The analysis of transgenic mice performance in the Rota Rod revealed significant differences in motor coordination compared with its non transgenic progenitors.. Nevertheless the cord test did not show any difference between normal and transgenic mice. Daily oral administration of 100 mg/kg of MiE markedly improved motor performance of SCA2 mice. At the same dose MiE recovered from the loose of weight of SCA2 transgenic mice. In all test developed, there were differences between male and female animals. Finally, footprinting test corroborated that MiE (100-10 mg/kg) improve the lack of motor coordination of SCA2 transgenic mice. Our findings provide the first pre-clinical evidences of MiE and mangiferin beneficial effects against SCA2. Further experimental and clinical studies will be needed to clarify the effect of MiE and its main polyphenol mangiferin in SCA2.

P-16 Distrofia Miotónica. Reporte de Caso.

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La distrofia miotónica o enfermedad de Steinert es la distrofia muscular más común en adultos y la segunda distrofia muscular más frecuente después de la distrofia muscular de Duchenne. Es un trastorno genético autonómico dominante que afecta a uno de cada 8000 individuos. El debut es usualmente en la segunda o tercera década y la esperanza de vida es de seis décadas. Se caracteriza por debilidad y atrofia de los músculos voluntarios de los ojos, la cara, el cuello, brazos y piernas, miotonía, cataratas, posteriores subcapsulares, defectos en la conducción nerviosa, trastornos endocrinos, déficit cognitivo y calvicie frontal. Los músculos relacionados con las actividades involuntarias como deglutir y respirar, así como los que rodean los órganos internos como el tracto digestivo alto y bajo, la vejiga urinaria y el útero, pueden ser afectados también cuando progresa la enfermedad en el individuo. La enfermedad presenta los fenómenos genéticos de anticipación y potenciación. Presentamos un caso que acude a nosotros con atrofia muscular de más de 15 años de evolución. Por las características clínicas se sospecha de la enfermedad de Steinert. Se realiza un repaso de la clínica más habitual de estos pacientes y se examina el mismo por las especialidades de Neurología, Oftalmología, Neurofisiología, y Medicina General Integral; encontrándose que tanto las manifestaciones clínicas y estudios electromiográficos corroboran el diagnóstico de Enfermedad de Steinert. Se encontró al interrogatorio que el padre y un hermano varón mayor padecían los mismos síntomas de la enfermedad, que habían comenzado cerca de los 40 años. Ambos ya habían fallecido por otras causas.

P-17: La rehabilitación integral y didáctica: Una alternativa terapéutica para pacientes y descendientes en riesgo de SCA2.

Pérez Ávila Ilbedy, Velázquez Manresa Mercedes, Prieto Ávila Lisandra

Las Ataxias Hereditarias constituyen un serio problema de salud en Cuba y específicamente en Holguín donde se reporta la prevalencia más elevada del mundo (43×10^5 habitantes), con predominio de la forma molecular SCA2. Las implicaciones médicas, sociales y culturales que derivan de este problema de salud han determinado la necesidad de un *programa de rehabilitación integral*. Con el propósito de extender la efectividad del mismo, en este estudio se proponen métodos, medios y procedimientos didácticos que faciliten la sistematicidad y sostenibilidad de la mejoría alcanzada durante el periodo de rehabilitación integral institucionalizada, reforzando la misma a través de este sistema, destinado a que pacientes y descendientes en riesgo aprendan a auto rehabilitarse, sin necesidad de un especialista. Hasta ahora no se cuenta con ningún medicamento capaz de contrarrestar la pérdida de las capacidades funcionales de estos pacientes. Por ello el impacto científico de este estudio radica en que la aplicación de este sistema constituye una alternativa para atenuar, conservar y retardar el momento de aparición de la enfermedad, obteniéndose una mejoría significativa en la preparación psicológica y física desde estadios presintomáticos. Desde el punto de vista social, esta investigación esta destinada a incrementar significativamente la calidad de vida de los pacientes afectados por la ataxia espinocerebelosa tipo2.

P-18: Emotional Characteristics and Consequences of Acquired Hearing loss According to gender.

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Introduction: Human Language is the vehicle of communication, which is based in verbal and auditive behavior. When audition is affected language is limited, which leads to some emotional and social deficits. There are 10 million Mexicans that have hearing deficits from different etiology and grade. It is a fact that people with Hearing Loss is in risk to present elevated adverse emotional estates , more than people without the loss of a physical function (Benderly,1980; Giolas,1982; Levine,1981; Meadow Orlans, 1985). With the hearing Loss it is identified communication dysfunction and social isolation. (David y Trehub, 1989; Glass & Elliot, 1992; Luey, 1980). The duel process (fear and negation) delay and interferes with the specialists attending and increase the impact of the hearing loss in their lives. (Shein& Delk, 1974.) The analysis is from the point of view of the patients, when referring to the feelings originated by the Hearing loss as in the mourning process reported by Kübler-Ross, **Objective:** To analyze the emotional behavior of mourning/ sorrow /duel of the people with acquired hearing loss and its variants according to gender. Gender variants. **Method:** A questionnaire was applied to 79 patients who assisted for the first time to the Audiology Service at INR (National Rehabilitation Institute).In order to evaluate the emotional and social characteristics. Patients with prelingüistic hearing loss were excluded, also the ones that didn't answered the entire questionnaire. **Results:** Women n= 42 tend to sadness and depression, while Men n=37 tend to anger and annoyance. 100% of the sample is worried because of understanding language. 33 % of the researched are women who noticed that relatives had hearing loss. In the other hand 16 % detected the hearing loss by themselves, 80 % are women and 20 % are men. **Discussion:** Men and women respond in different ways to acquired hearing loss. It was observed that women tend to sadness and in the other hand, men to anger. Also women have more self consciousness about sensations and feelings, men tend to avoid feelings. Patients report not to perceive hearing loss at the first symptom, because it is painless and asymptomatic. **Conclusions:** Emotional factors are linked with the capacity of communication and interaction, if the last one is vulnerated the implications increase. The obtained results follow the process of mourning / of Kübler-Ross (1996).

P-19: Selective effects of Piracetam on the brainstem evoked potentials and spontaneous cortical electrical activity in infant non-human primates.

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There is relatively little research about the piracetam (PIR) mechanisms affecting the electroencephalographic (EEG) activity and also a lack of knowledge about the effects on the auditory processing. Its pharmacologic features lead to the hypothesis of that PIR facilitates the conduction of the electrical stimulus in the brainstem auditory area and also synchronizes the spontaneous activity in diverse cortical regions. Our aim was to study the effects of the PIR on the brain auditory level and in the information processing of the superior cortical areas recording through EEG and brainstem auditory evoked potentials (BAEP). Two female, infant primates (*Maccaca mulatta*) were used for the comparison of either, the basal or experimental recordings. They were anesthetized (Zolacepan-tiletamine 0.4 u.i.ip) and the EEG was recording using the 10-20 system for neonates. One EEG and two BAEP recordings were performed. During another session, the PIR was orogastrically administered (8g), and the EEG and BAEP recordings were taken after 2 hours under anesthesia. The results where

analyzed using a Wilcoxon for the power spectra and BAEP's latency and amplitude. Results showed that PIR has effects in F3 theta ($p= 0.029$) and alpha ($p= 0.040$) and F8 beta1 ($p= 0.015$). In the parietal P3 delta ($p= 0.022$) and beta2 ($p= 0.037$), en P4 delta ($p= 0.009$) and Pz delta ($p= 0.018$). In temporal lobe in T3 theta ($p= 0.045$), T4 alpha ($p= 0.030$) and T6 alpha ($p= 0.018$). Finally in occipital lobe in O1 beta1 ($p= 0.036$) and O2 beta1 ($p= 0.022$). In the PEATC the PIR elicited effects in the symmetry between both ears and effects in the latency in left ear 50 dB ($p= 0.041$) and 30 dB ($p= 0.019$). There were also changes in peak V on left ear at 50 dB ($p= 0.034$) and right ear at 50 dB ($p= 0.044$) and 30 dB ($p= 0.020$). We conclude that the PIR produces selective effects on the power spectra observed in the cortical areas related with learning processing. In the other hand, auditory processing favors the symmetry between both ears. In the latency the PIR provoke effects in acoustic nerve and inferior colliculus. The results also support future research in the auditory area about the PIR on the auditory process for the development of treatment on people with progressive hearing loss and in suddenly hipoacusis patients.

3rd October 2008
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P-20: Molecular diagnosis of Friedreich ataxia in cuban families.

Cruz Mariño Tania, González Zaldívar Yanetza, Almaguer Mederos Luis, Almaguer Gotay Dennis, Laffita Mesa Jose Miguel, Velázquez Pérez Luis, Canales Ochoa Nalia, Rodríguez Labrada Roberto, Aguilera Raúl (Cuba)

Friedreich Ataxia is the most frequent of all hereditary ataxias with a world prevalence of 1 to 2 affected individuals for every 50 000 inhabitants and a carrier's frequency of 1 in 110 persons. In Cuba, the most frequent hereditary Ataxia is the SCA2, nonetheless a number of families affected by some kind of ataxia with an autosomic recessive pattern and presumptive clinical diagnosis of Friedreich Ataxia have been identified. This resulted in the implementation of this research work aiming at identifying those individuals actually affected by Friedreich ataxia and determining the molecular characteristics of the FRDA gene in these cases, for which purpose the selected families once obtained their written consent to be included in the research, were admitted in the Center for Research and Rehabilitation on Hereditary Ataxias. The medical interview, physical examination, clinical, laboratory, and neurophysiological studies, as well as molecular studies were carried out in order to identify the mutations present in the FRDA gene. The characteristics of the gene in the studied individuals is presented here together with the clinical behaviour of the disease in the individuals affected by Friedreich Ataxia, thus being settled the genetic and molecular diagnosis of the disease in Cuba.

P-21: Reproductive Attitudes and Predictive Test for SCA2.

Jorge Cedeño Humberto, Cruz Mariño Tania, Paneque Herrera Milena, Arguelles Hernández Ivian, Quevedo Batista Yudith, Reynaldo Armiñán Rubén, González Zaldívar Yanetza, Almaguer Mederos Luis, Velázquez Pérez Luis (Cuba)

The SCA2 is a late onset disease. A wide number of individuals have completed their families before finding out if they carry this gene. Aiming at identifying the main reasons for

which individuals decide to register for the Predictive Program, and determining the number of families with descendants at the moment of the registration, and describing the attitude of both couples and individuals at risk towards Pre-natal Studies, as well as some of the factors possibly related to such attitudes, a retrospective study was carried out by means of reviewing the surveys included in the clinical records of the individuals at risk. Concerns about the risk for their offspring were the main reason for applying. Most of the individuals were not willing to have more children and faced with the prospect of positive diagnosis would do likewise. Most of them were women between the 2nd and 4th decade of their lives and had a level of education between secondary school or higher. Pregnancy was not a compelling reason for the registration. Predominantly, the couples were stable and practiced no religion. All of the individuals studied consider proper the prenatal diagnosis for having healthy descendants and most of them would terminate the pregnancy in case of being positive to the tests.

P-22: Characterization of the Prenatal Diagnosis for Spinocerebellar Ataxia Type 2 in Holguín, Cuba, and Associated Ethical Dilemmas.

Tamayo Chang Víctor Jesús, Jorge Cedeño Humberto, Reynaldo Armiñán Rubén Darío, González Zaldivar Yanetza, Cruz Mariño Tania, Almaguer Mederos Luis, Velázquez Pérez Luis (Cuba)

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Spinocerebellar Ataxia Type 2 is the most frequent Autosomal Dominant Ataxia in Cuba. After having established the Program of Prenatal Diagnosis for this disease in Holguín Province; 29 diagnoses have been made for 7 years. In the present investigation, 24 of them are characterized from the sociodemographic, clinic and genetic points of view. The results of the Prenatal Diagnosis are analyzed as well as the reproductive option chosen by the couples, and the main associated ethical dilemmas are expressed. Most of the couples are from Holguín province, and they had no children. The average age of the member of the couple with antecedents of the disease was 25 ± 5 years old, most of them had no religious beliefs, the women predominated and the predominant educational level was high school. The average gestational age at the beginning of the Program was 13 ± 4 weeks and fetal samples were taken by means of amniocentesis at 17 ± 2 week. Only one of the couples had two prenatal diagnoses. 9 fetuses were diagnosed with the mutation that causes the disease and in 3 cases the diagnosis was no informative. The main associated ethical dilemmas were the making of a presymptomatic diagnosis to obtain the prenatal diagnosis in sick patients who denied being sick and in patients with associated mental retardation as well as the decision to continue the pregnancy in cases with Prenatal Diagnosis positive or no informative.

P-23: Ethical Dilemmas in the Predictive Diagnosis of SCA2. Presentation of a series of cases.

Cruz Mariño Tania, Jorge Cedeño Humberto, Paneque Herrera Milena, Reynaldo Armiñán Rubén, Arguelles Hernández Ivian, Quevedo Batista Yudith, González Zaldivar Yanetza, Almaguer Mederos Luis, Velázquez Pérez Luis

Individuals at risk of suffering from SCA2 are exposed to a series of ethical dilemmas while under predictive studies, resulting additionally in other conflicts impairing the decision making process. Aiming at identifying some of these dilemmas, a series of cases involved in

the programs for presymptomatic and prenatal diagnosis for SCA2 are presented here, as well as the conducted genetic counselling and the decision made by the individuals. Being one of a monozygotic female twins couple and at the same time being pregnant with twins, or being one of two siblings where only one is affected, bearing the CGA trinucleotidic expansion capable of producing the disease, deciding whether or not to interrupt de pregnancy once known that the fetus is affected, knowing three generations in advance to the outbreak of the disease that you bear the mutation, and the carrying out in a short period of time of a positive diagnosis in a pregnant woman whose fetus also resulted positive, are complex situations both for the affected individuals and the professionals in charge of the predictive diagnosis program.

P-26: Spinocerebellar Ataxia type 2 and oral diseases

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There was carried out a descriptive study in 43 sick persons of ataxia espinocerebelosa type 2 with the objective of determining the grade of affection of these patients for dental caries, malocclusions and periodontopatis as well as to establish some of the possible etiologic factors and the necessities of stomatologists. **Patients and methods:** To all of they were carried out previous permission, questioning and oral exam. Was determined COP-D, Buccal Hygiene of Love and of as amended Russell index (IP-R), revised by the OMS, PDCB exam and was evaluated the existence of dental malocclusions and prosthesis and if there were functional or not. **Results:** The index COP-D of the sick persons of SCA₂ was high, prevailing the extracted teeth, 74,4% of the patients had faulty buccal hygiene and 65,12% they presented some type of periodontal illness. The most frequent malocclusions were the packing of frontal inferior teeth and the marking overjet with or without spaces, existed high-level of edent people, requiring in 25 of the studied patients some type of dental prosthesis. **Conclusion:** In the ataxic patient, the buccal hygiene is bad, existed high-level of edent persons and are frequent the periodontal illnesses, for that reason they require of stomatologists prioritized attention guided to improve the oral health of these people as well as of carrying out promotion activities and prevention.

P-27: Expanded CAG Repeat Sizes Influences Age at Onset and Electrophysiological Parameters of Spinocerebellar Ataxia Type 2.

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Background: The spinocerebellar ataxia type 2 is characterized by a high variability in its clinical and electrophysiological presentation, even intrafamiliarily. Factors' identification, which explains this variability, could lead to the findings of therapeutical ways that may retard the disease onset. Objective: To contribute to this phenotypic variability knowledge of the different structures and functions of the nervous system. **Methods:** We investigated 52 SCA2 patients. They were studied by motor and sensitive nerve conduction studies and multimodal evoked potentials. **Results:** By means of molecular and electrophysiological

studies we have found two groups well differentiated. The first one was characterized by CAG repeat expansions above 41 units and by the total blockade of the afferent conduction that is, basic electrophysiological alteration with axonal damage predominance. The second one was characterized by CAG repeat expansions lower or equal to 41 units and showed a high variability in its electrophysiological behavior with myelinic damage predominance. **Conclusions:** These findings suggest that for by CAG repeat expansions lower or equal to 41 units should be affecting other genetics and/or environmental factors that explain the variability found in this group which are not significant for clinical and electrophysiological presentation in individuals with CAG repeat expansions above 41 units.

P-28: Spinocerebellar ataxia type 2 olfactory impairment shows a pattern similar to other major neurodegenerative diseases

Velázquez Pérez Luis, Fernández Ruiz Juan, Díaz Rosalinda, Pérez González Ruth, **Canales Ochoa Nalia**, Medrano Montero Jacqueline, Sánchez Cruz Gilberto, Almaguer Mederos Luis Enrique, Martínez Góngora Edilberto, Hudson Robyn, Drucker Colin René

Olfactory function is affected in different neurodegenerative diseases. Recently, it has been found that some hereditary ataxias are also associated with significant olfactory impairment. However, the initial findings did not examine the nature of the olfactory impairment associated with these ataxias. In the present article the effect of spinocerebellar ataxia type 2 (SCA2) on olfactory function was studied in 53 SCA2 patients and 53 healthy control subjects from Holguín, Cuba. Several tests were applied to evaluate olfactory threshold, description, identification and discrimination. The results show significant impairment in SCA2 patients on all olfactory measurements, and the pattern of olfactory deficits found suggests that they have much in common with those reported for other neurodegenerative diseases such as Parkinson's and Alzheimer's diseases.

P-29: Preclinical neurophysiological markers in non-symptomatic first-degree relatives of SCA2 patients.

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Background: The SCA2 has a prevalence of 42 per 100 000 inhabitants in Holguín province, which is the highest one reported worldwide. *Objective:* To identified preclinical neurophysiological markers and evaluate the correlation between electrophysiological parameters and disease duration, polyglutamine expansion and ataxia score, and to judge its usefulness in assessing polyglutamine toxicity and clinical progression. *Methods:* We investigated fifty five non- symptomatic first-degree relatives of SCA2 patients. They were studied 6 times over a period of 20 years by motor and sensitive nerve conduction studies and multimodal evoked potentials.. *Results:* The most consistent findings were there reduction in amplitude or absent in sensitive potentials and the increase in absolute latency of P40 component. Our data shown the structures of the nervous system are involved before any clinical symptom and/or sign appear. This allows us defining four stages in the evolution of this disease: First stage: normal electrophysiological parameters. Second stage: decrease in

the amplitude of the sensitive potentials, increase in the absolute and interpeak latencies in the SSEPs, and abnormal morphology in the BSAEPs. Third stage: increase electrophysiological abnormalities, which correspond with the first clinical manifestation of the disease and four stage: both, peripheral and central afferent conduction blocks appear, expressed by absence of the response in sensory conduction nerve studies and in SSEPs. This classification of the disease in different stages allows knowing the degenerative process during the evolution of the disease of the afferent and efferent systems. Also, the existence of electrophysiological abnormalities in non-symptomatic subjects permits to choose the optimal moment for the evaluation of a specific therapeutic action in the SCA2. *Conclusions:* This is the first longitudinal study that is carried out in the world during 20 years in a large sample of presymptomatic relatives, with a defined molecular alteration from a degenerative disease. Therefore, sensitive amplitude and the central latency of the somatosensory evoked potentials appears to be a promising surrogate marker for research projects into the duration of the diseases and the modulation of polyglutamine toxicity by modifier genes.

P-30: Preclinical alterations of SCA2 in normal subjects with non pathological long repetitions.

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Centro para la Investigación y Rehabilitación de las Ataxias Hereditarias.

The Spinocerebellar Ataxia type 2 is a polyglutamine disorder caused by expansions above 32 CAG repetitions in the SCA2 gene. Based in the unstable nature of CAG repeat domain, the normal individuals with large, but no pathological, expansions are considered as founder reservoir for further pathological CAG expansions. In order to evaluate the neurotoxic effect of CAG repeat domain in premutated state, we performed a clinical and electrophysiological characterization of 9 non-SCA2 mutation carriers with large alleles (27-31 CAG units) and use a control group with 22 repetitions. No SCA2 clinical manifestations were observed in these subjects. We identify a significant reduction of sensory amplitudes of peripheral nerves and increase of P40 latency of SSEPs. No electronistagmographic alterations were detected. This study identifies the sensory neuropathy as preclinical alterations of SCA2 in normal subjects with premutated alleles, indicating that the neurotoxic effect of SCA2 polyglutamine expansions also extends to normal subjects with non pathological long repetitions, specifically to the afferent pathways of peripheral nervous system and spinal cord, but no in the cerebellum and brainstem. Our findings support that axonal neuropathy is the first subclinical manifestation of SCA2 and suggest that it onset depends of a smaller CAG repeats threshold than cerebellar syndrome. This is the first report of preclinical alterations in normal subjects with non pathological long CAG repetitions in the SCA2 gene, which extends the knowledge related to premutational state of SCA2.

P-31: Saccadic movements in SCA2. From disorders to electrophysiological biomarkers for genetic and clinical researchs.

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Saccadic eye movement is a rapid shift of eye position to capture an object in the environment. Saccade pathology is a common symptom of SCA2. Eighty two SCA2 patients, 53 asymptomatic carriers and 82 healthy controls were studied by an electronystagmographical technique To characterize the saccadic abnormalities related to SCA2 by electronystagmography; evaluate their correlation with disease duration, ataxia score and polyglutamine expansion. For a longitudinal study, 50 SCA2 patients and controls were studied three times each two years to evaluate the progression of alterations along time and judge its usefulness as clinical biomarkers of polyglutamine toxicity and clinical progression. SCA2 patients showed significant reduction of maximal saccade velocity (MSV), prolonged latencies and hypometric saccades to 60⁰ predictable amplitude. Significant saccadic slowing, but less severe than patients, was observed in presymptomatics. MSV was negatively correlated with the polyglutamine expansion in both groups. In SCA2 patients, all saccadic abnormalities were significantly accented along time. Compared with controls, the saccadic slowing and hypometria showed a larger magnitude of progression than latency prolongation. Saccade velocity is the most important electrophysiological research tool for the study of genetic determinants of SCA2. The progression patterns of saccade slowing and hypometria appear to be objective biomarkers that reflect the severity of neurodegenerative process in the brainstem and cerebellum along time in SCA2 patients. This is the first electronystagmographical study carried out in a large population of SCA2 patients and presymptomatic carriers. Also it includes the first longitudinal study of saccadic abnormalities in SCA2 patients. Those findings identify MSV as the most important endophenotype for the estimation of SCA2 mutation size.

P-32: Processing of saccadic electro-oculographic data in clinical studies of SCA2

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The development of a suitable algorithm for the identification of saccades in saccadic test performed to patients with severe SCA2 and a software to process the electro-oculographic data obtained in order to calculate the saccadic peak velocity, amplitude, latency and deviation. The electro-oculographic data obtained using the same tests and equipment of five healthy subjects and five severe SCA2 patients was processed using the algorithm and software developed to identify the set and unset of saccades. The results for healthy and SCA2 patients were compared with other two algorithms and the medical criteria by means of an ANOVA on repeated measurements. The software containing the algorithm developed is being used in the Center for Research and Rehabilitation of the Hereditary Ataxias of Holguín to process the electro-oculographic data in saccadic studies, with more than 150 study cases processed up to date. The algorithm determines significant quantitative variables and its statistic behaviour, used as quantitative markers in the diagnose and evolution of the disease. The algorithm presents better performance in the identification of set and unset points of saccades when compared to other methods, especially in the processing of severe SCA2 patients, with saccades deeply affected by the disease in terms of shape, slope and noise.

The software calculates the most significant parameters of the identified saccades, being very useful in electro-oculographic saccadic tests for diagnose and evolution of SCA2 patients. This is an unique tool for processing the electro-oculographic data in saccadic tests according

to the needs of the ataxia clinical studies in the Center for Research and Rehabilitation of the Hereditary Ataxias of Holguín, solving a lack of software suitable to obtain the significant variables for the research carried out in this institution.

P-33: Heart rate variability in type 2 Spinocerebellar Ataxia.

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Objective: To detect whether SCA2 patients may be affected with autonomic dysfunction. Methods: Ninety seven SCA2 patients who had genetic positive test results, were submitted to the standardized clinical autonomic Scale for Outcomes in Parkinson’s disease (SCOPA-AUT) and to HRV testing (resting condition). Time and frequency domain HRV measures were obtained; all indexes were compared (t-student, $p < 0.05$) with those of 97 healthy subjects (age and sex matched) used as a control group. Results: SCA2 patients showed significantly more symptoms than subjects in the control healthy group, mainly referred to gastrointestinal and urinary systems. HRV measures in the time domain (mean cardiac period, range, standard deviation, root mean square of successive differences and the triangular index of RR intervals) and frequency domain indexes (spectral power density of the low, medium, high and total frequency range) were significantly lower in SCA2 patients. The autonomic stress index and the frequency LF/HF ratio were increased in SCA2 patients. On the other hand, normalized measures of the power spectral density (%) showed that the main reduction in the 0.04 to 0.15 spectral frequency range was observed for frequencies in the 0.1-0-15 range, intimately related with the arterial pressure Mayer waves. Detected differences in all cases were statistically significant for p level < 0.001 . Conclusions: Results of comparisons between SCA2 patients and healthy controls suggest a definite autonomic dysfunction in the group of patients. This report is the first one showing autonomic dysfunction in SCA subjects genetically diagnosed.

P-34: Electrophysiological neuromuscular evaluation of eccentric and concentric muscle training

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INTRODUCTION. The main fact determining the histological and functional skeletal muscle development is the contraction type that it performs. The effects of the training carried out with eccentric contractions are controversial. In the one hand it has been reported that the training with eccentric contractions can to prevent lesions and improve the muscular effectiveness (Fox 1992), while there are reports about lesions provoked by eccentric muscular contractions (Mark 1999). The rehabilitation treatments in different neuro-muscular alterations not include systematized training programs with simple eccentric and concentric muscular contractions or in different combinations.

OBJECTIVE. To report the initial results obtained from the frequency electromyography (EMG) analysis of patient’s anterior recto low limb muscle (knee extensor). The EMG records were obtained before and after different exercises programs (eccentric and concentric contractions). MATERIAL AND METHOD. Nine male patients (25 to 34 years age) after 3 months of the reconstruction of the crossed ligament of knee were studied. Three different training programs were performed by patients during three months: One group (G1) ($n=3$)

carries out concentric contractions on a running treadmill at 2.6 Km/h and 8° inclination, during 20 minutes, 3 times a week. Other group (G2) (n=3) carries out low limbs concentric contraction (Vs gravity), rise up 15 Kg weight, 3 series of 12 repetitions each, with recovery intervals of 1.5 minutes, 3 times a week. The last one (G3) (n=3) carries out low limbs eccentric contraction (in favor of gravity) get down 15 Kg weight, 3 series of 12 repetitions each, with recovery intervals of 1.5 minutes , 3 times a week. The EMG record was carried out by means of surface electrodes (5 mm of diameter) during 20 seconds of maxim voluntary contraction against resistance. The electrodes were connected to a EMG (100C) of MP150 BIOPAC System module. The EMG registers were made before, during (45 day) and after (91 day) training exercise program. In order to evaluate the EMG frequencies recorded from the patients' anterior recto muscles, the frequency analysis BIOPAC's program was used at three seconds epoch (from 10 to 13 seconds of record). RESULTS. The EMG frequency analysis showed a significant difference in G1 (the frequency was lower, from 50 to 35 Hz) in the healthy low limb and G2 (the frequency was bigger, from 50 to 95 Hz) in the healthy low limb also, in the final record. Furthermore the EMG amplitude register was bigger in G1 (from 13 to 42 mv). DISCUSSION. The results suggest that the aerobic exercises program (G1) provoked an important change of motor-unit type, from small to big ones, and on the other hand the concentric exercises program (G2) had contrary effect. The anterior effects were important in the healthy low limb. The changes in G3 (eccentric exercises program) were not significant. CONCLUSION. The results suggest that there is a functional change of motor units when the training program is the aerobic type, frequency decrease and amplitude increase suggest the development of bigger motor units. But it seems to be better the concentric exercises program, because increase frequency only.

P-35: Post-operative image guided assessment of subthalamotomy: clinical and neurophysiological implications .

E. Rodríguez (1), R. Rodríguez (2), N. Pavón (2), M. Carballo (2)

(1) ELACM. (2) CIREN.

Introduction: The subthalamic nucleus (STN) has been established by choice as target of neurosurgical treatment of Parkinson disease (PD). The extent of clinical improvement depends of the location and size of the lesion. In this paper, an assessment of STN lesions is made using post-operative MRI. Methods: The lesion plans were used as reference. The post-operative studies were carried out in an MRI equipment of 1.5 T within 96 hours after surgery. 3D axial and coronal images weighted at T1 and T2 were obtained and were transmitted to a station equipped with STASSIS software. Reference and real lesions were compared regarding their location, total lesion size and nuclear lesion size. Three patient groups were formed according to the clinical results. Results: No significant differences were found between the total volumes and locations of the reference lesions and those obtained with MRI. Significant differences were found ($p < 0.05$) in the mean of the spatial location in the three patient groups. The statistical analysis also showed differences in the size of the nuclear lesions in the three groups. The lesions located in the central area of the nucleus had better anti-parkinsonian effect, perhaps because they affect the neurons of both the primary and supplementary motor areas cortices at once. Conclusions: The topographic features of the lesion are decisive for the clinical success of the procedure. Therefore, it is necessary to perfect the accessing techniques to the functional target in the STN.

WORKSHOP ON NEUROREHABILITATION

“Biomolecular Approach of the Neurological Restoration”

ROOM B
1st October 2008

General and basic approaches to the study of neuronal plasticity

Ruíz Flores Lidia (México)

Neuroplasticity consists of the ability of the nervous system to adapt its structural organization to new situations emerging from changes of developmental and environmental situations, as well as other factors affecting the conditions of the nervous system, and the whole organism. There is three different models that explain the neuroplasticity: Long term potentiation (LTP), Cross-modal compensational plasticity and the mammalian thalamus gateway. We will show the anatomical and neurochemical basis of these processes.

Neuroplasticity and the cerebellum

Bergado Rosado Jorge (Cuba)

The author will show a set of evidences that indicate the existence of neuroplasticity mechanisms in cerebellar damages, caused by physical agents, tumours, and neurodegenerative process.

Trends and Perspectives of neurorehabilitation.

González Roy Jorge L (Cuba)

The author will show the present strategies of rehabilitation that are developed at international level with emphasis in Cuba. Greater attention to the strategies of neurorehabilitation will occur in this lecture.

Clinical and molecular diagnosis of Ataxias. Anatomical and functional evaluation in rehabilitation.

Aguilera Rodríguez Raúl (Cuba)

Las Ataxias Hereditarias constituyen un problema de salud en Cuba, donde se concentra la mayor población mundial de enfermos y de sujetos en riesgo, estimándose una población de aproximadamente 10 mil individuos involucrados. Los estudios epidemiológicos demostraron la existencia de más 168 familias, que se relacionan con esta enfermedad, de las cuales el 75,7% pertenecen a la forma molecular tipo 2 (SCA2). En el país existen 757 enfermos y anualmente nacen 22 niños portadores del gen, enferman 35 nuevos casos y fallecen 15 enfermos. Estas patologías se incluyen dentro de las mutaciones inestables o amplificación de tripletes. La SCA2 presenta una gran variabilidad en la edad de inicio y en su expresión clínica, justificándose el 60% por las características estructurales del gen. Se presenta a edades más tempranas y con evolución más desfavorable en personas que tienen mayores repeticiones de CAG. El 40% restante de la variabilidad fenotípica pudiera explicarse por la presencia de otros genes (modificadores), factores no genéticos endógenos y factores medioambientales, cuya identificación podrá conducir al descubrimiento de vías de intervención para el tratamiento de estas enfermedades. En Cuba, a partir de 1998 las investigaciones sobre las ataxias hereditarias se concentraron en la implementación y evaluación de la neurorrehabilitación como única alternativa terapéutica para mejorar la calidad de vida de los pacientes. Posteriormente se desarrollaron otras líneas en el orden neuroquímico y en la búsqueda de genes modificadores de la edad de inicio y la expresión clínica de la enfermedad. Sin embargo, desde principios de los años 90 se desarrollaron investigaciones neurofisiológicas destinadas a la búsqueda de marcadores cuantitativos de la evaluación de la enfermedad, del daño genético y de la neurodegeneración que permitieron describir mejor a la enfermedad no sólo desde el momento de la aparición de los síntomas, sino desde los estadios presintomáticos. El Programa de Neurorrehabilitación integral que se ha aplicado a casi 400 enfermos de todo el país. Este programa mejora sustancialmente los indicadores neurológicos cuantitativos relacionados con el equilibrio, la marcha, la estabilidad postural, el lenguaje y las funciones cognitivas en los pacientes con SCA2. Tales hallazgos abrieron una promisoriosa perspectiva en el tratamiento de dichos pacientes a través del ejercicio físico sistemático combinado con la terapia neuroprotectora y de micronutrientes.

Rehabilitation of SCA2 patients: 10 year follow up.

Rodríguez Díaz Julio Cesar, Velázquez Pérez Luís, Sánchez Cruz Gilberto, Aguilera Rodríguez Raúl, Rodríguez Labrada Roberto, Almaguer Mederos Luis, Almaguer Gotay Dennis, Soto Garcés Osvaldo

Introduction: In the province of Holguín, the greater concentration of patients of Ataxia Espinocerebelosa is reported 2 type or Cuban Ataxia at level the International, 42x100 000 inhabitants. In the Center for the Investigation and Rehabilitation of the Ataxias Hereditarias (CIRAH), is developed a Program of Neurorehabilitación Multifactorial for the SCA2. Objective: To evaluate the mechanisms of Neurological Restoration in patients with SCA2

submissive 6 weeks of Neurorehabilitación. Patients and Methods: A study of cross-sectional type of prospectivo cut was made in 96 patients with clinical and molecular diagnosis of SCA2 rehabilitated in the CIRAH. The subjects were selected randomly, 34 of masculine feminine sex and 62 of, with ages between 16 and 68 years (average of 39.3 years) and time of evolution between 1 and 21 years (average of 12.4 years). Clinical, electrofisiológicas, biochemical variables were evaluated and of Quantitative Neurology, before and after rehabilitating themselves. Results: L to total score of the scale of Tinetti demonstrated significant improvement ($p=0,000$) of the parameters of the march and the balance. The quantitative evaluation of diadococinecia also demonstrated improvement with the rehabilitation, with an average of the integral of 131,58 before rehabilitating and 76,09 after rehabilitating ($p = 0,023$). The GST in the serum was increased significantly after the rehabilitation (Average of 0,000132 before rehabilitating and 0,000164 later, $p=0,010$). Although significant changes in any of the variables of intervalometría RR of the Independent Nervous System did not exist, there are modifications with tendency to diminish the likeable hyperactivity in basal conditions. A significant improvement of the latency of the sacádica speed ($p<0,005$) in the evaluation existed post rehabilitation. Conclusions: The program of Neurorehabilitación that is applied during six weeks to the patients of SCA2 with different evolutionary estadios contributes efficiently in the neurological restoration of such.

Sistema de neurorehabilitación multifactorial intensiva en Cuba. Origenes, desarrollo, actualidad y perspectivas.

Sentmanat Belisón Armando (Cuba)

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La concepción científico-metodológica general del Sistema de Neurorehabilitación Multifactorial Intensiva se originó en Cuba en el año 1987 a propósito de los primeros trasplantes de células nerviosas en el cerebro de pacientes con Enfermedad de Parkinson. Esta forma de trabajo se amplió a otras enfermedades neurológicas y se ha aplicado durante 19 años en el Centro Internacional de Restauración Neurológica (CIREN), donde se han rehabilitado más de 20 000 pacientes crónicos de más de 80 países dentro del Programa de Restauración Neurológica. Se muestran las bases científico-metodológicas que lo sustenta, la organización del proceso de neurorehabilitación, una síntesis de sus programas por grupos de enfermedades, el equipamiento actual que se utiliza y algunos de sus resultados más recientes. Como colofón, se expone la tecnología de última generación introducida recientemente en el sistema, y el proyecto de asimilación tecnológica de avanzada a corto plazo, que se está llevando a cabo dentro del Sistema de Neurorehabilitación Multifactorial Intensiva.

1st CENTRAL AMERICAN AND CARIBBEAN WORKSHOP ON SLEEP MEDICINE

2nd October.
ROOM A

Anatomical and physiological basis of sleep-wake cycle.

Próspero Garcia Oscar (Mexico)

The author will show the most important findings focused in the anatomy and physiology of sleep-wake cycle, emphasizing in the sleep regulation mechanisms and REM sleep generation.

Methods for recording and analysis of sleep: An update

Galicia Polo Maria de Lourdes (Mexico)
Clínica de Trastornos de Sueño UNAM, México

The human's beings spend us approximately one third of our lives asleep, nevertheless we don't know much about how or why sleep occurs. Essentially, as we drift off to sleep, our overall brain activity slows down. This is reflected in a general slowing of the frequency of electrical activity of the brain. For obvious reasons, we cannot investigate human neural activity using intracellular recording, for that we use external electrodes that measure electrical activity at the level of the scalp. This technical procedure is known as the Electroencephalogram or EEG. The main advantage of this technique is that it allows non-invasive human studies to be conducted. The method is often referred to as open-field or far-field recording, because the site of the recording electrode on the scalp is receptive to electrical fields from many neurons and is not located at the internal site of any one particular neuron or groups of neurons. Depending on the net frequency of our brain's electrical activity, we are classed as either "awake" or "asleep". Normal sleep can be divided into two states: rapid-eye-movement (REM) sleep and non-REM sleep. Based on the electroencephalographic features, the non-REM sleep is conventionally subdivided into four stages: stages I and II (light sleep) and stages III and IV (deep sleep or slow-wave sleep). Non-REM sleep is associated with a synchronous electroencephalographic (EEG) pattern with characteristic graphoelements such as sleep spindles, K complexes and high voltage slow waves. REM sleep is characterized by a tonic asynchronous EEG pattern that resembles the awake state (low amplitude waves, high frequency), and bursts of phasic activities such as PGO waves and saccadic like rapid eyes movements, from which the term REM is derived. These ocular movements are registered by the electroculogram, which allow us differentiates the REM sleep from the Non-REM sleep. The electromyogram aids in the detection of REM sleep, as net muscle activity or muscle tone normally decreases during REM atonia. It also acts as a useful indicator of bruxism and other sleep disorders such as the REM behavior disorder and Periodic Legs Movements Syndrome. The respiratory function during sleep may be studied by aeronasal, thoracic, abdominal and snoring sensors, which are very useful for

the polysomnographical diagnosis of Obstructive Sleep Apnea and other respiratory disorders related with the sleep.

Sleep disorders: classification, epidemiology and treatments

Haro Valencia Reyes (Mexico)

The author will show and discuss about the last version of International Sleep Disorders Classification. Also, he will show the most important and prominent therapeutical strategies for sleep disorders.

Parasomnias

Sánchez Narváez José Francisco (Mexico)

The author will show the updated information about parasomnias, emphasizing in the classification, epidemiology, diagnosis, characterization and treatments of these sleep disorders.

Sleep disorders in infants and adolescents.

Huerta Delgado Ángel Daniel (Mexico)

The author will talk about the sleep disorders in paediatric and adolescence ages with marked importance in early diagnosis and treatment. International epidemiology data will be presented.

Obstructive sleep apnoea syndrome in Adults

Labra Alberto (México)

Sleep apneas are ones of the most common sleep disorders at worldwide. In this lecture, the author will discuss about the epidemiology, clinical and polysomnographical features, diagnosis, pathological mechanisms and treatment options of these disorders.

Sleep disorders in neurodegenerative disorders

Velázquez Pérez L, Rodríguez Labrada R. (Cuba).

Centro para la Investigación y Rehabilitación de las Ataxias Hereditarias, Holguín, Cuba.

Neurodegenerative diseases are important health problems at worldwide due to the high values of prevalence, incidence and mortality rates. Many neurodegenerative diseases are associated with sleep disorders, which reinforce the clinic picture of the diseases and affect the life quality of the patients. Numerous cases of REM Behavior Disorder (RBD) have been reported in association with clinically diagnosed and preclinical Parkinson's disease, multiple system atrophy (MSA) and dementia with Lewis Bodies. In Cuban patients with Spinocerebellar Ataxia type 2 (SCA2) is observed a marked loss of atonia during REM sleep, which may represent a subclinical RBD. Fortunately, many drugs tend to decrease the frequency and/or severity of RBD, such as Clonazepam, Levodopa and Melatonin. Other

REM sleep pathologies, such as decrease of total percentage of REM sleep, are common in SCA2, SCA3, MSA, Progressive Supranuclear Palsy, Creutzfeldt–Jakob, Parkinson and Alzheimer disease. Sleep spindles and K complexes become poorly formed, of lower amplitude, shorter in duration and much less numerous in many neurodegenerations. The restless legs syndrome (RLS) and periodic limb movement disorder (PLMD) are characteristic features of various neurodegenerative diseases, especially in Charcot-Marie-Tooth disease type 2, SCA2, SCA3, MSA and Parkinson disease. Both motor sleep disorders normally respond well to dopaminergic therapy. Myotonic Dystrophy and Amyotrophic lateral sclerosis are commonly related with obstructive sleep apnea syndrome (OSAS), because these disorders produce significant neuromuscular alterations in oropharynx. Central apneas are more prevalent in neurodegenerations that affect the central control of ventilation, such as SCAs. The reviewed sleep disorders cause a constant fragmentation of normal sleep patterns, which gets worse the life quality of the patients and induce marked dysfunction of natural alternative to combat the neurodegeneration in these patients: the neurogenesis mechanisms.

Clinical and electrophysiological characterization of sleep disorders in SCA2. Identification of disease biomarkers and therapeutic targets.*

Roberto Rodríguez Labrada, Luis Velázquez Pérez, Inka Tuin, Lourdes Galicia Polo, Nalia Canales Ochoa, Reyes Haro Valencia, Gilberto Sánchez Cruz, Jose M. Laffita Mesa, Georg Auburger

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Clinic for sleep researchs. UNAM, Mexico

Background. The sleep disorders are common complaints of SCA2 patients and their relatives, fundamentally towards the final stages of the disease. **Objective.** To characterize the sleep pathology in SCA2, and to evaluate its relation with the main clinical, neurophysiological and molecular features of the disease. Also to identify novel disease biomarkers and therapeutic targets for treatment strategies. **Methods.** We performed an extensive clinical and electrophysiological characterization of sleep disorders in a large population of SCA2 patients, presymptomatics relatives and healthy controls by standardized sleep questionnaires, two all-night video-polysomnography (VPSG) recordings and Multiple Sleep Latency Test (MSLT). All subjects were examined by the same neurologists according to a standardized protocol, including a SARA scale. The CAG repeat number was assessed by PCR. We also performed electronistagmographical, neurocognitive and neurochemical evaluations. **Results.** Almost all patients and presymptomatics reported good subjective sleep quality, but reduced dream recall. SCA2 patients and presymptomatics show a significant reduction of sleep efficiency as well as increase of wake periods after sleep onset and arousals indexes. REM sleep was abnormal in approximately 60% of SCA2 patients and presymptomatics. The pathology of REM sleep was characterized by reduction of mean REM episodes duration, loss of muscle atonia and decrease of REM density. In patients, the percentage of REM sleep was negatively correlated with SARA score but no with polyglutamine expansion. Nevertheless the CAG repeats influenced on arousal index during REM sleep. REM density shows a negative correlation with SARA score. Periodic legs movements (PLMs) were observed in approximately 42% of SCA2 patients. This alteration was significantly accentuated in patients with longer SARA scores and disease duration. Although, the PLM index wasn't influenced by CAG repeats. SCA2 patients show a significant increase of central, but no obstructive sleep apnea-hypopnea index. The mean

Epworth scores of patients and presymptomatics were not significantly different from healthy controls, which were supported by the results of MSLT. **Discussion.** The early and progressive REM sleep reduction can be associated with the pons, nigrostriatal and thalamic degeneration and is considered a novel progression marker of the disease. The decrease of REMs density is agreed with saccadic pathology in SCA2. REM sleep without atonia may be interpreted as subclinical RBD and suggest neurodegenerative lesions in subceruleus region. PLMs may be related with a dysfunction of dopaminergic pathways, which could be complemented by deficits of serum iron concentration in some patients. Central apneas may reflex dysfunctions, related with neurodegenerative processes, at the respiratory center in the brainstem, nevertheless neuropathological studies are necessary to confirm it. The Epworth scores and MSLT findings are inconsistent with the sleep fragmentation and the reduction of sleep efficiency in SCA2 patients, which suggest possible alterations in the homeostatic sleep regulation mechanisms. **Relevance.** This is the most extensive and complete characterization of sleep disorders in SCA2 or any other hereditary ataxia. We identify PLMs as therapeutical target for a future treatment that improves the sleep quality of SCA2 patients.

Sleep and epilepsy

Stokes Henry B, Stokes Arla, Cabrera Francisco (Guatemala)
San Carlos University, School of Medicine, GUATEMALA

The purpose of the study is to show the clinical applications and benefits that the polisomnography can provide to the diagnoses and management of patients with Epilepsy in which nocturnal seizures could be undetected, and helps to differentiate parasomnias and sleep related paroxysmal events that are not Epilepsy. Over three percent of adults and ten to thirty percent of children have nocturnal events on a routine basis. The differentiation of a sleep related phenomenon, nocturnal seizures or psychogenic events could be difficult because of the frequent overlap of clinical descriptions and lack of diurnal findings. Nocturnal events can be categorized into three major groups: disorders of sleep, disorders occurring with sleep and disorders unrelated to sleep. We currently recognize three normal states of being, and a continuum of pathological status: The normal states of wakefulness, non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep. They are defined by the physiological measures of the electroencephalogram, eye movement and muscle tone. Our current measures are only a few of parameters that vary with the sleep-wake state. We know that a wide array of physiological parameters such as respiration, thermoregulation, blood pressure and variability in heart rate are altered by sleep stage. From monitoring these parameters we can conclude that change of state is not necessarily “a flick of a switch” phenomenon. The active neuronal processes, which culminate into state determination, can be disrupted to produce a mixture of these states. Thus, behaviours that would usually accompany one state intrude into another. Some patients will have seizures strictly only during sleep; seizures will occur approximately 25% strictly during sleep, 42% only during wake. Sleep Related Events: Distinguishing features of the status of being are: Wakefulness, NREM sleep and REM sleep are the result of activation of several neuronal processes of the central nervous system. Patients demonstrate behaviours usually associated with wakefulness while still asleep. Events such as sleepwalking sleep terrors and confusional arousals are common in children and to a lesser extent in adults. Approximately 30 % of children have sleep walking or sleep terror events, and the reported prevalence in adults ranges from 2 to 5 % The decrease in NREM events with increasing age raises the question that these disorders represent a maturational process of sleep/wake regulation. Frequently patients with a NREM parasomnia disorder will have a family history. Typically,

NREM events are more common in the first half of the night, and patients have no memory for the event retention of sleep in parts of the cerebrum. The partial waking state of the hypothalamus and autonomic structures may explain how these episodes can evoke a flurry of autonomic responses as seen in a sleep terror. Sleep disorders such as obstructive sleep apnoea, narcolepsy or periodic limb movements may also exacerbate the arousal process. Medical disorders such as gastro-esophageal reflux, congestive heart failure, pulmonary disorders and renal failure can increase the risk of arousals. Additionally, neurological insults and seizures need to be considered as possible factors perpetuating the arousal disorder.

Effects of endocannabinoids on memory and sleep.

Próspero García Oscar (México)

Sleep and memory are complex traits regulated by a wide variety of molecules. Mounting evidence has indicated that endocannabinoids are playing an important role in the regulation of these processes. Endocannabinoids are a family of lipids that binds to the CB1 receptor; the same receptor marijuana binds to when it enters the body. The discovery of the receptors to marijuana (CB1 and CB2) has been a major achievement in neuroscience and the study of drug addiction. Moreover, The description of the endogenous ligands to these receptor, the endocannabinoids (eCB), has shed light into the physiology of the brain and the mechanisms by which eCB regulate several behaviours including food intake, sex, memory and possibly consciousness itself. A nascent line of research focused on studying the function of eCB in the modulation of sleep and memory has indicate that these lipids induce non-rapid-eye movement (Non-REM) sleep as well as rapid-eye-movement (REM) sleep. Interestingly, ECB also regulate mnemonic processes. Thus, endocannabinoids have an exciting therapeutic potential in treating common and debilitating sleep disorders such as insomnia or narcolepsy as well as memory disorders.

Effect of carbamazepine on sleep patterns disturbed by epilepsy

Alfaro Rodríguez Alfonso (México)

Coordinación de Investigación Básica y Aplicada, Laboratorio de Neuroquímica, Instituto Nacional de Rehabilitación.

Sleep patterns are disturbed by epileptic attacks, due to the magnitude of the seizures in both humans and animals. Therefore the aim of this work is to analyze the effect of carbamazepine on sleep patterns.

The experimental protocols used 30 male Wistar Rats weighing between 280 and 320 g. Under general anaesthesia, animals were chronically implanted with stainless steel electrodes placed in the right sensorimotor cortex for EEG recording, and in the neck muscles to obtain the electromyogram (EMG). Three recordings for 10 continuous hours were made from 09:00 to 19:00 h under the following conditions: (I) Control; (II) after administration of kainic acid (10 mg/kg sc) alone; and (III) with kainic acid injected in carbamazepine-pretreated animals (25 mg/kg). Poly-graphic recordings were analyzed visually and three different states of vigilance were identified: Wakefulness (W), Slow Wave Sleep (SWS) and Rapid Eye Movement (REM) sleep. Total time spend by animals in each state of vigilance was obtained under the above conditions. Statistical analysis. The electrographic parameters of the

different phases of the sleep were analyzed by an ANOVA TEST. Differences of $*p \leq 0.01$ and $**p \leq 0.001$ were considered to statistical significant.

Our results showed that seizures induced a total inhibition of sleep, since both SWS and REM sleep were absent through out the first day after kainic acid administration. This effect was partially reversed by carbamazepine, since pretreated animals were able to show SWS. Our conclusion is that, besides decreasing seizure intensity, carbamazepine facilitates partial recovery of sleep.