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Pós-Graduação Genética UFMG

V Encontro de Genética de Minas Gerais

V ENGEN MG

Pesquisa e Pós-Graduação

17 a 19 de setembro de 2014

Belo Horizonte

V Encontro de Genética de Minas Gerais

V ENGEMIG

Pesquisa e Pós-Graduação

Sociedade Brasileira de Genética – Regional Minas Gerais

Pós Graduação em Genética – UFMG

Universidade Federal de Minas Gerais – UFMG

Instituto de Ciências Biológicas – UFMG

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V Encontro de Genética de Minas Gerais

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Pesquisa e Pós-Graduação

Coordenação Geral

Maria Raquel Santos Carvalho

Ana Lúcia Brunialti Godard

Anderson Miyoshi

Colaboradores

Ana Paula Vimieiro Martins

Bruno Campos Silva

Bruno Marçal Repolês

Carolina de Paiva Lima

Carolina Guimarães Ramos Matosinho

Daniel Almeida da Silva e Silva

Fernanda Caroline dos Santos

Fernanda Sales Coelho

Gabriela Chadid Salazar

Izinara Rosse da Cruz

Julliane Dutra Medeiros

Karina Talita de Oliveira Santana

Luana Martins de Carvalho

Luige Biciati Alvim

Marlene de Miranda

Mercedes Carolina Soares da Silva

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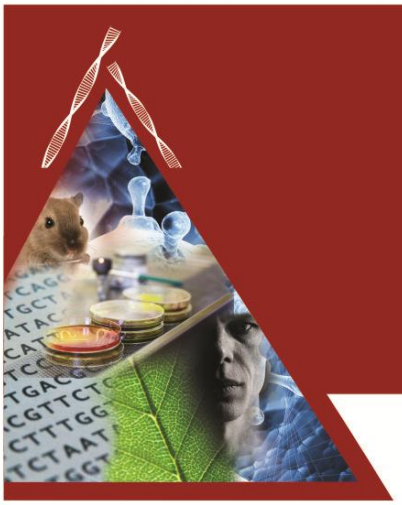

programação

17/09 quarta-feira

Horário	Tema	Participantes
10h - 13h	**Credenciamento**	
13h - 14h	**Almoço**	
14h - 15h30	**Pós Graduação em Genética em Minas Gerais**	**Coordenadora da mesa:** Prof.ª Dr.ª Ana Lúcia Brunialti Godard - **Prof. Dr. Rodrigo Antônio de Paiva Duarte** Pró-Reitor de Pós-Graduação da UFMG - **Prof. Dr. Márcio de Castro Silva Filho** Diretor de Programas e Bolsas no País – CAPES - **Prof. Dr. Augusto Schrank** Coordenador da área CB1 - CAPES
15h30 - 16h	*Coffee break*	
16h - 18h	**Apresentação dos Programas de Pós-Graduação em Genética de Minas Gerais: descrição, avaliação, perspectivas**	- **Prof.ª Dr.ª Ana Lúcia Brunialti Godard** Coordenadora do Programa de Pós-Graduação em Genética - UFMG - **Prof. Dr. Sérgio Yoshimitsu Motoike** Coordenador do Programa de Pós-Graduação em Genética e Melhoramento – UFV - **Prof. Dr. Paulo Afonso Granjeiro** Coordenador do Programa Multicêntrico de Pós-graduação em Bioquímica e Biologia Molecular - UFSJ - **Profª. Drª. Vânia Helena Techio** Coordenador do Programa de Pós-Graduação em Genética e Melhoramento de Plantas – UFLA - **Prof. Dr. Lyderson Facio Viccini** - Coordenador do Programa de Pós-Graduação em Ciências Biológicas - UFJF - **Profª. Drª. Renata Guerra de Sá Cota** - Coordenadora do Programa de Pós-Graduação em Biotecnologia - UFOP
18h30 - 19h30	**Abertura**	
19h30 - 20h30	**Palestra de abertura**	**Palestrante: Adrián Llerena** - CICAB Clinical Research Centre at Extremadura University Hospital and Medical School. Spain *"Farmacogenética de poblaciones iberoamericanas: impacto clinico en depresión y suicidio"*

18/09 quinta-feira

Horário	Tema	Participantes
8h - 9h20	Mesa redonda: **Genética Humana**	- **Profª. Drª. Maria Raquel Carvalho** - UFMG: Estudos Genômicos na Deficiência Intelectual - **Profª. Drª. Luciana Lara dos Santos** - UFSJ: "Estruturação do Centro de Controle do Câncer Hereditário em Minas Gerais: uma estratégia para acompanhamento clínico e análises moleculares de indivíduos em risco" - **Profª. Drª. Fernanda M. S. Jehee** - IEP-Santa Casa-BH: "Laboratório de Genética Humana e Médica do IEP -Santa Casa-BH: principais linhas de investigação"
9h20 - 9h30	Apresentação de **resumo selecionado**	
9h30 - 10h	*Coffee break*	
10h - 11h20	Mesa redonda: **Modelos animais de doenças humanas**	- **Profª. Drª. Ana Lúcia Brunialti Godard** - UFMG: "A genética do alcoolismo - o que aprendemos no estudo da regulação gênica em um modelo animal" - **Profª. Drª. Adriana Abalen** - UFMG: "Pentraxina 3: camundongos como modelos experimentais" - **Prof. Dr. Frederico M. Soriani** - UFMG : "Eosinophils - Induced TH17 response and mice lethality" during *Aspergillus fumigatus lung* infection"
11h20 - 11h30	Apresentação de **resumo selecionado**	
11h30 - 13h	Lunch in box Palestra técnica	Palestrante: **Raphael Fonseca, MSc** (Thermo Fisher Scientific) "A Genética em Chip: Novas Possibilidades para Descobertas Científicas"
13h - 14h	Apresentação de pôsteres (ímpares)	
14h - 15h20	Mesa redonda: **Genômica e Bioinformática**	- **Prof. Dr. Vasco Ariston de Carvalho Azevedo** - UFMG: "Omics of *Corynebacteriumpseudotuberculosis*: our pathogens workhorse" - **Dr. Marcos Vinícius Barbosa da Silva** - EMBRAPA Gado de Leite: "Aplicações e perspectivas da genômica e da bioinformática na pecuária" - **Prof. Dr. Guilherme Correa de Oliveira** - CPqRR/FIOCRUZ: "Transformando a genômica em ferramentas de controle para doenças parasitárias" - **Prof. Dr. Henrique Figueiredo** – UFMG: "Aplicações da genômica em laboratórios oficiais e em vigilância epidemiológica na pecuária"
15h20 - 15h30	Apresentação de **resumo selecionado**	
15h30 - 16h	*Coffee break*	
16h - 17h20	Mesa redonda: **Biotecnologia**	- **Prof. Dr. Alexandre Azenha Alves de Resende** - FACIP/UFU: "Avaliação mutagênica e recombinogênica de produtos naturais em células somáticas de *Drosophila melanogaster*" - **Prof. Dr. Sérgio Oliveira De Paula** - UFV: "Uso dos fagos: Terapia, Biocontrole e Microbiologia industrial" - **Prof. Dr. Anderson Miyoshi** - UFMG: "Bactérias Lácticas como veículos para vacinas de DNA"
17h30 - 18h30	Apresentação de pôsteres (ímpares)	

19/09 sexta-feira

Horário	Tema	Participantes
8h - 9h20	Mesa redonda: **Genética Evolutiva e de Populações**	- **Profª Drª. Maria Bernadete Lovato** - UFMG: "Genética de populações, evolução e conservação da flora nativa sul-americana" - **Prof. Dr. Fabrício Rodrigues dos Santos** - UFMG: "Genética evolutiva aplicada à história humana e conservação da biodiversidade" - **Profª Drª. Renata Acácio Ribeiro** - UFVJM: "Filogenia molecular de *Eriocaulaceae* e suas implicações taxonômicas" - **Profª Drª. Gisele Pires M. Dantas** - PUC-Minas: "Filogeografia dos Passeriformes da Mata Atlântica: uma abordagem genética e morfológica"
9h20 - 9h30	Apresentação de **resumo selecionado**	
9h30 - 10h	*Coffee break*	
10h - 11h20	Mesa redonda: **Genética, Evolução Ecologia**	- **Profª. Drª. Vânia Helena Techio** - UFLA: "Sítios frágeis e sequências repetitivas funcionais em *Lolium spp*" - **Prof. Dr. Gustavo Kuhn** - UFMG: "Dissecando a organização e evolução de DNAs satélites em genomas sequenciados de *Drosophila*" - **Prof. Dr. Renan Pedra** - UFMG: "Susceptibilidade genética a fenótipos secundários em pacientes com HIV/AIDS" - **Profª. Dra. Marta Svartman** - UFMG: "Citogenômica de Mamíferos do Brasil"
11h20 - 11h30	Apresentação de **resumo selecionado**	
11h30 - 13h	**Palestra técnica**	Palestrante: **Raphael Fonseca, MSc** - (Thermo Fisher Scientific) "Ion Torrent = Transcriptoma, Exoma, Genoma, CNV, miRNA, ChipSeq, Aneuploidias, GBS e mais"
13h - 14h	Apresentação de pôsteres (pares)	
14h - 15h20	Mesa redonda: **Genética Funcional**	- **Profª Drª. Diana Bahia** - UFMG: "*Trypanosoma cruzi*: Sinalização celular e molecular na interação com células hospedeiras" - **Profª Drª. Karina Braga Gomes Borges** - UFMG: "Polimorfismos, inflamação e diabetes: qual é a relação?" - **Profª Drª. Daniella Bartholomeu** - UFMG: "Variabilidade antigênica em *Trypanosoma cruzi*" - Prof. Dr. Gustavo Menezes - UFMG: "Hepatic DNA deposition: a new feature of drug-induced liver injury"
15h20 - 15h30	Apresentação de **resumo selecionado**	
15h30 - 16h	*Coffee break*	
16h - 17h20	Mesa redonda: **Melhoramento Genético**	- **Prof. Dr. Fabyano Fonseca e Silva** - UFV: "Propostas de novas metodologias para estudos de seleção e associação genômica" - **Prof. Dr. José Airton Rodrigues Nunes** - UFLA: "Uso de abordagem de modelos mistos no melhoramento genético" - **Drª. Claudia Teixeira Guimarães** - Embrapa Milho e Sorgo: "Estratégias genético-moleculares para seleção assistida e identificação de genes em milho e sorgo"
17h20 - 17h30	Apresentação de **resumo selecionado**	
17h30 - 18h30	Apresentação de pôsteres (pares)	
18h30 - 19h30	**Palestra de encerramento**	- **Prof. Dr. Romeu Cardoso Guimarães** - UFMG: "Origem do Sistema Genético"
19h30	**Encerramento das atividades**	Posse da nova diretoria da SBG-MG e entrega do Prêmio Romeu Cardoso Guimarães

Sumário

V Encontro de Genética de Minas Gerais

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Pesquisa e Pós-Graduação

Biotecnologia

ISOLATION AND IDENTIFICATION OF LACTIC ACID BACTERIA AS PROBIOTIC CANDIDATES FOR PREVENTION OR TREATMENT OF MASTITIS IN CATTLE

RS Steinberg1, LCS Silva1, LB Alvim1, MR Souza3, JR Nicoli2, E Neumann2, AC Nunes1
1 Departamento de Biologia Geral – Instituto de Ciências Biológicas – UFMG - Av. Antônio Carlos, 6627 - Pampulha -
Belo Horizonte - MG; CEP 31270-901. Brasil.
2 Departamento de Microbiologia – Instituto de Ciências Biológicas – UFMG - Av. Antônio Carlos, 6627 - Pampulha -
Belo Horizonte - MG; CEP 31270-901. Brasil.
3 Escola de Veterinária – UFMG - Av. Antônio Carlos, 6627 - Pampulha - Belo Horizonte - MG; CEP 31270-901. Brasil

Mastitis is the most impactful disease on milk production in cattle. Due to strategic importance of dairy livestock in food production, control of this disease is of utmost importance. To address international pressure to reduce the use of antimicrobials in animal production, development of alternative methods of treatment and prevention of mastitis should be implemented to replace the traditional antibiotic therapy. Due the increasing use of probiotic bacteria in the control of different infectious diseases, bacteriotheraphy offers a possible strategy for control of mastitis. The lactic acid bacteria are members of the natural microbiota of bovine mammary gland, have a long history of safe use, present several proven beneficial health effects on the host, and therefore are strong candidates for selection of probiotics for the mammary gland. The aim of this work was to isolate and identify lactic acid bacteria from milk samples and intramammary swab of healthy and subclinical mastitis cows. Samples of 123 cows (87 healthy and 36 with subclinical mastitis) were collected and submitted of serial dilution and plated out MRS agar and incubated under anaerobic conditions. Gram-positive and catalase-negative colonies from different morphotypes were collected and subjected to molecular identification by DNA sequencing of 16S rRNA gene. It was obtained 193 isolates of lactic acid bacteria belonging to 30 different species, with the following abundance: *Weissella paramesenteroides* (15), *W. confusa* (3), *W. cibaria* (3), *Streptococcus lutetiensis* (15), *S. bovis* (2), *S. salivarius* (2), *S. infantarius* (2), *S. henryi* (1), *S. gallolyticus* (1), *S. equinus* (1), *Pediococcus pentosaceus* (69), *P. stilesii* (1), *Lactococcus lactis* (16), *L. garvieae* (13), *Lactobacillus plantarum* (6), *L. pentosus* (1), *L. mucosae* (1), *L. brevis* (1), *L. paracasei* (1), *Enterococcus hirae* (23), *E. camelliae* (3), *E. casseliflavus/gallinarum* (3), *E. faecalis* (2), *E. italicus* (2), *E. saccharolyticus* (1), *E. faecium* (1), *E. pseudoavium* (1), *E. durans* (1). There was a tendency to reduction of species diversity when comparing healthy and diseased animals. This finding will be confirmed by a more robust analyses comparing total composition of the microbiota of mammary gland, through a comparative study of the microbiome of healthy and animals with subclinical mastitis. Out of 193 isolates, 94 were identified by rep-PCR as belonging to different strains, 82 obtained from healthy animals and selected for evaluation as potential probiotics using *in vitro* screening tests. These potential probiotic strains isolated in this work may in future be used in the production of probiotic products to be used in commercial herds for prevention and treatment of mastitis and improving the health status of the mammary gland.
Supported by: FAPEMIG, CAPES and CNPq.

A NOVEL ESAT-6 DNA MUCOSAL DELIVERY SYSTEM IS ABLE TO GENERATE AN IMMUNE RESPONSE TO TUBERCULOSIS IN MICE

PEREIRA, VB1; SOUZA, BM1, SARAIVA, TDL1; AZEVEDO, MSP1; DE CASTRO, CP1; MIYOSHI, A1.
1 Departamento de Biologia Geral, ICB, UFMG, Belo Horizonte, MG.

Introduction: The use of non-pathogenic bacteria, such as lactic acid bacteria (LAB), constitutes an attractive and safer alternative for plasmid DNA vaccine deliver. *Lactococcus lactis*, the model LAB, is considered GRAS (Generally Recognized As Safe) and, therefore, has been extensively used for the production and delivery of antigens and cytokines at the mucosal level. Now, *L. lactis* arises as an attractive alternative for the delivery of DNA vaccines. In this context, invasive *L. lactis* strains (*L. lactis* FnBPA) as well as a plasmid that replicates in this bacterium and contains an eukaryotic expression cassette (pValac) were constructed. Thus, it is believed that the use of the *L. lactis* FnBPA strain, containing the pValac vector, for eukaryotic expression of the ESAT-6 antigen (6-kDa Early Secreted Antigenic Target) of *Mycobacterium tuberculosis* could represent a new strategy for controlling Tuberculosis; an infectious disease that affects, in its latent form, 1/3 of the world's population. Thus, this work aims to construct a *L. lactis* FnBPA(pValac:ESAT-6) and evaluate the immune response generated by this strain in mice.
Methods: For this purpose, the pValac:*ESAT-6* was constructed and had its functionality tested by confocal microscopy and flow cytometry. Besides, it was constructed the *L. lactis* FnBPA(pValac:ESAT-6) strain and was performed an *in vivo* murine immunization by oral administration of such strain, in order to know the profile of the immune response generated. Thus, after immunization, the levels of cytokines and immunoglobulins were measured by Enzyme-linked immunosorbent assay (ELISA) and the percentage of T CD8+ and T CD4+ INF-γ producing cells were measured by flow cytometry.
Results: There was high INF-γ production by *L. lactis* FnBPA(pValac:ESAT-6), statistically different from controls, besides no detection of the anti-inflammatory cytokines IL-4 and IL-10 in the stimulated spleen. This strain was also able to increase the percentage of T CD4+ and T CD8+ INF-γ producing cells, compared to the saline control. Thus, the results, here obtained, denote a Th1 type immune response, needed for immunization against *M. tuberculosis* infection. Furthermore, *L. lactis* FnBPA(pValac:ESAT-6) was able to increase the levels of anti-ESAT-6 secretory IgA in the feces and the colon of the immunized animals, indicating the presence of a specific mucosal immune response in mice.
Conclusion: This work constitutes the first step towards validation of the effectiveness of a new DNA vaccine based on genetically modified LAB.
Financial support: FAPEMIG, CAPES and CNPq

FUNCTIONAL CHARACTERIZATION OF THE *WEISSELLA PARAMESENTEROIDES* WPK4 STRAIN AS A NOVEL PROBIOTIC FOR PIGLETS

LB Alvim1, BC Silva1, SHC Sandes1, RME Arantes2, JR Nicoli3, E Neumann3, AC Nunes1
1 Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais
2 Departamento de Patologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais
3 Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais

Diarrhea in piglets by *Salmonella* spp. and other pathogens can be a serious problem and require treatment. Nondrug therapies such as probiotic microorganisms administered orally have various effects on the gastrointestinal microbiota dysbiosis and host immune system stimulation. The aim of this study was to select autochthonous strains of lactic acid bacteria isolated from healthy piglets according to their probiotic properties and safety. Lactic acid bacteria were isolated from oral and nasal mucosa and feces of piglets and identified by 16S rRNA gene sequencing. Strains were *in vitro* tested for functional properties of potential probiotics and *in vivo* model of experimental salmonellosis in mice (approved by CETEA/UFMG under agreement number 203/09 and 96/11). Out of 37 isolates, 24 strains belonging to the *Weissella* and *Lactobacillus* genera were analysed *in vitro* for desirable probiotic characteristics. Three strains, *W. paramesenteroides* WpK4, *L. plantarum* LpG1, and *L. acidophilus* LaH4, were highly resistant to acidic pH and bile salts. In addition, the WpK4 strain also had a highly hydrophobic cell surface, exhibited antagonism against five of the six tested bacterial pathogens, showed H2O2 production and exopolysaccharide secretion and exhibited intrinsic resistance to only two antibiotics. Mice with complex intestinal microbiota fed daily doses of WpK4 for ten days showed no signs of bacterial translocation to the liver or spleen. Significantly, WpK4 colonization attenuated the weight loss, fostered the preservation of intestinal architecture and integrity and promoted survival in mice following infection with *Salmonella* Typhimurium at ten days post-challenge. These findings validate the suitable probiotic properties of the *W. paramesenteroides* WpK4 strain and provide for its eventual use in piglets.

Supported by: CAPES, FAPEMIG and CNPq.

CONSTRUCTION AND FUNCTIONAL EVALUATION OF A DNA VACCINE CODING FOR A FUSION PROTEIN COMPOSED OF ESAT-6 AND AG85A ANTIGENS OF *Mycobacterium tuberculosis* USING *Lactococcus lactis* AS A CARRIER VEHICLE

CC Prósperi1, P Mancha-Agresti1, VB Pereira1, SY Leclercq2, JSC Santos2, A Miyoshi1
1 Departamento de Biologia Geral, Instituto de Ciências Biológicas, UFMG, Belo Horizonte, MG.
2 Fundação Ezequiel Dias, Belo Horizonte, MG

Introduction: *Lactococcus lactis* is considered the Lactic Acid Bacteria model and has long been used for the production and delivery of antigens and cytokines at mucosal level. Recently, the potential of this microorganism as vehicle for delivery of DNA vaccines has been investigated as safe alternative to the use of attenuated pathogenic bacteria. In this context, our research group developed a eukaryotic expression vector, called pValac (Vaccination using Lactic acid bacteria), for use in genetically modified *L. lactis* strains (*L. lactis* FnBPA+), capable of invading mammalian cells and deliver the mentioned plasmid. In this regard, it is believed that the use of *L. lactis* FnBPA+ containing the pValac vector for expression of the fused immunodominant antigens of *Mycobacterium tuberculosis*, ESAT-6 and Ag85A, could represent a first step towards a new strategy to control tuberculosis, an infectious disease that leads to 1.3 million deaths per year worldwide. Therefore, this work had as objective to construct and functionally evaluate the pValac:*e6ag85A* vaccine plasmid.
Methodology: For this purpose, the ORFs coding for ESAT-6 and Ag85A were first isolated by PCR. Following, the ORFs were fused through an artificial restriction site added to the extremities of their sequences and cloned into the pValac vector. In order to evaluate the functionality of the pValac:*e6ag85A*, CHO (Chinese Hamster Ovary) cell lines were transfected with the referred plasmid and the ESAT-6 and Ag85A expression were evaluated by Confocal Microscopy and Flow Cytometry. Finally, pValac:*e6ag85A* was transformed into the invasive strain *L. lactis* generating the *L. lactis* FnBPA+(pValac:*e6ag85A*) strain.
Results: The construction of plasmid pValac*:e6ag85A* was confirmed by enzymatic digestion and PCR, as well as its functionality, through which the above-mentioned methodology. At the end, it was possible to observe the expression of the hybrid protein (ESAT-6::Ag85A).
Conclusion: The construction of pValac:*e6ag85A* opens perspectives for the development of an innovative immunization strategy against tuberculosis.
Supported by: CAPES, CNPq, FAPEMIG.

EQUINE LACTIC ACID BACTERIA MOLECULAR IDENTIFICATION

Silva B.C. 1; Alvim L.B. 1; Sandes S.H.C. 1; Nunes A.C. 1;
1 Programa de pós-graduação em Genética, Departamento de Biologia Geral – Instituto de Ciências Biológias (ICB)/Universidade Federal de Minas Gerais (UFMG) Laboratório de Genética Molecular de Protozoários Parasitas.

Background/Introduction. In equine production the indiscriminated use of antibiotics as growth factor brougth the problem of the antibiotic resistance. A probiotic is a bacteria which promote health benefits to its host. In equine production the probiotics are used as improvement of zootechnical performance and intestinal diseases prevention. The propose of this work was isolate and identify latic acid bacteria (LAB) from equine feces for subsequent characterization of strains with probiotic potential. LAB are generally Gram-positive bacteria, catalase negative, capable of fermenting glucose and other sugars with its final major product been lactic acid. They must have several characteristics to make them potentially good probiotics.
Methodology. Fifty four (54) bacteria were isolated from the feces of equine puppies, around one month and one year old, and were submitted to molecular identification at the genus level. Of these 54 isolates, thirty three (33) showed a typical pattern with three amplicons of 16S-23S rDNA intergenic spacers corresponding to *Lactobacillus/Weissella/Pediococcus* genera. After that, these amplicons were digested by twelve restriction endonucleases for LAB typing at species level (PCR-ARDRA).
Results. Using this methodology we could identify two species de *Lactobacillus* (two isolates of *L. reuteri* and two isolates of *L. crispatus*). We were not able to identify some isolates at the species level through 16S-23S rDNA ARDRA because some restriction patterns did not match any *Lactobacillus*-specific profile presented in our data bank. Thus, these bacteria were submitted to sequencing reaction of 16S rRNA gene. The sequencing reaction was performed by dideoxi method by automatic capilary sequencer MegaBace 1000 (APBiotech). Twenty one (21) isolates were recognized as *Weissella confusa*, three isolates were typed as *Enterococcus casseliflavus*, three isolates were typed as *Pediococcus pentosaceus* and the other two isolates were typed as *Lactobacillus equi*. There was a possibility that these isolates were clonally related because some of them came from the same animal and this was verified by rep-PCR (Repetitive Extragenic Polymorphic) genomic fingerprinting using primer GTG5. These equine LAB isolates were divided in eighteen (18) different strains based on GTG5 profiles.
Conclusions. The most prevalent species found in these equine fecal samples was *W. confusa*, corresponding to about 64% of the isolates. From the species found in this study, *W. confusa*, *L. reuteri*, *L. crispatus* and *L. equi* had already been identified in feces by others authors in literature.
Key-words: Latic Acid Bacteria, Molecular identification, 16S-23S rDNA ARDRA, GTG5.
Financial Support: FAPEMIG, CAPES.

CONSTRUCTION AND FUNCTIONAL EVALUATION OF THE THERAPEUTIC PLASMID pValac::*dts*::*IL-4*: DEVELOPMENT OF AN ALTERNATIVE STRATEGY FOR CROHN'S DISEASE TREATMENT

Tatiane Melo Preisser1; Bianca Mendes Souza1; Vanessa Bastos Pereira1; Meritxell Zurita Turk1; Vasco Azevedo1; Anderson Miyoshi1
1Laboratório de Genética Celular e Molecular, Departamento de Biologia Geral, Instituto de Ciências Biológicas,
Universidade Federal de Minas Gerais, Minas Gerais, Brazil

Inflammatory bowel diseases (IBDs) are multifactorial autoimmune disorders of the gastrointestinal tract, which present incidence and prevalence worldwide, with the largest number of affected people in Western countries. One of the most important types of IBDs is Crohn's Disease (CD), whose etiology is not completely understood. However, it is already known that environmental, psychogenic, microbiological, genetic and immunological factors are associated with its development. Analyzing the immunological features of CD, it was suggested that a mutation in the IL-4 coding gene is associated with the development of IBD. Thus, so far, the goals of this study were to construct and evaluate the functionality of a therapeutic plasmid for the expression of IL-4 in mammalian cells. This alternative strategy, for CD treatment, proposes the use of the lactic acid bacterium, *Lactococcus lactis*, for the delivery of the constructed plasmid. To this end, the *IL-4* ORF of *Mus musculus* was cloned into the commercial vector pCRTM-Blunt in order to be, then, subcloned into the eukaryotic expression vector pValac::*dts* for the construction of the therapeutic plasmid pValac::*dts::IL-4*. To evaluate the functionality of this plasmid and to verify the production of the recombinant IL-4 (rIL-4), Flp-InTM-CHO (InvitrogenTM) cells were transfected with pValac::*dts::IL-4* by the use of the Lipofectamine® 2000 (InvitrogenTM) system and techniques such as fluorescence confocal microscopy, flow cytometry and ELISA were performed. In the first, presence of rIL-4 was observed in the cytoplasm of cells transfected with the therapeutic plasmid, whereas it was not observed in non-transfected cells and cells transfected with the empty plasmid. In the second, it was observed that 2.26% of the cells transfected with pValac::*dts::IL-4* produced the rIL-4, while only 0.24% of the nontransfected cells and 0.38% of the cells transfected with the empty plasmid produced the protein. In the third, the rIL-4 was detected in values of 5.25±0.1 ng/mL in the culture supernatant of cells transfected with the therapeutic plasmid; however, it was not detected in the culture supernatant of non-transfected cells and of cells transfected with the empty plasmid. These results confirm that the therapeutic plasmid pValac::*dts::IL-4* is functional, since rIL-4 was transcribed, translated and secreted by the eukaryotic host cell machinery. Additionally, the perspective of this study is to transfer the pValac::*dts::IL-4* vector to non-invasive (*L. lactis* MG1363) and invasive (*L. lactis* MG1363 FnBPA+) strains of *L. lactis* to make it possible to perform tests with animals subjected to the TNBS model of intestinal inflammation. Finally, this study elucidates that gene therapy using a lactic acid bacterium containing an eukaryotic expression vector should be considered as an innovative strategy for the treatment of diseases, such as CD, since it takes advantage of the mucosal immunity to modulate the immune system of the gastrointestinal tract. Thereby, further studies in the biotechnology field are required for this promising tool to assist us in the treatment and prevention of diseases with its full potential.

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MOLECULAR, IMMUNOLOGICAL, AND BIOLOGICAL CHARACTERIZATIONS OF *TITYUS SERRULATUS* VENOM HYALURONIDASE: NEW INSIGHTS INTO ITS ROLE IN ENVENOMATION

Horta2, CCR; Magalhães1, BF: Oliveira-Mendes1, BBR; Carmo1, AO; Duarte3, CG; Felicori3, LF; Machado-de-Ávila3, RA; Chávez-Olórtegui3,C; Kalapothakis1, E.
1 Departamento de Biologia Geral, ICB, UFMG, BH, MG, Brazil; 2 Programa de Pós-Graduacão em Fisiologia e Farmacologia, ICB, UFMG, BH, MG, Brazil; 3 Departamento de Bioquímica-Imunologia, ICB, UFMG, BH, MG, Brazil.

Scorpionism is a public health problem in Brazil, where *Tityus serrulatus* (Ts) is primarily responsible for severe accidents. The main toxic components of Ts venom are low molecular weight neurotoxins; however, the venom contains high molecular weight enzymes which have been poorly studied. Among these molecules, hyaluronidase is known as a "spreading factor", but characterization of its role in Ts venom requires more investigation.
We examined clones from a cDNA library of Ts venom gland and described two novel isoforms of hyaluronidase: TsHyal-1 and TsHyal-2. The isoforms are 83% identical. The predicted amino acid sequences of both isoforms were aligned with other hyaluronidases, showing conserved residues even between evolutionarily distant organisms. We performed gel filtration followed by reversed-phase chromatography to isolate hyaluronidase from Ts venom. Native Tshyaluronidase was used to produce serum in rabbits.
Anti-hyaluronidase serum neutralized the *in vitro* hyaluronidase activity of Ts venom, by turbidimetric assay, and the minimum neutralizing serum volume for 1LD50 (13.2 μg) was 0.94 μl. Meanwhile, *in vivo* neutralization assays showed that 121.6 μl of anti-hyaluronidase serum inhibited 100% mice death, whereas 60.8 and 15.2 μl of serum delayed mice death. Death inhibition effect was also reproduced by the hyaluronidase pharmacological inhibitor, aristolochic acid. Interestingly, addition of native Ts hyaluronidase (0.418 μg) to pre-neutralized Ts venom (13.2 μg + 0.94 μl of anti-hyaluronidase serum) restored mice survival time. By using Spot method, we mapped epitopes of TsHyal-1 and TsHyal-2, in which TsHyal-1 had more peptides recognized by anti-hyaluronidase serum than TsHyal-2. Common epitopes to both isoforms included residues from the enzyme active site. Hyaluronidase inhibition and immunoneutralization reduced the toxic effects of Ts venom. Our results challenge the notion that only neurotoxins are important to the envenoming process, and may have implications in scorpionism therapy.
New generation sequencing was used to profile the transcriptome of the venom gland. The analysis revealed the presence of five new hyaluronidase isoforms present in the venom of *T. serrulatus*, in addition to the other two previously described. The sequences of the new isoforms are distinguished by the presence of SNPs, which confer missense mutations and can lead to differentiation in specificity and enzymatic activity. Thus, NGS technology provides a wider coverage and detailing of sequences present in the venom gland, contributing significantly to a better understanding of the molecules involved in the envenomation by scorpions.

MANIPULATION OF PRIMER AFFINITY CORRECTS PCR BIAS AND IMPROVES HIGH RESOLUTION MELTING ACCURACY FOR IMPRINTED GENES.

Rubatino FVM1, Carobin NV1, Oliveira VT1, Freitas ML1, Pietra RX1, Bosco AA1, Jehee FS1. Instituto de Ensino e Pesquisa da Santa Casa de Belo Horizonte - IEPSCBH, Belo Horizonte, MG.

High Resolution Melting studies have been considered to be cheap, fast and an attractive methodology to methylation analyzes. Amplification efficiencies for methylated and unmethylated templates are imbalanced towards the unmethylated one, this phenomenon is known as PCR Bias. Wojdacz et al proposed that primers should be fully complementary to the methylated sequence and have a mismatch to the unmethylated to overcome PCR bias. However, genes mapped within imprinted regions usually present higher methylation levels, therefore, an unusual PCR bias towards the methylated samples can arise. We hypothesized that the manipulation of primer affinity could overcome this problem. The aim of this work is show that one mismatch at the methylated binding site of the primer can improve High Resolution Melting assay in samples with high methylation level. Two sets of primers for the imprinted *MAGEL2* gene were designed, one with mismatch to the unmethylated binding sequence and another with mismatch to the methylated one. Reactions had prepared with the commercially available bisulfite converted methylated and unmethylated control DNA (EpiTect PCR control DNA set, Qiagen). PCR reactions had carried out in a final volume of 10uL containing 2ng DNA either totally methylated (100%), totally unmethylated (0%) or mix of both (50%, 75%, 90% and 99%) DNA controls, 1X MeltDoctor Master Mix Applied Biosystems. All reactions were performed in duplicates using the ABI 7500 Fast System. Amplification conditions were 95ºC for 10min for enzyme activation followed by 45 cycles of 95ºC for 30s and varying annealing and extension temperatures for 60s as detailed below. Primers that are fully complementary to the methylated sequence originated a very small plotting area between samples 50% and 100%, figure 1. When using primers that are fully complementary to the unmethylated sequence, we are able to increase the plot area between 50% and 100% and to distinguish among samples with small methylation fraction differences (90%, 99% and 100%), figure 2 and 3. Other important point is the PCR temperature of annealing and extension. When primers with mismatch to unmethylated were used, a lower temperature improves the unmethylated template amplification. Therefore, it can give rise to more unspecific products, which makes analysis impossible. Differently, reactions carried out using primers with mismatch to the methylated template don´t require lower temperatures because primers must favor unmethylated template amplifications at high temperatures too. To HRM methylation analyzes, when the samples have high methylation levels, such as genes mapped in imprinted regions, we propose that the primers must be able to favor the amplification of rarest sequence, unmethylated. The primers must be designed fully complementary to unmethylated binding site.

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MYCOTA ASSOCIATED TO NATIVE *HEVEA SPP.* IN THE BRAZILIAN AMAZON REGION

PLC Fonseca1, ABM Vaz3, F Badotti2, EM Silva1, A Góes-Neto3
1Centro de Pesquisa René Rachou
2Centro Federal de Educação Tecnológica de Minas Gerais
3Universidade Estadual de Feira de Santana

The fungi kingdom is highly diverse and plays various ecological roles in ecosystems. The endophytic fungi are those which reside in plant tissues and it is believed that present huge biotechnological potential. Plants of the genus *Hevea spp.*, are known for their ability to produce látex. The properties presented by natural látex cannot be replaced by synthetic latex and still widely used by the industry. It is known that the fungus *Mycroclyclus ulei* is a fitopathogen that affects this genus of plant and previous studies showed that endophytic fungi can be used to contain these infections. This study aimed to characterize the diversity of the fungal community in leaves of the genus *Hevea spp.* in the amazon region. A total of 198 endophytic fungi were isolated on malt extract agar (MEA) and 24 isolated were identified by sequencing the internal transcribed spacer (ITS) region of the rRNA gene revealing 9 different groups in different isolation regions of leaves of 4 individuals of *Hevea spp.* collected. In addition, all isolates were preserved in two different preservation techniques and were deposited in the Culture Collection of Microorganisms of Bahia (CCMB). The study of the endophytic diversity in *Hevea spp.* are still very scarce, but the results of this work suggest a diversity of endophytic fungi associated with *Hevea spp* that can be used to select fungal candidates in the control of *Mycrocyclus ulei* .

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STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF HOMOLOGOUS EXFOLIATIVE TOXIN D OF *STAPHYLOCOCCUS AUREUS* INVOLVED IN SUBCLINICAL MASTITIS OF SMALL RUMINANTS

NR Tartaglia1, RF Silva1, KTO Santana1, TLP Castro1, RB Mariutti2, RK Arni2, RJ Meyer3, A Silva4, Y Le Loir5, V Azevedo1, N Seyffert1 1Laboratório de Genética Celular e Molecular, Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais - Brasil 2Laboratório de Biologia Molecular, Universidade Estadual Paulista Júlio de Mesquita Fillho, São José do Rio Preto, São Paulo - Brasil. 3Laboratório de Imunologia e Biologia Molecular, Universidade Federal da Bahia, Salvador, Bahia- Brasil 4Laboratório de Polimorfismo do DNA, Universidade Federal do Pará, Belém, Pará - Brasil.
5Institut National de la Recherche Agronomique Institution (INRA), Rennes - France.

Mastitis is the most common and costly disease affecting dairy cattle and *S. aureus* is the most frequently isolated microorganism from clinical cases of this infection. This disease is characterized by inflammation of the mammary gland and presents local symptoms that can result in systemic infection. A better understanding of the factors involved in the pathogenicity, as well as the host response to infection by this microorganism, are key points for an efficient and satisfactory development of therapies to prevent and combat mastitis. Although considered an opportunistic pathogen, certain strains of *S. aureus* are more prone to cause pathologies than others, due to the variety of virulence factors associated to them. In this regard, through serological proteome analysis (SERPA) that enables the identification of differentially expressed immunoreactive proteins, the pathogenic profiles of *S. aureus* strains O46 - characteristic of subclinical mastitis - and O11 - characteristic of gangrenous mastitis - were evaluated. Based on these data, the aim of this study was to characterize a protein, encoded by the ORF O46_2740, immunoreactive only in *S. aureus* O46 and homologous to exfoliative toxin D. This protein is considered an epidermolytic toxin and has been associated to bullous impetigo and staphylococcal scalded skin syndrome (SSSS). A three-dimensional homology model was designed *in silico* using an ETB mold deposited in databases. For *in vivo* characterization, we obtained recombinant strains of *Lactococcus lactis* containing the ORF O46_2740 coupled with the XIES expression system; however, Western blotting did not confirm the production of the
ETD-like protein. Alternatively, the ORF was introduced in *E. coli* under control of the T5 promoter and the expression of ETD-like was successfully confirmed by Western blotting. Following, the ETD-like was purified and applied on the back of newborn Balb/c mice to evaluate its effects on the skin. Preliminary results of *in vivo* assessments indicated the formation of bullous reactions that were also caused by the epidermolytic activity of *S. aureus* ETD; however, no exfoliative activity was observed. Supported by: CAPES, CNPq, FAPEMIG, FAPESP, FAPESPA e FAPESBA.

SURVEY OF ANIMAL SPECIES OF INTEREST FOR FORENSIC GENETICS IN MINAS GERAIS STATE, BRAZIL

Aline Torres de Azevedo Chagas1,2, Evanguedes Kalapothakis1
1 Laboratório de Biotecnologia e Marcadores Moleculares Instituto de Ciências Biológicas Universidade Federal de Minas
Gerais. Programa de Pós Graduação em Genética.
2 Polícia Civil de Minas Gerais Superintendência de Polícia Técnico-Científica

Themes related to environmental questions have been routinely discussed by the scientific community and society. In general, we note an increasing concern in enlargement of its legal protection, through the establishment of laws that inhibit degrading practices. Regarding wildlife, the brazilian law describes several detrimental conducts, such as illegal hunting and fishing. In Minas Gerais State, the materiality of crimes committed against wildlife it's impaired, mainly due the lack of institutional structures that allows the routing of samples seized in enforcement actions to future forensic analyses. Thus, it would be desirable the establishment of techniques that allow the correct identification of the seized samples, through simplified protocols that enables the routing to reference labs. Unfortunately, there is not a complete understanding about this subject by the governmental institutions, and we found no data about the most seized species that, therefore, would be of interest for forensic analyses. The aim of this work was to evaluate datasets provided by the Environmental Military Police of Minas Gerais State and collect qualitative and quantitative information about the seizures related to illegal hunt and fishing. We analyzed 114 reports elaborated in 2013 and first half of 2014, 50 related to illegal hunt and 64 related to fishing. For the former, in 27 reports the weighing of the material was carried out, totaling 308.159kg of meat seized. The most common species were: spotted paca - *Cuniculus paca* (20), capybara - *Hydrochoerus hydrochaeris* (16), armadillo - *Dasypus novemcinctus* (6), anteater - *Myrmecophaga tridactyla*, deer - *Mazema* sp. and peccary - *Pecari tajacu* (3); in four situations there were no information about the species classification. Regarding fish, in 49 reports the weighing of the material was carried out, totaling 1,354.75kg. The most common species were: trahira - *Hoplias* sp. (15), curimata - *Prochilodus* sp. and piau - *Leporinis* sp. (14), mandi - *Pimelodus* sp. (12), pacu – Serrasalminae subfamily and tilapia - *Oreochromis niloticus* (8) and tucunare *Cichla* sp (7). Another 17 species were cited, but with reduced number of seizures.

In 18 reports related to meat seizures, the identification of the species was done by the offender, 25 by the policemen and 3 by a biologist or veterinarian. For the fishing reports, in all the 53 ocasions that the identification was done, it was informed by the policemen. We conclude that Minas Gerais State do have demand for identification of seized animals.

Future prospects for our group are based on the establishment of molecular markers for the interest species that were identified in this study. Such protocols would help to improve the investigative process, through techniques that will enable us to state if the material seized belongs, in fact, to a specie whose hunting or fishing is not allowed.

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MULTIDETECTION OF UNAUTHORISED GENETICALLY MODIFIED ORGANISMS USING SITEFINDING-PCR AND NEXT-GENERATION SEQUENCING

APV Martins1,2, JP Dijk1, M Staats1, M Voorhuijzen1, A Silva1 E Kalapothakis2, EJ Kok1
1 RIKILT Wageningen UR, Wageningen, The Netherlands
2 Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, MG, Brazil.

Abstract

In recent years, it is noticeable a shift in the interest in detection of genetically modified organisms (GMOs) from the mere purpose of correct labeling towards the detection of unauthorized GMOs (UGMOs). This shift is motivated by the growing number of biotech crops with novel genetic elements that increasingly difficult the detection of GMOs in food and feed samples using qPCR-based screening methods. Some incidents where UGMOs were found in seed batches or harvests have reinforced an international concern. PCR-based screening methods usually infer the presence of an unauthorized GMO by the absence of an authorized one that explains the presence of detected elements. However, in case of suspicion of UGMO presence, stablishment of the combination of GM elements and events that are not reported for authorized GM constructs is only truly possible through subsequent sequence-based analysis of unknown regions adjacent to known GM elements. Nowadays, such procedure involves a strategy of genome-walking and Sanger sequencing starting from the identified GMO element(s). This procedure, however, is very time-consuming for complex food and feed samples containing multiple GMOs. Next-generation sequencing (NGS) promises to offer enhanced possibilities for detecting genetic modifications, even in complex or unauthorized events. However, besides its potentiality, any NGS strategy has not yet been used for simultaneous detection and identification of multiple GMOs and UGMOs in a single sample. Moreover, the high sensitivity of high throughput methods require bioinformatic tools able to differenciate between very similar GM constructions reads and identify even small amounts of sample contamination with other non-GM organisms. In the present study we assessed the performance of correctly identifying GMOs in samples of different complexities using NGS data generated with Illumina HiSeq 2000 and PacBio *RS*. Genome-walking approach was used for targeted amplification and bioinformatics tools were applied in a pipeline for reducing the redundant dataset and indicate possible UGMO constructions. Five samples were produced varying the number and concentration of GMOs for which GM element sequence data had previously been reported, hence allowing us to confirm whether GMOs were correctly identified. Four enrichment targets (P35S, TNOS, Barstar, Barnase) were amplified using modified SiteFinding- PCR strategy. Paired-end 75-cycle multiplex sequencing was performed using Illumina HiSeq2000 technology and PacBio sequencing libraries were generated by ligating PacBio SMRTbell™ adapters to both ends of SiteFinding-PCR products. Dedicated bioinformatics pipelines were setup for analyzing the sequence data. For Illumina data, unambiguously BLAST identification was achieved for only one or two GMOs in each sample. For PacBio data all GMOs successfully amplified could be unambiguously identified. Furthermore, even if it was not thought to be a quantitative approach, the pipeline applied was able to detect GMOs in concentration as low as 1%. Although the SiteFinding-PCR enrichment strategy needs further optimisation, combining genome walking protocols and long high throughput sequencing platforms proved to be very promising for multidetection and characterization of GMOs and UGMOs in complex samples.
Supported by: CAPES

DISCOVERY AND RECOMBINANT EXPRESSION OF NEW METALLOPROTEASE TSMS1 FROM VENOM GLAND OF *TITYUS SERRULATUS* SCORPION

Chaves, L. M.; Carmo, A. O.; Oliveira-Mendes, B. B. R; Kalapothakis, E.

Tityus serrulatus is popularly known as the yellow scorpion, is about seven centimeters long and in the penultimate segment of the tail has a pair of serrations, that is why this species gained the name "*serrulatus*". The venom of *T. serrulatus* is a rich mixture of proteins, peptides and other substances that may have multiple functions, and the main classes of toxins are peptides that act on ion channels. However, one of the protein classes that are found in the venom, which are not very well characterized, are proteases. Ten new sequences related to mettaloproteases were identified by sequencing a cDNA library from the venom of *T. serrulatus* gland. The sequence found in most of the clones in the cDNA library is TsMS1. Obtaining protease TsMS1 from total venom *T. serrulatus* is difficult because an expensive purification process to achieve a small amount of this protease is necessary. To overcome this problem and achieve satisfactory amount of protease we can use a system of heterologous expression of proteins in bacteria. This work aimed the production of the protease TsMS1, closer to the native conformation, to study the characterization of this protein. We used the methodology for expression in *Escherichia coli* with the aid of two types of plasmids for efficient cloning and expression of this gene and tested in different *E. coli* strains, BL-21 and Shuffle, submitted in different expression condition of temperature expression, and, also, IPTG concentration. We also made a growth curve comparing the *E. coli* optical density in the same variable tested. The results shown the best expression condition was at 37 ºC and 0.6 mM IPTG. However, unfortunately, the TsMS1 were insoluble and didn't had the same function that the native one. Methods of denaturation and refolding are being studied and tested to turn the recombinant protease into a soluble protein.

IN SILICO CHARACTERIZATION, CLONING AND HETEROLOUGOUS EXPRESSION OF FIVE C. PSEUDOTUBERCULOSIS PROTEINS PROBABLY INVOLVED IN VIRULENCE

KTO Santana1, NR Tartaglia1, RF Silva1, RB Mariutti2, EL Aguiar1, RWD Portela3, RK Arni2, RJ Meyer3, A Silva4, V Azevedo1.
1 Laboratório de Genética Celular e Molecular, Instituto de Ciências Biológicas – Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais – Brasil.
2 Laboratório de Biologia Molecular, Universidade Estadual Paulista Júlio de Mesquita Fillho, São José do Rio Preto, SãoPaulo - Brasil.
3 Laboratório de Imunologia e Biologia Molecular, Universidade Federal da Bahia, Salvador, Bahia – Brasil.
4 Laboratório de Polimorfismo do DNA, Universidade Federal do Pará, Belém, Pará - Brasil.

Corynebaterium pseudotuberculosis is the causative agente of Caseus lymphadenitis (CL), disease affecting small ruminants and results in decreased productivity and loss in agribusiness. Different approaches aim the control of this disease by identifying new drug or vaccine development and diagnostic tests. However, there is not yet an satisfactory and efficiency method for the control of LC; nevertheless, the sequencing of the genomes of Corynebacterium pseudotuberculosis has enabled the identification of new virulence factors of this microorganism that may contribute to a better understanding of its biology and host-pathogen relationship. In this work, proteins PLD, PknG, SpaC, SodC and NanH were selected as targets of the study based on in silico analysis and literature data mining. PLD is the major virulence factor of C. pseudotuberculosis whereas the others are pointed as potential virulence factors. In silico characterization of these proteins were performed from predictions of physicochemical parameters, signal peptide, conserved domains, assessment of their conservation in eukaryotes and epiotopes. In addition, the proteins were also expressed using a heterologpus expression system in Escherichia coli and five different strains were tested for expression. By using the strains that showed the highest level of expression of each protein, we assessed the expression kinetics and solubility of the expression protocol that aimed to obtain the protein in the soluble fraction. Its purification was confirmed by Western blot. Following the purification of the other proteins, we will now evaluate them regarding their antigenicity and protection against infection. Since virulence factors are important candidates for drug targets, diagnostic tests or even vaccine targets, obtaining these proteins is important to perform studies that may contribute to the development of a control strategy against LC.
Supported by: CAPES, CNPq, FAPEMIG, FAPESP, FAPESPA, FAPESBA.

EVALUATION OF THE POTENTIAL PROTECTION OF STREPTOCOCCUS PNEUMONIAE SORTASE A PROTEIN USING TWO VACCINE STRATEGIES: RECOMBINANT PROTEIN AND DNA VACCINE

JAM Souza1, MA Azevedo2, A Miyoshi1, SY Leclercq2
1 Universidade Federal de Minas Gerais
2 Fundação Ezequiel Dias

Streptococcus pneumoniae is a gram-positive pneumococcus that affects the human respiratory system and is the most common cause of bacterial meningitis, otitis media and pneumonia all over the world, mostly affecting children under five years. The virulent strains of S. pneumoniae are surrounded by a polysaccharidic capsule and these polysaccharides are the basis for their serotyping, characterizing over ninety serotypes. Currently, there are four available vaccines against S. pneumoniae: the pneumococcal 23-Valent and the conjugated pneumococcal 7, 10 and 13-Valent; all based on polysaccharides antigens. The inclusion of conjugated vaccines is interesting because it can trigger a T-dependent response and consequently immunological memory. However, although these vaccines are effective, they do not provide protection against all the existing serotypes and has been observed an arising of disease caused by serotypes not included in the vaccines. Therefore, in order to overcome these disadvantages and provide a wide protection, it is sought to develop a vaccine based on conserved protein antigens that are present in different serotypes. In this study, we have selected the Sortase A protein, a membrane-anchored transpeptidase of the highly conserved S. pneumoniae, which has an important role in pneumococcus surface protein processing and it is involved in the adhesion and colonization in the host. Therefore, the objective of this work was to evaluate the protective potential of this protein, using two vaccine strategies: recombinant protein and DNA vaccine, in Swiss Webster mice. The mice immunization experiment was submitted to the ethic committee of animal research of Fundação Ezequiel Dias and approved under protocol 060/2013. For the DNA vaccine strategy, the srtA gene was amplified from S. pneumoniae genome by PCR and cloned into the pVAX 1 vector. In order to confirm the functionality of the construction, hamster ovary cells (CHO- Chinese Hamster Ovary) were transfected and the Sortase A expression was proved by flow cytometry. The construction pVAX 1/srtA was stored for future immunization experiments. For the recombinant protein strategy, the coding sequence of Sortase A protein was amplified by PCR and cloned into the expression vector pET-21a. After that, the protein was expressed in Escherichia coli and purified through liquid affinity chromatography. After Sortase A protein purification, Swiss Webster mice were intraperitoneally immunized and the humoral response was analyzed by ELISA. The results have shown that the protein induced an IgG response characterized by a high rate of IgG1 and IgG2a. After the challenge with S.pneumoniae virulent strain, there was a partial protection in the immunized group comparing with the control group. In conclusion, it was possible to show that Sortase A protein is highly immunogenic and the experiments with DNA vaccine will be shortly started for comparison between the two immunization strategies.

MOLECULAR CHARACTERIZATION OF CPB2 GENE (BETA2 TOXIN) OF THE CLOSTRIDIUM PERFRINGENS REVEALS ATYPICAL FORMS, 'SLIPPERY CODON SEQUENCE' DELETION AND ASSOCIATION WITH PLC GENE (ALPHA TOXIN).

Almeida M.O.1; Silva R.O. 2; Lobato F.C.F. 2; Kalapothakis E1.
1- Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais
2- Escola de Veterinária, Universidade Federal de Minas Gerais

Keywords: Clostridium perfringens, polymorphisms, alpha toxin, beta2 toxin, plc gene, cpb2 gene.

Clostridium perfringens is a Gram-positive anaerobic sporeforming bacterium which has a great importance in medicine and veterinary medicine as a pathogen in humans, domestic animals and wildlife. The C.perfringens pathogenicity is associated with the production of several exotoxins and differs in disease that they cause due to different genotypes and toxinotypes. All strains possess the plc gene which encodes the alpha toxin and the differential possession of other major toxin genes allows the discrimination into five toxinotypes from A to E. Each of these types has been linked to specific diseases that cause great economic losses to livestock. A novel toxin, the beta2 toxin, and its encoding gene cpb2 has been described and was detected in all C.perfringens toxinotypes, in different animal species and food isolates. Clear conclusions about the prevalence of cpb2 gene and the beta2 toxin's contribution to the disease cannot be drawn from existing studies. However, in some animal species, a strong correlation between the presence of cpb2-harboring C. perfringens, beta2 toxin and enteric disease has been reported. The present study aims to evaluate the genetic diversity of the cpb2 gene from different sources. Moreover, the study aims to investigate the correlation between polymorphisms in cpb2 and plc genes - which determine the different isoforms of the beta2 and alpha toxins, respectively - and health status. Hundred and twelve bacterial isolates were obtained from environment or from feces of different animals - wild birds, bovines, bubalines, caprines, canines, wild felines, rabbits, swines. The feces were collected directly from the intestine or of the environment, of domestic animals and wildlife, healthy or sick. The bacterial samples were subjected to DNA extraction and classified according to their toxigenic type by a multiplex PCR assay. The plc and cpb2 gene were subject to sequencing reaction and bioinformatics analysis. Most isolates used in this study were identified as toxinotype A. Molecular characterization of cpb2 gene of the C. perfringens reveals atypical forms, 'slippery codon sequence' deletion - cryptic consensus cpb2 gene - and association with plc gene. Cryptic consensus cpb2 gene seems to have epidemiological evolution importance and may be the result of selective pressure of the antibiotic use modulating the expression of the beta2 toxin. The porcine isolates showed specific alleles of the plc gene, which suggeststhe clonal relationships among porcine isolates. This work contribute to clarify the role of beta2 toxin in diseases proving, for instance, if cpb2 gene, and/or their atypical form, is a virulence factor or a simple biomarker of lineage.

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CLONING AND HETEROLOGOUS EXPRESSION OF THE VOX GENE AND PURIFICATION OF THE ENZYME VANILLYL ALCOHOL OXIDASE FROM TRICHODERMA HARZIANUM IN ASPERGILLUS NIGER

RM de Souza1, RM Cadete1, VAL Rocha2
1 Laboratório de Taxonomia, Biodiversidade e Biotecnologia de Fungos, Departamento de Microbiologia, Instituto de Ciências Biológicas, UFMG, Belo Horizonte, MG.
2 Laboratório de Desenvolvimento de Bioprocessos, Núcleo de Biocombustíveis, de Petróleo e de seus Derivados, Escola de Química - CT/UFRJ, Rio de Janeiro, RJ.

The current quest for a sustainable production of fuels has been led for environmental, economic, political, geographical and social reasons. Through the use of lignocellulolytic enzymes is being sought to increase the degradation of plant biomass substrates and consequently the obtainment of fermentable sugars as feedstock, to make possible the production of second-generation biofuels. Fungi from the genus Trichoderma have been highlighted as good producers of such enzymes (e.g. cellulases) evoking interest for biotechnological purposes. In this context, the aim of this project is based on the heterologous expression of the VOX gene, encoding the vanillyl alcohol oxidase (VAO, a lignocellulolytic enzyme) from Trichoderma harzianum (strain IOC 3844) in Aspergillus niger as an eukaryotic model. In a practical perspective, in a way to be possible to use VAO on large scale industrial applications, is necessary to reach the production of this enzyme in high yields. It can be achieved through the utilization of microorganisms as expression systems of recombinant proteins, like the fungus A. niger.
The methodology was based on molecular biology and microbiological techniques as well as biochemical tools. The VOX gene, encoding VAO in T. harzianum IOC 3844, was cloned and integrated in the genome of both Escherichia coli and A. niger strains through transformation. For the enzyme expression, A. niger was grown in a specific fermentation medium; SDS-PAGE and Western Blot were made to verify the presence of the protein, which was purified through FPLC using nickel column, since the fragment is labelled with a histidine tag. Firstly, the gene was cloned in a prokaryotic system (E. coli) due to its ease of manipulation. Sixteen E. coli clones were positive for the VOX gene, and this number decreased by half when the transformation was conducted in the eukaryotic system (A. niger). At the end of the experiments, after confirming the protein production by the SDS-PAGE and Western Blot, two A. niger clones could be identified expressing the enzyme and showing the his-tag marker on the protein structure. This tag has an important role for the enzyme purification through nickel column in the chromatography assay. The enzyme was purified in 12 fractions and then eluted with a buffer containing imidazole, which is the responsible for competing with the enzyme for the binding sites in the nickel column, by ionic force. Both the expression and purification were succeed and for future studies the enzyme can be tested against different lignin sources, for the analysis of its activity in this substrate. Given this success in both expression and purification, it was possible to conclude that A. niger served as a good eukaryotic model for the expression of the VOX gene from T. harzianum IOC 3844, which opens perspectives for future works aiming the optimization of this gene expression, and also for the expression of genes encoding other proteins of interest.
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TARGETING HISTONE MODIFYING ENZYMES OF SCHISTOSOMA MANSONI AS DRUG CANDIDATES

Paula, NCCST; Mourão, MM; Fernandes, NMGS; Oliveira, GC,
1 - Laboratório de Parasitologia Celular e Molecular, CPqRR/Fiocruz- Belo Horizonte, MG
2 - Centro de Excelência em Bioinformática, CPqRR/Fiocruz-Belo Horizonte, MG

Schistosomiasis is a tropical parasitic disease caused by a Trematode of the genus Schistosoma, being the second most prevalent parasitic disease in the world. Currently, Praziquantel (PZQ) is the drug of choice and, due to the identification of drug resistant parasites, the WHO has encouraged the development of new drugs against schistosomiasis. Histone modifying enzymes (HMEs) play a central role regulating epigenetic modifications in the chromatin which motivated the study of these enzymes as targets for therapy. Crystallographic studies show that the HME Histone Deacetylase 8 (HDAC8) of the parasite presents a different catalytic site compared to the mammalian enzyme, which confers specificity and enhance its potential as target. Therefore, this work, we study the role of HMEs as drug targets in the parasite through the use of specific inhibitors for S. mansoni HDACs in vitro (inhibitors MS30 and MS142) and in vivo (inhibitors J1036 and PE24/8) tests. In in vitro tests, schistosomula cultures were exposed to inhibitors and after 12, 24, 48 and 72 hours the mortality of the parasites was counted. Higher mortality (~ 19%) was observed in parasites exposed to the MS30 inhibitor from 48h exposure. In in vivo assays, schistosomula exposed to inhibitors were inoculated in mice, after infection perfusion was performed to count the worms, livers were weighed and eggs retrieved were counted. Mice inoculated with parasites exposed to the inhibitor J1036 (20μM) showed a small amount of worms (37% compared to the control group) and there was also a decrease in oviposition (~ 67% compared to the control group) in liver samples obtained from mice infected with parasites treated with the inhibitors, which may indicate that the inhibitor influence the viability of the parasites against the host immune system. The results showed that HDAC8 may play an important role in oviposition and/or reproductive system of the parasite, indicating the potential of these enzymes as drug targets. This project has the approval of the Ethics Committee for Animal Use (CEUA), Oswaldo Cruz Foundation, under the number P49/12-5.

V Encontro de Genética de Minas Gerais

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Pesquisa e Pós-Graduação

Genética, Evolução e Ecologia

COMPARATIVE GENOMIC *IN SITU* HYBRIDIZATION AND MAPPING OF TRANSPOSABLE ELEMENTS IN THE KARYOTYPES OF AKODONTINI

NP Araújo, GCS Kuhn, M Svartman

Laboratório de Citogenômica Evolutiva – Departamento de Biologia Geral – Instituto de Ciências Biológicas – Universidade Federal de Minas Gerais – *Av. Presidente Antônio Carlos, 6627, 31270-901 – Belo Horizonte-MG Brasil.*

The Akodontini tribe is characterized by a high level of chromosomal polymorphisms due to Robertsonian rearrangements and pericentric inversions. In this work, we compared the GTG- and CBG-banded karyotypes of three species of this group: *Akodon cursor* (2n=14, FN=19), *A. montensis* (2n=24; FN=42), and *Necromys lasiurus* (2n=34; FN=34). A great homeology between the chromosomes of the three species was confirmed after GTG banding. CBG-banding revealed few centromeric heterochromatic regions in most chromosomes of the three species.

Interspecific genomic comparisons through fluorescent *in situ* hybridization (FISH) using total genomic DNA of each species as probes (GISH) showed conservation of the euchromatic portion of the genomes of the two *Akodon* species and of *Necromys.* Furthermore, shared repetitive sequences comprising the heterochromatic regions of pairs 1, 3 and 6 and the X chromosome of *A. cursor* and pair 11 and the X chromosome of *A. montensis* were observed. These results evidence extreme conservation among the genomes of the species analyzed and suggest that their main differences are related to heterochromatic regions.

FISH with the telomeric sequence (TTAGGG)n revealed signals in the terminal regions of all chromosomes in the three karyotypes. Additionally, interstitial signals that suggest the origin by fusion in pairs 3, 4 and 7 of *A. montensis* and intensely bright signals associated with the constitutive heterochromatin of chromosomes 3, 15 and X and in the euchromatin of pair 16 of *N. lasiurus* were observed. Thus, the karyotypes analyzed differed mainly due to Robertsonian rearrangements, pericentric inversions, centromere repositioning and heterochromatin variation.

To investigate the relationship between transposable elements and karyotype evolution in the analyzed species, we amplified LINE-1 (long interspersed repetitive elements), SINE-B1 (short interspersed repetitive elements) and an endogenous retrovirus mysTR by PCR. The PCR products were purified, cloned and recombinant plasmids were sequenced. The distribution of the three transposable elements was analyzed after FISH in *A. cursor, A. montensis* and *N. lasiurus*. The results suggest that these transposable elements may be related to the great chromosomal variation found in the tribe.

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RESPONSE GENE EXPRESSION OF CYANOBACTERIAL TO SAZONAL VARIATIONS IN ENVIRONMENT

JSM Pimentel1, A Giani1, AC Nunes2
Instituição 1: departamento de Botânica, ICB – UFMG
Instituição 2: departamento de Biologia Geral, genética, ICB – UFMG

Introduction: Cyanobacteria have a high physiological capacity to adapt to stress, triggered by rapid changes that occur in the environment. These changes cause inductions of specific genes required for acclimatization or for cell protection to unfavorable conditions. The aim of this study was to measure the expression of genes linked to nitrogen, phosphorus, iron and oxidative stress, following the natural seasonal variation in two different aquatic environments. An additional goal was to measure the gene expression of some cyanopeptides and check whether these compounds may be linked to the process of cell acclimation.
Methodology: Therefore, eight samples were collected in Pampulha reservoir and four, at three different stations, in Volta Grande Reservoir. Biotic and abiotic parameters were measured and correlated with gene expression data.
Results: Despite the varying degrees in trophy in these environments, the results were similar in both: temperature and nutrients variations were the main factors related to stress. However, nitrogen was more important in the oligotrophic and phosphorus in the hypereutrophic environment. We also observed that cyanopeptolin responded similarly to the stress genes, indicating a possible role of this peptide in the cell acclimation process.
Conclusions: The molecular methods used here were very sensitive to detect changes in these organisms against the stress caused by environmental modifications. The application of these methods in environmental studies may help understanding the success of cyanobacteria and assist procedures for environmental control and recovering.
Supported by: CAPES, CNPQ, CEMIG

CHROMOSOME NUMBER ANALYSIS OF (*CYPRINUS CARPIO* LINNAEUS, 1758) (CYPRINIFORMES, CYPRINIDAE) COLLECTED FROM FISHFARMING IN CONTAGEM

Matosinho, C. G. R.[12]; Franco, T. R. L. R.[2]; Campolina, S. S.[3]
[1] Departamento de Biologia Geral, Universidade Federal de Minas Gerais, Belo Horizonte, 31.270-901, Brasil
[2] Graduada no Centro Universitário UNA, Belo Horizonte, 30180-100, Brasil
[3] Professora no Centro Universitário UNA, Belo Horizonte, 30180-100, Brasil

Introduction: The order Cypriniformes, to which the species *Cyprinus carpio* L belongs, is composed by approximately 2.500 species, distributed over 256 genus and 6 families. They occur throughout Southeastern Asia and live in estuaries. The family Cyprinidae presents 194 genus and 2,070 species, being one of the largest known families of fishes in the world.
In Brazil, carps were introduce in 1882 brought from USA and has been used in fish farms since 1934, mainly for ornamental purposes. Fish farming may endanger genetic variability, because animal are kept mostly in closed systems. In Nature, family Cyprinidae presents polymorphism of the chromosomal number. Therefore, chromosomal number and structure are useful resources for characterizing species, genetic diversity, as well as to monitor the appearance of chromosomal abnormalities in a population, and may be helpful as a sentinel in the control of environmental impacts. In Cyprinidae, chromosomal number is n=50 in most of the diploid species, but n=52, 48, 46 and 44 have also been frequently reported. *Cyprinus carpio* L presents n=50, half of their chromosomes are metacentric or submetacentric and half acrocentric and subtelocentric. Most cytogenetic studies in fish are based on Giemsa, NOR or C banding techniques, but real banding patterns, as seen in humans, are not achieved and the interpretation is not straightforward, because the chromosomes are usually quite small. Even considering these difficulties, to establish methodologies for *Cyprinus carpio* L is important, once it provides a resource for monitoring environmental impacts of pollutants. The objective of this study was to characterize the karyotype (chromosomal number and chromosomal structures) of the *Cyprinus carpio* L produced in a fish farm in Contagem, MG.
Methodology: Twenty individuals were collected in two separate collection efforts. Initially, karyotype was developed as described by Bertollo, Takahashi e Moreira-Filho (1978). Animals collected in the second samplig were injected intraperitoneally with colcemid (50 mg). Animals were observed for a week and then euthanasied in ice bath.
Results and Discussion: The results obtained with the protocol described by Bertollo, Takahashi e Moreira-Filho (1978) did not allowed chromosome identification. Using colcemid it was possible to obtain a small number of usable metaphases, but even so a clear characterization of chromosome structures was not feasible. The chromosomal number observed n=53 has not been previously described. This chromosomal number may exist in the Nature but has not yet been described, but it may reflect artifacts introduced by the methodology.
Conclusion: This study allowed a first characterization of the chromosome number *Cyprinus carpio* L breed in this fish farm. A refining of its chromosomal structure was not possible with the methodology applied here. A fine characterization of chromosomal number and structure is a fundamental step, if a fish species is going to be used as a sentinel for environmental impacts. Therefore, additional efforts should be set in order to confirm our results and to improve the karyotyping methodology for this species.

TEMPORAL AND SPATIAL DYNAMICS OF BACTERIOPLANKTON COMMUNITY STRUCTURE FROM TWO TROPICAL FRESHWATER LAKES

MP Ávila, FAR Barbosa E Chartone-Souza, AMA Nascimento
Laboratório de Genética de Microrganismos – Departamento de Biologia Geral - Instituto de Ciências Biológicas

Currently, it is recognized the critical role of bacterioplankton in freshwater environments, especially in nutrient and carbon cycling, and as biomass source for the trophic chain. However, most of the studies on freshwater lake bacteria particularly focus on temperate regions, whereas the bacterioplankton of tropical lakes is yet understudied. The present work explored the bacterioplankton community structure from two tropical freshwater lakes, using next generation sequencing (Illumina Hiseq 2000) of V6 16S rDNA amplicons. The lakes chosen in this study, Carioca and Gambazinho, are located in the Rio Doce state park (PERD, Minas Gerais state, Brazil), the largest Atlantic Rainforest remaining area of the State. The total DNA was extracted from water samples collected at different depths during dry (August) and rainy (December) seasons in 2013. After screening, 16S rDNA sequences were clustered at a distance of 97% in Mothur software and classified against Greengenes database. A total of 63.098 OTUs (operational taxonomic units) were obtained of 13.996.398 reads, and 77,2% of the reads were classified, belonging to 42 different phyla. Both lakes exhibited a predominance of the phyla Cyanobacteria, Proteobacteria, Actinobacteria and Verrucomicrobia. All bacterial communities showed a high number of rare OTUs (singletons, doubletons and tripletons) and dominance of a few abundant OTUs. Carioca Lake and the rainy season exhibited the highest diversity, and communities from deepest depths were more rich and diverse. Beta-diversity measures revealed great dissimilarity between the samples from different lakes. During the dry season, when water columns are mixing, communities from different depths were similar, while the stratification occurring in the rainy season promoted communities dissimilarity. Gambazinho Lake communities presented higher dissimilarity during the rainy season as well as a enourmous community composition turnover between seasons, this is probably a consequence of the stratification polimytic pattern of this lake. Our data are in agreement with niche diversification hypothesis, as community diversity enhanced along with environmental heterogeneity. OTUs dominance and taxon composition across samples were correlated to environmental variables, especially phosphate and temperature. Anoxic associated groups were detected exclusively during the rainy season, when the bottom of the lakes is oxygen-depleted. The phylum Verrucomicrobia, which is poorly documented in the literature, was highly abundant and seems to co-occur with other bacterial groups as the picocyanobacteria *Synechococcus*, especially during the dry season. Our data support the hypothesis that lake bacteria communities are composed of many well adapted taxa, common in distinct lakes. However, each lake harbors its innate community, which is shaped by the lake's inherent ecological features

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Genética Evolutiva e de Populações

ORIGIN AND DYNAMICS OF ADMIXTURE IN BRAZILIANS: A POPULATION-BASED FINE-SCALE APPROACH

Gouveia, M, H1; Kehdy F,S,G1; Magalhães W,C,S1; Horimoto, A2; Scliar, M, O1; Soares-Souza, G, B1; Machado, M1; Leal, T, P1; Rodrigues-Soares, F1; Moreira, R1; Araújo, G1; Zamudio, R1; Santana de Plá, H1; Marrero, A, R3; Berg, D,E4; Yeager, M5; Gilman R, H6,7; Beleza, S, S8; Ghirotto, S9; Gonçalves, V, F12; Horta, B, L10; Víctora, C10; Lima-Costa, M, F11; Barreto, M12; Rodirgues M, R1; Tarazona-Santos, E1 and The Brazilian EPIGEN Project Consortium

1 Department of General Biology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.
2 Heart Institute, University of São Paulo. São Paulo, São Paulo, Brazil.
3 Department of Celular Biology, Embriology and Genetics, Federal University of Santa Catarina, Florianópolis, Santa Catarina, Brazil.
4 Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri and Department of Medicine, University of California San Diego, California, USA.
5 Cancer Genomics Research Laboratory, SAIC-Frederick, Inc.,NCI-Frederick, Frederick, MD, USA.
6 Bloomberg School of Public Health, Johns Hopkins University. Baltimore. Maryland. USA.
7 Universidade Peruana Cayetano Heredia. Lima. Peru.
8 Department of Genetics, University of Leicester, Leicester, UK.
9 Dipartimento di Scienze della Vita e Biotecnologie. Università di Ferrara. Italy
10 Post-Graduate Program in Epidemiology, Federal University of Pelotas, CP 464, 96001-970 Pelotas, RS.

Here we present results of the EPIGEN-Brazil Initiative (Genomic Epidemiology of Complex Diseases in Population-based Brazilian Cohorts, http://epigen.grude.ufmg.br). We performed a population-based genome-wide analysis of nearly 2.2 million SNPs (Omni2.5M-Illumina) from the three most populated regions of Brazil: Salvador-Bahia (n=1309), Bambui (n=1442) and Pelotas (n=3736) from Northeast, Southeast and Southern Brazil respectively. While Amerindian ancestry was low (5-7% at population level, with no individual with > 30% of this ancestry), the three populations showed individuals with all possible combinations of African and European ancestry. At population level, African ancestry ranged from 14-15% in Pelotas and Bambui to 51% in Salvador. The EPIGEN cohorts also exemplify how kinship and inbreeding coefficients may be differently structured in small (Bambuí), medium-size (Pelotas) and large (Salvador) admixed Latin American populations. Model-based ADMIXTURE analysis revealed an unknown cluster of African ancestry, likely Bantu or Eastern Africa-associated more common in Southern Brazil that in any other Latin America population, probably related with slave trade from regions such as Angola-Mozambique. Genetic diversity of European origin in Brazil recapitulates a considerable portion of the whole diversity of this continent, not being restricted to the Iberian Peninsula. At a local ancestry level, the distribution of chromosomal segments of distinct ancestry do show evidence of very recent European admixture into the South East and Southern Brazil but not in the Salvador cohort (NE), while African admixture into admixed populations fits a model of continuous gene flow across the three cohorts. In conclusion, the inferred genetic structure of Brazilian populations has implications for the design of genetic association studies in these and other admixed populations. First, assortative mating by ancestry implies that when performing association studies in admixed populations, Hardy-Weinberg disequilibrium is actual and relevant, and filtering SNPs in Hardy-Weinberg disequilibrium in quality-control analyses conceals real aspects of the genetic structure of these populations. Second, we

identified relevant components of genetic structure at sub-continental level, both for European and African ancestry. This implies that when mapping the genetic basis of diseases in Brazilians, spurious association may arise if the genetic structure within continents is not accounted for. Interestingly, the two within-African ancestry clusters in the Brazilian populations (characterized by an average FST between them of 0.02) are characterized by a set of 3318 SNPs (the 10% of total SNPs with the highest FST [>0.06], which include 38 SNPs that are GWAS hits.

CHARACTERIZATION OF SKIN BACTERIA OF INDIVIDUAL WITH LEPROSY

PES Silva1, MLS Suhadolnik1, PS Costa1, MP Reis1, APC Salgado1, MFR Lima2, EC Souza1, AMA Nascimento1

1Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais; Av. Antônio
Carlos 6627 Belo Horizonte, Minas Gerais, Brazil, CEP: 31270-901.

2Laboratório Hermes Pardini, Rua Aimorés, 66 Belo Horizonte, Minas Gerais, Brazil, CEP: 30140-070.

From biblical times to the modern period leprosy has been described as a horrifying disease due to the deformation caused by the skin lesions displayed by infected individuals. This disease is caused by an infectious agent, *Mycobacterium leprae,* being transmitted person to person. Although the reported number of registered cases worldwide has declined in the last two decades the leprosy remains endemic in some developing countries. In spite of numerous studies on leprosy none study characterized the leprosy skin microbiota. Currently, is increasing the reports on the importance of microbiota for human general health. Important questions concerning the differences of the microbiota between healthy and diseased individuals are being addressed. In this study we used culture-independent molecular approaches, cloning and sequencing of the 16S rRNA gene, to characterize the skin microbiota of individuals with leprosy and analyzed whether it differs from the skin bacterial composition of healthy individuals. To achieve this, total DNA was extracted from paraffin block and fragment of the 16S rRNA gene of bacteria was obtained for the construction of library clones. A total of 44 clones were analyzed. Phylogenetic analysis revealed a moderate diversity, but complex. The bacterial 16S-rDNA clone sequences were distributed into 27 OTUs (operational taxonomic unity) spanning four phyla: Proteobacteria, Firmicutes, Bacteriodetes and Actinobacteria, represented 18 genera. The first two phyla were enriched and the latter markedly diminished in skin from leprous lesions when compared with healthy skin. Moreover, the genus *Propioniobacterium* (Actinobacteria), indigenous bacteria of healthy skin, was drastically reduced in lesional skin of individuals with leprosy. Most of the taxa found in skin from leprous lesions are not typical of human skin and potentially pathogenic. This is the first study of bacterial diversity in skin from leprous lesions, suggesting significant change in the abundance of dominant taxa of normal skin. Our data suggest significant shifts of the microbiota with emergence and competitive advantage of potentially pathogenic bacteria over skin resident taxa. Taken together, these findings show that the microbiota of leprous lesions is not typical of human skin, and it seems to favor the colonization of potentially pathogenic bacteria, negatively impacting the abundance of bacteria that populate healthy skin. This is the first report of the taxonomic diversity of the microbiota of the leprous lesion.

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BRAZILIAN POPULATION STRUCTURE INFERRED BY X-CHROMOSOME DATA

Sant'Anna, HP1; Gouveia, M1; Tarazona-Santos, E1; Kehdy, F1; The Brazilian EPIGEN consortium1,2,3,4,5

1 Departamento de Biologia Geral, Universidade Federal de Minas Gerais, Caixa Postal 486, 31270-901 Belo Horizonte, MG, Brazil

2 Instituto do Coração - Hospital das Clinicas – FMUSP, 10o. Andar, 05403-000, São Paulo, SP, Brazil

3 Universidade Federal da Bahia, 40110-040 Salvador, BA, Brazil

4 Departamento de Medicina Social, Universidade Federal de Pelotas, 96001-970 Pelotas, RS, Brazil

5 Centro de Pesquisas René Rachou, Fundação Oswaldo Cruz, Caixa Postal 1746, 30190-002 Belo Horizonte, MG, Brazil

hanaisaa@hotmail.com

Keywords: X chromosome, Native American, African, European, sex-bias

The Brazilian population, one of the most heterogeneous in the world, is a result of mixture between Africans, Native Americans and Europeans. Little is known about how those interethnic crosses are revealed by the Brazilian X-chromosome, whose diversity can show differences in the evolutionary history of males and females. In this study, the sex-biased gene flow among Native American, European and African in the recent admixture population of Brazil was evaluated in 4,192 females' samples genotyped with the Illumina's HumanOmni2.5-8v1 and HumanOmni5-4v1 arrays. The autosomal and X-chromosome ancestry composition was estimated using principal component analysis (PCA) and ADMIXTURE with a SNPs dataset of 331,790 in autosomes and 5,792 in the X-chromosome. To compare those results, we performed a Wilcoxon Signed-Rank Test. We observed that: (i) The distributions in PCA space of each individual obtained from PC 1 and 2, the only informative clustering pattern for X-chromosome, as well as for autosomes, suggest differences in the evolutionary history of males and females. For all cohorts, we observed that, compared with autosomal data, a larger number of females X-chromosome cluster near the Native American and African parental populations. This is consistent with the lower effective recombination rate of the X-chromosome that result in a larger number of X-chromosomes with a unique continental ancestry. This differential pattern between X-chromosome and autosomal markers is not evident for European ancestry because it is the predominant continental ancestry in our sample, and therefore there is a high number of individuals with both high autosomal and X-chromosome European ancestry. (ii) Both PCA and ADMIXTURE analyses show that compared with autosomal data, the X-chromosome evidences a larger Native American and African contribution to extant Brazilian genomic diversity than at genome wide level. This is due to a historical pattern of sex-biased preferential mating between males with predominant European ancestry with women with predominant African or Native American ancestry. (iii) On average, the sex-bias in admixture was larger in Salvador, and lower in Bambuí and Pelotas, and it was higher for Native American ancestry than for African ancestry. Overall, our results suggest a significantly higher contribution from Native American and African ancestors into the composition of the X chromosome of Brazilian women, supporting the presence of sex-biased gene flow during the process of admixture.

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HETEROGENEOUS GEOGRAPHIC DISTRIBUTION OF βS-GLOBIN GENE CLUSTER HAPLOTYPES IN SICKLE CELL DISEASE PATIENTS FROM MINAS GERAIS, BRAZIL

PS Rodrigues1, VR Souza1, ML Martins1, MCF Silva-Malta2
1. Serviço de Pesquisa – Fundação Hemominas
2. Núcleo de Inovação Tecnológica – Fundação Hemominas.

Introduction: During the Colonial period, Brazil received slaves from different regions of Africa, which generated differences in the geographic distribution of sickle cell disease (SCD) and βS-globin haplotypes (CAR: Bantu or Central Africa Region; Ben: Benin; Sen: Senegal; Cam: Camarões; Asi: Asiático). The βS-globin haplotypes are important markers in the study of population compositions of SCD patients, since they allow the inference of the geographical origin of the β globin gene. In this study we evaluated the frequency of βS-globin haplotypes in patients with SCD in different regions of Minas Gerais, the second most populous state of Brazil.
Methodology: βS-globin gene haplotypes were studied in 125 non-related SCD patients by PCR-RFLP techniques to analyze six polymorphic sites from the beta cluster.
Results: We find that CAR haplotype is the more frequent (59.6%) in Minas Gerais, followed by Ben (33.6%) and Sen (0.4%). Sixteen chromosomes analyzed (6.4%) have been found with atypical pattern. Of all the patients studied, 37.6% were identified with genotype CAR/CAR, 32.8% CAR/Ben, 16% Ben/Ben, 0.8% CAR/Sen and 14.4% CAR, Ben or Cam heterozygous with an atypical haplotype.
Conclusion: Regions of Minas Gerais with distinct haplotype frequencies were identified. Benin haplotype was predominant in regions North West and North East while in other geographical regions (Central, West, South and North) the CAR haplotype was the most frequent. The high prevalence of haplotypes CAR and Benin in Minas Gerais is in accordance with historical records that describe the flow of slaves from the South Central, East and Center West regions of the African continent to Bahia and Rio de Janeiro, from where they migrated to different Brazilian regions. Our results emphasize the genetic heterogeneity of the population of Minas Gerais. This study was approved by the ethic committee of the Fundação Hemominas (OF 14/2013).
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CROSS-AMPLIFICATION OF MICROSATELLITE MARKERS DEVELOPED FOR *BYRSONIMA CRASSIFOLIA* (MALPIGHIACEAE) IN OTHER CONGENERIC SPECIES

LC Resende-Moreira1, MM Enoque1, MB Lovato1
1 Laboratório de Genética de Populações, Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais

Microsatellites (SSR) are multiallelic molecular markers commonly used in studies of population genetic diversity and structure. These markers are very informative and easy to amplify through PCR and to genotype. In addition, more than one SSR locus can be amplified in a single multiplex PCR, which reduces genotyping costs. However, the primers required for amplification of SSRs must be first designed for each locus of each species, which requires extensive work and makes the whole process very expensive. Still, many studies have shown that primer pairs designed for one species can often be used to amplify the same SSRs in other correlated species. Thus, our work aimed to verify the cross-amplification of ten SSR loci previously developed for *Byrsonima crassifolia* in three other congeneric species: *B. coccolobifolia*, *B. pachyphylla* and *B. verbascifolia*. The genus *Byrsonima* has 48 species endemic to Brazil, many widely distributed in the Brazilian Cerrado with economic and ecological interest. Several different PCR conditions were tested varying mainly the primer annealing temperature, MgCl2 concentration and the addition of bovine serum albumin (BSA). *B. crassifolia* was always used as a positive control. In addition, we tested the multiplexing of SSR loci based on PCR amplifying conditions, type of fluorescent label and the possibility of dimer formation between primers, which could hinder the amplification. All ten microsatellites developed to *B. crassifolia* were transferred to the species *B. pachyphylla* and *B. verbascifolia*; and seven primer pairs were cross-amplified in *B. coccolobifolia*. Regarding the PCR multiplexing, not all multiplex groups tested were efficient, but those with positive results enabled considerable reduction in the costs of genotyping. The SSRs were characterized in one population of each species, and all loci were in linkage equilibrium. The mean number of alleles (A), mean expected (HE) and observed (HO) heterozygosities were estimated for each species: *B. coccolobifolia* (A=4.6; HE= 0.458; HO= 0.337), *B. pachyphylla* (A=3.6; HE= 0.481; HO= 0.322) and *B. verbascifolia* (A=5.2; HE= 0.604; HO= 0.477). The fixation index was not significant. Our results reveal that these SSRs are useful in population genetic studies in these species. We are currently using these markers to investigate the genetic diversity and structure of central and peripheral populations.

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STATISTICAL COMPARATIVE PHYLOGEOGRAPHY OF TWO SPECIES OF TREES IN THE CERRADO CORE AREA AND COASTAL SAVANNAS OF BRAZIL

VASCONCELOS, PN 1 *; RESENDE, LC 1; LEMOS-FILHO JP 2; LOVATO, MB 1.
1. Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais
2. Departamento de Botânica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais
*pnvasc@gmail.com
Keywords: comparative phylogeography, coalescent, cerrado, coastal savannas, *Byrsonima coccolobifolia*, *Curatella americana*

The current existence of Cerrado enclaves in the coastal regions of Brazil - the coastal savannas - is an unexplored phenomenon. There are plant species which occur in both the core Cerrado area and in the coastal savannas, having a disjunct distribution indicative of a previous connection between these two regions. To date, there have been no studies investigating the possible evolutionary link between them, despite all the clues as to this.
The phylogeographic approach has been widely successful in elucidating the evolutionary history of species and - when applied in its comparative version - biomes. However, despite its broad implementation throughout Europe and North America, where it has brought to light the processes responsible for the current distribution of species, the phylogeographic approach is still underutilized in Brazil, especially in plant research.
Our work aims at investigating the historical relationship between the core Cerrado area and the coastal savannas of Brazil by conducting comparative phylogeographic studies under a statistical framework on two species of trees that occur in both areas and presently show a disjunct distribution: *Byrsonima coccolobifolia* and *Curatella americana*. Both species are very common in the first area and less so in the second, and the fact that they have similar dispersion and pollination mechanisms allows us to better compare their outcomes.
To accomplish our goals, we have sampled a total of ten populations of each species: five from the Cerrado core area and another five from the coastal peripheries, covering a significant portion of its current distribution. This work combines population genetic analyses with coalescent simulations to model past demographic events in order to infer the evolutionary history of these two areas and possible connections between them. We sequenced one intergenic spacer in 5 individuals of each population for *B. coccolobifolia* and two intergenic spacers in 5 individuals of each population for *C. americana*. Coalescent simulations were run in the software ms (Hudson, R. R., 2002) under five different demographic models, including branching, admixture, expansion and bottleneck. The fifth model was a 'null hypothesis', corresponding to an absence of demographic events.
Preliminary results point towards a bottleneck event which took place sometime during the last glacial period (roughly corresponding to the end of the Last Glacial Maximum) as the most probable scenario for explaining the present pattern of genetic variation, given our current data. However, two other models also presented high posterior probabilities. These three models will be investigated in more detail with added complexity and the inclusion of a nuclear marker to account for the history of biparental lineages, and also to make up for the stochasticity associated with a single locus.
Supported by: CAPES

FUNCTIONAL GENETICS OF GASTRIC CANCER CANDIDATE GENE IN PERUVIANS WITH PREDOMINANT NATIVE AMERICAN ANCESTRY

R Zamudio1, L Pereira1, H Sant'anna1, TM Queiroz1, F Lyon1, C Zolini1, FB Leão2, CD Rocha2, AM Silva2, P Herrera3, L Cabrera3, RH Gilman3,4, E Tarazona-Santos1, F Kehdy1
1Laboratório de Diversidade Genética Humana, Departamento de Biologia Geral, Instituto de Ciências Biológicas Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil.
2Laboratório de Genes Inflamatórios, Departamento de Morfologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil.
3Asociación Benéfica PRISMA, Lima, Perú and Laboratorio de Investigación en Enfermedades Infecciosas, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Perú.
4Department of International Health, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, US.

Gastric cancer is the most lethal cancer in Peru and in the world it is the third leading cause of cancer death in both sexes (723,000 deaths in 2012). Infection with *Helicobacter pylori* is the major risk factor; however environmental factors, diet and genetic background of the host also contribute to the susceptibility to gastric cancer. The colonization of *H. pylori* in the gastric mucosa leads to gastric inflammatory response and the pathogenesis of cancer is driven by this inflammation. The severity of the gastric inflammatory response may vary, depending on the host genetic background; thus, much research has focused on host SNP of cytokine and other genes that mediate the inflammatory response. Previous studies have shown the overexpression of Interleukin-8 receptor beta (*IL8RB*) increase susceptibility to gastric cancer. For this reason, we aim to study the genetic variability of the candidate gene *IL8RB* and their expression in Peruvian population with predominant Native American ancestry. Our results showed (i) from the sequencing analysis, that two SNPs (rs3890158 and rs4674258) located in the *IL8RB* promoter which together form two haplotypes (AT and GC) have very different frequencies between Native Peruvians, Europeans and Africans, with the AT haplotype being unique in Native Peruvians. (ii) From *in silico* analysis using the TRANSFAC database, the allele A of rs3890158 (much more common in Native) creates a binding site for the FOXO3 transcription factor (suggested as relevant in carcinogenesis); (iii) from *in vitro* expression assays (luciferase reporter gene), using TNF-α (Tumor Necrosis Factor alpha), that the AT Haplotype (exclusive in Native Peruvians) of *IL8RB* promoter upregulates expression of the luciferase reporter gene, meanwhile the GC haplotype has no effect on reporter gene expression; (iv) the SNP rs1126579 present in the 3'UTR region of *IL8RB* is in high linkage disequilibrium (LD) (r2 = 90%) with cis-regulatory SNP rs3890158. Consequently, this SNP will be used for Allelic Imbalance assay to understand the effect of cis-regulatory SNPs rs3890158 on expression levels of the *IL8RB* in normal gastric tissues, pre-malignant and malignant lesions in Peruvian patients with high native ancestry. For all that, we believe in the potential causal impact of differential gene expression on complex disease risk and that divergence in gene expression can be mainly explained by cis-regulatory variants.

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MOLECULAR CHARACTERIZATION AND PHYLOGENETIC OF THE CO-CIRCULATING DENV1 AND DENV4 IN DIVINOPOLIS, MG, BRAZIL, 2013.

Dutra, KR1; Lopes, DO1; Drumond, BP3; Nogueira, ML4; Ferreira, JMS2; Santos, L.L1.
1Laboratório de Biologia Molecular da Universidade Federal de São João del-Rei (Campus Centro Oeste Dona
Lindu), Divinópolis/MG, Brasil.
2 Laboratório de Microbiologia da Universidade Federal de São João del-Rei (Campus Centro Oeste Dona
Lindu), Divinópolis/MG, Brasil.
3Laboratório de Virologia da Universidade Federal de Juiz de Fora, Juiz de Fora/MG, Brasil.
4Laboratório de Pesquisa em Virologia, Departamento de Doenças Dermatológicas, Infecciosas e Parasitárias,
Faculdade de Medicina de São José do Rio Preto (FAMERP), São José do Rio Preto/SP, Brasil.

Introduction: The *Dengue virus* (DENV) is an enveloped virus belonging the family Flaviviridae with four distinct serotypes: DENV1, DENV2, DENV3, DENV4. Currently, these four serotypes are circulating in almost all states of Brazil and an increase in genetic variability between each serotype has been described. In Minas Gerais, the number of cases has been grown, therefore the phylogeny provides information on dengue virus distribution in human populations over time and place, identifying mutations and recombination events accumulated over the years. The most common gene used in studies of molecular epidemiology of DENV is the *Glycoprotein E* gene, due to the importance of its product in the binding and entry of the virus into the cell, especially in the humoral response of the human host. Mutations in this gene may be related to the increased virulence of viral strains and influence on disease severity.
Methodology: 100 blood samples of patients with acute febrile illness during the 2013 outbreak of dengue in Divinópolis/MG were analyzed. This study was approved by the internal review board of the Ethics Committee of the UFSJ under the register number 012/2010. The Viral RNA was extracted from the serum with QIAamp ® Viral RNA Kit (QIAGEN ®, USA) and viral detection was performed by reverse transcription (RT - PCR). The identification of serotypes was performed by sequencing the viral fragment of 511 pairs obtained by RT-PCR. The sequences obtained were compared to reference sequences deposited in GenBank using the BLASTN software. One second RT - PCR was performed with specific primers for each serotype to amplify the envelope *Glyprotein* gene. Nucleotide sequencing of a fragment of 1485 pairs for the region of E gene of 5 samples was performed (3 of DENV1 and 2 of DENV4). Nucleotide sequences were aligned using ClustalW (version 1.6), implemented in the MEGA program, with sequences already reported to the Genbank. Neighbor joining phylogenetic trees will be generated for each DENV serotype using MEGA software (version 6.0) with 1000 boodstrap replications.
Results: Of the 100 blood samples analyzed, 26 were positive for DENV. For the viral typing, 12 samples were sequenced, 10 were positive for DENV 1 and 2 positive for DENV 4. The phylogenetic analyses will report the circulating genotypes of DENV1 and DENV4.
Conclusions: These results explain the epidemic occurred of DENV in Divinópolis/MG, since DENV1 do not circulate in the municipality for almost 10 years, and there was no report of circulation of DENV4 in Divinópolis before 2013. The cocirculation of diferents serotypes and/or genotypes has become common. The evolution of DENV genotypes may lead to phenotypic changes in the virus and their potential to cause outbreak of severe or attenuated disease. Phylogenetic studies may constitute an important tool to monitor the introduction, evolution and spread of viruses as well as to predict the potential epidemiological

consequences of such events. This study will also provide the screening of molecular markers of special interest, associating them with the pathogenicity and immunogenicity of DENV.
Supported by: FAPEMIG
karina_rocha10@hotmail.com

GENETIC STUDY AND REPRODUCTIVE MIGRATION OF CURIMATÁ-PIOA (*PROCHILODUS COSTATUS*) AND CURIMATÁ-PACU (*PROCHILODUS ARGENTEUS*) IN THE REGION OF INFLUENCE OF TRÊS MARIAS DAM, MINAS GERAIS, BRAZIL

Motta-Quintão,G., Ribeiro, T.C., Kalapothakis, E.

Migratory fishes of genre *Prochilodus* (Prochilodontidae) are iliophogous and have an important role in the cycle of nutrients of ecosystem. Migration connects reproduction the site to the feeding site. This connection is important for migratory fish life cycle. Dams break this connectivity, and it often can compromise the maintenance of migratory fish in such area. Frequently, fish passages are insufficient to avoid high rate of mortality of migratory species. However, the identification and conservation of spawning sites may be the most effective way to preserve migratory fish populations. Two Prochilodus species occur at the São Francisco river: *P. argenteus* (curimatá-pacu) and *P. costatus* (curimatá-pioa). Both species are commercially important at this basin and its stock has been seriously affected by dam's construction along the São Francisco river basin. Site spawning identification is essential to establish Permanent Preservation Area (PPA). In the present study, we collected ichthyoplankton between November and January, in River Paraná, tributary of São Francisco. Samples were fixed in 70% alcohol. Screening of eggs and larvae were performed using sieves of different meshes, followed by a manual search using stereomicroscope. Eggs and larvae were placed individually in 96-well plate for DNA extraction. For DNA extraction we used Lifton buffer and Proteinase K. Purification was performed using a GTS extraction kit pht (Phoneutria). To test the presence of *Prochilodus* among collected samples in River Paraná, specific primers for genus were used in polimerase chain reaction (PCR) tests. The methodology proved to be efficient for screening of several samples shortly and effective for molecular identification of larvae of Prochilodus. Yet, 31% of analyzed larvae of one tributary corresponds the genus. This result places Paraná River as a candidate to become a PPA in environment conservation programs.

IDENTIFICATION OF POLYMORPHIC MARKERS FOR PHYLOGEOGRAPHIC STUDIES IN *BYRSONIMA COCCOLOBIFOLIA* (MALPIGHIACEAE) AND *SALVERTIA CONVALLARIODORA* (VOCHYSIACEAE)

AP Souto1, LC Resende-Moreira1, APA Menezes1, PN Vasconcelos1, JP Lemos-Filho2, MB Lovato1

1Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Departamento de Biologia Geral, Laboratório de Genética de Populações, Belo Horizonte, MG, Brasil;

2Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Departamento de Botânica, Laboratório de Fisiologia Vegetal, Belo Horizonte, MG, Brasil.

The Brazilian Cerrado, the largest tropical savanna of the world, is an important area of endemism of several groups of organisms and one of the most biodiverse biomes. Nevertheless, it was identified as one of the most threatened ecosystems of the world. For these reasons it is considered a hotspot for conservation priorities. The core area of the biome occurs in the central area of Brazil but disjunctive sites also occur in North and Northeast regions of the country, and in Southern states such as São Paulo and Paraná. Its current distribution and the great floristic variation observed across its range might have been influenced by past climatic fluctuations. Phylogeographical studies are an efficient tool for investigating the effects of past climate change on the genetic structure and diversity of plant and animal species, and the comparative perspective might contribute to elucidate the evolutionary history of the biomes on which these species occur. However, these studies are still scarce especially with tree species from the Cerrado biome. Therefore, we aimed at identifying polymorphic chloroplast DNA (cpDNA) markers to perform a comparative phylogeographic study with tree species from Cerrado. The species *Byrsonima coccolobifolia* and *Salvertia convallariodora* were selected as model organisms because of their wide range distribution in the biome (including disjunctive sites) and because they display distinct pollination and seed dispersal syndromes. First, in order to obtain high quality DNA, five different DNA extraction protocols were tested. Then, individuals from different Brazilian states (Goiás, Mato Grosso, Minas Gerais, Pará, Roraima and São Paulo) were used to identify cpDNA markers with informative polymorphism. The extraction DNA protocol described by Novaes *et al.* (2009) yielded high quality DNA for both species. After testing twenty universal cpDNA primers previously reported in the literature, two polymorphic regions were selected for each species: *trnS-trnG* and *trnK-trnH* for *B. coccolobifolia,* and *trnK1-trnK2* and *ndhJ-tabE* for *S. convallariodora*. The polymorphic cpDNA regions selected in this study will be useful in the comparative phylogeographic study of both species and are currently being used to compare the Cerrado core area and its disjunct sites with over twenty populations of each species already sampled.

Supported by: CNPq

HETEROGENEOUS GEOGRAPHIC DISTRIBUTION OF β^S-GLOBIN GENE CLUSTER HAPLOTYPES IN SICKLE CELL DISEASE PATIENTS FROM MINAS GERAIS, BRAZIL

PS Rodrigues1, VR Souza1, ML Martins1, MCF Silva-Malta21. Serviço de Pesquisa – Fundação Hemominas

2. Núcleo de Inovação Tecnológica – Fundação Hemominas.

Introduction: During the Colonial period, Brazil received slaves from different regions of Africa, which generated differences in the geographic distribution of sickle cell disease (SCD) and βS-globin haplotypes (CAR: Bantu or Central Africa Region; Ben: Benin; Sen: Senegal; Cam: Camarões; Asi: Asiático). The βS-globin haplotypes are important markers in the study of population compositions of SCD patients, since they allow the inference of the geographical origin of the β globin gene. In this study we evaluated the frequency of βS-globin haplotypes in patients with SCD in different regions of Minas Gerais, the second most populous state of Brazil.

Methodology: βS-globin gene haplotypes were studied in 125 non-related SCD patients by PCR-RFLP techniques to analyze six polymorphic sites from the beta cluster.

Results: We find that CAR haplotype is the more frequent (59.6%) in Minas Gerais, followed by Ben (33.6%) and Sen (0.4%). Sixteen chromosomes analyzed (6.4%) have been found with atypical pattern. Of all the patients studied, 37.6% were identified with genotype CAR/CAR, 32.8% CAR/Ben, 16% Ben/Ben, 0.8% CAR/Sen and 14.4% CAR, Ben or Cam heterozygous with an atypical haplotype.

Conclusion: Regions of Minas Gerais with distinct haplotype frequencies were identified. Benin haplotype was predominant in regions North West and North East while in other geographical regions (Central, West, South and North) the CAR haplotype was the most frequent. The high prevalence of haplotypes CAR and Benin in Minas Gerais is in accordance with historical records that describe the flow of slaves from the South Central, East and Center West regions of the African continent to Bahia and Rio de Janeiro, from where they migrated to different Brazilian regions. Our results emphasize the genetic heterogeneity of the population of Minas Gerais. This study was approved by the ethic committee of the Fundação Hemominas (OF 14/2013).

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V Encontro de Genética de Minas Gerais

V ENGEN IG

Pesquisa e Pós-Graduação

Genética Funcional

THE ROLE OF CLASS III PHOSPHATIDYLINOSITOL 3-KINASE (PIK) TCVPS34 IN *TRYPANOSOMA CRUZI* INVASION

Soares-Silva, M.1; Ferreira, E.R.2; Oliveira, P.2; Cortez, D.R.2; DA Silveira, J.F.2; Bartholomeu, D.C.3; Mortara, R.A.2; Bahia, D.1,2.
1. UFMG, Departamento de Biologia Geral, Programa de Pós-Graduação em Genética, Belo Horizonte - MG - Brasil;
2. UNIFESP, Escola Paulista de Medicina, Departamento de Microbiologia, Imunologia e Parasitologia, São Paulo - SP - Brasil;
3. UFMG, Departamento de Parasitologia, Belo Horizonte - MG - Brasil.
Keywords: Phosphatidylinositol kinases; invasion, signaling and TcVps34.

Abstract
Trypanosoma cruzi, the etiological agent of Chagas disease, has a complex life cycle and responds to a variety of environmental changes, such as pH changes and nutrient availability. Many different proteins, including protein kinases, are involved in the regulation of response of *T. cruzi* to environmental changes. Among the protein kinases, phosphatidylinositol kinases (PIK) phosphorylate phospholipids regulating important cellular functions such as metabolism, cell cycle and survival. PIK is at the heart of one of the major pathways of intracellular signal transduction. Through the development and use of specific inhibitors, PIK and its lipid products have been demonstrated to play a pivotal role in a diverse array of essential cellular processes. Wortmannin strongly binds *in vitro* to all subtypes of PIK being therefore a potent antiproliferative agent. Recent studies highlighting a role for host cell PIKs in the *T. cruzi* invasion process have revealed surprising new insights into the mechanism of host cell invasion by this pathogen. Moreover, the treatment of trypomastigotes with class I PIK inhibitors prior to infection reduced parasite entry, indicating that PIK activation in parasites, as in host cells induced by *T. cruzi*, is an early invasion signal required for successful trypomastigote internalization. Vps34, a class III PIK, has been shown to regulate autophagy, trimeric G-protein signaling, and the mTOR (mammalian Target of Rapamycin) nutrient-sensing pathway. In *T. cruzi*, TcVps34 has been related to morphological and functional changes associated to vesicular trafficking. TcVps34 overexpression affects enzymatic activities related to acidification of intracellular compartments whereas its inhibition interferes with the parasite recovery after severe hypo-osmotic stress. With the aim of to characterize the TcVps34 role in the invasion of HeLa cells by *T. cruzi*, invasion assays were performed with metacyclic trypomastigotes (MTs) and extracellular amastigotes (EAs) overexpressing TcVps34 and with EAs treated or not with TcVps34 inhibitor 3-methyladenine. Invasion rates of parasites overexpressing TcVps34 and pretreated with the Vps34 inhibitor were compared to wild type or non-treated parasites. EAs overexpressing TcVps34 showed significant increase in the invasion rate while parasites treated with TcVps34 inhibitor exhibited a lower invasion rate. By contrast, MTs overexpressing TcVps34 showed a lower invasion rate compared to control, suggesting a down regulation by TcVps34 in this developmental form. Taken together, these results suggest that TcVps34 plays key role in *T. cruzi invasion*. Further studies are needed to determine the mechanisms by which TcVps34 acts in the invasion of these different developmental forms.
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FUNCTIONAL CHARACTERIZATION OF AN ASPERGILLUS FUMIGATUS GTP BINDING PROTEIN RAS2 HOMOLOGUE - RHEBA.

Fernandes, MF[1]; Ferreira, PHS[1]; Soriani, FM[1].
[1]Biologia Geral, Instituto de Ciências Biológicas; Universidade Federal de Minas Gerais, Belo Horizonte, Brasil.
marina-faria@hotmail.com

Keywords: Aspergillus fumigatus, TOR Kinase, functional characterization

Aspergillus fumigatus (Afu) is a ubiquitous saprophytic filamentous fungus that recycles carbon and nitrogen from organic matter in soil. During last decades, this ascomycete became one of the major human pathogen. In lower eukaryotes, cell signaling is important to maintain physiological processes and TOR kinase mediated signaling is central for cell cyclekinetics, vegetative growth, and cellular proliferation. In this sense, the aim of this work is the functional characterization of TOR kinase pathway in A. fumigatus. In order to verify the role of this pathway during cell development, we analyzed the expression levels of aktA, torA and rhebA in vegetative and reproductive growth of A. fumigatus. Results show that there is a higher expression of these genes after 24 hours at 37°C, during vegetative and reproductive growth. In this way, we selected the rhebA gene as a starting point for the characterization of TOR pathway. rhebA gene (AFUA_5G11230) is located at the chromosome 5 and its 942 base pairs ORF (4 exons and 3 introns) codes for a small GTPase essential for TOR kinase activation. We used homologous recombination for deletion of the entire ORF and the A. niger pyrG gene for selection. The ΔrhebA mutant strain demonstrated a marked growth deficiency in different conditions such as temperature and nutrient availability. Microscopically, ΔrhebA show a marked decrease in conidia production demonstrated by sparse conidiophores on the surface of the fungal mass, which was quantified by counting as 2.99±0.35 x104 in ΔrhebA compared to 2.24±0.11 x107 conidia/mL/mm in WT strain. Moreover, ΔrhebA mutant was subjected to various challenges such as ions (50 mM CaCl2, 10 mM MgCl2, 2 mM FeSO4, 25 mM LiAc), drugs that cause DNA damage (50 µM camptothecin and 0,005% MMS) and free radical donor (0,25M menadione). Curiously, the mutant growth was maintained, even in the presence of drugs, demonstrating that the severe phenotype observed by the absence of RhebA GTP binding activity is upstream of all the stress adaptation mechanisms that take place during challenges. As TOR signaling is involved in the sensing of nitrogen nutrients, we analyzed the phenotype of the mutant ΔrhebA in the presence of different nitrogen sources. Again, there was no difference in growth rates of the mutant strain compared to WT in the presence 1,41 mol NaNO3, 200 mM histidine, 200 mM lysine, 150 mM leucine, showing that even though it has been described that TOR kinase senses nitrogen nutrients, the lack of activated GTP bound RhebA in crucial for cellular development. Our evidences show that rhebA gene of A. fumigatus takes place on the control of vegetative and reproductive growth, which supports its already described function, in mammalian cells, in which TOR is responsible for control of protein synthesis.

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STUDY OF CYTOTOXIC AND GENOTOXIC EFFECTS OF ROUNDUP ON STEM CELLS DERIVED FROM HUMAN ADIPOSE TISSUE

MIA Melo[1], E Novato-Silva[2], AM Góes[3]
1 ICB- UFMG
2 ICB- UFMG
3 ICB- UFMG

INTRODUCTION Glyphosate is the most commercialized herbicide in the world, known commercially as Roundup®, and many studies was conducted to investigate the potential damage caused by it. To carry out this work we chose stem cells derived from human adipose tissue (hASC). These cells are able to differentiate into chondrogenic, adipogenic and osteogenic lineages, which enables an investigation of the toxic effects of Roundup® in undifferentiated and differentiated state of these cells. Furthermore, *in vitro* studies with human embryonic umbilical and placental cells have shown that the formulations of glyphosate are capable of generating damage to the genetic material, which reinforce the need to test this compound in other types of human cells. The main goal of this work is to investigate the citotoxicity and genotoxicity of Roundup® on hASC in short and long term exposure.

METHODOLOGY Stem cells were evaluated for the presence of surface markers to defining multipotent mesenchymal stromal cells. Immunophenotyping was performed using the technique of flow cytometry. Viability assays were performed periodically using the MTT method, in order to assess the toxicity of glyphosate throughout the days of culture**.** The alkaline phosphatase activity was evaluated by BCIP-NBT assay. This assay is used to detect activity of bone cells, which are derived from hASC in the differentiation process**.** Another test was conducted to detect the difference necrosis from apoptosis in cells exposed to Roundup® for short and long time. For this assay, we used flow cytometry with Annexin V-FITC kit. hASC were grown in medium supplemented with differentiation factors for osteogenic and adipogenic lineages. The ability of differentiation of hASC exposed to glyphosate formula was also verified by observation of morphological changes and the expression of genes related to the differentiation.

RESULTS AND CONCLUSION We observed that cells exposed to Roundup® die through apoptosis mechanism and also undergo morphological changes when subjected to a mixture of Roundup® within adipogenic and osteogenic differentiation medium. Moreover, these cells exhibit a shift of MTT metabolism and production of alkaline phosphatase when exposed to herbicide for long term. Gene expression is also affected, with inhibition of some important that are expressed when differentiation occurs. In conclusion, Roundup® is cytotoxic and genotoxic for adult stem cells derived from adipose tissue.

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CYANIDE EFFECTS ON CELL CYCLE OF *LACTUCA SATIVA* L.

AS Freitas1, LF Andrade-Vieira1, VH Techio1
1Federal University of Lavras

Background/Introduction: Cyanide is a common soil contaminant, being usually found in association with manufactured gas and precious metal mines. It is known to be toxic to all organisms by inhibited the cell respiration. Short-term exposure to the cyanide compounds can cause tremors, breathing difficulties and numerous neurological symptoms for humans. Risks increase with prolonged exposure, and weight loss, nerve-cell damage, thyroid disorders and death may occur. However, there are few reports about the effects of cyanide in plant species. A study about the effects of cyanide solutions on cell cycle of *Lactuca sativa* L. (2n=2x=18) demonstrated the presence of C-metaphases evidencing the aneugenic action. Thus, this study aimed to understand the mechanism of action of cyanide by evaluating the distribution of phosphorylation of Histone H3 at serine 10 (H3S10ph) on cell cycle of the plant model *L. sativa.*

Methodology: Seeds of *L.sativa* with roots length of about 1 a 2mm were exposed with test solution for 48h. The cyanide solution of 0.0031mg/L was obtained by diluting of cyanide in a solution of CaCl2 (0.01M). A solution of CaCl2 (0.01 M) was used as negative control. Roots were fixed in 4% paraformaldehyde solution and washed in PBS buffer. Slides were prepared with squash technique, blocked in 3% BSA, incubated with primary antibody Rabbit Polyclonal IgG against H3S10ph, which was detected by a secondary antibody Goat anti-rabbit IgG-FITC. The alterations on cell cycle due to the cyanide treatment were compared with negative control cells.

Results: It was observed that the cyanide induces alterations in all phases of the cell cycle as demonstrated in this study by distribution and number of abnormal signals of the H3S10ph. The H3S10ph is a (peri) centromeric marker and we observed a number less than 18 marks (peri) centromeric in prophases and C-metaphases, demonstrating that probably occurs inactivation of some centromeres in cells treated with cyanide. The sticky metaphases presented weak signals of phosphorylation of the H3, outnumbered and scattered throughout the cell, rather than aligned to the equatorial plate of the cell as in a normal metaphase. Laggard chromosomes, not oriented in the mitotic spindle and without the immunosinal were also observed in cells exposed to cyanide. Polyploid cells with number above 18 marks (peri) centromerics also were observed in these cells. In anaphase, chromosome fragments without signs of phosphorylation were recorded. In addition, in some telophases the signal of H3S10ph was stronger than the expected, evidencing that the centromere remained active even after chromatid segregation. Moreover, cells with micronuclei without signs of phosphorylation were noticed, indicating that acentric chromosome fragments or chromosomes with inactive centromere are being eliminated.

Conclusions: The present research contributes to the understanding of the mechanisms of action of cyanide on plant cell cycle. It was demonstrated that the some alterations could be related to the inactivation of the centromeres. Future studies with other cell signalizers are being developed. Then, the wide action of cyanide on cell could be accessed.

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METHYLATION PATTERN OF *SNRPN* AND ITS POSSIBLE ASSOCIATION WITH OBESITY

[1]Carobin, N.V., [1]Rubatino F.V.M., [1]Oliveira V.T,. [1]Rocha, P.P.O., [1]Pietra, R., [2]Reis, G.M.F., [2]Petruceli, E.J.P., [2]Almeida, M.A.P., [2]Vieira, C.M.A.F., [1]Bosco, A.A., [1]Jehee, F.S.,
[1]Instituto de Ensino e Pesquisa, Santa Casa Belo Horizonte, MG
[2] Ambulatório de Obesidade Clínica do Centro de Especialidades Médicas da Santa Casa de Belo Horizonte, MG.

Introduction: Defective genomic imprinting is often associated with syndromes that include abnormal growth and severe obesity as a clinical phenotype. Prader-Willi syndrome occurs as a result of a loss of expression of paternal genes from chromosome 15q11-13. Several maternally silenced, imprinted genes within this region, including *MKRN3, MAGEL2, NDN* and *SNRPN*, were associated with this complex disorder. Numerous studies in human patients and mouse models have led to the suggestion that *SNRPN*, which have an imprinting center, act regulating the pattern of DNA methylation in this region. This suggests that *SNRPN* is a positive regulatory element required for establishment and maintenance of paternal imprinting.
Objective: Study the methylation levels of *SNRPN* in obese and non-obese individuals and verify a possible association with BMI (Body Mass Index).
Methods: We developed a methylation-sensitive high-resolution melting-curve assay (MS-HRM) to measure methylation levels of bisulfite-treated blood DNA samples from obese (n=15 adults and n=10 children) and non-obese (n=15 adults and n=10 children) individuals. Reactions were carried out with MeltDoctor™ HRM reagents on the Applied Biosystems® 7500 Fast Real-Time PCR System and analyzed with the High Resolution Melt (HRM) software. Statistical analyses were performed using IBM SPSS Statistics 201113. The student's *t* test and Levene's were carried out to determine whether methylation levels have correlation with BMI and age.
Results: The methylation analysis for *SNRPN* by MS-HRM, showed that the methylation pattern of obese and non-obese adults individuals was 45.7% and 46.6% respectively. They did not differ statistically (p=0,672). The same was true for obese and non-obese children (p=0,653), with methylation patterns of 39.7% and 39.1% respectively. Interestingly, within the control group there is significant statistical difference in relation to methylation pattern among adults and children. The values are p=0,002 to difference between 46.6% and 39.1%. When comparing obese adults and obese children the same is true. We notice that adults have a statistically significantly higher methylation level than children 45.7% and 39.7% respectively (p=0,004).
Conclusion: The methylation pattern of *SNRPN* does not show positive association with obesity between the groups. On the other hand, the methylation pattern of *SNRPN* seems to be influenced by the age. In other words, the increase of age is positively correlated with the increase of methylation pattern of this gene.
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CELLULAR MAP KINASE PATHWAY IS DIFFERENTLY INDUCED BY G AND CL STRAINS OF *TRYPANOSOMA CRUZI* EXTRACELLULAR AMASTIGOTES (EAS)

Peters, L.[1]; Lima, B. R[2].; Bonfim-Melo, A[2].; Ferreira, E. R.[2]; Soares-Silva. M. [1]; Florentino, P. T. V.[2]; Bartholomeu, D. C[3].; Mortara, R. A.[2]; Bahia, D[1,2].

1Departamento de Biologia Geral, Universidade Federal de Minas Gerais, Brasil.

2Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de São Paulo, Brasil.

3Departamento de Parasitologia, Universidade Federal de Minas Gerais, Brasil.

Abstract

Chagas disease is caused by *Trypanosoma cruzi* and this disease is endemic in at least 21 countries. It is estimated that 8 million people in Latin America are infected with this parasite and based on this fact it is crucial to understand the parasite biology and its interaction with the host cells. *T. cruzi* has a complex life cycle which includes two intermediate hosts (triatomine insects and mammals) and four distinct developmental stages (epimastigotes, metacyclic trypomastigotes, amastigotes and bloodstream trypomastigotes). Extracellular amastigotes (EAs) are alternative infective forms of *T. cruzi* and together with bloodstream trypomastigotes, sustain the parasite cycle in mammalian hosts. Differences in the infectivity of EAs from two strains, G (derived from sylvatic type I) and CL (derived from the vector *Triatoma infestans*, type VI) have been widely acknowledged: G strain EAs are much more infective *in vitro* than CL parasites. Mitogen-Activated Protein kinases (MAPKs) are involved in cellular responses to a diverse stimuli such as osmotic stress, heat shock, mitogens and proinflammatory cytokines. MAPKs pathways are activated in mammalian cells by a variety of extracellular stimuli and mediate signal transduction from the cell surface to the nucleus. Activated MAPKs phosphorylate transcription factors and other targets to regulate gene transcription and immune responses. Recent studies have provided new insight into the upstream and downstream components of the MAPK pathway that facilitate the activation and propagation of MAPK signaling in immune responses. Moreover, MAPK activity is negatively regulated by MAPK phosphatases (MKPs), a group of dual-specificity phosphatases that dephosphorylate and inactivate the MAPKs. There are four MAPKs cascades: the extracellular signal-regulated kinases (ERK1/2), c-Jun NH2-terminal kinases (JNK-1/2/3), p38 (p38α/β/γ/δ) and ERK5. With the aim to study whether MAPK pathway of the host cells is activated by the interaction with *T. cruzi* and the differences in the phosphorylation profile between G and CL EAs, we have analyzed host proteins phosphorylation in the extracts of HeLa cells previously incubated with EAs from both strains, using a phospho-kinase array kit®, and Western blots assays. G strain induced a bimodal phosphorylation of MAPKs (p38, ERK) (increased activation followed by rapid decline and increased again, ending in a further decline of activation). The phosphorylation of protein phosphatase MKP1 was also observed, at the opposite points of greater ERK activation. CL parasites, however, did not induce MAPK bimodal activation. G strain also activated JNK1, MKK3 and MKK6 (p38 activators) and MSK2 (activated by p38 and ERK1/2) whereas CL not. Taken together these results could raise two hypotheses: first, G and CL strains induce different MAPK signaling responses in Hela cells; second, G strain EAs -but not CL- seem to induce the MAPK-based danger response pathway which ultimately might lead to immune activation and secretion of proinflammatory cytokines. Further studies are needed to verify these hypotheses.

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DISCOVERY OF METLLOPROTEASES FROM VENOM GLAND OF THE SCORPION TITYUS SERRULATUS BY CDNA LIBRARY.

Carmo, A.O.; Oliveira-Mendes B.B.R.; Horta, C.C.R.; Magalhães, B.F.; Dantas A.E.; Chaves, L.M; Chávez-Olórtegui, C.; Kalapothakis, E.

Tityus serrulatus is a Brazilian scorpion species with great medical significance. While the effects of neurotoxins have been extensively studied, little is known about the proteases expressed in the venom gland of this arthropod. In this study, clones from a *T. serrulatus* (Ts) venom gland cDNA library were selected according to homology to proteases. The sequences were aligned in the database and classified by homology. Similarity and identity analyses of the sequences were carried out, and a phylogenetic tree was constructed with the sequences of other proteases. These cDNA sequences correspond to ten different metalloproteases, named metalloserrulases (TsMS). TsMS 1–9 belong to the metzincin family, which has three domains: signal peptide, propeptide, and metalloprotease domain; while TsMS 10 belongs to the gluzincin family. The proteolytic activity of the venom was inferred from the cleavage of fibrinogen, and the residues recognized by the proteases were determined by cleavage of a tripeptide library using a fluorescence resonance energy transfer assay. The Ts venom showed proteolytic activity on fibrinogen and preferential cleavage close to the basic residues K and R. Its activity could be inhibited by EDTA, indicating that the venom from this scorpion predominantly consists of metalloproteases.

Keywords: *Tityus serrulatus*, metalloprotease, metalloserrulase, venom, scorpion, envenomation.

EVALUATION OF CYTOTOXIC AND GENOTOXIC EFFECTS OF TEA FROM *MENTHA PULEGIUM* ON *ALLIUM CEPA* BIOASSAY

RCA Rosa[1], SS Silva[1], LR Ribeiro[1]
[1] Centro Universitário de Formiga, UNIFOR – MG, Formiga, MG.

Introduction: *Mentha pulegium* L., known as "poejo", is a medicinal plant widely used in alternative treatments of a variety of human diseases. However, the tea from this species lacks studies that elucidate about its security of consumption. In this context, it is noteworthy that plant bioassays, mainly *Allium cepa*, are globally recognized in the study of effects of plant extracts, aiming at detecting of genotoxicity. Thus, this study aimed to evaluate the cytotoxic and genotoxic effects of *M. pulegium* tea on the cell cycle and chromosomal structure of *A. cepa*.

Methodology: The experimental design was completely randomized with three replicates of fifty seeds for each treatment. The commercial tea decoction was prepared following the manufacturer's recommendations, producing concentrations of 2; 10 and 20 g/L. Distilled water was used as negative control. For cytogenetic tests, seeds of *A. cepa* were submitted to each treatment for 72 hours. The roots obtained were fixed in Carnoy (3:1) and stored at -4°C for subsequent cytogenetic analysis. The slides were prepared by the squash method and stained with aceto-orcein (2%). For each treatment, five slides were analyzed with 1000 cells each, totaling 5000 cells per treatment. The mitotic index was determined and the presence of chromosomal abnormalities was evaluated.

Results: There was no statistically significant difference observed for the mitotic index or to the presence of chromosomal abnormalities between treatments and control group ($p<0.05$ - Tukey test).

Conclusion: Due to the reliability of the *A. cepa* test, it was concluded that the tea from *M. pulegium*, in the concentrations evaluated, has no cytotoxic or genotoxic effects.

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V Encontro de Genética de Minas Gerais

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Pesquisa e Pós-Graduação

Genética Humana

COMPLEX CHROMOSOMAL REARRANGEMENTS IN PATIENTS WITH INTELLECTUAL DISABILITY AND CONGENITAL MALFORMATIONS AND/OR DYSMORPHISMS

Salazar GC1, Chami AM2, Viana MM2, Martins AAS1, Leão LL2, Aguiar MJB2, Carvalho MRS1
1Departamento de Biologia Geral, ICB, UFMG;
2Serviço Especial de Genética Médica do Hospital das Clínicas, UFMG.

Background: Complex chromosomal rearrangements (CCRs) are structural rearrangements involving at least three breakpoints on two or more chromosomes. These rearrangements can be *de novo* or familial. Although presumably rare in humans, CCRs can produce intellectual disability and congenital malformations (ID+CM). Multiplex ligation probe amplification (MLPA) is a very promising technique for screening patients with ID. However, MLPA kits were developed to focus on recurrent rearrangements and to detect a small number of CCRs. Therefore, additional genome-wide methods are required, such as array comparative genomic hybridization (aCGH). The purpose of this study is to ascertain the contribution of recurrent microdeletions/microduplications and CCRs to the ID+CM phenotype.
Methodology: We used a sample of 92 individuals of both sexes presenting with ID+CM and normal molecular analysis of Fragile X Syndrome. Karyotypes were obtained for all patients. Recurrent interstitial and subtelomeric microdeletions/microduplications were screened using the SALSA MLPA P245-B1 MICRODELETION SYNDROMES and SALSA MLPA P070-B2 HUMAN TELOMERE kits, respectively. Putative CCRs were further investigated using the Human Genome CGH Microarray 180K platform (Agilent Technologies, Santa Clara, CA, USA). Selection criteria for aCGH were abnormalities on conventional karyotype (marker chromosome and autossomal translocation) deletions in the MLPA (including a patient with a syndrome typically caused by microduplications) and clinical suspicion of genetic imbalance.
Results: MLPA was used in 90/92 patients and 18 of them (20%) presented microdeletions/microduplications. Interstitial microdeletions observed were: del2q33.1 (1 patient), del5q35.3 (1), del7q11.23 (2), del11p13 (1) and del22q11.21 (2). Additionally, two double microdeletions were identified: del1p36.33 and del22q11.2 (1); del17p13.3 and del22q11.21 (1). The subtelomeric microdeletions found were del1q44 (1); del4q35 (1); del9q34.3 (2); del11p15.5 (1); del15q11.2-q1 (1); PAR2, X/Yq (1). The microduplications found were dup8p23.3 (1) and PAR1, X/Yp (1). aCGH allowed the detection/refinement of rearrangements in 5/8 patients tested. The patient with a t(17;22)(p13.3;q11.21) presented deletions of 4.2Mb at 22q11.1 and 2.3Mb at 17p13.3. The patient with the del5q35.3 presented a 3.9Mb deletion at 5q35.3 and, in addition, a 559kb duplication at 11p15.5. The size of the del2q33.2 was refined to 8.4Mb. The del11p15.5 revealed a 570kb terminal deletion at 11p15.5; a 20.7Mb, apparently contiguous, interstitial duplication at 11p15.1; and, a 3.4Mb terminal duplication at 18q23. The patient with a marker chromosome presented a 13Mb interstitial deletion at 1p21.2. Three patients had normal results at aCGH. Therefore, CCRs were present in at least 4/92 patients (4.3%). The frequency of microdeletions/microduplications detected by MLPA is similar to results previously described in the literature. The frequency of CCRs among patients having these selection criteria has occasionally been published, but the present results suggest that they may be more frequent than previously suspected.
Conclusions: Approximately 20% of the patients with DI+CM present recurrent microdeletions/microduplications detectable by MLPA. CCRs, with a frequancy of at least 4.3%, are probably not as rare as suspected before the introduction of aCGH. **Supported by:** CAPES, FAPEMIG, CNPq, PPSUS/FAPEMIG, PRPq/UFMG, CNPq 307975/2010-0

MOLECULAR DIAGNOSIS AND RECLASSIFICATION OF VON WILLEBRAND PATIENTS

FREITAS, Samuel da Silva1; HALABI, Natan Namen1; NAMEN-LOPES, Maria Sueli1; VELLOSO-RODRIGUES, Cibele2; CHAVES, Daniel Gonçalves1
1. Serviço de Pesquisa - Fundação HEMOMINAS
2. Universidade Federal de Juiz de Fora - Campus Governador Valadares

INTRODUCTION: von Willebrand disease (VWD) is a congenital hereditary coagulopathy caused by qualitative or quantitative deficiency of von Willebrand factor (vWF), presenting a high degree of clinical and allelic heterogeneity. There are three types of the disease (type 1, 2 and 3) and four subgroups for type 2 (2A, 2B, 2M and 2N). The VWD is the most common coagulopathy in human populations and its prevalence varies from 0.8 to 2.0%. The diagnosis of VWD requires a series of high cost laboratory procedures that are often unavailable in the Brazilian treatment centers. Difficulties of diagnosis of VWD are an important problem because the diversity of patients´ phenotype, inaccuracy of some laboratory tests, and the limit between normal and abnormal phenotype of the coagulation process. There is a big challenge for hematologists to distinguish the correct diagnosis and to treat the suspicious cases of VWD. This leads to a relative neglecting of VWD diagnosis in many centers of hematology and transfusion medicine. To minimize the difficulty of performing tests to properly diagnose VWD and its subtypes, this study aimed to standardize reactions for DNA sequencing to verify specific mutations in five exons of the vWF gene. **METHODOLOGY:** Nine pairs of primers were designed to amplify and sequence exons 17, 18, 19 and 20 of the vWF gene. The sequencing reactions were performed on the 3130 DNA Analyzer from Applied Biosystems equipment. The sequences were compared to the sequence available in the public database (NCBI - National Center for Biotechnology Information) using the software BioEdit Sequence Alignment Editor version 7.2.5. **RESULTS:** Patients with unclassified VWD (n = 168) were selected in the ambulatory of the Fundação Hemominas in Belo Horizonte. Genomic DNA from all samples was extracted using commercial kits. The sequencing of exon 17 of the vWF gene was standardized and the analysis of transition mutations rs2228317 (type 1 and subtype 2M), rs61748466 (subtype 2N) and rs61748467 (subtype not identified) and rs61748465 deletion (type 3) were developed. PCR was performed for 52 samples for amplification of exon 17 of the vWF gene and 16 samples were sequenced and analyzed with the aid of. Until now three strains were identified as heterozygous for the mutation transition rs2228317. **CONCLUSIONS AND PERSPECTIVES:** The implementation of the analysis of exon 17 and the standardization of sequencing of all proposed exons will allow the molecular diagnostics of von Willebrand disease and the specific classification of diagnosed patients. This study was approved by the ethics committee of the Fundação Hemominas (CEP306). **FINANCIAL SUPPORT:** CNPq, FAPEMIG and Fundação Hemominas.

ETIOLOGIC INVESTIGATION OF INTELLECTUAL DISABILITY, CONGENITAL MALFORMATION AND/OR SEIZURES BY CYTOGENOMIC TECHNICS

GS Vianna1, PPO Rocha1, M Gonçalves1, VT Oliveira1, ML Freitas1, RX Pietra1, R Sturzeneker2, CMB Carvalho2, PFV Medeiros3, TO Silva3, BSV Ribeiro4, JG Giannetti4, FS Jehee1.

1 Instituto de Ensino e Pesquisa da Santa Casa de Belo Horizonte/MG.
2 Centro de Pesquisas René Rachou.
3 Universidade Federal de Campina Grande.
4 Laboratório Ambulatorial do Hospital das Clínicas da UFMG.

Introduction and Objectives: Intellectual disability, congenital malformation and/or seizures, in many patients can be attributed to chromosomal alterations. This study aimed to search for submicroscopic chromosomal alterations by MLPA and Microarray genomic technics. **Methods:** One hundred twenty two patients of both sexes were assessed with ages varying from a couple of days to adult age. All patients were first clinically evaluated either by a clinical geneticist, neuropediatrician or neonatal doctor. Blood samples were collected from the proband and his/her parents. DNA was extracted and investigation was carried out using two MLPA kits for subtelomeric regions (P036 and P070) and one MLPA kit for microdeletions and microduplications syndromes (P064). For the 20 patients with seizures the MLPA kit P343 was used, focusing a couple of the most commonly altered regions in patients with epilepsy. Microarray genomic test was performed using Affymetrix 750k SNP array for 15 selected patients who had no alterations in the MLPA tests. Alterations of at least 30 Kb and 5 consecutives altered probes were considered in order to investigated the pathogenicity in databases of benign and pathogenic alterations (ISCA, DGV and DECIPHER). Ethical Committee approval - CAAE 01969512.9.0000.5138. **Results:** MLPA identified alterations in 19 patients (15,6%): four Williams syndrome, three Prader Willi Syndrome, one Velocardiofacial syndrome, one Smith Magenis syndrome, one duplication 17q, one duplication 18p and 18q, two deletions 4p (Wolf Hirschhorn syndrome) associated to duplication in 8p and 8q, one deletion 5p (Cri-du-Chat syndrome), one deletion 3p associated to a duplication 20p, one deletion 13q and two duplications 22q13.3 and one deletion in LAT gene (16p11). In 15 patients studied by microarray 17 rearrangements were identified as possibly pathogenic: five duplicated segments on chromosomes 7, 11, 17, 22 and X and twelve deleted segments on chromosomes 2, 7, 8, 10, 17, 22 and X. These CNVs will need further confirmation by other technics. **Conclusion:** Our preliminary results confirm that submicroscopic chromosomal alterations are an important cause of intellectual disability, congenital malformation and/or seizures and cytogenomics technics are essential diagnostic tools to elucidate the etiology of this group of patients.

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ATYPICAL PRADER-WILLI SYNDROME IS NOT DUE TO DYSREGULATION OF METHYLATION PATTERNS IN THE 15Q11.2-Q13 REGION IN TWIN GIRLS.

Valdirene Teodoro de Oliveira1, Rafaella Xavier Pietra1, Fernando Víctor Martins Rubatino1, Natália Virtude Carobin1, Patrícia Pereira Oliveira Rocha1, Beatriz Silva Vilela Ribeiro2, Juliana Gurgel Giannetti2 e Fernanda Sarquis Jehee1.
1.Instituto de Ensino e Pesquisa Santa Casa de Belo Horizonte, MG, Brasil
2. Faculdade de Medicina da Universidade Federal de Minas Gerais, MG, Brasil

Introduction: Prader-Willi syndrome (PWS) is characterized by mild to moderate mental retardation, hyperphagia and obesity and is caused by the loss of expression of paternally inherited alleles in the 15q11.2-q13 region. Angelman syndrome (AS) is characterized by impaired motor development, severe speech delay and seizures resulting from the lack of the maternal *UBE3A* gene also mapped in15q11.2-q13. For this study we identified 8 month-old monozygotic twins, presenting severe neurodevelopmental delay and seizures of difficult control, which resembled AS. **Aim:** Study of methylation patterns of genes in15q11.2-q13 region. **Methods and Results:** Genetic testing performed by microsatellite markers, methylation-sensitive PCR for *SNRPN* and FISH revealed that both girls had paternal deletions of the 15q11.2 - q13 region, a result consistent with PWS. At 11 years old, both probands still presented with severe neurodevelopmental retardation, seizures and had not developed hyperphagia and obesity. Array-CGH determined that their deletion size was 4.9 Mb and mapped between break points 2 and 3 (type 2), one of the most common among PWS patients. Direct sequencing of the *UBE3A* gene did not identify pathogenic mutations. We then tested methylation patterns of other genes in the region by designing primes for methylation -sensitive PCR for *ATP10A, NDN* and *MKRN3*. Results were compared with normal controls and positive controls (PWS individuals). In our patients, the methylation pattern for *NDN* and *MKRN3* was paternal and consistent with those of PWS individuals. Normal controls have a biallelic pattern for these two genes. On the other hand *ATP10A*, a maternally expressed gene, showed a biallelic pattern of methylation in all subjects. This evidentiates a somatic methylation mechanism occurring even in the single imprinted non-methylated allele of PWS individuals. Additionally, High Resolution Melting Analysis (HRM) was used to quantify methylation levels of *NDN, SNRPN, MAGEL2* and *MKRN3* in the twins. All results showed a methylation pattern similar to PWS. **Conclusion:** We conclude that the atypical clinical presentation in the twin is not due to changes in methylation patterns of imprinted genes in region 15q11.2-q13. **Financial support:** CAPES, PROSUP, CNPq e FAPEMIG.

SCREENING OF CHROMOSOMAL ABNORMALITIES BY MLPA PROVES TO BE AN IMPORTANT DIAGNOSTIC TEST IN PATIENTS WITH CONGENITAL MALFORMATION AND INTELLECTUAL DISABILITIES

2Gonçalves, M.*, 1Rocha, P. P. O.*, 1Oliveira V. T., 1Freitas M. L., 1Pietra R. X., 3Medeiros, P. F. V., 3Silva, T. O., 4Ribeiro, B.S.V., 4Giannetti J. G.,1Jehee, F. S.
1 Instituto de Ensino e Pesquisa da Santa Casa de Belo Horizonte/MG. 2 Hermes Pardini, MG. 3Universidade Federal de Campina Grande, Paraíba. 4 Ambulatório de Doenças Neurogenéticas do Hospital das Clínicas da UFMG.
*both authors contributed equally for this work

Introduction and Objectives: Intellectual disability associated with multiple congenital malformations (ID/CM) comprise a large and extremely heterogeneous group of diseases with an incidence of 2-3% in live births and cause a great impact on the lives of patients and their families. Understanding the etiology of ID/CM is critical for guidance and genetic counseling of families. This study evaluated the combination of three MLPA kits from MRC-Holland for the diagnosis of chromosomal abnormalities in a group of patients with ID/CM. **Methods:** 122 patients of both sexes and ages ranging from birth to early adulthood were referred by intensive care pediatricians, pediatric neurologists and geneticists. All patients were clinically evaluated and presented with dismorphisms, congenital malformation and/or intellectual impairment. Blood samples were collected from probands and their parents. DNA was extracted and the investigation was carried out using MLPA kits P036, P070 (subtelomeric regions) and P064 (most commom microdeletion and microduplications syndromes). Ethical Committee - CAAE 01969512.9.0000.5138. **Results:** A total of 18 chromosomal abnormalities were found in our cohort of 122 patients (14.8%). When we analyzed our data per referral center we found the following abnormality detection rates: three out of the nine patients referred by pediatric intensivists (33.3%), a trisomy18, one 17qter duplication and one 13qter deletion; 14 out of the 84 patients referred by geneticists (16.7%), four Williams syndrome, two Prader-Willi syndromes, one Smith-Magenis syndrome, one Velocardiofacial syndrome, two 22q13.3 duplications, one 5pter deletion, and three unbalanced translocations (4pter deletion/8qter duplication, 4pter deletion/8pter duplication, 3pter deletion/20q duplication); and only one Prader-Willi syndrome (monozygotic twin girls) out of the 29 patients (3.4%) referred by pediatric neurologists. Follow up with most patients showed that only the trisomy 18 was also diagnosed by conventional karyotype. **Conclusion:** Our results show that the screening of chromosomal abnormalities is an essential tool for the diagnosis of IC/CM. Furthermore, we reinforce the importance of including molecular techniques in this screening, once 17 out of the 18 patients would remain without diagnosis had MLPA not been performed. As expected, detection rates are higher in the group of patients referred by intensive care units and geneticists, due to their more severe clinical features. The low detection rate found in patients referred by pediatric neurologists probably reflects the milder dismorphologies and fewer congenital malformations of these patients. Nonetheless, we believe that as we increase the number of pediatric neurology referral centers, a larger number of abnormalities will be detected. **Financial support:** CAPES, PROSUP, CNPq e FAPEMIG.

ASSOCIATION OF THE POLYMORPHISMS OF CYP2C9, VKORC1, MDR1, APOE AND UGT1A1 GENES WITH THERAPEUTIC WARFARIN DOSE IN PATIENTS WITH THROMBOSIS

VCOAlmeida 1,2,DDRibeiro 3, KBGomes 1,4, ALBGodard1.
1. Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais; Minas Gerais, Brasil.
2. Departamento de Genética Molecular, Hermes Pardini, Minas Gerais, Brasil.
3. Hospital das Clínicas, Universidade Federal de Minas Gerais, Minas Gerais, Brasil.
4. Departamento Análises Clínicas e Toxicológicas, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Minas Gerais, Brasil.

Abstract

Introduction: Warfarin is an oral anticoagulant widely used for prevention and treatment of thromboembolic diseases. The prediction of an effective and safe dose is compromised because of the narrow therapeutic range and large inter-individual variability in dose requirements. There are several pharmacogenetic algorithms to determine the warfarin doses required in individuals, but they only explain 60% of dose variation, suggesting that other genes may also influence the warfarin dose required. This study aimed to evaluate the impact of clinical factors and CYP2C9*2, CYP2C9*3, VKORC1 -1639G>A, MDR1 3435C>T, APOE* ε4, and UGT1A1(TA)n polymorphisms on the warfarin dose required in Brazilian patients taking anticoagulants to prevent thromboembolism (TE).

Methodology: We studied 116 Brazilian patients who received warfarin anticoagulation therapy for thromboembolism (TE), which were genotyped for the aforementioned polymorphisms. Associations between dose variability and age, BMI, gender, use of warfarin antagonists, and genetic polymorphisms were also evaluated.

Results: The warfarin dose decreased with age increasing (r2= –0.049, p=0.017). Individuals older than 60 y required lower doses of warfarin (28.8□14.1 mg), compared with those under 60 y (mean 52.5□34.6 mg) (p=0.009). The use of warfarin antagonists showed a positive correlation with dose (r2=0.049, p=0.003). Therefore, 21% patients required a warfarin dose higher than 70 mg/week, which was associated with a BMI > 25, use of warfarin antagonists, and the presence of the MDR 3435T allele and UGT1A1(TA)7 polymorphism. Individuals with the MDR1 3435TT genotype required a dose 21% (12 mg) higher than that required by individuals with the 3435CT and 3435CC genotypes. The UGT1A1(TA)7 allele was also positively correlated with the warfarin dose. CYP2C9*2, CYP2C9*3, VKORC1 -1639G>A, and APOE *ε4 were associated with lower warfarin doses. Also, 5.2% of our population showed complete resistance to warfarin, i.e., they needed a high dose of warfarin without achieving a stable INR. In addition, 15.5% of our population showed partial resistance to warfarin, and required a high dose to achieve a stable INR. This percentage is much higher than expected, but the use of antagonists observed in 10% of our population could help to explain this finding.

Conclusions: MDR1 3435C>T and UGT1A1(TA)n genotypes are associated with a requirement for a high warfarin dose. To our knowledge, this is the first study to evaluate APOE *ε4 and UGT1A1(TA)n genotypes in the Brazilian population, and the association of these genotypes with warfarin dose required.

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SCREENING OF THE *BRCA2* GENE IN PATIENTS WITH BREAST AND/OR OVARIAN CANCER BY HIGH RESOLUTION MELTING

BRITO, A. M1; SOARES, B. L1; LEMOS, S2; RODRIGUES, A. N2; LOPES, D. O1; SANTOS, L.L1
1Universidade Federal de São João Del Rei
2Associação de Combate ao Câncer do Centro Oeste de Minas Gerais
amandabrito.bqi@gmail.com
Keywords: *BRCA2*, screening, mutations, HRM, HBOC

INTRODUCTION: The breast and ovarian cancers are the second and the seventh most common types of cancer, respectively, in Brazil. In 2014 these cancers will be responsible for 22.9 % of new cancer cases in women. It is estimated that 5-10 % of all cases has a hereditary profile, being responsible for the Hereditary Breast and Ovarian Cancer Syndrome (HBOC). *BRCA1* and *BRCA2* genes are the most frequently associated with HBOC. Methods for genetic screening are important and effective in the early diagnosis of cancer in families suspected to have HBOC syndrome, and the High Resolution Melting (HRM) technique has been sensitive to this purpose. The aim of this study is to characterize the *BRCA2* gene of 20 probands in cancer treatment at the Cancer Hospital of Divinópolis,MG, with suspected HBOC syndrome using the HRM assay. **METHODOLOGY:** Patients were evaluated through clinical criterias and risk calculation of *BRCA* mutations using the HughesRiskApps software. Patients at increased risk for HBOC were informed about the study and invited to participate of the project. All the patients involved in the project signed the consent form and 5 ml of their blood were collected for the molecular analysis of *BRCA2* gene. DNA was extracted using the Qiamp DNA Mini Kit (Qiagen). Forward and reverse primers were designed for the 26 coding exons of the *BRCA2* gene using the Primer3 software. Real-time PCR Reaction followed by the HRM to each exon was performed. Altered profiles in HRM reaction were confirmed and mutations identified by sequencing of the fragment amplified. Exons 10 and 11 were directly sequenced. **RESULTS:** So far 12 mutations were found: 10 nonpathogenic and 2 pathogenic mutations, c.9154C>T and c.2T>G, in accordance with the BIC (*Breast Cancer Information Core*) and LOVD (*Leiden Open Variation Database*) database. Proband's first-degree relatives with positive results in molecular test have been screened for the same mutations. **CONCLUSIONS:** The results have shown that HRM technique has an innovative potential in mutation screening and diagnosis of HBOC, decreasing costs of the test and being sensitive and effective in detecting genetic changes. The identification of individuals with pathogenic mutations in *BRCA2* gene will allow better monitoring of these families, been possible to reduce risk and to prevent the development of cancer of these individuals.

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MODULATION OF EXPRESSIVITY IN *PDGFRB*-RELATED INFANTILE MYOFIBROMATOSIS: A ROLE FOR *PTPRG*?

ND Linhares1, MCM Freire2, RGCCL Cardenas1, M Bahia3, E Puzenat4, F Aubin4 and SDJ Pena1,2,5

1 Laboratório de Genômica Clínica, Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil

2 Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil

3 Divisão de Gastroenterologia Pediátrica, Hospital das Clínicas da Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil

4 Service de Dermatologie, Centre Hospitalier Universitaire, Université de Franche Comté, Besançon, França

5 Gene – Núcleo de Genética Médica, Belo Horizonte, MG, Brasil

Introduction: Infantile myofibromatosis is a rare genetic disorder characterized by the development of benign tumors in the skin, muscles, bones, and in exceptional cases, visceral organs. The incidence is 1/150,000 live births and the disease is the most common cause of fibrous tumors in infancy. The molecular pathogenesis is still incompletely known. An autosomal dominant form had been reported as causally related with c.1681C>T mutations in the gene for platelet-derived growth factor receptor beta (PDGFRB). **Methodology:** While trying to establish the culpable gene in cases of autosomal recessive infantile myofibromatosis we have studied a French family with two affected children and their healthy non-consanguineous parents. Whole-exome sequencing was performed in samples from the siblings and their parents by the Centre for Applied Genomics, Hospital for Sick Children (Toronto, Canada) using the Agilent SureSelect Human All Exon V4 kit and the SOLiD 5500xl platform. Sanger sequencing was performed for validation of the variants of interest identified in exome analysis using the BigDye Terminator v3.1 Cycle Sequencing kit and the Applied Biosystems (ABI) 3730 Genetic Analyzer. **Results and Discussion:** Over 100,000 variants were identified in each individual by exome sequencing. After filtering for common polymorphisms and non-pathogenic variants, we identified a heterozygous c.1681C>T mutation in *PDGFRB* in both siblings. However, the unaffected mother also had the same *PDGFRB* mutation. Since some variants may require the presence of additional pathogenic variants at other loci for full penetrance, we searched the affected siblings of our family for paternal mutations in genes known to interact with PDGFRB. Indeed, we found that both children had also inherited from their healthy father a c.1276G>A heterozygous mutation in the gene for receptor protein tyrosine phosphatase gamma (PTPRG), an enzyme known to dephosphorylate PDGFRB. According to the NHLBI Exome Sequencing Project (ESP6500), the *PTPRG* mutation has an allele frequency of 0.0001. The mutation is located in a region well conserved throughout evolution in PTPRG orthologues. It was predicted to be deleterious by SIFT, probably damaging by PolyPhen-2 and disease causing by Mutation Taster. **Conclusions:** This is the first documentation of incomplete penetrance in *PDGFRB*-related infantile myofibromatosis. We suggest that in this family, the additional mutation in *PTPRG* may explain the full phenotypic penetrance in the siblings affected, in comparison with the unaffected mother. It remains to be established whether the *PTPRG* mutation is a private feature of our family, or whether it is a more general phenomenon.

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FETAL RHD GENOTYPING IN MATERNAL PLASMA AS A NONINVASIVE TOOL IN THE MANAGEMENT OF RHD NEGATIVE PREGNANT WOMEN

SCHMIDT, Luciana Cayres1; FARIA, Marina Alves2; CABRAL, Antonio C. Vieira3; TARAZONA-SANTOS, Eduardo4; MARTINS, Marina Lobato2

1- Laboratório de Imunohematologia, Fundação Hemominas; 2- Serviço de Pesquisa, Fundação Hemominas; 3- Faculdade de Medicina, UFMG; 4- Departamento de Biologia Geral, ICB, UFMG.

Introduction: The management of RhD negative pregnant women is performed starting from the principle that the fetus is RhD positive and therefore at risk of developing hemolytic disease. However, it is possible to infer fetal RhD phenotype by *RHD* genotyping of the fetus from maternal plasma. This approach allows to review the management of pregnancy in order to avoid it be considered high risk in case of RhD negative fetus, and indicates the need for antenatal anti-D immunoprophylaxis in case of RhD positive fetus.

Methods: It were obtained from the plasma of RhD negative pregnant women 141 samples of fetal DNA whereas 21 DNA were obtained from pregnant women sensitized and 121 of sensitized women. It was performed two PCR in real time using the TaqMan system. One of them for amplification of the human albumin gene (130 bp) to confirm the recovery of free DNA in plasma and the other one for simultaneous amplification of a region of exon 5 (82 bp) and 7 (75 bp) of the *RHD* gene, in duplicate. Samples with amplification for both exons 5 and 7 in the duplicate were considered positive and samples with no amplification in any exons were considered negative. Samples with amplification of only one of the exons were considered inconclusive. The sensitivity, specificity, accuracy, negative and positive predictive value of the test was taken by comparing the conclusive results of fetal *RHD* genotyping with the results of RhD phenotype of the newborn.

Results: Of the 142 samples genotyped, 93 (65.5%) were *RHD* positive, 44 (31%) were RHD negative and five (3.5%) were inconclusive due to amplification of only exon 7 of the *RHD* gene. Of the 21 sensitized women, 16 (76.2%) had positive fetus and 5 (23.8%) *RHD* negative. Of the 121 non sensitized women, 77 (63.7%) had *RHD* positive fetus, 39 (32.2%) had *RHD* negative fetus and 5 (4.1%) showed inconclusive fetal *RHD* genotyping. So far, 69 fetal *RHD* genotyping results could be compared with the RhD phenotype at birth. Of these, 48 samples (69.6%) were positive *RHD* and 21 (30.4%) samples were negative *RHD*. We had two discordant results between *RHD* genotyping and RhD phenotype of the newborn, as were genotyped as *RHD* positive and phenotyped as RhD negative. Thus, the accuracy of molecular test was 97.1%, the sensitivity was 100% and the specificity was 90.5%. The positive predictive value was 96% and the negative predicitve value was 100%.

Conclusion: Noninvasive fetal *RHD* genotyping showed that regardless of the state of sensitization of pregnant women, about 30% of pregnancies could be considered risk-free, since the fetal RhD phenotype was also negative. Moreover, approximately 70% of women could be properly oriented on the use of anti-D antenatal immunoprophylaxis. Regarding sensitized pregnant women, the study has shown that about 24% of pregnancies could be considered risk-free, since the fetus was RhD negative. To ensure the accuracy of *RHD* genotyping is crucial to analyze more than one region of the *RHD* gene to avoid erroneous inferences of fetal RhD phenotype.

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ANALYSIS OF *KRAS* MUTATIONS IN NON-SMALL CELL LUNG CANCER BRAZILIAN PATIENTS

A.J.F.D Bicalho1; F. G. R. de Oliveira1 ; H. C. Sarubi1 ;L. B. Rodrigues1 ;P. G. P. Couto1; L. A. C. de Marco1
1FM-UFMG

Keywords: Lung Cancer; KRAS; Molecular Biology.

Introduction: Lung cancer is the leading global cause of cancer-related mortality. To improve the survival rate of lung cancer patients, a better understanding of tumor biology is required as well as the subsequent development of new therapeutic strategies. The RAS family has been implicated in the development of human malignancies. The KRAS oncogene resides on chromosome 12p12 and encodes a protein (p21RAS) involved in the MAP-kinase signal transduction pathway modulating cellular proliferation and differentiation. *KRAS* mutations, mainly codons 12, 13 and 61 (exons two and three) are present in roughly 25% of NSCLC tumors but the overall impact of these mutations on clinical outcome in NSCLC remains unclear. **Objective:** To ascertain the molecular mechanisms related to the development and progression of cancer. **Methods:** To determine the presence of KRAS mutations in a Brazilian population, we studied 50 patients with NSCLC. The target regions of exons 2 and 3 of KRAS were amplified using specific primers. The PCR samples were then purified and sequenced. We also evaluated the patients using 40 ancestry informative indels ancestry. **Results:** No mutations in KRAS were found and the analysis revealed higher African ancestry component among cancer patients than controls. **Conclusions:** According to the literature, our study is the first to examine the association between mutations in the KRAS gene and NSCLC in the Brazilian population. The lack of mutation can be explained by the fact that the adenocarcinoma (the main subtype of cancer associated with mutations in KRAS), is less prevalent in our sample (24%) than other subtypes. These findings suggest that other genes besides the KRAS are related to NSCLC. Therefore, it is necessary to investigate possible molecular alterations in other genes related tumors.

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Eixo: a.3) Genética Humana

PARVOVIRUS B19 AND CYTOMEGALOVIRUS INFECTION IN DIALYZED PATIENTS HYPORESPONSIVENESS TO ERYTHROPOIETIN THERAPY

MT Alves1,2, SS Vilaça3, LC Godoi4, MG Carvalho1, LMS Dusse1, KB Gomes1,2
1Instituto de Ciências Biológicas, UFMG, Belo Horizonte, MG
2Faculdade de Farmácia, UFMG, Belo Horizonte, MG
3Hospital Felício Rocho, Belo Horizonte, MG
4Labtest Diagnóstica, Lagoa Santa, MG

Introduction: viral infections are an important cause of morbidity in dialyzed patients with chronic kidney disease. Parvovirus B19 (B19) has a marked tropism for erythroid progenitor cells, leading to transient inhibition of red cell production. In addition, cytomegalovirus (CMV) infection has been associated with negative regulation of hemoglobin production. **Methodology:** we investigated the frequency of B19 and CMV infection and their association with hematological finds in 60 dialysis patients hyporesponsive (hemoglobin <11g/dL) and 60 patients responsive (hemoglobin ≥ 11g/dL) to the recombinant human erythropoietin (rHuEPO) treatment. The plasma samples were tested for specific antibodies by ELISA and viral DNA by nested-PCR. **Results:** our data showed that 10 patients (7 patients of the hyporesponsive group and 3 of the responsive group) were B19 DNA positive, suggesting active infection. Three of them had no detectable levels of anti-B19 IgG, probably due to a recent viremic period. The anti-B19 IgG test was positive in 67.5% (81/120) of the dialyzed patients. Seventy four patients were positive only for anti-B19 IgG, suggesting a past infection or immunization. No difference was observed between hyporesponsive and responsive groups regarding B19 frequency. There was also no difference between anti-B19 IgG or B19 DNA positive and negative patients and erythrocytes count and hemoglobin levels. It was observed a tendency of correlation between active CMV infection and decreased hemoglobin levels and hematocrit value, which suggest that CMV infection could affect erythropoiesis. A positive correlation between anti-CMV IgG and red cell distribution width (RDW) and a negative correlation between this antibody and mean corpuscular hemoglobin concentration (CHCM) was also obtained. These finds reflecting the disturbance of erythrocytes production associated to a compromised hemoglobin synthesis. In our study, 33/120 (27.5%) patients had active CMV infection demonstrated by viral DNA. Our data also revealed a high prevalence of anti-CMV IgG (94.2%). In 80 of 120 positive patients, only anti-CMV IgG was detected, similarly for B19 virus. **Conclusions:** B19 and CMV infection do not seem to be the major cause of resistance to rHuEPO treatment in patients on dialysis, however, these infections should be investigated after exclusion of common cause of rHuEPO resistance. Besides, additional studies are necessary to examine the relationship between CMV and B19 infection and their effects in eritropoiese in dialyzed patients.

Support by: Fapemig and CNPq

ALLELIC FREQUENCY IN CGG REPEAT OF THE *FMR1* GENE IN INDIVIDUALS WITH TYPICAL DEVELOPMENT AND IN INDIVIDUALS WITH MATH LEARNING DISABILITY

Martins AAS1; Salazar GC1; Coelho FF1; Fonseca PAS1; Julio-Costa A2; Haase VG2, Carvalho MRS1
1Departamento de Biologia Geral, ICB, UFMG
2Departamento de Psicologia, FAFICH, UFMG

Introduction: Math learning disability (MLD) is a learning disorder characterized by lower performance in arithmetic, when compared with other learning areas, in persons with normal intelligence and that have been exposed to proper formal education. With frequency estimates varying from 1 to 13%, MLD can be caused by neurological, environmental and genetic factors. One of the genetic factors is familial mental retardation 1 (FMR1) or fragile-X syndrome (FXS). This syndrome is caused by the expansion of a CGG repeat in the 5'-UTR of the *FMR1* gene. According to the number of CGGs, *FMR1* alleles are classified as normal, gray zone, premutation or full mutation alleles. Different phenotypes have been associated with gray zone, premutation and full mutation alleles in both sexes. One of the behavioral phenotypes observed in girls is MLD, even in the presence of premutations and normal intelligence. To date, only one study has investigated the contribution of *FMR1* mutations to MLD. No significant contribution was detected, but the sample was small. This study aimed to ascertain the contribution of *FMR1* premutations to the MLD phenotype. This project was approved by COEP/UFMG decision ETIC42/08.
Methodology: The sample comprised 103 boys (47 cases, 56 controls) and 123 girls (40 cases, 83 controls). Initially, the children were tested using school achievement, numerical transcodification and neuropsychological tests. Normal and premutation alleles were screened using a Gene-Specific PCR. This technic allows the identification of normal and premutation alleles, but not of full mutations. Therefore, a methylation sensitive PCR (mTP-PCR) was used to differentiate true homozygous girls from heterozygous ones that present one allele outside the Gene-Specific PCR detection range. mTP-PCR is based on the conversion of unmethylated citosines by sodium bisulfite and allows the detection of the loss of AGG monomers which, interspersed with CGG ones, preclude expansion. Statistical analyses included the Hardy-Weinberg Equilibrium test and establishment of confidence intervals to estimate the contribution of premutation alleles to MLD.
Results: Normal alleles were identified for all the boys in both case and control groups. Among the girls, a normal, heterozygote profile was detected for 24 girls in the MLD group and 54 in the control group. One girl in the control group presented a profile with three alleles, all of them in the normal range. Sixteen girls in the MLD group and 28 in the control group presented a homozygous profile and were submitted to the mTP-PCR. All of them presented alleles in the normal range and were considered true homozygotes. The most frequent alleles identified had 26, 27 and 28 CGG repeats and there were no statistically significant differences between groups. The 95% confidence interval estimate, based on the observed frequency of premutation alleles in the case group (0/87), was 0 - 2.3%.
Conclusions: The results suggests that premutations in the CGG repeats in the *FMR1* gene do not present a high contribution to MLD phenotype.
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DELETIONS INVOLVING *PLP1* GENE MAY BE ASSOCIATED WITH INVERTED LOW-COPY REPEATS

FS, Coelho1,2; F, Zhang3; JA, Lee4; GC, Oliveira2; JR, Lupski,5,6,7; CM, Carvalho1,2,4.
1Programa de Pós-Graduação em Genética Departamento de Biologia Geral - UFMG, Belo Horizonte, Minas Gerais, Brazil; 2 Grupo de Genômica e Biologia Computacional, Centro de Excelência em Bioinformática, Centro de Pesquisas René Rachou – FIOCRUZ, Belo Horizonte, MG, Brazil; 3State Key Laboratory of Genetic Engineering and Ministry of Education Key Laboratory of Contemporary Anthropology, School of Life Sciences, Fudan University, Shanghai, China; 4 Greenwood Genetic Center, Greenwood, SC; 5Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 6Department of Pediatrics, Baylor College of Medicine, Houston, TX; 7Texas Children's Hospital, Houston, TX.

Keywords: *PLP1*, Pelizaeus-Merzbacher, disease, deletion, inverted low-copy repeats

Pelizaeus-Merzbacher Disease - PMD (OMIM: #312080) is a recessive X-linked leukodystrophy characterized by dysmyelination of the central nervous system (CNS). The genetic cause of PMD is the mutation of proteolipid protein 1 (*PLP1)* gene. *PLP1* contains 7 exons spanning around 17 kb at Xq22.2. The genomic architecture surrounding *PLP1* is laden with numerous low-copy repeats (LCRs) in direct and inverted orientation (IP-LCRs). The inverted paralogous low-copy repeats (IP-LCRs) have been shown to render susceptibility to genomic instability, either mediating or facilitating formation of structural variants (SVs) in the genome. Non-allelic homologous recombination (NAHR) between IP-LCRs can generate balanced inversions of the intervening genomic intervals and lead to a pathogenic phenotype for instance as that observed in 45% of severe hemophilia A. IP-LCRs can facilitate formation of complex rearrangements such as triplication flanked by duplication (DUP-TRP/INV-DUP) structures through replicative-based mechanisms such as fork stalling and template switching (FoSTeS)/microhomology-mediated break-induced replication (MMBIR) which may lead to more severe clinical neurodevelopmental phenotypes such as those seen in patients who carry triplications involving the *MECP2* and *PLP1* genes. The presence of LCRs and IP-LCRs at both loci has been associated with duplications in over 85% and 50-76% of cases involving *MECP2* and *PLP1*, respectively. Whereas gain of copy number has been extensively studied at those loci, deletions involving *PLP1* are very rare and the mechanism of formation is still not resolved. Here we studied *PLP1* deletion breakpoints in seven patients, three of whom have not yet been reported. Precise mapping of the deletion-associated breaks using customized high-resolution array comparative genomic hybridization (aCGH) data was performed. Surprisingly, we observed that in four of the patients (57%), one breakpoint falls within an inverted segmental duplication with at least 90% nucleotide similarity. These analyses suggest that inverted repeats may play a role in the formation of deletions involving *PLP1*, potentially by providing susceptibility to a DNA break resulting in double-strand breaks that require further repair. The underlying mechanism for repair of such breaks is currently under investigation.

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ASSOCIATION OF POLYMORPHISM TGF-β1 CODON 25 C/G WITH RETINOPATHY IN TYPE 2 DIABETES MELLITUS PATIENTS

Rodrigues, K. F.1; Pietrani, N. T.1; Bosco, A. A.2; Gomes, K. B.3
1 - ICB, UFMG, Minas Gerais, Brazil 2 - Ambulatório de Endocrinologia, Santa Casa de Belo Horizonte, Minas Gerais, Brazil 3 - FAFAR, UFMG, Minas Gerais, Brazil

INTRODUCTION: Diabetic retinopathy (DR) is the most frequent cause of newly cases of blindness in adults. In the retina the transforming growth factor beta 1 (TGF-β1) modulates cell migration, production of others growth factors and extracellular matrix deposition. The polymorphism TGF-β1 codon 25 C/G causes change in the amino acid sequence of the signal peptide sequence (arginine to proline), and the homozygous genotype (GG) is associated with increased production of this cytokine. The aim of this study was to evaluate the association on this polymorphism with DR. **MATERIALS AND METHODS:** This study was conducted with 102 patients (19 men and 83 women), mean age 54.99 ±8.97 years, with a diagnosis of type 2 diabetes mellitus (T2DM). Of these, 66 patients had DR. The diagnosis criteria of T2DM and DR are according to American Diabetes Association (ADA). Genotyping was performed using Cytokine Genotyping Kit (One Lambda) followed by electrophoresis on a 2.5% agarose gel with GelGreen Nucleic Acid Gel Stain (Biotium). Statistical analyzes were performed with SPSS v. 13.0 and was used the Chi-square test. Values of $p<0.05$ was considered statistically significant. **RESULTS:** Genotypes and alleles frequencies were distributed as follows: T2DM with DR - GG 0.924, GC 0.061, CC 0.015, G 0.955 and C 0.045 - versus T2DM without DR - GG 0.722, GC 0.278, CC 0, G 0.861 and C 0.139. We observed that DR is associated with the GG genotype ($p=0.004$) and G allele ($p=0.018$) in TGF-β1 codon 25 C/G polymorphism. DISCUSS: These results showed that the genotype GG, associated to high expression of TGF, is more frequent in patients with DR. Therefore, this data suggest that TGF-β1 and consequently inflammation events are involved in the etiopathogenesis of this microvascular complication in T2DM.

TANDEM DUPLICATION OF THE SHORT ARM OF CHROMOSOME 7: METASTASIS RISK, CANCER SUSCEPTIBILITIES AND ETHICAL IMPLICATIONS

MP Almeida1, M Miranda1, TB Teixeira3, ACA Prado3, PAS Fonseca1, LL Leão3, MJB Aguiar3, VG Haase2, MRS Carvalho1

1 Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG.

2 Departamento de Psicologia, Faculdade de Filosofia e Ciências Humanas, Universidade Federal de Minas Gerais, Belo Horizonte, MG.

3 Serviço Especial de Genética Médica, Hospital de Clínicas, Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte, MG.

In the last few years, the advent of powerful tools enabled the identification of new deletions/duplications and the discovery of dosage sensitive genes, which are implicated in the manifestation of intellectual disability and/or dysmorphic features in many patients that suffer from a genetic disorder. These new technologies have opened new practical and ethical questions related to the functional implication of some chromosomal variants, introducing complex questions in the genetic counseling. Specifically, these questions include the incidental findings. Recently, we evaluated a 17-year-old girl with a history of developmental delay, borderline intelligence, dysmorphic features, triphalangeal thumbs, attention deficit, hyperextensibility, besides motor coordination and language difficulties. A large 7p chromosome insertion was detected in the karyotype. Using CytoSNP-850K BeadChip (Illumina Technologies), we were able to identify a previously undescribed 16.4 Mb duplication of 7p21.3p14.3 region (arr[hg19]7p21.3-p14.3 (13.265.700- 29.668.638)x3). Genes related to cancer and epithelial-mesenchymal transition (and therefore, metastasis) map to this chromosomal region, including the *HOXA* cluster, *TWIST1* (OMIM 601622) and *HOTAIRM1*. The *HOXA4* (OMIM 142953), *HOXA5* (OMIM 142952), *HOXA7* (OMIM 142950), *HOXA9* (MIM 142956) and *HOXA13* (OMIM 142959) have been related to leukemia. Overexpression of these five *HOXA* genes due to the duplication of chromosome 7 in a gastric cell line has been associated with tumorigenicity. Most notably, *HOXA9* increased expression has been described in the accelerated phase of chronic myeloid leukemia (OMIM 608232). Overexpression of *TWIST1* has been related to metastatic cancer in general, advanced oral squamous cell carcinoma and with poor survival rates. At the duplicated region there are also some lincRNAs (e.g. *HOTTIP*) which may coordinate long-range activation of vital genes. Since large 7p duplications have been only rarely described, it is impossible properly to establish the risks and management protocols for cancer surveillance or other possible health problems of this patient. Moreover, high metastasis risk unrelated to one specific type of cancer represents a complex situation in genetic counseling. In conclusion, the incidental findings associated with this specific chromosomal duplication, particularly the high metastasis risk, puts forward ethical dilemmas concerning the right not to know, as well as a strategy to care the patient.

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DESIGN AND VALIDATION OF AN ASSAY TO INVESTIGATE *CALR* GENE MUTATIONS IN MYELOPROLIFERATIVE NEOPLASM PATIENTS WHO ARE NEGATIVE FOR *JAK2* MUTATIONS.

BL Soares 1, C Lancor1, FB De Abreu1, L Lara Dos Santos2, GJ Tsongalis1
1Departments of Pathology and Medicine, Geisel School of Medicine at Dartmouth, DHMC and NCCC, Lebanon, NH, USA
2 Universidade Federal de Sao Joao Del Rei, Divinopolis, MG, Brasil.

Introduction: Mutations in *JAK2* gene are well characterized in patients with The Philadelphia chromosome–negative myeloproliferative neoplasm. Although *JAK2* mutations are very frequent in these patients, there are other additional and less frequent mutations responsible for the myeloproliferative neoplasm. Recent studies have identified genomic alterations in exon 9 of the encoding calreticulin gene (*CALR*) in patients negative for *JAK2*. Frameshift mutations (deletions and insertions) are the most frequent type of mutations founded in *CALR* gene. The aim of this study was to validate an assay to screen *CALR* mutations using Fragment analysis. **Methods:** Using the EZ1 DNA Blood Kit (Qiagen), the Genomic DNA from 60 whole blood samples was extracted. Of the 60 samples, 14 were positive and 46 were negative samples for *JAK2* mutations. Primers flanking the exon 9 region of the CALR gene were designed and the AmpliTaq Gold 360 Mastermix (Applied Biosystems) was used for the Conventional PCR. A commercial Human Genomic DNA Male (Promega) was used as an assay control. Fragment analysis assay was performed using the ABI 3500 Genetic Analyzer with POP-7TM polymer and 50 cm capillary with the PCR products. The results were analyzed using Applied Biosystems GeneMapper® 4.1 software. Limit of detection (LOD) was assessed in terms of DNA input, by varying the amount of gDNA in the reaction (100ng, 50ng, 10ng, 5ng, 1ng). Precision was performed in order to confirm the reproducibility of this assay. **Results:** The established LOD for our assays was 10ng of DNA, since the electropherograms showed lower peak intensity at 5ng of DNA input. 2 out of the 60 samples tested were positive for *CALR* mutations and negative for *JAK2*. Both samples presented a 52 bp deletion (c.1092_1143del). The reproducibility of the assay was demonstrated by Precision, when identical calls (100% precision) were made for 8 duplicate samples (2 positive, 5 negative and the gDNA) at four independent runs for each assay (fragment analysis and qPCR), generated by two different operators. **Conclusions:** Patients who carry myeloproliferative neoplasms and are positive for *CALR* mutations tends to present a more indolent and benign clinical course than the ones who carry *JAK2* mutations. Therefore, the development of clinical assays that can identify these individuals is important for the prognostic of the disease. This Fragment Analysis assay showed to be a highly sensitive and specific genotyping assay, being able to identify *CALR* mutations in patients with myeloproliferative neoplasm who are negative for *JAK2*.

ASSOCIATION BETWEEN VAL158MET COMT POLYMORPHISM, GENDER AND MATH ANXIETY

Authors: Annelise Júlio-Costa[1 2], Andressa Moreira Antunes[2], Gabrielle Vianna[3], Guilherme Wood[4], Maria Raquel Santos Carvalho[3], Vitor Geraldi Haase[1 2]

1. Programa de Pós-graduação em Neurociências, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Brazil

2. Laboratório de Neuropsicologia do Desenvolvimento, Faculdade de Filosofia e Ciências Humanas, Universidade Federal de Minas Gerais, Brazil

3. Programa de Pós-graduação em Genética, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Brazil

4. Department of Neuropsychology, Institute of Psychology, Karl-Franzens-University of Graz, Austria

ABSTRACT: Math anxiety is a specific phobia that appears in response to math tasks which are perceived as a threat. Little is known about the genetic basis of this construct. The aim of this study was to investigate how math anxiety may be associated to val158met COMT polymorphism in children aged 7-12 years. There is no differences regarding genotypes, but we found a main effect of gender (girls are more anxious). We also found an interaction effect which indicates that boys homozygous for valine allele had the best emotional regulation regarding mathematical anxiety. This study was the first to point out the impact of the COMT polymorphism on math anxiety, however further investigations must be conducted in order to clarify this relationship.

Keywords: Catechol O-Methyltransferase COMT, math, children, math anxiety

V Encontro de Genética de Minas Gerais

V ENGEMIG

Pesquisa e Pós-Graduação

Genômica e Bioinformática

NOVEL POLYMORPHISMS IN GENES ASSOCIATED WITH MILK AND MEAT PRODUCTION AND DISEASE RESISTANCE IN THE GUZERÁ BREED IDENTIFIED BY WHOLE-GENOME SEQUENCING

IC Rosse1, JG Assis1,2,3, FS Oliveira1,2,3, LR Leite1,2,3,F Araujo2,3, A Zerlotini4, BC Lopes5,WA Arbex6, MAMachado6, MGCD Peixoto6, RS Verneque6, MFM Guimarães6, MVGB Silva6, RS Coimbra7, G Oliveira2,3, MRS Carvalho1
1 Instituto de Ciências Biológicas – UFMG
2 Genomics and Computational Biology Group - FIOCRUZ-MG
3 Center for Excellence in Bioinformatics – FIOCRUZ-MG
4 EMBRAPA Informática Agropecuária - SP
5 EPAMIG – MG
6 EMBRAPA Dairy Cattle – MG
7 Biosystems Informatics, Research Center René Rachou – FIOCRUZ

Background: Currently, livestock accounts for 9% of internal product in Brazil and the herds that take part in milk selection programs have achieved gains of up to 1% per year. Genotyping arrays composed by hundred-thousands of SNPs were developed aiming the use in genome wide
association studies and genomic selection. These genotyping arrays contain variations observed in several commercial breeds, but do not include the Guzerá (*Bos taurus indicus*). Guzerá is a dualpurpose breed, well adapted to the tropical climate and that presents resistant to parasites and low susceptibility to mastitis. In this context, the objective of this work was to sequence and assemble the genome of one Guzerá bull in order to identify breed-specific variations that might be included in the genotyping arrays and be useful in breeding programs.
Results: We performed the genome assembly of the sequence of a Guzerá bull produced with SOLiD v4 second-generation sequencing using mate-paired libraries with 1-2 and 3-4 kb inserts. The sequences were mapped to the publicly available reference genome of *Bos taurus taurus* (UMD 3.1) using the LifeScope software. The average depth of coverage achieved from mapping was 26X for each chromosome and 87% of the reference genome was covered. A list of putative SNPs and INDELs were generated from the mapped reads, using the LifeScope and SAMtools. After filtering for quality, 4,200,936 SNPs and 664,704 INDELs were identified. Sixty-five percent of the SNPs and 89% of the INDELs were previously unknown. Additionally, 2,676,067 (64%) of the SNPs and 466,005 (70%) of the INDELs were homozygous and may represent true differences between Guzerá and *Bos taurus taurus*. Ninety percent of the over 4 million SNPs detected in this individual have not been included in the current commercial genotyping arrays and will be useful for the construction of more effective genotyping arrays for the Guzerá breed. Of 3,142,072 genetic differences in Guzerá, 2,041 variations were classified functionally according to NGS-SNP in nonsynonymous SNPs, splice-site variants, frameshift and coding INDELs. There variations were detected in 1,690 genes. Of the genes containing these variations, 54 were assigned as QTL for milk and meat quality and disease resistance based on dbQTL and literature search.
Conclusions: This study provided us with a large number of new SNPs, which may be used in order to enhance SNP genotyping arrays. Additionally, potentially functional variants were identified, that map to regions where QTLs were already detected and that constitute valuable markers to be used in association studies.
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ANALYSIS OF THE REPRESENTATIVENESS OF SNV (SINGLE NUCLEOTIDE VARIANTS) PRESENT IN GENES OF IMUNOHEMATOLOGIC INTEREST: INTEGRATING CLINICAL AND GENOMIC DATA

NM Araújo1, TP Leal1, GB Soares-Souza1, MCFS Malta2, EM Tarazona-Santos1, WCS Magalhães1

1 Laboratório de Diversidade Genética Humana - Universidade Federal de Minas Gerais

2 Laboratório de Pesquisa - Fundação Centro de Hematologia e Hemoterapia de Minas Gerais

The immunohematology is an important area of transfusion medicine, considering that an incompatible transfusion may have serious consequences for the receiver as transfusion reactions that may even be fatal. The genotyping of blood groups has become an important tool in blood banks, and many experts support the idea that molecular tests may in the future replace the hemagglutination tests. Currently, there are several specific commercial platforms for genotyping of blood groups. However, genotyping is still expensive when compared to current phenotyping techniques. In this context, becomes important the study of the direct representation of the polymorphisms responsible for blood group antigens in large, non-specific genotyping platforms widely used in Genome Wide Association Studies. The BGMUT (The Blood Group Antigen Gene Mutation Database) is a specific database for mutations in blood group antigens. Due to the absence of interaction between communities of immunohematologists and geneticists there is a lack of data integration between BGMUT and genomic databases, this can be seen in the absence of the identifier #rs, an essential identifier to search for more information about the polymorphism in dbSNP. This shortness of integration difficults and impedes further use of the information deposited in BGMUT. In this project, we developed and implemented in a pipeline a methodology able to retrieve the identifier #rs of the variants described in BGMUT with the objective of making available the segmented information from immunohematology for genomic and clinical application. Among the 9 genes of blood groups used in HEMOMINAS Foundation clinical routine, it was possible to retrieve the #rs for approximately 70% of the recoverable variants described by BGMUT and among the variants able to discriminate the major antigens, approximately 79% of those were recovered. It was also possible to evaluate the informativeness of genome wide arrays in the area of immunohematology. The arrays used for genome wide association studies (Omni 2.5M and 5M from Illumina) had enough coverage for direct sampling of variants responsible for discriminating the major antigens of the Duffy, Kell and Kidd systems. The same was not seen for ABO, MNS and Rh systems. In addition, 6 systems used in transfusion practices have 9 variants described as responsible for the distinction between the main antigens, a total of 19, represented in the arrays used for genome wide association studies and the 1000 Genomes Project (1KGP). It shows a poor representation of major variants in arrays for genome wide association studies, when compared to 1KGP and demonstrates the insufficiency of these arrays to relate, by direct approach of SNPs, most clinically important blood group variants to diseases by association studies.

Supported by: CAPES, CNPq, FAPEMIG

COMPLETE MITOCHONDRIAL GENOME OF *PROCHILODUS LINEATUS* (CHARACIFORMES, PROCHILODONTIDAE)

Pimenta, R. J. G.; Carmo, A. O.; Brandão-Dias, P. F. P.; Martins, A. P. V.; Bedore, A. G.; Kalapothakis, E.1

1Departamento de Biologia Geral; Instituto de Ciências Biológicas – Universidade Federal de Minas Gerais, Avenida Presidente Antônio Carlos, 6627 – Pampulha, Belo Horizonte – MG, 31270-901 – Brasil.

Prochilodus lineatus (Valenciennes, 1836) is a fish from the Prochilodontidae family (Characiformes, Prochilodontidae), popularly known as Curimba, Curimbatá, Curimbatã, Curimatã or Papa-terra. It occurs in the basin of Paraná-Paraguai, Paraíba-do-sul River and is one of the most important fishes for fishery in the Grande River. It reaches the maximum length of 60-70cm and measures up to 6 Kg, but most individuals are 30-40 cm. *P. lineatus* also migrates upstream in the rainy season (from November to March) so as to reproduce, being the most studied potamodromous in the Neotropics. The fish has iliophag habits, what means it feeds moving the substrate, eating algae, crustacean larvae and many other organisms they find on mud. This makes the Curimbatá a very important species for the ecosystem. The genomic DNA was extracted from the muscular tissue of a *P. lineatus* individual collected in Grande River, at the Minas Gerais, Brazil (20°01'53.8" S, 48°13'27.5" W) and purified by the phenol/chloroform method. One paired-end library was prepared with 50 ng of total DNA using the Nextera Kit (Illumina) according to manufacturer's protocol. The library was sequenced using Illumina Miseq technology in a 250- cycle strategy using MiSeq Reagent V2-500 (Illumina). Plots of the per base sequence quality were generated using FastQC version 0.10.1, and quality plots were visually inspected. Sequencing resulted in 18.125.238 reads, and we obtained a uniform coverage of 296x for the mitogenome. A *de novo* assembly performed using Mira 4.0 revealed that the size of mtDNA genome of *P. lineatus* is 16.669 in length and has 45.5% of GC content. The length is according to the mtDNA size range described for others species in the Characidae family.Gene coding regions were annotated using MitoFish web server, using the vertebrate mitochondrial genetic code. Similarly to a typical vertebrate mitogenome, the 37 found genes are divided between the two strands; they comprised 22 tRNA genes, 2 rRNA genes, 13 protein-coding genes and a 1041 bp-long control region (D-loop).With the exception of the COI gene, which starts with GTG codon, all other protein-coding genes had the usual ATG start codon. Six of the 13 protein-coding genes have TAA stop codon, while four have incomplete stop codon T-- and two have a TA-. Seven of the 22 tRNAs and one protein-coding gene were encoded by the light strand whilst the others genes were encoded by the heavy strand. All tRNAs were within the size range of of 67 to 75 bp, and all of them were folded into a typical secondary structure. Twelve genes presented overlapped coding sequences, most of them overlapped in one base.

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COMPLETE MITOCHONDRIAL GENOME OF *SALMINUS BRASILIENSIS* (CHARACIFORMES,
CHARACIDAE).

BRANDAO-DIAS, P. F. P.1; CARMO, A. O.1; MARTINS, A. P. V.1, PIMENTA, R. J. G.1; ALVES, C. B. M.2; KALAPOTHAKIS, E.1.

1 Departamento de Biologia Geral; Instituto de Ciências Biológicas – Universidade Federal de Minas Gerais, Avenida Presidente Antônio Carlos, 6627 – Pampulha, Belo Horizonte – MG, 31270-901 – Brasil.

2 Nuvelhas-Projeto Manuelzão, Universidade Federal de Minas Gerais, Avenida Presidente Antônio Carlos, 6627 – Pampulha, Belo Horizonte – MG, 31270-901 – Brasil.

Salminus brasiliensis (Cuvier, 1816), popularly known as dourado, is the largest fish in the order Characiformes, with adults weighing more than 20 Kg. It lives mainly in the Paraná-Paraguay and Uruguay rivers basins in Brazil, Argentina, Uruguay, and Paraguay, but it also occurs in other places, such as Lagoa dos Patos, Brazil, and in Rivers Chaporé and Mamoré, Bolivia. *S. brasiliensis* is an important predatory piscivore that migrates upstream in the rainy season to reproduce. During migration, some populations are affected by dam constructions, because it blocks both the upstream movement of adults and the downstream movement of eggs and larvae. *S. brasiliensis* is also prized by both commercial and amateur fisherman due to its flesh flavor and its resistance when caught, jumping several times out of water. For the present work, a *S. brasiliensis* was caught in the Grande River (20°01'53.8" S, 48°13'27.5" W) and DNA was extracted from its muscular tissue. A genomic paired-end library was built using Nextera Kit (Illumina) according to manufacturer's protocol, and the library was then sequenced using MiSeq sequencer (Illumina) with a 500 cycles paired-end strategy. We performed a *de novo* assembly using the software MIRA assembly 4.0, and obtained 161,407 contigs with more than 500 bp. A single 17,721 bp contig with 148× coverage was extracted. Alignment against Genbank deposited sequences using Basic Local Alignment Seach Tool (BLAST) revealed it to be the mitochondrial DNA of *S. brasiliensis.* We used the Mitoannotator feature from Mitofish website so as to annotate it. The molecule contains 13 protein-coding genes, 2 rRNA genes, 22 tRNA genes, and a major non-coding control region, as in most animal's mitochondrial DNA and in all vertebrates. All protein-coding genes (PCGs), except for *nd6*, are coded on the complementary (heavy) strand. The gene order and coding strand were consistent with the order Characiformes, and AT proportion was 55.7 %. As in most mitochondrial genomes, some alternative start and stop codons can be used. In the present study, cox1 gene has the GTG start codon instead of ATG, and only five PCGs out of 13 have the stop codon TAA (*nd2, cox2, atp6, nd4* and *cytb* have T--, *cox3* and *nd3* have TA-, *nd6* has TAG and *cox1* has AGG as a stop codon). A tRNA inference analysis using MITOS webserver revealed that 21 out of 22 tRNAs, fold into the typical cloverleaf structure. The exception, tRNASer(GCT), may lack the D-arm. The control region is between tRNAPro(TGG) and tRNAPhe(GAA), and is 2,128 bp long. Of the 18 complete control regions published for the order, the average length is 1 020bp. The longest currently known is from *Lebiasina astrigata* and is 1,222 bp long. The big D-loop in *S. brasiliensis* is due to an insertion of 1 019bp, which has no significant similarity in BLAST alignment.

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TAXONOMIC AND FUNCTIONAL INVESTIGATION OF A MICROBIAL COMMUNITY FROM A TROPICAL METAL-IMPACTED STREAM

Patrícia S. Costa1, Mariana P. Reis1, Marcelo P. Ávila1, Laura R. Leite2, Flávio M.G. de Araújo2, Anna C.M. Salim2, Guilherme Oliveira2, Francisco Barbosa1, Edmar Chartone-Souza1, Andréa M.A. Nascimento1*.

1 Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais; Av. Antônio Carlos 6627 Belo Horizonte, Minas Gerais, Brazil, CEP: 31270-901.

2 Centro de Pesquisas René Rachou Fundação Oswaldo Cruz; Av. Augusto de Lima 1715 Belo Horizonte, Minas Gerais, Brazil, CEP: 30190-002.

Abstract

The Iron Quadrangle (QF, Minas Gerais, Brazil) is one of the world's largest mining regions, being historically explored for over 300 years. Since then, many toxic metals and metalloids were released into the environment leading to contamination of water bodies. Since prokaryotic community influences the bioavailability of these toxic elements and many have revealed to be important members for the healthy and/or ecological balance of various environments, we describe the taxonomy of the microbial community and its functional composition in historically metal-contaminated tropical stream sediment. The sediment was collected from the Mina Stream (MSS, QF). The environmental DNA extracted was sequenced using SOLiD and Ilumina platforms for the metagenome and microbiome analysis, respectively. Taxonomic profile obtained by Greengenes database revealed a complex microbial community, with dominance of *Proteobacteria* and *Parvarcheota*. Bacterial and archaeal genomes were reconstructed based on SEED subsystems database, especially *Candidatus Nitrospira defluvii* and *Nitrosopumilus maritimus*, and their presence implicated them in C and N cycling in the MSS. Functional reconstruction revealed a large diverse set of genes for ammonium assimilation and ammonification. These processes have been implicated in the maintenance of N cycle and healthy of the sediment. SEED subsystems functional annotation unveiled a high diversity of metal resistance genes, suggesting that the prokaryotic community is adapted to metal contamination. Furthermore, a high metabolic diversity was detected in MSS, suggesting that the historical As contamination is no longer affecting it. Finally, the results reported herein may contribute to expand the current knowledge of the microbial taxonomic and functional composition of tropical metal-contaminated freshwater sediments.

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PARTIAL TRANSCRIPTOME ANALYSIS OF THE *TRIATOMA BRASILIENSIS* ANTERIOR MIDGUT BY EXPRESSED SEQUENCE TAGS (ESTs) LIBRARIES

PEREIRA, M.A.1; BORONI, M.1; ALVES, C.L.1; DALSECCO, L.S.2; DRUMMOND, M.G.2; PEREIRA, M.H.3; MOURÃO, M.M.4; ARAÚJO, R.N.3; FRANCO, G.R.1.
1 Departamento de Bioquímica e Imunologia – UFMG – BH/MG
2 Escola de Veterinária – UFMG – BH/MG
3 Departamento de Parasitologia – UFMG – BH/MG
4 René Rachou/FIOCRUZ – BH/MG

Introduction *Triatoma brasiliensis* is a hematophagous arthropod and the main vector responsible for the transmission of the protozoa *Trypanosoma cruzi* in the semiarid areas of the Northeast region of Brazil. Since *T. cruzi* life cycle is influenced by the insect digestive tract, is of utmost importance to understand the factors that influence the insect feeding performance. The success in the feeding is directed related to the success of *T. cruzi* transmission. Very little is known about regulation of gene expression in *T. brasiliensis* and what genes are induced in the insect gut after feeding. Furthermore, the available *Triatoma* transcriptome information is also very limited. Must studies analyzed the salivary glands transcriptome of different triatomines. In this work we report the characterization of the anterior midgut transcriptome of *T. brasiliensis* using Expressed Sequence Tags (ESTs). **Methodology and Results** We have isolated the mRNA from the anterior midgut of 101 fifth-instar nymphs and generated a cDNA library. A total of 768 clones were randomly selected and sequenced with M13 forward and reverse primers. ESTs were edited in silico to remove low quality regions and sequences of vector, adapters and poli(A) tail using DNA Baser and SeqClean software. Sequences were also clustered using the CAP3 software and submitted to similarity searches, functional annotation and classification with blastn, blastx and blast2GO. We have obtained 311 uniques (138 contigs e 170 singletons). Some transcripts were considered as contaminants and further removed from analysis. From the remaining transcripts, 180 uniques did not present similarity with sequences deposited in databases and are, possibly, *T. brasiliensis*-specific genes. The library exhibited a high expression level of secreted transcripts and transcripts related to energetic metabolism, protein synthesis and modification machinery, immunity and viral transcripts. A considerable variety of transcripts was isolated and these are consistent with their tissue of expression (anterior midgut) and organism. For instance, we observed transcripts coding for defensin, lysozyme, brasiliensin and digestive enzymes. Five transcripts were submitted to RT-qPCR analysis. The expression of three transcripts tends to be influenced by fasting and the other two, by blood feeding. **Conclusion** The characterization of the *T. brasiliensis* midgut transcriptome will help us to understand the feeding evolution of arthropod vectors, as well as to provide useful information to develop new strategies for vector control and to understand the processes involved in vector/*T. cruzi* interaction.

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CHARACTERIZATION OF THE STIMULONS OF *CORYNEBACTERIUM PSEUDOTUBERCULOSIS* STRAIN 258 FROM AB INITIO ASSEMBLY AND RNA-SEQ

MP Santana1, AC Pinto1, R Ramos2, P Sá2, F Aburjaile1, A Carneiro2, A Silva2, V Azevedo1
1 Federal University of Minas Gerais
2 Federal University of Pará

Background: *Corynebacterium pseudotuberculosis* is a pathogenic bacterium responsible for different pathologies, which bring losses livestock. Ulcerative lymphangitis, one of these pathologies, may cause lameness and deformity of the limbs in affected animals. This disease manifests mainly on horses and have different causative agents, being *C. pseudotuberculosis* (biovar equi) the main causative pathogen. Aiming a better understanding of the microorganism biology, as well as the dynamic of the gene expression during exponential growth phase, a transcriptional study was realized on the strain 258 of *C. pseudotuberculosis* isolated from a horse with ulcerative lymphangitis in Belgium.
Methodology: Through RNA-seq it was possible to identify the transcripts on osmotic stress (2M), acidity (pH), and heat shock (50°C), which simulate the environment encountered by the pathogen during the infection process, and compare them to the physiological condition (N). After sequencing through the next generation technology SOLiD™ V3 plus, the transcripts were assemblies by ab initio methodology and data processed on Blast2go. The coding sequences were organized by each category and Gene Ontology term through the CoreStlmulon software, and the "biological process" category was selected for gene analysis.
Results: Among the stimulons, a group of genes regulated by a certain environmental condition, acidity stress contributed further for the genic catalog. Genes related to combat against reactive oxygen species and heat shock stress were identified. The heat shock stimulon involved the heat shock proteins as expression modulators, as well as iron uptake genes and cell wall synthesis. On osmotic stimulon, no gene were described only in this condition, but genes that may be involved on virulence and pathogen survival were described, like MraZ e SDH. Among genes shared on two or three conditions, we highlight *fts* genes, involved on cell division and cycle, and genes related to osmoprotection synthesis. According to the Blast2go results, it is possible to highlight the oxidation-reduction process, transport, response to heat and response to oxidative stress as being the more representative between the 24 transcripts described only in the stress condition, and between the proteins considered important for the bacterium survival during the adverse conditions. The codifying genes of the proteins MraZ, Trx, TrxR, Dps, Ferritin like protein, Drp1 e Drp2 were selected as being possible virulence factors in *C. pseudotuberculosis*, since they have already demonstrated as being relevant on other organisms and therefore must be focused in future studies.
Conclusions: The results described in this work may contribute to efficient solutions against *C. pseudotuberculosis* disease and minimize livestock economic losses.
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STUDIES ABOUT THE HOMOLOGOUS RECOMBINATION IN DIFFERENT STRAINS OF *TRYPANOSOMA CRUZI*.

Repoles, BM1; Santos-Silva. S1; Alves. C. L1; Franco, GR1; Macedo, AM1; Pena, SDJ1; Machado, CR1

1- Departamento de Bioquímica e Imunologia , Instituto de Ciências Biológicas – UFMG, Belo Horizonte, M.G.

Trypanosoma cruzi is the etiological agent of Chagas Diease. Philogeny studies recently subdivided *T. cruzi* into six discrete taxonomic units, named *T. cruzi* I to *T. cruzi* VI. The parasite presents a complex life cycle which includes an obligate intracellular stage in a mammalian host. It was already proven that the *T. cruzi* has a high resistance to ionizing radiation (IR). Although the parasite does not face high doses of radiation on his life cycle, it can resist to doses 100x higher than humans. When the parasite is exposed to doses as high as 500Gy, occurs DNA fragmentation, but this is gradually repaired 48 hours later exposure. One of the effects of IR is the generation of double strand breaks (DSB's). In *T. cruzi*, homologous recombination repair (HRR) is the pathway responsible for the processing of these DSB's. In this process a central protein, Rad51, polymerizes into single-stranded DNA and acts in the search for homology in the other DNA molecule. The function of Rad51 is regulated and depends on his interaction with the BRCA2, a protein with homologues widespread in eukaryotes. This protein has a domain, named BRC, which interacts with Rad51, on his loading to the single-stranded DNA, although other regions on BRCA2 can interact with Rad51. To verify the response to IR in the *T. cruzi* DTU's I, II and VI, the strains CL Brener (T. cruzi VI), Sylvio (T. cruzi I) and Esmeraldo (T. cruzi II) were exposed to 500 Gy of radiation. It was demonstrated that CL Brener strain resume its growth faster than Sylvio and Esmeraldo . Two hypothesis emerges to explain this phenotype: a difference between the proteins/proteins interactions involved on DSB repair in each strain; an differential expression of this proteins by each strain. The analyses of the proteins involved in DSB response showed the most relevant difference is on the BRCA2 protein, while Rad51 is equal for all strains analysed. The predicted models of the respective BRCA2 proteins shows that the position and accessibility of the BRC domain is different for each DTU. Using the Haddock WebServer, we generated models that show the interaction is more stable for the CL Brener protein complex than for the other complexes using Esmeraldo and Sylvio proteins, when analysed for the first BRC domain. To further investigate this difference, we will study the expression of the main proteins involved in this interaction by Real-Time PCR. So, in this work we showed that a hybrid strain (CL Brener) responds better to a genotoxic treatment that needs the homologous recombination process, which could suggest that recombination is key process to hybrid generation.

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REVERSE VACCINOLOGY TO PREDICTED VACCINE TARGETS IN *C. UREALYTICUM*

Luis Carlos Guimarães1, Siomar de Castro Soares2, Leandro J. Benevides1, Marcus V. C. Viana1, Edson L. Folador1, Vasco Azevedo1.
1Department of General Biology, Institute of Biological Sciences, Federal University of Minas Gerais, Avenue Antônio Carlos, 6627, Belo Horizonte, Minas Gerais, Brazil.
2Department of Preventive Veterinary Medicine, School of Veterinary Medicine, Federal University of Minas Gerais, Avenue Antônio Carlos, 6627, Belo Horizonte, Minas Gerais, Brazil.

Corynebacterium urealyticum is an opportunistic pathogen living normally on skin and mucous membrane in humans and may cause acute or encrusted cystitis, encrusted pyelitis, and pyelonephritis in immunocompromised patients. The medical treatment requires administration of multiple drugs and additional invasive intervention, once *C. urealyticum* is a multi-drug resistant bacterium. In this work, we used two strains of *C. urealyticum* (DSM 7109 and DSM 7111) to attempted identify new targets that can be used in drug or vaccine development against this pathogen. Antigenic targets were predicted using reverse vaccinology strategy. This method based on 4 steps: (I) prediction of subcellular location once the majority of antigenic proteins are those proteins exposed to the host, which can be promptly recognized by the immune system, like secreted proteins, surface-exposed proteins, and membrane proteins; (II) proteins with MHC I and II binding properties with adhesion probability greater than 0.51 and absence of similarity to host proteins; (III) protein conservation among different genomes; (IV) proteins with virulence factors present in genomic islands. However, the rule IV does not exclude the targets from step III. According to the step I, the prediction of the sub-cellular location classified 590 and 579 putative candidates for *C. urealyticum* strains DSM 7109 and DSM 7111 respectively. Further, these proteins classified in rule I, 54 and 57 proteins (DSM 7109 and DSM 7111 strains respectively) showed adhesion probability proprieties greater than 0.51 and absence of similarity to host (step II). The step III was considered only proteins that are shared by both strains, resulting in 46 candidates. The step IV, a screened was done for proteins which are shared by both strains that were harbored by genomic islands and we found 19 vaccine candidates. Two of these 19 candidates are proteins encoded by *spaD* and *spaE* genes part of a cluster that encode adhesive pilus structures that are surface-anchored in corynebacterial cell wall and probably facilitate the adhesion of the pathogen to host tissue. The presence of the *spaF* gene was detected in each *C. urealyticum* genome, as well as the sortase coding genes *srtB* and *srtC*. The genome organization of the *spaDEF* cluster is similar to *C. diphitheriae* NCTC 13129 and *C. ulcerans* 809 and BR-AD22 strains. The results of virulence factors show a basis for therapeutic strategies development. However, additional strains have to be added to create effective vaccines against this bacterium.

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V Encontro de Genética de Minas Gerais

V ENGEMIG

Pesquisa e Pós-Graduação

Melhoramento Genético

CANDIDATE GENES AND CHROMOSOMAL REGIONS FOR SPERMATIC AND TESTES ALTERATIONS IN BOVINES: ADDITIONAL CLUES FOR AN X-CHROMOSOME COMPONENT

Fonseca, P.A.S[1]; Moura, G.S[2]; Santos, F.C[1]; Santos, D.J[3]; Rosse, I.C[1]; Oliveira,G.C4; Andrade, V. J.[2], Vale-Filho, V. R[2]; Silva, M. V. G. B[3]; Carvalho, M.R.S[1]

[1] Departamento de Biologia Geral, Universidade Federal de Minas Gerais, Belo Horizonte, 31.270-901, Brazil

[2] Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, 31.270-901, Brazil

[3] Embrapa Gado de Leite, Juiz de Fora, 36038-330, Brazil

4 Centro de Pesquisas René Rachou (CPqRR) - FIOCRUZ, Belo Horizonte- MG, 30190002, Brazil

Introduction: In the last few decades, increase in milk production within selection programs has been associated with decrease in fertility rates. In some breeds, losses of 1% a year in fertility traits have been reported. Therefore, the identification of genetic causes of reproductive disorders is fundamental in order to ensure better results in animal breeding. Although most studies focused on females reproductive phenotypes, male factors must be taken into account, considering the larger contribution of bulls to the genetic pool of the next generation, when comparing to the cows. Additionally, abnormalities present in the bulls may quickly spread to many different herds. Gonadal hypoplasia, particularly when associated to spermatic abnormalities may reduce bull fertility and cause economical losses. However, its genetic causes remain unclear. The aim of the present study was to investigate the genetic basis of gonadal hypoplasia and spermatic abnormalities.

Material and methods: An in-family GWAS was carried out for sperm defects and testes hypoplasia in a sample composed by 30 affected and 30 normal bulls of the Dairy-Gir breed (*Bos taurus indicus*). The phenotypes ascertained were gonadal hypoplasia and spermatic defects. Animals were genotyped using Axiom Genome-Wide BOS 1 Array from Affymetrix, composed by 648K SNPs. Phenotypes were corrected for fixed effects (father, age, farm, and season). Pairwise Association Study (PAS) was developed for quantitative and numeric continuous phenotypes. For Haplotype Association Study (HAS) two dichotomic variable were construct: testes morphology abnormalities *plus* spermatic defects (ALL) and spermatic defects (Sperm Only). GWAS was conducted using SNP and Variation Suit 7 (Golden Helix, Inc.).

Results and discussion: X-chromosomal associations were detected in both PAS and HAS. In HAS, two significant, Bonferroni corrected associations were detected: 1. Cullin 4B (*CUL4B*) gene for ALL phenotype; 2. Zinc finger and BTB domain containing 33 (*ZBTB33*) for Sperm Only phenotype. *CUL4B* plays a crucial role in ubiquitination of proteins involved in cell cycle regulation, DNA replication and embryonic development. *CUL4A*, a *CULB4B* homologue, has been associated to spermatic development. *ZBTB33* encodes Kaiso zinc finger protein, which is associated with embryonic development, homeostasis of adult tissues, maintenance of progenitor cells, cell fate determination and differentiation and with reproductive traits in bovine males. Additionally, we were able to identify associations with three candidate regions, all located on the X-chromosome. These regions contain blocks of genes encoding proteins that are essential for gonadal cell functions like mitosis, meiosis, sperm development and compartmentalization of the sperm cells. The accurate selection of candidate genes and regions from a whole genome study reinforces that the *in family* case-control design used here is an efficient methodology to be explored for GWAS of pathological conditions. This is the first high density GWAS for gonadal hypoplasia and sperm abnormalities in Gir and one of the first for these phenotypes in bovines at all.

Candidate genes/regions identified here can help understanding the metabolic and physiological dynamics of reproductive phenotypes in bulls and may be useful in marker assisted selection strategies against spermatic abnormalities in the dairy-Gir and eventually for other bovine breeds.

TWO NEW CANDIDATE REGIONS FOR BOVINE BEHAVIOR IDENTIFIED THROUGH A GENOME-WIDE ASSOCIATION STUDY IN THE GUZERÁ BREED IN BRAZIL

Santos, F.C[1]; Peixoto, M. G. C. D[2]; Fonseca, P.A.S[1]; Pires, M. F. Á.[2]; Rosse, I.C[1]; Bruneli, F. A. T.[2]; Santos, G.G.[2]; Carvalho, M.R.S1
1Departamento de Biologia Geral, Universidade Federal de Minas Gerais, Belo Horizonte, 31.270-901, Brazil
2 Embrapa Gado de Leite, Juiz de Fora, 36038-330, Brazil

Background: Bovine behavior is a complex characteristic composed by many endophenotypes. Temperament is the most investigated of them and involves aggression, reactivity, passivity, physical movements, persistent habits, emotions, alertness and even curiosity. Temperament is a fundamental trait, because it reflects the fear felt by an animal while it is in human presence. Consequently, it influences directly on the animal-cowman relationship. When compared to calm animals, the angry ones exhibit lower weight gain, lower reproductive efficiency and lower milk production. Additionally, these animals produce meet of lower quality, are more susceptible to some diseases and are more frequently involved in work accidents. Therefore, ill-tempered animals increase the costs of herd maintenance and lead to losses of productivity. The definition of temperament is broad and subjective, leading to difficulties in its evaluation. Consequently, most of the studies, divide it in less complex components or endophenotypes, which can be evaluated quantitatively. Despite this, few studies on the genetics of temperament showed concordant results to date, partially due to the subjectivity of the behavior scoring resources. Reactivity can be less subjectively measured with devices such as REATEST®, which captures electronically the movements of the animal in a mobile cage for 20 seconds and attributes a score to it. This score reflects the intensity and the number of movements produced by the animal once catch in the mobile cage.
Here, we describe the results of a genome-wide association study (GWAS) for reactivity, as assessed with Reatest, in a sample of 794 cows of the Guzerá breed, using reactivity data collected in 5 farms in Brazil. The Generalized Linear Model (GLM) was used to extract effects of age, herd, weight and physiological status. The genotypes were obtained using the BovineSNP50 v2 DNA Analysis BeadChip. Association tests and quality control were performed using the SNP & Variation Suite software V7 Power Seat.
Results: Three SNPs were significantly associated to reactivity in Guzerá with highly significant P-values even after Bonferroni correction. P-values ranging from -log(p) 6.1 to 6.99 were obtained. Each one of the three associated SNPs explained almost 4.5% of the variance of the characteristic. One of these SNPs is located on BTA1 and the other two are located on BTA25. After analyzing the regions around these markers, we observed that most of the genes located in the BTA1 region are related with brain formation or neuronal function, while genes in BTA25 might be related with sensorial functions. These findings reinforce these regions as possible positional as well as functional candidates for bovine behavior.
Conclusions: The identification of variants associated to reactivity may yield valuable information for understanding the biology behind this trait in bovine breeds. Given the importance of temperament for animal production systems using indicine breeds, generally assumed as ill-tempered animals, the lack of studies in the area, and the importance of the Guzerá breed for milk production systems in Brazil, these results stand out as an important contribution for bovine temperament research in Brazil.
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THE FREQUENCY OF POLYMORPHISM IN THE KAPPACASEIN GENE IN ½ AND ¾ BLOOD GIROLANDO BREED

Paiva, A.E[1]; Fonseca, P.A.S[1]; Santos, F.C[1]; Rosse, I.C; Freitas, D.R[2]; Cerqueira, M.M.O.P[2]; Carvalho, M.R.S[1]
[1]Instituto de Ciências BiológicasUFMG
[2] Escola de Veterinária – UFMG

Keywords: Kappacasein;
Girolando; Polymorphism

Introduction: Brazil is the sixth milk producer in the world, with approximately 25 billion liters per year. The Girolando breed is responsible for approximately 80% of this total. This breed appeared when Brazilian ranchers started breeding animals of Gir (*Bos Taurus indicus*) and Holstein (*Bos taurus taurus*) in order to get animals that would associate the high milk production of the Holstein cattle and the rusticity of the Gir. These aims were largely achieved, leading to the dissemination of the Girolando breed throughout the country. Studies on genetic variation of bovine milk proteins started over 50 years ago and have intensively accelerated in recent years. Milk specific proteins in bovines include four caseins: Alpha s1casein; Alpha s2casein; Betacasein and KappaCasein. These proteins are associated with milk production and composition and cheese yield. Bovine KappaCasein constitutes approximately 12% of the whole milk caseins. This protein is located predominantly on the milk micelle surface. Its main function is to stabilize milk micelles, preventing their aggregation and helping to keep calcium phosphate in solution. A KappaCasein functional polymorphism, in bovine, induces a double amino acid substitution generating the allele A (136Thr and 148Ala) and the allele B (136Ile and 148Asp). The KappaCasein allele B has been associated with higher milk protein content, shorter rennet clotting time and higher curd firmness. The B allele of KappaCasein promotes an increase in cheese yield, improves cheese quality (cheeseability). The KappaCasein allele A has been associated with lower milk fat, calcium and pH. Thus, the aim of this study was investigate the frequencies of the alleles A and B of Kappacasein in Girolando breed and determinate how the blood degree influences on this variation.

Material and methods: Genomic DNA of 77 Girolando cattle samples (49 ½blood and 28 ¾ blood) were extracted from blood. Genotyping was based on a tetraprimer ARMSPCR designed by our research group.The allelic and genotypic frequencies; expected and observed heterozygosity were calculated for the ½ and ¾ groups such as for the all sample (1/2+3/4 blood sample).

Results and discussion: The allele A frequency was 0.75; 0.8 and 0.66 for all sample, ½ and ¾ subsamples, respectively. The less frequent genotype was the BB. In the all sample, the frequency of BB was 0.08 and for ½ and ¾ blood subsamples were 0.02 and 0.17, respectively. The expected/observed heterozygosity in the total sample and the two subsamples were 0.38/0.35 (all sample), 0.32/0.37 (1/2 blood) and 0.45/0.32 (3/4 blood). In the total sample, genotype frequencies weren't in the HardyWeinberg equilibrium (P<0,005).
The lower frequency of B genotype in zebu when compared with taurus may be the responsible by the higher B allele frequency in the ¾ blood. This is the first study to compare the allelic and genotypic frequencies of the A and B alleles of KappaCasein in different blood degrees of the Girolando breed and can be very interesting for cheese selection in Girolando.

THE FREQUENCY OF A POLYMORPHISM IN THE AS1-CASEIN GENE FOR 1⁄2 AND 3⁄4 BLOOD GIROLANDO BREED

Zebral, N. P2; Fonseca, P.A.S1; Santos, F.C1; Freitas, D.R2; Cerqueira, M.M.O.P2; Carvalho, M.R.S1
1 Instituto de Ciencias Biologicas- UFMG
2 Escola de Veterinaria – UFMG

Keywords: αS1-casein; Girolando; Polymorphism; Genotypic frequency; blood degree

Introduction: Brazil is the fifth country in the list of the greatest milk producers in the world. From all of its production, 80% come from the milk produced by Girolando, a synthetic breed obtained through the crossbreeding of Gir and Holstein. Animals from this breed can be found in all regions of Brazil, because of their characteristics of good adaptation to cattle rearing, such as hardiness, endoparasites and ectoparasites resistance and high milk productivity. However, not only the amount of milk produced is important, but also its composition. Milk composition is of paramount importance for a good nutrition of the calf and milk consumers and it is also important for the production of milk derivatives as different types of cheese and yogurts. Protein levels for example are very important for this production and it determines the good quality of the product. The casein content in milk is important for the dairy industry mainly because of its impact on the yield of cheese and it varies within breeds, nutrition type, stage of lactation, animal's health and feeding management program. The production of protein has high heritability. Among all the caseins present in milk, the αS1, αS2, β, and κ casein, the αS1 is the most abundant in bovine milk. And there is an important polymorphism in the proximal promoter region of the αS1-casein gene which causes an exchange at position -175 (A_G) determining the B and C alleles, respectively, for this locus. When compared to animals with the genotype BB and BC, the CC ones present higher milk yield as well as protein content. Higher levels of this protein in milk are related to the formation of firmer curds during coagulation and better chemical composition of it.

Material and methods: In order to investigate the genotype and allele frequencies for this polymorphism in a population sample composed of Girolando .H:Gir and Girolando 3/4H:Gir, 76 animals (49 1⁄2 blood and 28 3⁄4 blood) were genotyped for this locus, using genomic DNA extracted from peripheral blood. The B and C alleles of αS1-casein gene were determined using a tetra-primer ARMS-PCR. The allelic and genotypic frequencies were calculated for the 1⁄2 and 3⁄4 groups, such as for the all sample (1/2+3/4 blood sample). Additionally, a chi-square test was carried out to test the Hardy-Weinberg equilibrium.

Conclusions: The allele B frequency was 0.68; 0.65 and 0.74 for all sample, 1⁄2 and 3⁄4 groups, respectively. The less frequent genotype was the CC. In the all sample, the frequency of CC was 0.11 and for 1⁄2 and 3⁄4 blood groups were 0.12 and 0.07, respectively. The frequency of BC was 0.42 and for 1⁄2 and 3⁄4 blood groups were 0.45 and 0.37, respectively. The three samples, showed genotype frequencies equivalent to the expected frequencies in Hardy-Weinberg Equilibrium ($P<0,05$). Moreover, the C allele was the less frequent in the three groups. This is the first study to compare the allelic and genotypic frequencies of the B and C alleles of αS1-casein in different blood degrees of Girolando breed.

Supported by: FAPEMIG, CNPq and CAPES

MYOSTATIN AND LEPTIN GENE AND CARCASS TRAITS OF SANTA INES SHEEP

C.R. Quirino1; A.S. Azevedo1; A. Pacheco1; A.F. Madella-Oliveira2
1Universidade Estadual do Norte Fluminense Darcy Ribeiro.Laboratório Reprodução e Melhoramento Genético Animal
2 Instituto Federal de Espírito Santo – Campus Alegre

The aim of this study was to associate the myostatin and leptin genes in 86 Brazilian Santa Inês sheep with the carcass trait. At eight months of age the animals were weight (W) and slaughter. Were recorded hot carcass weight (HCW), cold carcass weight, loin weight, tenderloin weight and the fat thickness. The carcass yield was calculated with (HCW/W) x 100. Samples of hair were collected for each animal and this material was used for DNA extraction by alkaline protocol. The animals were genotyped for the mutation G>A at nucleotide 9827 of myostatin gene and three polymorphism in exon 3 of the leptin gene by PCR-RFLP. The PCR was performed for amplification of the 3'UTR region of the myostatin and exon 3 of the leptin gene. The variance analysis was performed. For myostatin, GG (homozygous for the absence of mutation), GA (heterozygous for the mutation) and AA (homozygous mutation) genotypes were found. Frequency of the A allele was 7.69% and the G allele was 92.31%. The frequency of GG genotype was 84.61%, for GA was 15.38% andfor AA was 0%.The genotypes for the polymorphisms in the leptin gene were homozygous GG (without mutation) and heterozygous GT. The TT homozygote animals for the mutations were not found. For EF534371 allele were digested with the restriction enzyme for fragments AleI, AciI e MspI. The frequency of the T allele was 24.60% and the G allele was 75.40% . The frequencies of the genotypes GG, GT and TT were 50.80%, 49.20% and 0% respectively.The mutant allele of the myostatin gene was higher associated with CCW and fat thickness and lower associated with the carcass yield. Among the three alleles of leptin gene, only one showed effect with higher CCW, the others alleles were not associated with the carcass traits.
Supported by: FAPERJ, CNpq.

TEMPORAL AND SPATIAL GENE EXPRESSION ANALYSIS OF MAIZE *PSTOL1* HOMOLOGS IN TWO P CONTRASTING GENOTYPES

Negri, BF1,2; Azevedo, GC1,3; Lana, UGP1; Barros, BA1; Guimarães, CT1; de Sousa, SM1
1Embrapa Milho e Sorgo, Sete Lagoas, MG
2Universidade Federal de São João del-Rei, UFSJ, São João del-Rei, MG
3Universidade Federal de Minas Gerais, UFMG, Belo Horizonte, MG

Maize is generally considered to have a high fertility soil requirement, so the development of phosphorus-efficient maize genotypes would be beneficial in low-input agroecosystems and would improve the sustainability of high-input agroecosystems. Plants developed several mechanisms to adapt to low phosphorus (P) conditions, indicating that this is a complex trait. The main mechanism that has been implicated with increased P acquisition efficiency involves changes in root morphology. In this context, *Phosphorus-starvation tolerance 1* (*Pstol1*) was identified as the gene underlying the *Pup1* locus, which encodes a protein kinase responsible for enhanced early root growth, P uptake and, consequently, grain yield in rice. Recently, we performed comprehensive QTL mapping in maize recombinant inbred line population (RIL) in nutrient solution under low-P conditions and pointed out candidate genes as maize homologs (*ZmPSTOL1*, *ZmPSTOL4*, e *ZmPSTOL6*) to the rice *PSTOL1* (*OsPSTOL1*) based on QTL co-localization with root and P efficiency traits. In the present study, we aimed to verify the spatial and temporal gene expression of these maize *Pstol1* homologs in two P contrasting maize genotypes (L3 – efficient and L22 – inefficient). Gene expression was determined through quantitative PCR (qPCR-RT) using TaqMan assays in adult plant grown in pots with soil at the greenhouse and plantlets grown in nutrient solution (low P – 2,5 □M) in a paper pouch system. First, the temporal expression revealed that all genes start to express, in nutrient solution, at 7 days after germination (DAG) and had their peak of expression at 17 DAG. Expression profile of the candidate genes was assessed in different maize tissues (tassel, leaves, stem, seeds and roots) that were harvest during flowering, revealing that *ZmPSTOL1* and *ZmPSTOL6* were more expressed in roots and tassel of the inefficient line (L22) while *ZmPSTOL4* was more expressed in these same tissues but of the efficient line (L3). Receptor-like kinases comprise the largest family of receptors in plants and the diverse structures in the receptor domains suggest that there are likely to be several biological functions for these proteins. We also harvested different root parts (primary, lateral, non-embryonic seminal, embryonic seminal, crown) of L3 and L22 grown in nutrient solution at 17 DAG. These results showed that *ZmPSTOL1* and *ZmPSTOL6* were more expressed in all root types of L22 line and *ZmPSTOL4* was more expressed in L3 primary root, especially at the differentiation zone. These results shed a light on the illusive *Pstol1* pathway; however, further functional studies are required to comprehend the actual pathway leading to root system modulation by *Pstol1*.

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SBMATE GENE FROM SORGHUM INCREASES AL TOLERANCE IN TRANSGENIC MAIZE

Mitre, LK1,3; Silva, LF2,3; Barros, BA3; Carneiro, AA3; Magalhães, JV3; Guimarães, LJM3; Alves, VMC3; Guimarães, CT3.
1Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, UFMG, Belo Horizonte, MG
2Universidade Federal de São João del-Rei, UFSJ, São João del-Rei, MG
3Embrapa Milho e Sorgo, Sete Lagoas, MG

Aluminum (Al) toxicity severely limits plant development on acid soil. Under these conditions, the rhizotoxic form of Al (Al3+) is solubilized in the soil solution and inhibits root growth, reducing water and nutrient uptake. The major Al tolerance mechanism in plants consists on the Al-activated release of organic acid (e.g. malate and citrate) from root apex, forming non-toxic complexes with Al3+. Some genes responsible for Al tolerance has been identified and characterized, as is the case *SbMATE*, the major Al tolerance gene of sorghum that encodes an Al-activated citrate transporter. Considering that heterologous expression of *SbMATE* increased Al tolerance in Arabidopsis and barley, transgenic maize lines expressing *SbMATE* were generated. The events were originally obtained in the temperate hybrid Hi II, and subsequently introgressed in the elite line L3 from Embrapa Maize and Sorghum. Additionally, a major Al tolerance QTL was mapped in the maize chromosome 6 (*qALT6*) that harbor the *ZmMATE1*, a maize functional homolog to *SbMATE*. The objective of this study was to develop genetic and physiological analyses of maize lines and single-cross hybrids expressing *SbMATE*, as well as the combinations of both Al tolerance genes *SbMATE* and *ZmMATE1*. Al tolerance were measured as the relative net root growth (RNRG) in nutrient solution containing {39} and {60} μM of Al3+ activity. The root morphology was evaluated in acrylic boxes filled with limed soil in the surface and with acid soil with 15% of Al saturation in subsuperficial layer. The gene expression of *SbMATE* was obtained in different tissues and time after Al treatment using quantitative real-time PCR with the endogenous control of 18S RNA. Our results demonstrated that the transgenic L3 presented high expression levels of *SbMATE*, which was consistently induced in roots and root apices after 24 hours of exposure to Al of the treatment. Additionally, the transgenic line improved significantly the Al tolerance in nutrient solution and presented a superior root system on acid subsoil, when compared with the conventional L3. Maize hybrids carrying the *SbMATE* in heterozygosity were less tolerant to Al toxicity than the parental transgenic line, but more Al tolerant than the means of both parental lines, indicating a partial dominant effect of the transgene. The expression level of *SbMATE* in the hybrids was similar to the lines, at least for most of the genotypes evaluated, indicating that the transgene expression could not explain alone the reduction in the Al tolerance in the transgenic hybrids compared with the transgenic line. Altogether, our results indicate that transgenic maize with superior Al tolerance would be expected to improved grain yield and yield stability under acid soil conditions. Additionally, these results will be included in the biosafety process for the commercial release of this transgenic by CTNBio.

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FREQUENCY OF K232A POLYMORPHISM IN DGAT1 GENE FOR ½ AND ¾ BLOOD GIROLANDO BREED

Grillo, LT; Fonseca, P.A.S; Freitas, D.R[2]; Santos, F.C[1]; Rosse, I.C; Paiva, A.E[1]; Cerqueira, M.M.O.P[2]; Carvalho, M.R.S[1]

[1] Instituto de Ciências Biológicas- UFMG

[2] Escola de Veterinária – UFMG

Keywords: DGAT1; K232A; Girolando; Polymorphism

Introduction: The Girolando breed (Gir x Holstein) is the main dairy producer in Brazil. Thus, it has become the focus of studies involving genotyping the molecular markers used in the genetic improvement process. Genotyping of polymorphisms closely associated with the synthesis of milk in cattle is the main tool for marker assisted selection.

The DGAT1 gene is part of a Quantitative Trait Locus (QTL) with a significant effect on the percentage of fat, protein content and volume of milk production. DGAT1 was selected as the functional candidate for this QTL, due to its involvement in the catalysis of triglycerides. Furthermore, significant results were observed in studies linking polymorphisms in this gene with milk fat content in cattle. The main polymorphism of the DGAT1 gene is K232A. The K allele has been associated with increased production of protein and fat, but with a smaller final volume of milk production. The A allele is associated with increased production of milk and protein, but with a smaller amount of fat. However, the A allele is present with very low frequency in zebu individuals.

Due to the great importance of the K232A polymorphism and low frequency of the A allele in zebu breeds, the aim of this study is to understand how the degree of blood influences allele and genotype frequencies of this polymorphism in Girolando individuals.

Materials and methods: The K232A polymorphism of the DGAT1 gene was genotyped using a tetra-primer ARMS-PCR designed by our research group. DNA of 77 Girolandos (49 ½ blood and 28 ¾ blood) was obtained from peripheral blood. The allele and genotype frequencies, the expected and observed heterozygosities were calculated for the total sample (½ + ¾) and for the ½ blood and ¾ blood sub-samples. A chi-square test was also performed in order to test the Hardy-Weinberg equilibrium.

Results: The frequency of the K allele was 0.51; 0.58 and 0.41 for the total sample and for the ½ and ¾ blood groups, respectively. For the total sample and the ½ blood individuals, the less frequent genotype was AA, while for the ¾ blood animals, the KK genotype was less frequent. The highest genotypic frequencies were found for heterozygotic individuals, with higher heterozygosity found in the ½ blood animals (0.71). These results are probably explained by the lower frequency of the A allele in zebu breed. As ½ blood individuals have a larger fraction of the zebu alleles when compared to ¾ blood individuals lower frequencies of the A allele are expected. For the total sample and the sub-samples the observed heterozygosities were higher than expected, suggesting an excess of heterozygotes. Moreover, for the total sample, the genotypic frequencies weren't in Hardy-Weinberg equilibrium ($p < 0.01$).

The results obtained here, may be useful in marker assisted selection strategies against milk production in the Girolando and eventually for other bovine breeds; and, highlight the importance of taking into account the allelic frequencies in the crossings of the formation of different blood degree individuals, since the allelic frequencies are different among the degrees of blood.

MAPPING QTLS FOR ALUMINUM TOLERANCE IN A KENYAN MAIZE POPULATION

Guimarães, RGN1, Matonyei, T2; Tinoco, CFS3; Ligeyo, D4; Mendes FF5, Magalhães, JV6; Ouma, E2; Gudu, S2,7; Guimarães, CT6
1Universidade Federal de São João del-Rei, Sete Lagoas, MG – Bolsista PIBIC, CNPq
2Moi University, Eldoret, Kenyan
3Centro Universitário UNIFEMM, Sete Lagoas, MG – Bolsista BAT, FAPEMIG
4Kenyan Agriculture Research Institute, Kitale, Kenya
5Embrapa Milho e Sorgo, Sete Lagoas, MG – Bolsista Recém Doutor, CNPq
6Embrapa Milho e Sorgo, Sete Lagoas, MG – Pesquisador
7Rongo University College, Rongo, Kenya.
roberta.gra@hotmail.com
Keywords: root growth, nutrient solution, *MATE*, SNPs, *Nrat1*

Agriculture is responsible for one third of gross domestic product in Kenya, where 84% of the total country, consists of arid and semi-arid lands. Additionally, acid soils are commonly distributed in the maize growing areas, reducing the potential of grain yield. In these soils, aluminum (Al) is solubilized in the form of trivalent cation (Al3+) inhibiting root growth, which limits by preventing the plant to obtain water and nutrient acquisition by the plants. Thus, our goal was to dissect the genetics of Al tolerance in 180 F2:3 families derived from a cross between Kenyan maize lines contrasting for Al tolerance, 203B-14, highly tolerant, and the Al-sensitive SCH3. Al tolerance was evaluated using the relative net root growth (RNRG) in nutrient solution with {39} μM Al3+ activity compared to the root growth without Al, after five days of treatment. Variance analysis of phenotypic data was performed using two common checks in six experiments with three replicates using Proc GLM (SAS Institute), revealing a high genetic variability, low coefficient of variation (8.82%) and high heritability (97.07%). A total of 152 SNP markers were genotyped in the population using KASP assay by LGC Genomics. These, 132 polymorphic markers were ordered along the 10 linkage groups and covered 1164.7 centiMorgans (cM) of the maize genome, with an average of 9 cM of interval between markers. Five QTLs for Al tolerance were mapped on chromosomes 1, 5, 8, 9 and 10, explaining together approximately 47% of the phenotypic variation for RNRG, including the epistatic effects. The contribution of each QTL ranged from 4 to 9.7%. New genomic regions associated with Al tolerance were identified on chromosomes 1 and 9, whereas the QTLs mapped on chromosomes 5, 8 and 10 were previously detected by other studies. Interestingly, no association was detected on chromosome 6, where the *ZmMATE1* was previously showed to underlie a large-effect Al tolerance QTL. However, the *ZmNrat1* was mapped within the QTL region on chromosome 5. *ZmNrat1* is a homolog to *OsNrat1*, which encodes an Al3+ specific transporter involved in rice Al tolerance. Thus, we revealed that this Kenyan maize line may harbor new QTLs and candidate genes for Al tolerance that can be further validated and used for breeding purposes.

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V Encontro de Genética de Minas Gerais

V ENGEMIG

Pesquisa e Pós-Graduação

Modelos animais de doenças humanas

ALCOHOLISM – IDENTIFICATION OF MOLECULAR TARGETS BY ANALYSIS OF TRANSCRIPT LEVELS OF AN ALCOHOL ADDICTION MODEL

Silva-e-Silva, D.A.1; Ribeiro, A.F.1; Rocha, C. S.2; Boerngen-Lacerda, R.3; Godard, A.L.B.1
(1) Federal University of Minas Gerais, Belo Horizonte, MG, Brazil
(2) Faculty of Medical Science, University of Campinas (UNICAMP), Campinas, SP, Brazil
(3) Federal University of Paraná, Curitiba,PR, Brazil
danielsilvasilva@hotmail.com.br

Introduction

Ethanol is a psychotropic drug whose consumption is allowed and even encouraged. Although genetic susceptibility to alcoholism is known to be an important factor, the molecular mechanisms remain to be elucidated. Thus, we propose the study of the transcriptome of a model exposed to a free choice treatment, established by Fachin-Scheit et al., 2006. The procedures employed in this study comply with the Universidade Federal de Minas Gerais guidelines of the Ethics Committee on Animal Experimentation (protocol number 159/2007).

Methodology

Heterogeneous Swiss mice were housed individually and exposed to a three-bottle free-choice self-administration treatment consisting of four phases: 1. free choice (10 weeks) from water, ethanol 5% or 10% v/v; 2.Withdrawal from ethylic solutions (two weeks); 3.Reexposure (2 weeks); 4. quinine-adulteration (2 weeks). A control group (C) had access only to water. Later, mice were behaviorally into three distinct groups: "Addict" (A: preference for ethanol and high levels of consumption during all phases); "heavy drinker" (P: preference for ethanol and high levels of consumption during AC and reduction in AD); "light drinker" (L: preference for water during all phases). Through the microarray technique, we compared the transcript levels between Heavy and Addict mice in striatum, a brain area related with addiction and reinforcement, followed by qPCR validation in some selected candidate genes.

Results

Microarray analysis showed 826 genes with transcript difference between Addict and Light group. The most part these genes is related with important process linked to alcoholism, like dopamine, GABA, and glutamate receptors, and genes related with alcohol metabolism in liver.

Enrichment analysis in that gene list showed classic pathway related with alcoholism too, like Dopamine D1A receptor signaling, Regulation of CDK5 in CNS, and Delta- and kappa-type opioid receptors signaling via beta-arrestin.

A pathway called Lrrk2 was chosen to future analysis. This pathway is related with Parkinson's disease and execute specific functions in phosphorylations in specific targets, and contribute to the synaptic vesicles reuptake. We hypothesized that this pathway can be disturbed in addicted animals, and could contribute to neuroadaptations seen in this addict animals.

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POSSIBLE INFLUENCE OF HYPERCALORIC DIET IN THE INGESTION OF ETHANOL AND ITS RELATION ON MOLECULAR MECHANISMS OF THE REWARD SYSTEM

LM Carvalho1, ASB Pedersen 1, IM Menezes 1, RE Moreira Júnior 1, D Correia 1, DA Silva e Silva1, ALB Godard 1

1Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Biologia Geral, Laboratório de Genética Animal e Humana.

The reward pathway has an important role in behavior's motivation, working as a core where several chemical messengers work together to release dopamine. This brain circuit is associated with the pleasure triggered by natural rewards, such as food, or artificial reward, such as ethanol, and it is also the neural basis for addiction and compulsion phenomena. Both ethanol and food have positive reward effects, which increase the probability of a certain behavior in the presence of the reward, and this is partly mediated by the increase of dopamine in this reward system. Knowing that the consumption of ethanol and a palatable diet is regulated by the reward system, our hypothesis is about the possibility of the consumption of such diet change the pattern of consumption of ethanol, due to a previous change in this system, which may be involved with mechanisms of gene regulation. To test our hypothesis, it has been done two experiments, the first with n= 20 mice C57BL/6 and the second with n=20 mice BALB/c, divided into the following groups: Group 1 (AING+H2O), Group 2 (AING+ET-OH), Group 3 (HSF+ H2O), Group 4 (HSF+ ET-OH) e Group 5 (HSF constant + ET-OH), in which AING consists in a pattern diet, with normal percentages of sugar and lipids, and HSF consists in a diet rich in sugar and lipids. In the first stage of the experiment, that lasts 4 weeks, the animals were divided into the 5 groups described above, which group 1 and group 2 received AING and groups 3, 4 and 5 received HSF, all of them had free access to water. In the second stage, that happened in the next four days after the end of the fourth week, groups 1 and 2 were still receiving AING and group 4 and 5 switched their diet from HSF to AING. Group 5 was constantly receiving HSF. Additionally to that, we performed the drinking in dark protocol in groups 2, 4 and 5, which consists in replacing the water drinking with water by another one with a 20% of ethanol solution. The replacement occurred during the four days about three hours after the dark cycle. In the first three days the water drinking with the 20% of ethanol solution remained for 2 hours, and in the fourth day it remained for 4 hours. In the fourth day after the 4 hours of ethanol access the mice were euthanized. With the data of ethanol ingestion obtained until now, it was observed that the groups that were treated with HSF diet showed a higher tendency of consuming ethanol when compared with groups treated with AING diet. However, it is necessary to increase the n of our sample to prove our hypothesis and demonstrate in a molecular level the alterations in the pattern of gene regulation involved in the reward system.

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DIFFERENTIALLY EXPRESSED GENES IN HIPPOCAMPUS OF WAR - ANIMAL MODEL OF AUDIOGENIC SEIZURES

Damasceno, S 1; Silva e Silva, DA 1; Martins, SF 2; Matos, AHB 3; Lopes-Cendes, IT 3; Martins, AS 2; Godard, ALB 1.
1 Departamento de Biologia Geral, UFMG, Belo Horizonte - MG.
2 Departamento de Fisiologia e Biofísica, UFMG, Belo Horizonte - MG.
3 Departamento de Genética Médica, UNICAMP, Campinas - SP.

The rat lineage WAR (Wistar Audiogenic Rats) is a genetic model of audiogenic seizures, established from colonies of Wistar rats that showed sensitive animals to sound stimulus. When submitted to high intensity sound the WAR animals exhibit generalized tonic-clonic seizures that require the activation of auditory pathways of the brainstem, characteristics of acute seizures. However, by repetitive sound stimulation occurs a phenotypic change in the pattern of seizures involving the recruitment of limbic structures such as amygdala, neocortex and hippocampus mainly. Our research group, by studying the gene expression profile by microarray identified several differentially expressed genes in the hippocampus of WAR animals compared to Wistar controls, including Gabra2 (Gamma-aminobutyric acid receptor subunit alpha 2) Gabbr1 (Gamma-aminobutyric acid B receptor, 1) and Bdnf (brain-derived neurotrophic factor), both selected for validation by real time quantitative PCR. For this, WAR and Wistar animals were subjected to sound stimulation (~120dB) for one minute at 70, 74 and 78 days of age and evaluated by severity index (IS). Were selected WAR animals with IS 0,85 and controls that did not respond to the stimulus. The collect of brain structures was made after 82 days of life. The RNA was extracted from the hippocampus of WAR (n=5) and Wistar rats (n=6), submitted to RT-PCR for cDNA synthesis. The qPCR reactions were performed in the CFX 96TM Real Time system (BioRad) equipment using the intercalating agent SYBR® Green PCR Master Mix (Applied Biosystems). The reference genes were Gdi1, Rps26 and β-actin and the primers were tested and showed amplification efficiency ~100%. The gene expression data were normalized according to the methodology of Vamdesompele and cols, and the difference in expression between transcripts was evaluate by test t student using the GraphPad Prism *5.0* program. The Gabra2, Gabbr1 and Bdnf and genes were validated as differentially expressed in the hippocampus of animals WAR compared to control, both downregulated. In this model is described the reduction in Gabaergic inhibitory chain, that may be related to low amount of transcripts specific subunits of GABA receptors. Although Bdnf gene has been shown upregulated during the epilepsy development, in WAR hippocampus this gene was downregulated.
Funding agency: FAPEMIG, FAPESP e CAPES.

CONNECTING PHENOTYPE TO GLYCOTYPE - ANALYSIS OF COG MUTATIONS IN *DROSOPHILA MELANOGASTER*

Erick Silva, Rita Sinka, John Sparrow, Daniel Ungar Department of Biology, University of York, York, and Department of Genetics, University of Szeged, Hungary; Capes: Science without Borders

The Conserved Oligomeric Golgi (COG) complex mediates retrograde tethering of vesicle within the Golgi apparatus, maintaining the unique enzymatic profile of each cisterna, required for the specific glycosylation of proteins. In the light of the fact that excess lobe A (COG2-4 subunits) may inhibit lobe B (COG5-8 subunits) mediated tethering at the trans-Golgi, the altered glycan profile of *Drosophila melanogaster* COG mutants and consequent phenotype were studied. Indeed, COG2 suppression, not apparent in the sterility, showed some possible signs in the flight and lethality tests. In the former, sterility tests showed that homozygous *fws* (*Drosophila* COG5 homologue) mutation flies, which are sterile, did not change with COG2 mutation. Secondly, 3-5 days old flies were released in the middle of a box with a light source on the top and the flight scored to the direction. COG mutation showed to affect flight; mainly the *fws* mutation. A genotype that flew better with COG2 mutation supported the rescue of the flight phenotype by releasing COG2 suppression when it was mutated. To test lethality, *fws* homozygous and heterozygous flies and larvae were counted in the presence and absence of COG2 mutation. It was observed an increase in the number of *fws* homozygous flies in the COG2 mutant, but this was absent in the larva stage, pinpointing the COG involvement in the pupal stage. In addition, N-glycan profile of whole flies (WT and fwsCOG2 mutant), head and thorax were generated by mass spectrometry (MS), after Filter-aided N-glycan separation (FANGS). N-glycans were predominantly oligomannose and paucimannose; Fuc1Hex3HexNAc2 and Hex5HexNac2 were increased in the mutant. Glycans found in higher levels or only in specific segments uncovered a possible glycan role in the respective body section. Finally, O-glycan profiles of the WT and COG3 and *fws* mutant were generated by MS of in gel released glycans from glue glycoproteins. The comparisons outlined some differences and the glue secretion showed to be useful for O-glycosylation studies.

MOLECULAR IDENTIFICATION OF AN ENU-INDUCED MUTATION RESPONSIBLE FOR A MICE NEUROMUSCULAR PHENOTYPE

Marques, Y. E. S.[1]; Silva-Silva, D.A[2].; Massironi, S. M. G[3]; Guénet, J.L.[4]; Godard, A. L. B.[2]
(1) Faculdade de Minas Gerais, MG (FAMINAS-BH)
(2) Universidade de Minas Gerais, MG (UFMG)
(3) Universidade de São Paulo, SP (USP)
(4) Instituto Pasteur, França

Introduction: Weakness mutation was generated by treatment of BALB /c mice with the mutagen ENU, ethyl nitrosourea. The mutation is inherited in an autosomal recessive manner and is characterized by progressive loss of muscle strength and motor coordination in tail and paws. The situation is observed from the first half of life and the animal dies around the twelfth week.

Methodology: For the purpose of genetic mapping, BALB /c animals heterozygous for the weakness mutation were crossed with C57BL /6 normal generating F1 animals, which were intercrossed generating recombinant F2 animals. To identify the mutation, it was chosen the sequencing of cDNA of all isoforms, thus covering the entire coding region of candidate genes. The expression analysis was made using real-time PCR.

Results: Linkage analysis was performed with recombinant animals by polymorphic microsatellite markers for both strains (BALB / c and C57BL / 6) and the mutation was mapped in chromosome 1 between D1MIT373 (10.92 cM) and D1MIT320 (18.64 cM) markers, delimiting a region of 7.72 cM, region where the Bpag1 gene is.

The gene encodes 107 exons spanning approximately 400 kb, is located at 12.91 cM and is responsible, among other functions, the integrity and organization of cellular architecture. The gene is introduced into four major isoforms: Bpag1a, Bpag1n, Bpag1b and Bpag1e being expressed in brain (1a, 1n), muscle and skin, respectively. The different isoforms are sourced by alternative splicing and perform similar functions in their respective tissues. The Bpag1a isoform was selected for preliminary analysis for being widely expressed in the nervous system and directly involved in muscular dystonia, a neurodegenerative disease in mice which is genetically very similar to the phenotype displayed by the weakness animal.

About 90% of these regions have been sequenced and no molecular alteration was observed so far, but analysis of gene expression by real-time PCR found a significant difference in neuronal isoform transcripts, Bpag1a, in weakness mice when compared with the control group.

Quantitative analysis of muscle and epithelial isoforms will be held to enrich the current data in parallel with continued sequencing of the Bpag1, to confirm the gene as responsible for the weakness phenotype.

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