HUMAN GENOME RESEARCH CENTER (HGRC)
Instituto de Biociências
Universidade de São Paulo

FAPESP/CEPID 98/14254-2

Coordinator: Mayana Zatz

REPORT - 2006

HUMAN GENOME RESEARCH CENTER (HGRC)
Instituto de Biociências
Universidade de São Paulo

REPORT - 2006

HUMAN GENOME RESEARCH CENTER (HGRC)
Instituto de Biociências
Universidade de São Paulo

Part 1. RESEARCH REPORT

1. SUMMARY

Our proposal for 2006 - 2008 was focused on two main projects:
a) Exploration of the causes and clinical variability of genetic disorders by the dissection of the genetics and architecture of the human genome.
b) Assessment of different approaches for future treatment and/or therapy of human genetic diseases, particularly neuromuscular disorders and craniofacial syndromes, focusing mainly on the potential of stem cells, from different sources to differentiate in specific tissues. Gene therapy approaches were also included.

2. RESULTS

I. INVESTIGATION OF GENETIC FACTORS UNDERLYING HUMAN DISEASES

I.1 IDENTIFICATION OF DISEASE GENES

One of the main goals of the CEPID project is to identify genes associated with human genetic disorders, particularly neuromuscular and developmental disorders. The general approach used to achieve this goal is linkage and positional cloning for Mendelian disorders where large families have been identified or association studies for complex disorders. Our progress in this last year regarded to this major aim is detailed below:

NEUROMUSCULAR DISORDERS

A) Linkage analysis in a new family: We have identified a new genealogy from Montevideo, with many patients affected by an autosomal dominant (AD) form of muscular dystrophy with some new clinical characteristics (distal weakness in lower limbs, cataract and face atrophy). Linkage analysis allowed mapping it to the region 4q21 where our group has recently mapped a novel form of AD-LGMD, named LGMD1G. Interestingly, patients from this new family have a different phenotype from those described as LGMD1G, which could be explained by the existence of a) a new gene in close linkage or b) a different mutation in the same gene. Gene identification is underway.
B) Intrafamilial variability in patients with the same mutation: Molecular analysis in a sibship with three sisters affected by the autosomal recessive form of LGMD (LGMD2I) caused by mutations in the FKRP gene (fukutin related gene) and a discordant phenotype revealed the underlying responsible mechanism in this family. FKRP gene sequencing showed that all three sisters carried a nonsense paternal mutation (W225X). The two oldest sisters with a severe phenotype carried two maternal mutations V79M and P89A. However, the youngest sister with a milder course carried only the V79M mutation, due to an intragenic recombination. (Vieira et al., 2006; these results were presented as late breakthrough in the 10th International Congress of the World Muscle Society.

In addition we have identified a mutation in the VAP-B gene, which has been shown to cause amyotrohic lateral sclerosis type 8 (ALS8) in a patient with a limb-girdle presentation. This case, presented in the international congress of the world muscle society (Lazar et al., 2006, manuscript in preparation) illustrates once more genetic heterogeneity in patients with a similar clinical course.
C) Identification of new mutations: In central core disease (CCD), a predominantly autosomal-dominant congenital myopathy, we identified two out of nine Brazilian CCD families, displaying

autosomal recessive inheritance. The patients were mildly affected, differing from the few autosomal-recessive (AR) cases described previously. AR inheritance in CCD may therefore be relatively common, which has important implications for genetic counseling and prevention of malignant hipertermia in affected families. In addition, mutations in the Dynamin 2 gene, which was recently identified, were found in 3 among 6 Brazilian families with Centronuclear Myopathy.

DEVELOPMENTAL DISORDERS

A) Syndromic mental retardation: We identified a *UBE2A/HR6A* mutation as the cause of a novel X-linked mental retardation syndrome (Nascimento et al., 2006). This is a gene of the ubiquitin proteasome pathway, encoding a conjugase. Angelman syndrome is the other known mental retardation syndrome that is caused by mutation in a ubiquitination gene, a ubiquitin-ligase. These findings demonstrate the involvement of altered protein ubiquitination in neurodevelopmental disorders. We are now undertaking functional studies on: (a) expression of the UBE2A normal isoforms in different adult tissues, including brain; (b) effect of the detected mutation on transcription, protein synthesis and cellular localization in lymphocytes and fibroblasts from carriers; (b) functional complementation of yeast *ΔRAD6* mutation (conserved ortholog) by normal and mutated human isoforms.

B) Auriculo-condylar syndrome (ACS): It is an autosomal dominant condition that involves structures derived from the first and second branchial archs. We have screened the genome with microsatellite markers in one out of two large ACS families and evidence of linkage was identified. Interestingly, the other family seems unlinked to this region, suggesting locus heterogeneity

C) Syndromic forms of craniosynostosis (SC): This is a very heterogenous group of disorders in which the etiology is known in less than 50% of the cases. We analyzed 47 patients with SC, by karyotyping, MLPA and comparative genomic hybridization. A total of 22 possibly pathogenic chromosomal rearrangements were identified. Eleven of these were microdeletions or duplications not detected through karyotype analysis. These results show that small chromosomal rearrangements are an important cause of SC. Part of these results were published (Jehee et al., Am J Med Genet 139A:221-226, 2005; Krepischi-Santos et al., 2006). We have also tested positional and functional candidate genes for SC in patients without any cytogenetic alteration; to date, no pathogenic mutation was identified (Jehee et al., 2006).

D) Syndromic obesity (obesity in association with phenotypic abnormalities and mental retardation) can represent a diagnostic challenge, because of the overlap of the phenotypes. We have reported cytogenetic and genetic studies, including screening of *S1M1* gene deletions, on 87 patients with a PWS-like phenotype. We also described the fifth case of syndromic obesity with an interstitial deletion of chromosome segment 6q16.1-q21 and established the contribution of this chromosome segment to the etiology of syndromic and non-syndromic obesity (Varela et al., 2006). The investigation of 1p36 deletion in 41 patients with delayed psychomotor development, hypotonia, obesity and /or hyperphagia, learning disabilities and behavioral problems, pointed out that the presence of a deletion of the chromosome segment 1p36.33-1p36.32, (between 2.4 and 2.48 Mb pter), smaller than usually seen in monosomy 1p36 patients, is a critical region for the manifestation of obesity and hyperphagia (D'Angelo et al., 2006). We are currently investigating patients with syndromic obesity with a specifically designed set of probes for testing subtelomeric chromosome imbalances, and we are planning to screen these patients with a SNP array to detect chromosome regions responsible for obesity.

E) Autism: This is a complex disorder, and a multifactorial inheritance is the most likely model to explain the involvement of genetic factors. We analysed four SNPs and one microssatelite within *HTR2C* gene in 200 controls, 220 patients and their mothers. A case-control analyses and TDT are being conducted.

F) Deafness: Oculocutaneous albinism and deafness have been previously described as a possible syndrome (OMIM 22090); in a Brazilian family, we demonstrated the independent segregation of these phenotypes, and the coincidental inheritance of the two disorders (Lezirowitz et al., 2006).

In a family, with a high level of inbreeding and presenting with autosomal recessive deafness, two novel *MYO15A* mutations were identified, in homozygosis and compound heterozygosis, thus characterizing allelic heterogeneity within one pedigree. In a large family with non-syndromic autosomal dominant deafness, we identified a new candidate region, on chromosome 2; the search for the mutated gene is under way.

We determined the prevalence of the A1555G (12SRNA) and tRNAser(UCN) mitochondrial mutations in hearing-impaired Brazilian patients (Abreu-Silva et al., Bioch 2006a and 2006b).

G) Ectrodactily/tibial hypoplasia syndrome: In a large pedigree with individuals affected by autosomal dominant ectrodactily associated to tibial hypoplasia , we mapped the candidate region to chromosome 17, the first mapped locus for syndrome. The search for the mutated gene is underway.

H) Syndromes associated with balanced translocations: Mapping of the breakpoints in two apparently balanced translocations led to the identification of candidate genes for Silver-Russel and Nager syndromes.

I) Sotos syndrome: Forty patients with clinical diagnosis of Sotos syndrome were investigated for submicroscopic deletions. Two patients, one with a partial deletion and the other with a complete deletion of 5q325.2-5q35.3 were identified. The search for mutations in *NDS1* gene is underway.

I.2 MECHANISMS MODULATING PHENOTYPIC EXPRESSION

A. ***A) Characterization of regulatory regions related to genes associated with developmental disorders:*** *We are also developing projects to elucidate regulatory regions and pathways related to* TCOF1, COL18A*1 and* FGFR2 *genes.*

A1) TCOF1: Our proposal was to verify if SNPs in the promoter region of *TCOF1* in trans with a primary pathogenic mutation contribute to the clinical variability of Treacher Collins syndrome (TCS), an autosomal dominant condition caused by haploinsufficiency of treacle, the protein product of *TCOF1* gene. We have characterized the promoter region of *TCOF1* and characterized a functional SNP in this region (Masotti et al., Gene 359:44-52, 2005). Unfortunately the number of TCS families and the low frequency of this SNP did not allow testing our hypothesis. We are currently testing other genetic (methylation) and environmental factors (retinoic acid) that might contribute to different expression levels of *TCOF1*. A database for *TCOF1* mutations has been constructed and is available at our website.

A2) COL18A1: We have characterized the second promoter region of *COL18A1* and found a functional SNP in this region (Armelin-Correa et al., Matrix Biol. 24:550-9, 2005). Further characterization of this promoter region is showing that CEBPalpha, a transcription factor associated with hepatocarcinoma, is also involved in the expression control of *COL18A1* gene. This project is being done in collaboration with Dr. Musso, France. In addition, the data obtained from our research on *COL18A1* over the past 5 years were recently published (Passos-Bueno et al., 2006, Review).

A3) FGFR2: Microarray analysis using RNA from 7 patients with Apert syndrome (AS)(mutation in *FGFR2 gene*) and 7 control individuals revealed an expression signature in Apert syndrome cells. The genomic analysis is being done in collaboration with Dr. Sergio Verjovski-Almeida and Dr. Eduardo Reis (IQ, USP). Besides, we have observed that this mutation seems to maintain the undifferentiated state of the fibroblastoid cells. The AS cells constitute a more homogeneous population with higher percentage of mesenchymal stem cell markers expression and present a higher potential to differentiate into bone tissue as compared to fibroblast cells without FGFR2 mutation (manuscript, in preparation). In an attempt to understand why this FGFR2 mutation is associated with both higher proportion of mesenchymal stem cells and higher potency

for bone differentiation, we will proceed towards better characterization of these cells at the molecular and cellular levels. Along with the expression profile studies, we are searching new mutations associated with unusual craniosynostosis syndromes phenotypes and we have recently reported a FGFR2 mutation associated with a severe form of craniosynostosis and sacrococcygeal eversion (Oliveira et al., 2006).

B) Clinical variability in facioscapulohumeral muscular dystrophy (FSHD): FSHD is an AD disorder characterized by great intra and interfamilial variability. Other groups and ours have reported that a significant proportion of mosaic carriers of the FSHD deletion are asymptomatic. Variability may be related to mosaicism being present only in peripheral blood lymphocytes (PBL) and germline, but not in other tissues such as muscle. In order to address this issue we compared different tissues (PBL, muscle and fibroblasts) from an asymptomatic mother of a patient with FSHD who was a mosaic carrier in PBL, but did not find any significant difference which could explain intrafamilial variability or why some FSHD carriers remain asymptomatic throughout life (Tonini et al., 2006). The next step attempting to understand phenotypic variability in this disorder will focus on metylation analysis comparing clinically affected and asymptomatic patients as well as expression microarray studies in collaboration with Dr. Sergio Verjovski-Almeida.

C) Clinical variability of fragile X-associated tremor ataxia syndrome *(FXTAS)*: The clinical manifestations of FXTAS are under investigation in fragile X families. We described unusual presentations of the syndrome: FXTAS radiological typical findings (a) in the absence of neurological manifestation (Capelli et al., Mov Disord, accepted for publication), or (b) associated with severe cognitive decline, but no neuromotor involvement (manuscript submitted).

D) Clinical variability in Robinow syndrome: Robinow syndrome is a genetically heterogeneous condition characterized by mesomelic limb shortening associated with facial and genital anomalies that can be inherited in an autosomal dominant or recessive mode. We characterized these two variants clinically, with the aim of establishing clinical criteria to enhance the differential diagnosis between them or other similar conditions. The manuscript has been accepted for publication (Mazzeu et al., Am J Med Genet, accepted for publication).

I.3 PROTEIN AND FUNCTIONAL ANALYSIS

A) Proteins involved in axonal guidance: Yeast two hybrid analysis have been used to identify proteins that might interact with the proteins collagen XVIII and collybistin, both involved in axonal guidance. The screening for the N-terminal region of COLl8A1 did not reveal any candidate interacting protein. On the other hand, three candidate collybistin binding partners have been identified through the first screen and further confirmed using the yeast two- hybrid approach. We are currently using other methods to confirm these potential interactions.

B) Protein in neuromuscular disorders:

B1) Characterization of protein complexes: A new multiplex DNA screening test for the more common mutations in the Brazilian population was established (Gouveia et al., 2006) and allowed the identification of a new patient with partial deficiency of only SG, and a milder clinical course (Gouveia et al., submitted). We are currently analyzing the possibility that over-expression of SG, might replace the missing protein. The multiplex method also allowed us to identify a new patient with a large deletion in the gama-SG gene. The analysis of this case opens a new avenue in the elucidation of the interaction among these complex of glycoproteins. The mechanism of glycosilation of alpha-dystroglycan in the muscle of patients with mutation in the FKRP gene is under analysis, with the study of galactins 1 and 3 in muscle biopsies from affected patients. The results were presented in the last meeting of the WMS.

B2) Proteins involved in muscle development: For the analysis of the degeneration/regeneration pattern as a control for cell therapies, the pattern of expression of the following genes in affected muscles was already standardized: TGF-B1, pro-collagen 1 and 2, Peptidil-prolil-isomerase A, telethonin (internal muscle control). The controls for regeneration

MyoD, and MYF5 were previously standardized in humans, and are being now standardized in mice.

B. **B3) Myostatin and muscle differentiation:** *A polyclonal antibody for myostatin was produced in rabbits, using a synthetic peptide. The first experiments using this antibody revealed a weak reaction, and different procedures for its purification and concentration are ongoing. A preliminary analysis of Myostatin expression in different muscles from one affected GRMD dog is showing a very low expression of this protein in all studied muscles, as well as in normal control.*

B4) Functional and structural analysis of mutations at the protein level: The cloning and expression (in Escherichia coli) of several domains of FKRP was performed. in collaboration. One fragment, from the C-terminal region was already expressed, and isolated in the insoluble fraction. This protein will be used for the generation of an antibody. Now, we are changing the expression conditions, mainly decreasing the temperature in which bacteria is cultivated, and host of bacteria strain, to try to improve the expression of the remaining fragments. Besides FKRP, recombinant telethonin is being expressed for structural analysis, and different domain of myostatin are being cloned, for structural analysis and co-transfection studies.

B5) Protein analysis in amyotrophic lateral sclerosis type 8 (ALS8): Our group has previously mapped and identified a new gene, VAP-B responsible for an autosomal dominant form of amyotrohic lateral sclerosis with great clinical variability in progression rate. All the Brazilian patients identified to date have a missense mutation, P56S, in the MSP (major sperm domain) of the VAP-B gene. *In vitro* analyses of the consequences of P56S mutation in human VAP-B protein were performed, in an attempt to correlate them with the mechanisms responsible for ALS8. The results demonstrate that P56S mutation does not seem to be related to a modification of MSP–VAP-B's state of oligomerization, but rather with the interactions between VAP-B and other cellular proteins, mainly tubulin and GAPDH (glyceraldehyde-3-phosphate dehydrogenase). Interestingly these two proteins have been previously related to other forms of neurodegenerative diseases and are potential key points to understand ALS8 pathogenesis and other forms of Motor Neuron Disease. The results of this investigation were submitted to publication (Mitne et al.).

C) Fragile X Mental Retardation Protein: Using a polyclonal antibody (3460) raised in rabbits against a peptide encoded by *FMR1* exon 12, we identified on Western blots of embryonic (E20) rat brain FMRP migrating as 50-, 60-, or 70-kDa bands. According to the presence or absence of the segment encoded by exon 14, which codes for FMRP nuclear export signal, the observed bands may correspond to nuclear (50 and 60 kDa) or cytoplasmic (70 kDa) isoforms. Cell fractionation experiments confirmed the subcellular distribution of those isoforms and additionally revealed a 28-kDa peptide enriched in supernatant fractions of posnatal brains. Preliminary testing suggests that the 28-kDa peptide is unrelated to FMRP, and its expression is regulated during brain aging.

D) Functional studies to evaluate oxidative stress in patients with hearing deficiency: In order to investigate the possible role of oxidative stress in the phenotypic expression of hearing deficiency, the establishing of an assay to measure the levels of peroxides in plasma, serum and lymphoblastoid cell lines is under way.

I.4 COMPLEX DISORDERS AND GENETIC VARIATION AT THE POPULATION LEVEL

A) Genetic basis of noise induced hearing loss: A sample of 234 individuals similarly exposed to noise were investigated, 93 of them affected by noise induced hearing loss. Common mutations already related to deafness were excluded, and association studies are being performed with polymorphisms in *GJB2* gene, and in *GSTM1* and *GSTT1* genes, both related to protection against oxidative stress. No significant associations were detected so far, but a significant excess of familial history of hearing loss was found in the affected group, suggesting that it may represent a risk factor for noise induced hearing loss.

B) Genetic factors associated to hypertension and obesity in Afro-Brazilian partially isolated population: 434 individuals were tested for polymorphisms in *ACE*, *eNOS* and *GNB3* genes, previously related to hypertension. Case–control analysis suggests an association between high blood pressure and the I/D *ACE* polymorphism in men, and C825T *GNB3* polymorphism in women. *LEP*, *LEPR*, *ADBR2*, *PPARG*, *PLIN* and *RETN* polymorphic sites previously associated to obesity were studied in 550 individuals, but case-control analysis revealed no significant association between genotypes and Body Mass Index (Mingroni-Netto et al., 2006 Am J Phys Anthropology, abstract).

C) Diabetic Retinopathy (DR): We have tested if SNPs mapped within genes in angiogenesis or vascular pathways are involved with diabetic retinopathy. We identified an association between a SNP in the promoter region of *VEGF* and diabetic retinopathy (Errera et al., 2006; manuscript submitted for publication) but not with SNPs at the *MTHFR* gene (Errera et al., 2006). We are currently concluding statistical analysis of SNPs at the *COL18A1* and *DR* genes.

I.5 GENOMIC ARCHITECTURE

A) Clinical impact of DNA sequence copy number variation

Array-CGH has been introduced and is being applied to the identification of chromosome regions relevant for the diseases in which our Department is reference. Until now, 160 syndromic individuals have been or are being investigated by array-CGH. These individuals were selected for presenting at least one of the following features associated to additional clinical signs:

- Mental retardation and negative for fragile-X syndrome (49 individuals).
- Craniosynostosis, negative for mutations in the *TWIST*, *FGR1*, *FGFR2* and *FGFR3* genes, and for deletions at *TWIST*, 9p22-24 and 11q23 regions (27 individuals).
- Short stature, hypoplastic genitalia and hypertelorism (autosomal dominant Robinow syndrome features) (14 individuals).
- Mental retardation, obesity and normal methylation pattern for the PWS region (11 individuals).
- Utero-vaginal aplasia with renal defects and negative for mutations in the *RAR-gamma*, *RXR-alpha*, and *WNT-4* genes (14 individuals).
- Heart defects, negative for 22q11.21 deletions by satellite marker analysis (25 individuals).
- Deafness, negative for mutations in *GJB2* (35delG e 167delT screening tests and SSCP), *GJB6* (delGJB6D13S1830 and delGJB6D13S1854) and the mitochondrial A1555G mutation (20 individuals).

Results from this investigation published in 2006: Cheroki et al.(2006), Krepischi-Santos et al. (2006), Kriek et al. (2006), Kriek et al. (in press), Rosenberg et al. (2006); Shaw-Smith et al. (2006), Varela et al. (2006a).

B) Evolutionary Aspects: Knowledge about variability and structure of CGG repeat of the *FMR1* gene (fragile X syndrome) in non-human primates could assist in the understanding of the repeat evolution, as well as in evidencing factors involved in its stability in humans. We have preliminary data on size and pattern of interruption of the *FMR1* CGG repeat in a sample of 183 non-human primates: 17 Catarrhini (Old World) specimens belonging to six genera, and 166 Platyrrhini (New World) specimens, belonging to 13 genera. The repeat size was determined in 17 Catarrhini and 106 Platyrrhini specimens. The repeat sizes in Catarrhini were closer to the human distribution than

those in the majority of Platyrrhini. The pattern of interruption of the CGG repeat was determined in four *Cercopithecus* (Catarrhini) and 102 Platyrrhini specimens. In the majority of genera, the repeat was short and uninterrupted. Among Platyrrhini, *Aotus* and *Lagothrix* presented CGG repeats interrupted by "G"; in *Saimiri*, CAG and CGA triplets occurred at the 5' end and at the middle of the sequence, respectively; in *Ateles* the CGG repeat was interrupted by AGG, a triplet so far observed only in humans and great apes. Interestingly, in this genera, differently from others Platyrrhini, we observed alleles in the human gray zone/permutation range - a male with $(CGG)_{43}$ and interruption pattern 9+1+5+25 ("+" = AGG) and a female with 43/60 CGG triplets. We are concluding the study of Platyrrhini genera.

II. DEVELOPMENT OF FUTURE THERAPEUTIC APPROACHES TO GENETIC DISEASES

Use of stem cells or other cellular types are being conducted with the future aim to ameliorate the phenotype in two groups of disorders, neuromuscular and craniofacial disorders, or to be used in therapeutic trials for induced deafness.

II.1 STEM CELL RESEARCH

A lot of effort has been put in stem-cell research by the group, which will probably result in publications in the next two years. Regarding human adult stem cells, during this first year we have established the conditions for culturing and characterize adult stem cells from different sources such as cord-blood, lipo-aspirate and dental pulp.

A) Neuromuscular Disorders

1) Human adult stem cells

Preliminary results attempting to differentiate adult mesenchymal stem cells, in muscle cells "in vitro" and "in vivo" have shown that :

a) Mesenchymal stem-cells from human cord blood have shown little potential to differentiate in muscle cells "in vitro". However, these cells were able to differentiate into myotubes and express dystrophin, after injected in the mdx mouse model, that is, only after exposure to *in vivo* muscle environment (Nunes et al., 2006, accepted for publication).

b) Adipose stem cells: Better results were obtained with mesenchymal stem-cells obtained from human lipo-aspirate. These cells were able to differentiate in myoblasts and myotubes and express dystrophin in vitro (Vieira et al., submitted).

c) Human mesenchymal stem cells from dental pulp were injected in affected GRMD dogs in a collaborative project with Dr. Irina and Alexandre Kerkis and the veterinarian group, under the responsibility of Dr. Maria Angelica Miglino. Different concentration of cells, as well as local versus systemic injections is being analyzed. The morphology and the differentiation potential of cells from different sources and tissues are being characterized mining to establish animal bank cells.

2) Mouse models for muscular dystrophy

During this year we have put a lot of effort establishing new colonies of mouse models with muscular dystrophy, which show muscle weakness in order to be able to evaluate clinical effects of stem-cell therapy in these models. After circumventing the difficulties of importation we were able to establish successfully new colonies of dysferlin deficient mice (SJL), little-little, glycosilation defects – Large, alpha2-laminin total deficiency (dy/dy) and partial deficiency (Dy2J). The standardization genotyping methods, as well as the analysis of the primary defect of each model were done, and the selection of the best antibodies for the study of the models deficient for dysferlin (SJL), merosin (dy/dy) and glycosilation (LARGE) were started.

a) *In vitro* studies: Co- culture using BM mesenchymal stem cells, extracted from gfp mice are ongoing, using primary myoblasts cultures from different animal models for NMD

diseases: the studies with the mdx (dystrophin deficient) and the SJL (dysferlin deficiendy) model were already started and the next step will be to study the other muscular dystrophy mice models.

b) *In vivo* studies: The therapeutic potential of mesenchymal BM stem cells is being tested, with the injection of gfp-labelled cells in the different models. As positive control for muscle cells, gfp-transfected C2C12 cells were also injected. The treated animals will be evaluated clinically blindly in comparison with untreated controls.

B) Craniofacial Disorders

We established dental pulp stem cell lineages from 10 individuals (three with Van der Woude syndrome, three with cleft lip and palate and four controls). These cells were characterized with mesenchymal and hematopoietic antibodies. Five of these cell lineages were submitted to differentiation into bone, adipose and muscle cells. All of them differentiated in these cell lines. In addition, we are testing if these cell lines can be used as in *in vivo* models for regeneration of large bone cranial defects.

II.2 ADENOVIRUS-MEDIATED TRANSDUCTION OF DNA REPAIR GENES RELATED TO HUMAN DISEASES

For the investigations on DNA repair with adenovirus vectors, during this period we concluded the studies with the new vector carrying the *XPV* gene, which codes for DNA polymerase eta (a translesion DNA polymerase). Although this gene was successful for complementation of XPV cells, it did not affect cells that are deficient in the removal of DNA lesions, such as XPA cells, indicating that this DNA polymerase is not a limiting factor in cells dealing with DNA damage, and confirms DNA polymerase models for translesion synthesis (Lima-Bessa et al., 2006). Also, we obtained data that clearly indicates that DNA synthesis blockage by lesions is involved in apoptosis induction by ultraviolet (UV) light (Batista et al., 2006).

II.3 PHARMACOLOGICAL AND THERAPEUTIC TRIALS APPROACHES

Therapeutic trials for induced deafness: We have established a protocol for growing guinea pig cochlear cells. Different cell types were obtained and they have been characterized with antibodies. The aim of this approach is to develop a therapeutic trial for induced deafness (with aminoglicosides), and to study cochlear cells protein network, and differentiation. These experiments resulted from a collaboration with the Otorhynolaringology Department of the Medical School – USP.

3. OBJECTIVES FOR 2007

I. INVESTIGATION OF GENETIC FACTORS UNDERLYING HUMAN DISEASES

I.1 Identification of disease genes

Mapping and identification of genes and new mutations associated with the following phenotypes: limb-girdle muscular dystrophy type 1G, SPOAN (spastic paraplegia optic atrophy and neuropathy), idiopathic scoliosis, auriculo-condylar syndrome, sindromic obesity, Sotos syndrome, autism, X- linked mental retardation syndromes, syndromic craniosynostosis, deafness and ectrodactily/tibial hypoplasia syndrome.

I.2 Mechanisms modulating phenotypic expression

1. Characterization of regulatory elements of genes involved with developmental disorders – *TCOF1* and *COL18A1*.
2. Characterization of cells from Apert patients at the cellular and molecular level in order to understand their increased osteogenic potential.

3. Comparison of gene expression in affected and asymptomatic FSH patients carrying the same mutation.

I.3 Protein analyses

1. Confirmation of the interaction of three candidate proteins, detected by yeast two- hybrid approaches, with collybistin.
2. Verify if over-expression of SG, might replace the missing SG protein in affected patients with sarcoglycanopathies with milder clinical course.
3. The contribution of galectins to the dystrophic process.
4. UBE2A (ubiquitin-conjugase 2A): (a) expression of the UBE2A normal isoforms in different human adult tissues, including brain; (b) effect of the *UBE2A* mutation detected in an XLMR family on transcription, protein synthesis and cellular localization in lymphocytes and fibroblasts; (b) functional complementation of yeast *ΔRAD6* mutation (conserved ortholog) by normal and mutated human isoforms.
5. FMRP (Fragile X Mental Retardation Protein): a) to determine the subcellular distribution of the three FMRP isoforms containing the segment encoded by FMR1 exon 12; b) to specifically inhibit exon-12-containing Fmr1 transcripts using RNAi; c) to identify *FMR1* genomic sequences as candidates for exon 12 splicing enhancers and silencers; d) to establish tridimensional (3-D) cultures of epithelial cells for cell migration studies, which may be extended later for neuronal and muscle cells analyses.
6. Mice double-mutants for deficiencies of two known muscle proteins will be created and characterized at the protein level and clinical course. Their potential use for cell therapy will be also evaluated.

I.4 Complex disorders and genetic variation at the population level

The following studies will be concluded:

1. Association studies between SNPs at functional candidate genes and diabetic retinopathy .
2. SNP genotyping and statistical analysis in association studies of hypertension and obesity in African-derived quilombo populations.
3. Genotyping of polymorphic markers and statistical analysis in the study of genetic basis of noise-induced-hearing loss in a sample from São Paulo.

I.5 Genomic architecture

1. In our search for chromosome regions/genes involved in the diseases listed in the report above, CGH screening of selected patients will continue, extended by the use of an X-chromosome array, produced at the University of Nijmegen, The Netherlands, particularly in the study of mental retardation and premature ovarian failure.
2. The investigation of the mechanisms underlying the formation of at least three structural chromosome rearrangements that appear to involve non-allelic homologous recombination should be completed.

II. DEVELOPMENT OF THERAPEUTIC APPROACHES TO GENETIC DISEASES

II.1 Stem cell analysis and animal therapeutic trials

1. Evaluate the potential of adult pulp stem cells to reconstruct large cranial defects in rats, using the model already developed in the pilot study.
2. Evaluate if adult stem cells obtained from umbilical cord and umbilical cord blood, adipose tissue and dental pulp have the same potential of tissue regeneration *in vivo.* In order to address this issue, we will test the plasticity of adult mesenchymal stem cells to differentiate into bone and muscle in *in vivo* models. The potential to differentiate into bone will be tested in cranium rat defects of 5x8mm and we will compare the time of regeneration of this defect among the different cell

lineages as well as the quality of the bone formed. The potential to differentiate into muscle will be tested in different mice models (mdx, dysferlin deficient, dydy, large) as well as in the GRMD dog.
3. Compare the potential of regeneration of adult versus embryonic stem cells in the *in vivo* models described in the above item.
4. Evaluate the potential of hair cells obtained from cultivated cochlea tissues to regenerate induced deafness in the guinea pig.

II.2 Growth hormone inhibition and the progression of muscular dystrophies

The effect of growth hormone (GH) inhibition will be tested using pharmacological inhibitors as well as through the generation of double mutant mice models (little-little versus muscular dystrophy models).

II.3 Adenovirus-mediated transduction of DNA repair genes related to human diseases

During the last period we had problems to grow knock-out mice for XP genes, but these problems have been solved and now the first results on direct therapy have been obtained. For the next year, we plan to have the first data concerning the use of adeno-associated vectors, which are non-pathogenic and do not trigger immunologic responses. This will be tested searching for long-term transgene expression and vector re-administration. Moreover, we plan to continue our approaches to understand the signals for apoptosis in human cells damaged by UV irradiation, and the effects of DNA repair in this phenomenon. Initial experimental approaches using siRNA to knock down DNA repair genes are also underway.

Part 2. EDUCATION/PUBLIC INFORMATION REPORT

1. RESULTS

I. Education

Our main focus for the 2006 - 2008 period is the High School Visiting Program. In 2006 the activities comprised two related aspects:

I.1. HIGH SCHOOL VISITING PROGRAM

The program of visiting public high schools was implemented and is fully operational: approximately 7000 students from 16 schools participated (Chronogram, Annex 6). Prior to the start of the visiting program, teachers of these 16 schools attended a workshop and received specific training pertaining to the educational material to be used during the visit. We focused on the concept of the cell. A giant cell (amplified 130,000 times) was constructed especially for this program and mounted in each school, allowing the immersion of the students in the three-dimensional compartments of an animal cell (DVD, Annex 7). Complementary activities addressed specific issues such as: how 1.5 meters of DNA can fit inside the nucleus, the relation between the cell membrane and the cytoskeleton, the molecule structure of DNA and the amplification power of microscopes. Through the use of three educational games, the students learned about organelles and cell functions. After our visit, teachers were able to carry on activities related to the subjects, since we supplied educational material and follow-up. Undergraduate and postgraduate students from the Biosciences Institute (IB-USP) acted as monitors in this visiting program (Annex 8).

The program is evaluated using two types of questionnaires, aiming at:

- evaluating students' perception of the activities (pleasantness, importance as a learning process);
- evaluating the learning process itself; before the activities and 30 days afterwards.

Although the evaluation process has not been concluded it is already evident that most students became motivated to study the cell.

I.2. Training professionals to develop the current and future educational projects

Two new disciplines for post graduation students have been offered to 24 students, namely:

-"Ensaios Pedagógicos no Ensino de Biologia". (BIO-5741 – Pedagogic essays in biology teaching – (Coordination: Regina C. Mingroni-Netto). Students selected a theme, organized and taught a training course on the subject to high school teachers.

-"Ensino de Genética": (BIO-5727 – Teaching Genetics – Coordination: Eliana M. Belluzzo Dessen). This course provides pedagogical support for the production of teaching material to be used in the visiting program.

I.3. Difficulties in the implementation of the Visiting Program:

While these disciplines are complementary to give support to the High School Visiting Program, they do not fill the need of people formally engaged in this activity. A far-reaching such as the High School Visiting Program requires at least two qualified full-time people. The post-graduation students develop the pedagogic activities, but the production of accessible material requires specialized people. In addition, the monitors for the visiting program are insufficient and work as volunteers. This structure results in a lack of personnel and a high turnover, not compatible with the

task of organizing and managing a program involving approximately three visits per week to schools. Another complicating factor is the grant rules for acquiring consumables and paying personnel, which are not adaptable to the proposed activities.

II. PUBLICATIONS

II.1 -DIRECTED TO HIGH SCHOOL TEACHERS

1. Dessen, E. M. B. (2006) – Gripe aviária: seguindo as pegadas de um novo vírus. Genética na Escola Vol 1; 4-7.2006 www.sbg.org.br
2. Mingroni-Netto, R. C. e E.M.B. Dessen (2006) Células-tronco: o que são e o que serão. Genética na Escola Vol 1: 12-15, 2006. www.sbg.org.br

II.2 OTHER

3. Vianna-Morgante, A.M. – Temos 46 cromossomos. *Ciência Hoje, São Paulo, p.75-78, 2006.*

III. OTHER ACTIVITIES

The Internet site

The CEGH Internet site is being revised and will be ready in January, 2007

Symposium

- "Embryonic and adult stem cells: research before treatment (June 9th, 2006; Centro de Estudos do Genoma Humano, Instituto de Biociências, USP). Coordinators: Mayana Zatz (USP), Maria Rita Passos-Bueno (USP), Irina Kerkis (Instituto Butantan). Speakers: Marco A Zago, Irina Kerkis, Steven Reher, Sponsorship: Invitrogen/FAPESP-CEPID. 103 participants.

Workshop

- Teaching biology using pedagogic material (September 3rd, 2006) – Coordinator: Eliana Maria Beluzzo Dessen – Speakers: Eliana Maria Beluzzo Dessen and Maria Ligia Coutinho Carvalhal -35 participants

Training Courses:

- **Human Genetic disease: what is new?**- 40 hours - July (17-21st , 2006) – participation of 21 teachers from public high schools. Eliana Maria Beluzzo Dessen and Regina Célia Mingroni-Netto.
- **Genetics of Neurological Diseases** – 8 hours – Satellite course to the 11th National Meeting of Pediatric Neurology. November 2nd, 2006. Coordination: Fernando Kok – 119 participants. Speakers: Fernando Kok, Angela M. Vianna-Morgante, Célia P. Koiffman, Carla Rosenberg, Lúcia Inês Macedo-Souza, Maria Rita Passos-Bueno and Mayana Zatz .
- **Molecular Cytogenetics in the Investigation of Genomic Alterations** – 30 hours – November 21-24th, 2006. Coordination: Carla Rosenberg – 13 participants. Speakers: Carla Rosenberg, Ana Cristina Krepischi-Santos, Angela M. Vianna-Morgante, Cleide Borovik, Regina C. Mingroni-Netto, Peter L. Pearson.

Participation in "The National Science and Technology Week":

- Exhibit of the Giant Cell – Instituto Oceanográfico/USP, October 22nd, 2006.

Seminars:

- Mayana Zatz - Escola Superior de Advocacia/SP (Law School)- Genetic Tests. September 19th, 2006.
- Mayana Zatz - Espaço Cultural-CPFL (Cultural Center)/Campinas – Stem cells. October 10th, 2006.

Debates:

- Mayana Zatz - Subject: "Cloning" - TUCA ARENA – (Catholic University São Paulo), TV Network - Sesc/Senac, November 27th, 2006

Media (newspapers, magazines, TV):

- (Annex 9)

Educational activities in scientific meetings:

- Dessen, E.M.B. - 52º Congresso Nacional de Genética – September 3-6, 2006 – Foz do Iguaçu, Paraná. Coordination: Genética na Praça (activities for high school students and teachers).
- Dessen, E,M.D. – Coordination: Open Workshops – X EPEB Encontro "Perspectivas do Ensino de Biologia" – July 2006
- Mingroni-Netto, R.C. - Short-course "Organização do Genoma Humano", 17º Encontro de Biólogos do CRBio-1, April 9-12, 2006
- Mingroni-Netto, R.C. - Short course "Mecanismos atípicos de herança das doenças genéticas humanas, Semana de Reunião de Biologia, Universidade Metodista de Piracicaba, August 29th, 2006.
- Vianna-Morgante AM – Short Course "As Alterações Cromossômicas Humanas Origem e Conseqüências Fenotípicas", 17º Encontro de Biólogos do CRBio-1, April 9 - 12, 2006.
- Rosenberg, C. - Short-course: "Variability of the Human Genome", 52º Congresso Nacional de Genética – September 3 - 6, 2006 – Foz do Iguaçu, Paraná

2. PLANS FOR 2007

Objectives of the diffusion program in 2007:

- Implement the partnerships with 13 public high schools established this year with the Diretoria de Ensino Norte 2 (Educational Directory for the northern area of the city of São Paulo). The partner schools will include the Giant Cell in their teaching projects; students will build a giant cell concomitant with classes on the topic. The School Visiting Program will provide monitors and didactical material.
- Place the giant cell exhibit (and related educational activities) on permanent display at "Estação Ciência" (Center for Scientific, Technological and Cultural Information of the Administration Office for Culture, Extension Courses, University of São Paulo).
- Make the produced educational material available in the web
- Organize two training courses for high school teachers, two workshops for geneticists, and a short course for media professionals.
- Implement "scientific coffee" talks - dialogues in cultural spaces, such as bookshops, between geneticists and individuals who are interested in a clearer comprehension of genetics and its impact on society.

PART 3. TRANSFER OF TECHNOLOGY/ TECHNOLOGY APPLICATIONS

Our original proposal included the set up of two services: DNA sequencing and molecular diagnostic tests of genetic disorders. Recently, microsatellite genotyping has been added. The progress of these services, based on the increased number of tests done and incomes (Figures 3.1 and 3.2) is clearly observed. The genetic tests are being performed and conducted at the core lab at the Genome center leaded by MR Passos-Bueno and Vanessa Naomi and by six research groups (Drs. Angela Vianna-Morgante, Carla Rosenberg, Célia Koiffmann, Mariz Vainzof, Regina C. Mingroni-Netto, and Mayana Zatz). Details of these services are available at http://genoma.ib.usp.br/index_exames_diagnosticos.php). Below we present the development of the novel proposals submitted in the last project:

1. DEVELOPMENT OF BIOINFORMATIC SYSTEM: A software to evaluate the work flow of DNA analysis is under development by Dr. João E. Ferreira and his team, at the Institute of Mathematics/USP in collaboration with CEPID/Genoma. A preliminary version can found at http://143.107.45.175:8083; **user**: fapesp; **password** (senha): fapesp2006.The following main items are planned to be included to finalize the software: reports of the genetic tests, how to execute tests in parallel, how to send samples to external laboratories, inclusion of bar codes for control of the DNA samples.

Our goal for 2007 will be to validate this system. Once this is achieved, we will be able to expand the service and we will work out on the advertisement of these services.

2. METHODOLOGICAL UPDATE: We have validated the use of MLPA (Multiple Ligation-dependent Probe Amplification) for the diagnosis of the following genetic alterations: a) duplication and deletion of the *PMP22* gene, causative of Charcot-Marie-Tooth type 1A (CMT1A) and HNPP ; b) deletion of the *SMN1*(Survival motor neuronal one) gene and determination of the number of copies of the *SMN2* gene; analysis of these genes are important respectively for detection of healthy carriers of deletions at the *SMN1* gene and to evaluate prognosis in very young affected patients; c)deletions or duplications at subtelomeric regions for diagnosis of the causes of mental retardation and multiple malformation syndromes The first two tests are already being regularly offered for the interested families while we will launch the test to detect subtelomeric rearrangements to the general public at the first semester of 2007.

3. PROJECT IN PHARMACOGENETICS: We did not get support from the *Secretaria da Saúde do Estado de São Paulo, SS-ESP* (São Paulo State Health Department) for the development of our original proposal. Based on the available resources of the CEPID/ 2006, we have focused on:

a) Characterization of deletions, duplications and functional polymorphisms in the cytochrome P-450 -*CYP2D6* in subjects of our population, as several polymorphisms in this gene are already known to be responsible for adverse drug response.

b) Standardization of the analysis of the SNPs c.430C>T (p. Arg144Cys) and c.1075A>C (p.Ile359Leu) at *CYP2C9* and determination of their allelic frequencies in our population. These SNPs are well known to be involved with adverse response to Warfarin, one of the most commonly used anti-clotting drugs.

The frequencies of the most common functional polymorphisms are depicted in Table I. Some alleles showed a higher frequency in African-Brazilians, as expected based on available data of the literature. We will genotype 20 individuals of African and 20 of European descent to estimate the frequencies of the alleles most frequent for *CYP2D6* in our population.

The use of genetic tests before prescribing a drug is yet not a reality in Brazil. Therefore, in this next year, besides concluding the molecular analysis of the *CYP2D6*, we will work on the diffusion of the use of pharmacogenetics, with inclusion of adverse response to the most

common polymorphisms found in the Brazilian population for *CYP2D6* and *CYP2C9* genes.

Table 1 – Allelic frequencies of the most common polymorphism of the CYP2D6 and CYP2C9 genes in the São Paulo population.

Gene	Mutation	Functional effect	Frequency		Total
CY2D6			European	African	
gene	Deletion	No activity	7/60 (11.67%)	3/52(15.38%)	10/112 (8.9%)
gene	Duplication	Excess of activity	4/60(6.67%)	8/52(5.77%)	12/112 (10.7%)
CYP2D6/exon					
Exon 1	Gly42Arg	No activity	0/ 13*	2/8	2/21 (9.5 %)
Exon 3	Trp152Fs	No activity	0/13	1/11*	1/24 (4.2%)
Intron 3	c.1846G>A	Splicing/No activity	2/13	1/11	3/24 (12.5%)
Exon 6	Arg296Cys	Activity decreased	5/13	7/11	12/24 (50%)
Exon 8	c.3790C>T	Activity decreased	5/13	7/11	12/24 (50%
CYP2C9					
	Arg144Cys		5/48(10.42%)	3/38(7.89%)	8/86 (9.3%)
	Ile359Leu		2/48(4.17%)	1/38(2.63%)	3/86 (3.5%)

*the number of individuals tested were 7 and 6 respectively in European and African-Brazilians, however, two individuals has a deletion of CYP2D6. Therefore, the total number of alleles were 13 and 11 respectively.

A)

B)

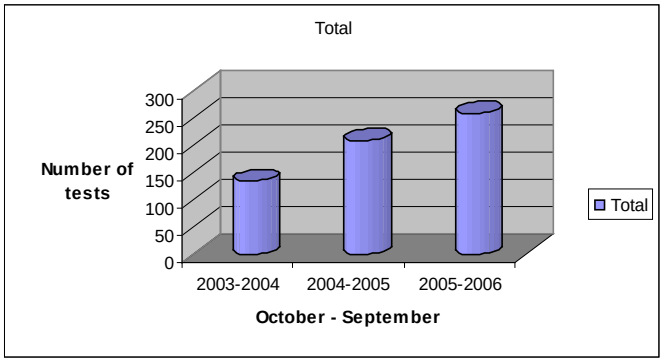

Figure 3.1: Income (A) and number of tests (B) in the last 3 years. It is clearly observed an increase in the number of tests.

Figure 3. 2: Income pear year since the set up of the sequencing service.

ANNEX 1. RESEARCH TEAM

1. Researchers

Name	Participation	Research Subproject
Mayana Zatz	Coordinator	Neuromuscular disorders: I.1 Identification of disease genes. *I.2 Mechanisms modulating phenotypic expression* II.1 Stem cell research
Maria Rita Passos-Bueno	**Coordinator:** **Transfer of Technology**	I.1 Identification of disease genes: craniofacial disorders; autism. I.2 Mechanisms modulating phenotypic disorders: craniofacial disorders I.3 Protein Analysis: craniofacial disorders I.4 Complex disorders: diabetic retinopathy II.1 Stem cell research: craniofacial disorders
Eliana M. Belluzzo Dessen	**Coordinator: Education/Public Information**	**Education**
Angela M. Vianna-Morgante	**Full-time investigator**	Mental retardation/congenital anomalies: I.1 Identification of disease genes **I.2 Phenotypic variability** I.3 Protein and functional analysis 1.5 Genome Architecture: impact of copy-number variation; chromosomal structural abnormalities
Célia P. Koiffmann	Full-time investigator	Mental retardation/neurobehavioral diseases (obesity, overgrowth syndromes) I.1 Identification of disease genes **I.2 Phenotypic variability** 1.5 Genome Architecture: impact of copy number variation; chromosomal abnormalities

Mariz Vainzof	Full-time investigator	Neuromuscular disorders: I.3. Protein and function analysis: proteins involved in muscle development II.1- Stem cells research: muscular dystrophies in mice models. Animal models
Regina Célia Mingroni-Netto	Full-time investigator	I.1 Identification of disease genes: deafness; ectrodactily I.3 Protein and Functional Analysis: deafness I.4 Complex disorders in Afro-Brazilian "isolates": obesity and hypertension II. 1. Animal therapeutic trials: deafness
Carla Rosenberg	Full-time investigator	Mental retardation/ congenital anomalies: I.1 Identification of disease genes 1.5 Genome Architecture: impact of copy number variation
Luciana Haddad	Collaborator	Neurodevelopmental disorders: I.3. Protein and functional analysis
Fernando Kok	Collaborator	Neuromuscular and developmental disorders: clinical aspects
Carlos F. Menck	Collaborator	II.2 Adenovirus-mediated transduction of DNA repair genes related to human diseases
Maria Angélica Miglino	Collaborator	II.1 Stem cell research: animal model in neuromuscular disorders
Luis Eduardo Netto	Collaborator	I.3 Protein and functional analysis

2. Post-doctoral fellows			
Name	Agency	Supervisor	Research Subproject
Ambrósio,CE	FAPESP	Zatz, M	I.3 Protein analyses
Jehee, FS	FAPESP	Passos-Bueno, MR	I.2 Mechanisms modulating phenotypic expression
Krepischi-Santos, ACV	CNPq	Rosenberg, C	I.5 Genomic Architecture
Mazzeu, JF	FAPESP	Vianna-Morgante, AM	I.1 Identification of disease genes I.2 Mechanisms modulating phenotypic expression
Monteiro, G	FAPESP	Netto, LES	I.2 Mechanisms modulating phenotypic expression I.3 Protein analyses
Oliveira, MA	FAPESP	Netto, LES	I.3 Protein analyses
Sertié, AL	FAPESP	Passos-Bueno, MR	I.2 Mechanisms modulating phenotypic expression I.3 Protein analyses
Tatiana Jazedje da Costa Silva	FAPESP	Miglino, A	II.1 Stem Cell Research
Varela, MC	FAPESP	Koiffmann, CP	I.1 Identification of disease genes I.5 Genomic Architecture
Yamamoto, LU	FAPESP	Vainzof, M	I.3 Protein analyses

3. PhD Students			
Name	Agency	Advisor	Research Subproject
Abreu-Silva, RS	FAPESP	Mingroni-Netto, RC	I.4 Complex disorders and genetic variation at the population
Angeli, CB	FAPESP	Mingroni-Netto, RC	I.4 Complex disorders and genetic variation at the population
Arashiro, P	FAPESP	**Zatz, M**	**I.2 Mechanisms modulating phenotypic genes**
Araújo, KP	CAPES	Miglino, MA	II.1 Stem cell analysis and animal therapeutic trials
Armelin, L	FAPESP	Passos-Bueno, MR	I.2 Mechanisms modulating phenotypic expression
Azevedo, NF	FAPESP	**Vianna-Morgante, AM**	**I.5 Genomic Architecture**
Berra, CM	FAPESP	Menck, CFM	II.3 Adenovirus-mediated transduction of DNA repair genes related to human diseases
Bueno, DF	CAPES	Passos-Bueno, MR	II.1 Stem cell analysis and animal therapeutic trials
Capelli, LP	FAPESP	Vianna-Morgante, AM	I.2 Mechanisms modulating phenotypic expression
Carvalho, MD	FAPESP	Zatz, M	I.3 Protein analyses
Casagrande, CF	FAPESP	Miglino, MA	II.1 Stem cell analysis and animal therapeutic trials
Catelani, ALPM	Laboratório Fleury	Rosenberg, C	I.5 Genomic Architecture
D´Angelo, CS	FAPESP	Koiffmann, CP	I.1 Identification of disease genes I.5 Genomic Architecture
Errera, F	CNPq	Passos-Bueno, MR	I.4 Complex disorders and genetic variation at the population level
Fanganiello, R	FAPESP	Passos-Bueno, MR	I.2 Mechanisms modulating phenotypic expression
Gifalli-Iughetti, C	FAPESP	Koiffmann, CP	I.5 Genomic Architecture
Horta, BB	FAPESP	Netto, LES	I.2 Mechanisms modulating phenotypic expression I.3 Protein analyses
Kague, E	FAPESP	Passos-Bueno, MR	I.2 Mechanisms modulating phenotypic expression
Kohl, I	CAPES	Koiffmann, CP	I.1 Identification of disease genes
Leite, RA	FAPESP	Menck, CFM	II.3 Adenovirus-mediated transduction of DNA repair genes related to human diseases
Lezirovitz, K	FAPESP	Mingroni-Netto, RC	I.1 Identification of disease genes

Lima-Bessa, KM	FAPESP	Menck, CFM	II.3 Adenovirus-mediated transduction of DNA repair genes related to human diseases
Luppi, MMRC	CNPq	Miglino, MA	II.1 Stem cell analysis and animal therapeutic trials
Magalhães, ML	FAPESP	Zatz, M	I.1 Identification of disease genes
Martins, DS	FAPESP	Miglino, MA	II.1 Stem cell analysis and animal therapeutic trials
Martins, PCM		Vainzof, M	I.3 Protein analyses
Masotti, C	FAPESP	Passos-Bueno, MR	I.1 Identification of disease genes I.2 Mechanisms modulating phenotypic expression
Nascimento, RMP	FAPESP	**Vianna-Morgante, AM**	**I.2 Mechanisms modulating phenotypic expression**
Neto, MM	FAPESP	Zatz, M	I.1 Identification of disease genes
Orabona, G	FAPESP	Passos-Bueno, MR	I.1 Identification of disease genes
Schlesinger, D		Zatz, M	I.1 Identification of disease genes
Silva, GM	FAPESP	Netto, LES	I.2 Mechanisms modulating phenotypic expression I.3 Protein analyses
Soltys, D	FAPESP	Menck, CFM	II.3 Adenovirus-mediated transduction of DNA repair genes related to human diseases
Souza, LIM	FAPESP	Zatz, M	I.1 Identification of disease genes
Vieira, NMS	FAPESP	Zatz, M	I.3 Protein analyses
Wenceslau, CV	FAPESP	Miglino, MA	II.1 Stem cell analysis and animal therapeutic trials
Yeh, E	FAPESP	Passos-Bueno, MR	I.2 Mechanisms modulating phenotypic expression
Zucconi, E	FAPESP	Zatz, M	I.3 Protein analyses

4. MSc Students			
Name	Agency	Advisor	Research Subproject
Alencastro, G	FAPESP	Passos-Bueno, MR	I.3 Protein analyses
Barros, DA	FAPESP	Vainzof, M	II.1 Stem cell analysis and animal therapeutic trials
Batissoco, AC	CNPq	Mingroni-Netto, RC	I.2 Mechanisms modulating phenotypic expression
Brólio, MP	CNPq	Miglino, MA	II.1 Stem cell analysis and animal therapeutic trials
Cavaçana, N	----	**Zatz,M**	*II.1 Stem cell analysis and animal therapeutic trials*
Fagali, CQ	FAPESP	Koiffmann, CP	I.1 Identification of disease genes; I.5 Genome Architecture
Fogaça, LQ	FAPESP	Vainzof, M	I.3 Protein analyses
Fontes, L	FAPESP	**Vianna-Morgante, AM**	**I.1 Identification of disease genes;** **I.5 Genome Architecture**
Kimura, L	FAPESP	Mingroni-Netto, RC	I.4 Complex disorders and genetic variation at the population level
Mortari, AC		Vainzof, M	I.3 Protein analyses
Oliveira, KG	CNPq	Passos-Bueno, MR	I.1 Identification of disease genes
Oliveira, MA	FAPESP	Koiffmann, CP	I.5 Genome Architecture
Oliveira, NA	FAPESP	Passos-Bueno, MR	I.2 Mechanisms modulating phenotypic expression
Onofre, PCG	FAPESP	Vainzof, M	II.1 Stem cell analysis and animal therapeutic trials
Passos, J	CAPES	Miglino, MA	II.1 Stem cell analysis and animal therapeutic trials
Reis, SBA		Vianna-Morgante, AM	I.1 Identification of disease genes; I.5 Genome Architecture
Romanos, J	CNPq	Mingroni-Netto, RC	I.2 Mechanisms modulating phenotypic expression
Secco, M	FAPESP	**Zatz, M**	**I.3 Protein analyses**
Velloso, FJ	CNPq	**Haddad, LA**	**I.3 Protein analyses**
Vieira, LCZ	FAPESP	Vianna-Morgante, A.M.	I.1 Identification of disease genes; I.5 Genome Architecture

Formatado

5. Specialization and Technical Training Students			
Name	Agency	Advisor	Research Subproject
Alvez, SV	Procontes	**Netto, LES**	I.3 Protein analyses
Costa, SS	LGH	**Vianna-Morgante, AM**	**I.2 Mechanisms modulating phenotypic expression**
Lopes, VF		Vainzof, M	I.3 Protein analyses
Quayle,C	USP	Menck,CFM	II.3 Adenovirus-mediated transduction of DNA repair genes related to human disease

6. Undergraduate Students			
Name	Agency	Advisor	Research Subproject
Coqueti, KN	FAPESP	**Vianna-Morgante, AM**	**I.5 Genomic Architecture**
Cruvinel, EM	FAPESP	**Koiffmann, CP**	**I.5 Genomic Architecture**
Izzo, G		Vainzof, M	I.3 Protein analyses
Maia, LS		Vainzof, M	I.2 Mechanisms modulating phenotypic expression
Montalbano,G	FAPESP	**Zatz,M**	I.1 Identification of disease genes
Rincón, D	CNPq (PIBIC)	Mingroni-Netto, RC	I.4 Complex disorders and genetic variation at the population level
Sarmento, BC	CNPq (PIBIC)	**Koiffmann, CP**	**I.1 Identification of disease genes**
Sell, K	CNPq (PIBIC)	Vainzof, M	I.2 Mechanisms modulating phenotypic expression
Valadares,MC	-------	**Zatz,M**	**I.1 Identification of disease genes**
Zilberstein, D	FAPESP	Vainzof, M	I.3 Protein analyses

ANNEX 2. List of Publications 2006

A. **Directly related to the Project**

Abreu-Silva RS, **Lezirovitz K**, Braga MCC, Spinelli M., Pirana S., Della-Rosa VA, Otto PA., **Mingroni-Netto RC**. Prevalence of the A1555G (12SRNA) and tRNAser(UCN) mitochondrial mutations in hearing-impaired Brazilian patients. Braz J Med Biol Res 2006a; 39:219 - 226.

Abreu-Silva RS, **Batissoco AC**, **Lezirovitz K**, **Romanos J**, **Rincon D**, Otto PA, **Auricchio MTBM, Mingroni-Netto RC**. Correspondence regarding Ballana et al.: "Mitochondrial 12S rRNA gene mutations affect RNA secondary structure and lead to variable penetrance in hearing impairment ". Bioch Bioph Res Comm 2006b**;**.343: 675-676 .

Armelini MG, Lima-Bessa KM, Marchetto MCN, Muotri AR, Chiganças V, Leite RA, Carvalho H and **Menck CFM.** Exploring DNA damage responses in human cells with recombinant adenoviral vectors. Hum Exp Toxicol. *In press*.

Batista LFZ, Chiganças V, Brumatti G, Amarante-Mendes GP and **Menck CFM.** Involvement of DNA replication in ultraviolet-induced apoptosis of mammalian cells. Apoptosis 2006; 11:1139-1148.

Cheroki C, **Krepischi-Santos AC**, **Rosenberg C**, **Jehee FS**, **Mingroni-Netto RC**, Filho IP, Filho SZ, Kim CA, Bagnoli VR, Mendonca BB, Szuhai K, Otto PA. Report of a del22q11 in a patient with Mayer-Rokitansky-Kuster-Hauser (MRKH) anomaly and exclusion of WNT-4, RAR-gamma, and RXR-alpha as major genes determining MRKH anomaly in a study of 25 affected women. Am J Med Genet 2006;140:1339-1342.

Costa SS, Fonseca AM, Bagnoli VR, **Vianna-Morgante AM**. The *FMR1* premutation as a cause of premature ovarian failure in Brazilian women. Genet. Mol. Biol 2006; 29:423-428.

D'Angelo CS, Paz JA, Kim CA, Bertola DR, **Castro CIE**, **Varela MC**, **Koiffmann CP**. Prader-Willi-like phenotype: investigation of 1p36 deletion in 41 patients with delayed psychomotor development, hypotonia, obesity and/or hyperphagia, learning disabilities and behavioral problems. Eur J Med Genet 2006; doi:10.1016/j.ejmg 2006.02.001 (ahead of print).

Errera FI, Silva ME, Yeh E, **Maranduba CM**, Folco B, Takahashi W, Pereira AC, Krieger JE, **Passos-Bueno MR**. Effect of polymorphisms of the MTHFR and APOE genes on susceptibility to diabetes and severity of diabetic retinopathy in Brazilian patients. Braz J Med Biol Res. 2006;39:883-8.

Gouveia TL, Paim JFO, **Pavanello RCM**, **Zatz M**, **Vainzof M**. Sarcoglycanopathies: A multiplex analysis for the most common mutations. Diag. Mol. Pathol 2006; 15:95-100, 2006.

Jehee FS, Alonso LG, Cavalcanti DP, Kim C, Wall SA, Mulliken JB, Sun M, Jabs EW, Boyadjiev SA, Wilkie AO, **Passos-Bueno MR**. Mutational screening of FGFR1, CER1, and CDON in a large cohort of trigonocephalic patients. Cleft Palate Craniofac J. 2006;43:148-51.

Krepisch-Santos AC, **Vianna-Morgante A, Jehee FS, Passos-Bueno MR**, Knijnenburg J, Szuhai K, Sloos W, **Mazzeu JF**, Kok F, **Cheroki C**, Otto PA, **Mingroni-Netto RC, Varela MC, Koiffmann CP**, Kim CA, Bertola DR, Pearson P, **Rosenberg C**. Whole-genome array-CGH screening in undiagnosed syndromic patients: old syndromes revisited and new alterations. Cytogenet Genome Res. 2006; 115:254–261.

Kriek M, Szuhai K, Kant SG, White SJ, Dauwerse H, Fiegler H, Carter NP, Knijnenburg J, Den Dunnen JT, Tanke HJ, Breuning MH, **Rosenberg C.** A complex rearrangement on chromosome 22 affecting both homologues; haplo-insufficiency of the Cat eye syndrome region may have no clinical relevance. Hum Genet 2006; 120:77-84.

Kriek M, Knijnenburg J, White S, **Rosenberg C**, Den Dunnen JT, van Ommen GJ, Tanke H, Breuning M, Szuhai K. Diagnosis of Genetic Abnormalities in Developmentally Delayed Patients: a New Strategy Combining MLPA and Array-CGH. Am J Med Genet. (*In press*).

Kossugue PM, Paim JFO, Silva HC, **Pavanello RCM**, Gurgel Giannetti J, **Zatz M**, **Vainzof M**. Central Core disease due to recessive mutations in RYR1 gene: is it more common than described? Muscle & Nerve. *In press*.

Lezirovitz K, Nicastro FS, **Pardono E**, **Abreu-Silva RS**, **Batissoco AC**, Neustein I, Spinelli M, **Mingroni-Netto RC**. Is autosomal recessive deafness associated with oculocutaneous albinism a coincidence syndrome? J Hum Genet 2006; 51:716 – 720.

Lima-Bessa K, Chiganças V, Stary A, Kanouche P, Sarasin A, Armelini MG, Jacysyn JF, Amarantes-Mendes GP, Cordeiro-Stone M, Cleaver JE and **Menck CFM**. Adenovirus mediated transduction of the human DNA polymerase eta cDNA. DNA Repair 2006; 5: 925-934

Maranduba CM, Friesema EC, **Kok F,** Kester MH, Jansen J, Sertie AL, **Passos Bueno MR**, Visser TJ. Decreased cellular T3 uptake and metabolism in Allan-Herndon-Dudley syndrome(AHDS) due to a novel mutation in the MCT8 thyroid hormone transporter. J Med Genet 2006; 43:457-60.

Miglino MA, Giglio RF, Baliero JCC, Sterman FA, Pinto ACBCF, **Zatz M**, Ferrigno CRA (2006). Estudo longitudinal da densidade mineral óssea em cães jovens da raça Golden Retriever: Correlações com idade e peso corpóreo. Brazi J Vet Res Anim Sci 2006; 43:681-687.

Nascimento RM, Otto PA, de Brower AP, **Vianna-Morgante AM**. UBE2A,which encodes a ubiquitin-conjugating enzyme, is mutated in a novel X-linked mental retardation syndrome Am J Hum Genet. 2006; 79:549-555.

Nunes VA, **Vainzof M**, **Canovas M**, Strauss BE, **Zatz M**. Stem cells from human umbilical cord blood were able to differentiate into myotubes and express dystrophin in vitro only after exposure to in vivo muscle environment .Biology of the Cell .*In press*.

Oliveira NA, Alonso LG, Fanganiello RD, **Passos-Bueno MR.** Further evidence of association between mutations in FGFR2 and syndromic craniosynostosis with sacrococcygeal eversion. Birth Defects Res A Clin Mol Teratol. 2006;76:629-33.

Padorno E, **Mazzeu JF**, **Lezirovitz K**., Auricchio MTBM, **Iughetti P**, **Nascimento RMP**, **Mingroni-Netto RC**, Otto PA. Waardenburg syndrome: description of two novel mutations in the PAX3 gene , one of which incompletely penetrant. Genet Mol Biol 2006; 29: 601 - 604.

Passos-Bueno MR, **Suzuki OT**, **Armelin-Correa LM**, **Sertie AL**, **Errera FI**, **Bagatini K, Kok F**, Leite KR. Mutations in collagen 18A1 and their relevance to the human phenotype. An Acad Bras Cienc. 2006; 78:123-31. Review.

Rosenberg C, Knijnenburg J, Bakker E, **Vianna-Morgante A**, Sloos WC, Otto PA, Kriek M, Hansson K, **Krepisch-Santos AC**, Fiegler H, Carter NP, Bijlsma EK, Van Haeringen A, Szuhai K, Tanke HJ. Array-CGH detection of micro rearrangements in mentally retarded individuals: Clinical significance of imbalances present both in affected children and normal parents. J Med Genet 2006; 43:180-186.

Rozenberg R, **Kok F**, Burin MG, Sa Miranda MC, Vasques C, Henriques-Souza AM, Giugliani R, **Vainzof M**, Pereira LV. Diagnosis and molecular characterization of non-classic forms of Tay-Sachs disease in Brazil. J Child Neurol. 2006;21:540-4.

Silva MC, Alves Meira ZM, Gurgel-Gianett J, Moreira da Silva M, Oliveia Campos AF, Melo Barbosa M, Starling Filho GM, Aguiar Ferreira R, **Zatz M**, Rochitte CE (2006) Myocardial delayed enhancement by MRI in patients with muscular dystrophy J. Am. Acad. Cardiology. *In press*.

Shaw-Smith C, Pittman AM, Willatt L, Martin H, Rickman L, Gribble S, Curley R, Cumming S, Dunn C, Kalaitzopoulos D, Porter K, Prigmore E, **Krepischi-Santos AC**, **Varela MC**, **Koiffmann CP**, Lees AJ, **Rosenberg C,** Firth HV, de Silva R, Carter NP. Microdeletion encompassing MAPT at chromosome 17q21.3 is associated with developmental delay and learning disability. Nat Genet. 2006;38:1032-1037.

Tonini MM, Lemmers RJ, **Pavanello RC**, **Cerqueira AM**, Frants RR, van der Maarel SM, **Zatz M.** Equal proportions of affected cells in muscle and blood of a mosaic carrier of facioscapulohumeral muscular dystrophy. Hum Genet. 2006; 119:23-28.

Valdmanis PN, Meijer IA, Reynolds A, Lei A, MacLeod P, Schlesinger D, **Zatz M**, Reid E, Dion PA, Drapeau P, Rouleau GA (2006) Mutations in the KIAAO196 gene at the SPG8 locus cause hereditary spastic paraplegia .Am. J. Hum. Genet. Epub 2006 Dec 1. PMID: 17160902

Valente KD, **Koiffmann CP**, Fridman C, **Varela, MC**, **Kok F**, Andrade JQ, Grossmann RM, Marques-Dias M J. Epilepsy in patients with Angelman syndrome caused by deletion of the chromosome 15q11-13. Arch Neurol 2006, 63: 122-128, 2006.

Valente KD, Freitas A, Fridman C, **Varela MC**, Silva AE, Fett AC, **Koiffmann CP** . Inv dup(15): Is the electroclinical phenotype helpful for this challenging clinical diagnosis?. Clin Neurophysiol 2006; 117:803-809.

Varela MC, **Krepischi-Santos ACV**, Paz JA, Knijnenburg J, Szuhai K, **Rosenberg C**, **Koiffmann CP**. A 17q21.31 microdeletion encompassing the *MAPT* gene in a mentally retarded patient. Cytogenet Genome Res 2006a; 114:89-92.

Varela MC; Sato, AYS, Kim CA; Bertola DR, Castro CIE, **Koiffmann CP**. A new case of interstitial 6q16.2 deletion in a patient with Prader-Willi-like phenotype and investigation of SIM 1 gene deletion in 87 patients with syndromic obesity. Eur J Med Genet 2006b; 49:298-305.

Vieira N.M., Schlesinger D, de Paula F, **Vainzof M, Zatz M**. Mutation analysis in the *FKRP* gene provides an explanation for a rare cause of intrafamilial clinical variability in LGMD2I. Neuromuscul Disord 2006; 16:870-873.

B. Not directly related to the project

Barbosa, J.A.R.G., **Netto LES**., Farah, C.S., Schenkman, S. & Meneghini, R. "The Structural Molecular Biology Network (SMolBNet) of the State of São Paulo, Brazil." Anais da Academia Brasileira de Ciências 2006;78: 241-253.

Becker C, Lacchini S, Muotri AR, da Silva GJ, Castelli JB, Vassallo PF, **Menck CF**, Krieger JE. Skeletal muscle cells expressing VEGF induce capillary formation and reduce cardiac injury in rats. Int J Cardiol. 2006, (in press).

Bertola DR, Cao H , Albano LM, Oliveira DP, **Kok F**, Marques-Dias MJ, Kim CA, Hegele RA.Cockayne syndrome type A: novel mutations in eight typical patients. J Hum Genet. 2006;51:701-5. Epub 2006 Jul 25

Cardoso J, Molenaar L, de Menezes RX, van Leerdam M, Rosenberg C, Moslein G, Sampson J, Morreau H, Boer JM, Fodde R. Chromosomal instability in MYH- and APC-mutant adenomatous polyps. Cancer Res 2006; 66:2514-2519.

Demasi, A.P.D., Pereira, G.AG. & **Netto, LES** "Yeast oxidative stress response: influences of cytosolic thioredoxin peroxidase I and of the mitochondrial functional state", FEBS J. 2006; 273:805-16.

Dijkman R, Tensen CP, Jordanova ES, Knijnenburg J, Hoefnagel JJ, Mulder AA, **Rosenberg C**, Raap AK, Willemze R, Szuhai K, Vermeer MH. Array-based comparative genomic hybridization analysis reveals recurrent chromosomal alterations and prognostic parameters in primary cutaneous large B-cell lymphoma. J Clin Oncol 2006; 24:296-305.

Godoi PH, Galhardo RS, Luche DD, Van Sluys MA, **Menck CF**, Oliva G (2006). Structure of the Thiazole Biosynthetic Enzyme THI1 from *Arabidopsis thaliana*. J Biol Chem. 2006; 281:

30957-30966.
Maegawa GH, Stockley T, Tropak M, Banwell B, Blaser S, **Kok F**, Giugliani R, Mahuran D, Clarke JT. The Natural History of Juvenile or Subacute GM2 Gangliosidosis: 21 New Cases and Literature Review of 134 Previously Reported.Pediatrics. 2006 Oct 2; [Epub ahead of print]
Malavazi I, Savoldi M, Di Mauro SM, **Menck CF**, Harris SD, Goldman MH, Goldman GH (2006). Transcriptome Analysis of Aspergillus nidulans Exposed to Camptothecin-Induced DNA Damage. Eukaryot Cell 2006; 5: 1688-704.
Marchetto MCN*, Correal RG*, **Menck CFM** and Muotri AR. Functional lentiviral vectors for xeroderma pigmentosum gene therapy. J. Biotechnology, (*in press).* *both have equally contributed
Medina-Silva R, Barros MP, Galhardo RS, **Netto LES**, Colepicolo P, **Menck CFM**. "Oxidative stress and mitochondrial instability after heat shock in yeast deficient in the thiazole biosynthesis", Res Microbiol 2006; 157:275-81.
Medina-Silva, R., Barros, MP, Galhardo, RS, **Netto, LES**, Colepicolo, P and **Menck CFM** (2006) Heat stress promotes mitochondrial instability and oxidative responses in yeast deficient in the thiazole biosynthesis, Research in Microbiology 2006; 157: 275-281.
Netto, LES, Oliveira MA, Monteiro G, Demasi APD. Cussiol JRR, Discola KF, Demasi M, Monteiro-Silva G, Alves SV, Faria VG, Horta BB. Reactive Cysteine in Proteins: protein folding, antioxidant defense, redox signaling and more Comparative Biochemistry & Physiology, *in press*.
Ogusucu R., Rettori D, Munhoz, DC, **Netto LES**, Augusto O. Reactions of yeast thioredoxin peroxidases I and II with hydrogen peroxide and peroxynitrite: Rate constants by competitive kinetics." Free Radic Biol Med 2006; *in press*.
Oliveira, M.A., Guimarães, BG, Cussiol, JRR, Medrano F.J., Gozzo FC, **Netto LES**. "Structural Insights into Enzyme-Substrate Interaction and Characterization of Enzymatic Intermediates of Organic Hydroperoxide Resistance Protein from Xylella fastidiosa." J Mol Biol 2006; 359, 433-445.
Pinheiro A.S., Amorim G.C., **Netto LES**., Valente A.P., Almeida F.C. (2006) "(1)H, (13)C and (15)N Resonance Assignments for the Reduced Forms of Thioredoxin 1 and 2 from S. cerevisiae." J Biomol NMR, 2006; Apr 11; [Epub ahead of print].
Roos WP, Batista LFZ, Naumann S, Weller M, **Menck CFM** and Kaina B Mechanism of apoptotic death triggered by methylating agent-induced DNA damage in glioma cells. Oncogene (i*n press*).
Rozeman L, Szuhai K, Schrage Y, **Rosenberg C**, Tanke H, Taminiau AH, Clenton-Jansen A, Bovee J, Hogendoorn P. Array-comparative genomic hybridization of central chondrosarcoma: identification of ribosomal protein S6 and cyclin-dependent kinase 4 as candidate target genes for genomic aberrations. Cancer 2006; 107(2):380-388.
Szuhai K, Ijszenga M, Tanke HJ, **Rosenberg C**, Hogendoorn PCW. Molecular cytogenetic characterization of four previously established and two newly established Ewing sarcoma cell lines. Cancer Genet Cytogenet 2006; 166:173-179.
Szuhai K, Ijszenga M, Tanke HJ, Taminiau AH, Schepper A, van Duinen SG, **Rosenberg C**, Hogendoorn PCW. Detection of a novel chromosomal rearrangement in a histological variant of Ewing sarcoma . The Journal of Pathology. *In press*.
Tahara, Erich B., Barros, Mario H., Oliveira, Graciele A., **Netto LES,** Kowaltowski, AJ "Dihydrolipoyl Dehydrogenase is a Substantial Source of Reactive Oxygen Species Leading to Aging in Saccharomyces cerevisiae: Role of calorie restriction." Faseb J., no prelo.
van Dekken H, Vissers K, Tilanus HW, Gray JW, Kuo WL, Tanke HJ, **Rosenberg C**, Ijszenga M, Szuhai K. Genomic array and expression analysis of frequent high-level amplifications in

adenocarcinomas of the gastro-esophageal junction. Cancer Genet Cytogenet 2006; 166:157-162.

Chapters in Book

Vainzof M, **Zatz M**. Muscular dystrophies and protein mutations. in: Uversky VN and Fink AL., Protein Misfolding, Aggregation and Conformational Diseases. Volume II: Molecular basis of Conformational diseases. Kluwer Academic/Pleunum Publishers, USA. *In press*.

Otto PA, **Mingroni-Netto RC**, **Vianna-Morgante AM** . Princípios de Genética Humana e Médica. In: Lopes AC; Amato Neto V. (Org.). Tratado de Clínica Médica. São Paulo: Editora Roca, 2006, v. I, p. 280-311.

ANNEX 3. Abstracts in Congress - 2006

International Meetings:

Bueno D, Lopes LD, Amaral CER, Passos-Bueno MR. Analysis of transforming growth factor B3 gene (TGFB3) in Pierre Robin sequence patients. 56th Annual meeting American Society of Human Genetics, New Orleans, 2006, 1623/B.

Capelli LP, Sampaio I, Harris E, Gifalli-Iughetti C, Koiffmann CP, Schneider H, Vianna-Morgante AM. Analysis of the (CGG)n repeat in the *FMR1* orthologue of non-human primates. 47th Annual Short Course on Medical and Experimental Mammalian Genetics, July 16-28, 2006, Bar Harbor, Maine USA.

Casagrande CF, Casals JB, Morini AC, Ambrósio CE, Miglino MA, Carvalho MAM. Morphological characterization of umbilical cord blood stem cells of Golden Retriever dogs to treatment of muscular dystrophy. XXVI Congress of The European Association of Veterinary Anatomists, 2006, Messina, Itália. Italian Journal of Anatomy and Embryology. Florence: Mozzon Giuntina S.p.A., 2006. v. 111. p. 86.

D'Angelo CS, Paz JA, Kim CA, Bertola DR, Castro CIE, Varela MC, Koiffmann CP. Prader-Willi-Like Phenotype: Investigation of 1p36 deletion in 41 patients with delayed psychomotor development, hipotonia, obesity and/or hyperphagia, learning disabilities and behavioral problems. In: 55th Annual Meeting of the American Society of Human Genetics, 2005, Salt Lake City, Utha. Annual Meeting of The American Society of Human Genetics - período de 25-29 outubro, 2005.

Fanganiello R, Sertie AL, Oliveira N, Reis EM, Yeh E, Bueno D, Alonso N, Cavalheiro S, Kerkis I, Verjovski-Almeida S, Passos-Bueno MR. Identification of a gene expression signature and increased plasticity in periosteal fibroblasts from Apert syndrome patients. 56th Annual meeting American Society of Human Genetics, New Orleans, 2006, 1926/B

Haddad LA and Vianna-Morgante AM. Developmentally Regulated Expression of a 28-kDa Peptide in the Rat Brain. The 46th Annual Meeting of the American Society for Cell Biology, 9-13 December 2006, San Diego, CA. Online Program No 518.

Kague E, Armelin-Correa L, Suzuki OT, Sogayar MC, Passos-Bueno MR. Identification of regulatory regions nearby COL18A1 gene. 56th Annual meeting American Society of Human Genetics, New Orleans, 2006, 1184/A.

Martins DS, Saraiva NZ, Garcia JM, Ambrósio, CE, Zatz M, Miglino MA. Characterization of canine embryonic germ cells: an experimental model for cell therapy . In: International Embryo Transfer Society, 2006, Orlando. Reproduction, Fertility and Develpment. Collingwood, Austrália: Csiro Publishing, 2006. v. 18. p. 209-209.

Masotti C, Kague E, Passos-Bueno MR. The influence of YY1 and retinoic acid in the Treacher Collins syndrome gene (TCOF1) regulation. 56th Annual meeting American Society of Human Genetics, New Orleans, 2006, 1891/C.

Mingroni-Netto RC, Kimura L, Cotrim NH, Pereira AC, Vicente JP, Auricchio MTBM, ANGELI CB. Association studies of obesity and high blood pressure to variants of the genes *LEPR*, *ADRB2* and *ACE* in afro-Brazilian populations In: 75th Annual Meeting of the American Association of Physical Anthropology, March 2006. Am J Phys Anthropol, 2006. v.149. p.132.

Romanos J, Fávero ML, Otto PA, Mingroni-Netto RC. Novel mutations in the *OTOF* gene in Brazilian subjects with auditory neuropathy. 56th Annual Meeting of the American Society of Human Genetics, Abstracts, p. 342. Hereditary Hearing Impairment Consortium, 9th October 2006.

Sertie A, Alencastro G, Passos-Bueno MR. Dissection of the roles of collybistin, a neuron-enriched GDP-GTP exchange factor in synapse formation and function. 56th Annual meeting American Society of Human Genetics, New Orleans, 2006,542/A.

Tonini MM, Lemmers RJLF, Arashiro P, Van Teijlingen C, Lazar M, Frants RR, van der Maarel SM, Zatz M. D4Z4 repeat exchanges between chromosomes 4 and 10 in different Brazilian ethnics: implications for FSHD prevalence? 11th International Congress of the World Muscle Society.Bruges,Belgium, 4- 7 October 2006. Neurom. Disord. 16: 712-713.

Vainzof M, Yamamoto LU, Ayub D, Kossugue P, Man NT, Morris GE. Utrophin in the dystrophic muscle: evaluation of the contribution of the connective tissue replacement. 11th International Congress of the World Muscle Society Bruges - Belgium - 4-7 October 2006. Neurom. Disord. 16: 697.

Varela MC, Kim CA, Bertola DR, Sato AYS, Castro CIE, Koiffmann CP. A new case of interstitial 6q16.2 deletion in a patient with Prader-Willi-Like Phenotype. In: 55th Annual Meeting of the American Society of Human Genetics, 2005, Salt Lake City, Utah. Annual Meeting of The American Society of Human Genetics, período de 25-29 de outubro, 2005.

Vasconcelos B, Albano LMJ, Castro CIE, Bertola DR, Sbruzzi IC, Koiffmann CP, Kim CA. Ring chromosome syndrome: Report of 12 cases. In: 55th Annual Meeting of the American Society of Human Genetics, 2005, Salt Lake City, Utah. Annual Meeting of The American Society of Human Genetics, período de 25 a 29 de outubro, 2005.

Yamamoto LU, Chamas R, Zatz M, Vainzof M (2006) Galectin-1 and -3 expression in patients with mutations in the FKRP gene. 11th International Congress of the World Muscle Society. Bruges, Belgium, 4-7 October 2006. Neurom. Disord. 16: 681

National Meetings:

Abatemarco A, Martins DS, Morini AC, Casagrande CF, Luppi MMCP, Gaiad TP, Oliveira CM, Santos FM, Watanabe P, Brólio MP, Ambrósio CE, Miglino MA. Implantação do manejo alimentar adequado ao modelo experimental canino afetado pela distrofia muscular (GRMD). 6º Congresso Paulista de Clínicos Veterinários de Pequenos Animais, 2006, São Paulo. Anais do 6º Congresso Paulista de Clínicos Veterinários de Pequenos Animais. São Paulo : Anclivepa, 2006. p. 252.

Abatemarco A, Martins DS, Morini AC, Gaiad TP, Casagrande CF, Gerger RPC, Alves MM, Oliveira, CM, Santos FM, Lupi MMCP, Miyazato LG, Carvalho AF; Ambrósio CE, Miglino MA. Como a ditrofia muscular no modelo experimental canino Golden Retriever afeta o esôfago? XXII Congresso Brasileiro de Anatomia-XXVII Congresso Chileno de Anatomia-VII Congresso de Anatomia Del Cono Sul, 2006, Florianópolis. Anais do XXII Congresso Brasileiro de Anatomia-XXVII Congresso Chileno de Anatomia-VII Congresso de Anatomia Del Cono Sul. Florianóplis: UFSC, 2006.

Alencastro G, Sertié AL, Passos-Bueno MR. Estudo funcional da proteína colibistina e investigação de seu envolvimento na etiologia de hiperecplexia em pacientes brasileiros. 52º Congresso Brasileiro de Genética, Foz do Iguaçu, PR, 3 a 6 de setembro, 2006, GH057.

Capelli LP, Gonçalves MRR, Barbosa ER, Vianna-Morgante AM. A pré-mutação do gene *FMR1* em pacientes com tremor essencial ou atrofia de múltiplos sistemas. I Reunião de Pesquisadores em Distúrbios do Movimento e Gânglios da Base, no período de 09 a 11/11/2006, Ribeirão Preto, SP.

Casagrande CF, Morini AC, Abatemarco A, Wenceslau CV, Brólio MP, Jarra J, Ambrósio CE, Martins DS, Zatz M, Miglino MA. Separação e caracterização morfológica de células tronco CD34- don cordão umbilical de cães golden retriever para o tratamento da distrofia muscular. XXII Congresso Brasileiro de Anatomia, 2006, Florianópolis, SC. XXII Congresso Brasileiro de Anatomia, 2006.

Catelani ALPM, Krepischi-Santos ACV, Mingroni-Netto RC, Rosenberg C. Identificação de regiões cromossômicas ou genes candidatos para surdez por array-CGH. In: 52o Congresso Brasileiro de Genética, Águas de Lindóia., September 2006. CD de Resumos, 2006. p. 355.

D'Angelo CS, Paz JA, Kim CA, Bertola DR, Castro CIE, Varela MC, Koiffmann CP. Diferentes mecanismos genéticos na origem da síndrome de monossomia 1p36. In: 52o. Congresso Brasileiro de Genética, 2006, Foz de Iguaçu. período de 3 a 7 de setembro. Ribeirão Preto, SP: Sociedade Brasileira de Genética.

D'Angelo CS, Paz JA, Kim CA, Bertola DR, Castro CIE, Varela MC, Koiffmann, CP. Fenótipo Prader-Willi-like: investigação de deleções em 1p36 em 41 pacientes com atraso do desenvolvimento neuropsicomotor, hipotonia, obesidade e/ou hiperfagia, dificuldade de aprendizado e problemas de comportamento. In: 51o Congresso Brasileiro de Genética, 2005, Águas de Lindóia, SP, 2005.

Fagali CQ, Kim CA, Bertola DR, Koiffmann CP. Pesquisa de mecanismos genéticos na síndrome de Sotos. In: 52o Congresso Brasileiro de Genética, 2006, Foz de Iguaçu - PR. período de 3 a 7 de setembro. Ribeirão Preto, SP: Sociedade Brasileira de Genética.

Gonçalves MRR, Capelli LP, Barbosa ER, Nitrini R, Kok F, Vianna-Morgante AM. Caso clínico da síndrome de tremor/ataxia ligada ao X frágil e revisão dos critérios diagnósticos da patologia. I Reunião de Pesquisadores em Distúrbios do Movimento e Gânglios da Base, no período de 09 a 11/11/2006, Ribeirão Preto, SP.

Griesi-Oliveira K, Orabona GM, Moreira AS, Takahashi VN, Vadasz E, Passos-Bueno MR. Estudo de polimorfismos nos genes HTR1A e HTR1B em pacientes com autismo. 52o Congresso Brasileiro de Genética, Foz do Iguaçu, PR, 3 a 6 de setembro, 2006, GH230.

Iughetti CG, Pereira VS, Koiffmann CP. Sintenia ancestral HSA 14/15 também presente em Alouatta sara (Platyrrhini). In: 52o Congresso Brasileiro de Genética, 2006, Foz de Iguaçu, PR. período de 3 a 7 de setembro. Ribeirão Preto, SP: Sociedade Brasileira de Genética.

Iughetti CG, Teixeira RHF, Nunes ALV, Pessutti C, Cassaro K, Fedullo JDL, Fries BG, Summa MEL, Silva AMJ, Koiffmann CP. Evolução cromossômica na família Atelidae: Sintenia dos cromossomos humanos 14/15. In: III Congresso Brasileiro de Mastozoologia, 2005, Aracruz, ES. período de 12 a 16 de outubro, 2005. p. 79.

Izzo G, Martins PCM, Mori CMC, Vainzof M. Estudos histológicos e genotipagem do comundongo LARGE: um modelo para distrofias musculares com defeitos de glicosilação. SIICUSP 2006.

Kohl I, Kim CA, Varela MC, Koiffmann CP. Caracterização molecular dos pontos de quebra e da extensão da deleção em 20 pacientes com síndrome de Cri-du-Chat. In: 51o Congresso Brasileiro de Genética, 2005, Águas de Lindóia, SP. período de 7 a 10 de setembro. Ribeirão Preto, SP: Sociedade Brasileira de Genética, 2005.

Kossugue PM, Muniz VP, Pavanello RCM, Silva HC, Gurgel Giannetti J, Paim JFO, Zatz M, Vainzof M. Mutações na região C-terminal do gene RYR1 e padrão de herança autossômica recessiva em pacientes brasileiros com miopatia de Central Core. 57a Reunião anual da SBPC, Fortaleza, CE, 17 a 22 de julho de 2005.

Kossugue PM, Muniz VP, Pavanello RCM, Zatz M, Silva HC, Gurgel Giannetti J, Paim JFO, Vainzof M. Screening for mutations in the C-terminal region of RYR1 gene identify high frequency of autosomal recessive form of Central Core Disease (CCD) in Brazil. V Simposio de Hipertermia Maligna, São Paulo, 7-8 de outubro de 2005. Neurociencias 3(13): 66-68, 2005.

Kossugue PM, Muniz VP, Vainzof M. Estudos Genéticos na Hipertermia Maligna e Miopatia de Central Core. V Simposio de Hipertermia Maligna, Sao Paulo, 7-8 de outubro de 2005. Neurociencias, 3 (13): 66-68, 2005.

Krepischi-Santos ACV, Vianna-Morgante AM, Rosenberg C. Whole-genome array-CGH screening in syndromic patients with mental retardation: new alterations and old syndromes revisited. 52o Congresso Brasileiro de Genética, Foz do Iguaçu, PR, 2006. CD-ROM 359.

Martins DS, Saraiva NZ, Ambrósio CE, Garcia JM, Miglino MA. Extração de células germinativas embrionárias caninas para uso em terapia celular no modelo GRMD (Distrofia Muscular do Golden Retriever). II Simpósio de Pós-Graduação & XV Semana Científica "Prof. Dr.

Benjamin Eurico Malucelli", 2006, São Paulo. Anais do II Simpósio de Pós-Graduação & XV Semana Científica "Prof. Dr. Benjamin Eurico Malucelli". São Paulo: FMVZ/USP, 2006.

Morini A, Martins DS, Abatemarco A, Casagrande CF, Luppi MMCP, Gaiad T, Ambrósio CE, Miglino MA, Zatz M. Análise das dosagens e concentrações séricas da ciclosporina a em cães da raça golden retriever normais ou afetados pela distrofia muscular. VI Congresso Paulista de Clínicos Veterinários de Pequenos Animais - VI CONPAVEPA, 2006, São Paulo. VI Congresso Paulista de Clínicos Veterinários de Pequenos Animais - Anais 2006, p. 251.

Morini A, Martins DS, Abatemarco A, Luppi MMCP, Gaiad T, Ambrósio CE, Miglino MA, Zatz M. Efeitos da Ciclosporina A sobre a função renal de cães da raça Golden Retriever normais ou afetados pela distrofia muscular. VI Congresso Paulista de Clínicos Veterinários de Pequenos Animais - VI CONPAVEPA, 2006, São Paulo. VI Congresso Paulista de Clínicos Veterinários de Pequenos Animais - Anais 2006. p. 252.

Morini AC, Miglino MA, Carvalho MB, Martins DS, Ambrósio CE, Souza AP, Wenscelau CV, Brólio MP, Jarra J, Morini Junior JC, Gaiad TP, Ferreira E, Zatz. Alterações na avaliação da função renal de cães GRMD submetidos a imunossupressão por ciclosporina A. II Simpósio de Pós-Graduação & XV Semana Científica "Prof. Dr. Benjamin Eurico Malucelli", 2006, São Paulo. Anais do II Simpósio de Pós-Graduação & XV Semana Científica "Prof. Dr. Benjamin Eurico Malucelli". São Paulo : FMVZ/USP, 2006. p. 120-120.

Morini AC, Miglino MA, Carvalho MB, Martins DS, Ambrósio CE, Souza AP, Wenceslau CV, Brólio MP, Jarra J, Morini Jr JC, Gaiad TP, Ferreira E, Zatz M. Concentrações séricas da ciclosporina A em cães GRMD. II Simpósio de Pós-Graduação & XV Semana Científica "Benjamin Eurico Malucelli", 2006, São Paulo. Anais do II Simpósio de Pós-Graduação & XV Semana Científica "Benjamin Eurico Malucelli". São Paulo: FMVZ/USP, 2006. p. 119.

Oliveira MA, Castro CIE, Kim CA, Bertola DR, Koiffmann CP. Duplicação 20p(p11.2-p12.1)mat e t(5;14)(q13;q32) de novo: estudo genético-clínico. In: 52o Congresso Brasileiro de Genética, 2006, Foz de Iguaçu, PR. período de 3 a 7 de setembro. Ribeirão Preto, SP: Sociedade Brasileira de Genética.

Rosseto AS, Silva TML, Eler G, Pereira LC, Passos-Bueno MR, Carvalho OS, Rabbi-Bortolini E, Errera FIV. Estudo do SNP45T>G no gene da adiponectina em pacientes obesos de Vitória-ES. 52o Congresso Brasileiro de Genética, Foz do Iguaçu, PR, 3 a 6 de setembro, 2006, GH196.

Santos JPA, Froes TR, Miglino MA. Ultra-sonografia muscular pélvica de cães da raça Golden Retriever portadores pela distrofia muscular e em terapia celular. II Simpósio de Pós-Graduação & XV Semana Científica "Prof. Dr. Benjamin Eurico Malucelli", 2006, São Paulo. Anais do II Simpósio de Pós-Graduação & XV Semana Científica "Prof. Dr. Benjamin Eurico Malucelli". São Paulo : FMVZ/USp, 2006. p. 31.

Sell K, Velloso FJ, Yamamoto LY, Zatz M, Zanotelli E, Chimelli LMV, Kashiwagi F, Vainzof M. Triagem de mutações no gene Dinamina 2 em pacientes brasileiros com miopatia centronuclear. SIICUSP 2006.

Vainzof M, Kossugue PM, Ayub D, Onofre PCG, Zilberztajn D, Sell K, Yamamoto LU, Martins PCM, Maia LS, Zatz M, Silva HCA, Miglino MA, Ambrosio CE, Xavier Neto J. Modelos animais para doenças neuromusculares humanas. V Simpósio Brasileiro de DNM/ELA, São Paulo, 19-20 de maio de 2006. Neurociências.

Vainzof M, Yamamoto LU, Kossugue PM, Fogaça LLQ, Velloso FZ, Ayub D, Muniz VP, Zatz M, Silva, HCA, Massironi SMG, Miglino MA, Ambrosio CE, Miyazato LG, Moraes JRE, D'Angeles, FHF, Lacerda Neto JC, Mortari AC, Borges AS, Rezende LAL, Rahal SC. Modelos Animais ajudando a decifrar doenças neuromusculares humanas. V Simpósio de Hipertermia Maligna, Sao Paulo, 7-8 de outubro de 2005. Neurociencias, 3(13): 19-21, 2005.

Varela MC, Kim CA, Bertola DR, Sato AYS, Koiffmann CP. Deleção intersticial 6q16.2 e fenótipo 'Prader-Willi-like': investigação da deleção do gene SIM1 em 87 pacientes com obesidade

sindrômica e apresentação de um novo caso. In: 51o Congresso Brasileiro de Genética, 2005, Águas de Lindóia, SP, 2005.

Varela MC, Kok F, Bertola DR, Koiffmann CP. Cromossomos marcadores inv dup(15): semelhantes no tamanho e origem, diferentes na manifestação clínica e comportamental. In: 52o Congresso Brasileiro de Genética, 2006, Foz de Iguaçu, PR. período de 3 a 7 de setembro. Ribeirão Preto, SP: Sociedade Brasileira de Genética.

Vieira LCZ, Krepischi-Santos AC, Antonini S, Rosenberg C, Vianna-Morgante AM. Recombinação homóloga não alélica na origem de deleções cromossômicas. 52o Congresso Brasileiro de Genética, Foz do Iguaçu, PR, 2006. CD-ROM 446.

Vlach LWD, Miglino MA. Separação e cultivo de hemangioblástos do saco vitelino de cães SRD e da raça Golden retriever normais e afetados pela distrofia muscular (GRMD). I Simpósio de Iniciação Científica do Programa de Pós-Graduação em Anatomia dos Animais Domésticos e Silvestres da FMVZ, 2006, São Paulo. Anais do I Simpósio de Iniciação Científica. São Paulo: FMVZ, 2006. v. 1.

Wenceslau CV, Martins DS, Ambrósio CE, Jarra J, Miglino MA. Análise de células mesenquimais de fígado, medula óssea e saco vitelino de fetos caninos. II Simpósio de Pós-Graduação & XV Semana Científica "Benjamin Eurico Malucelli", 2006, São Paulo. Anais do II Simpósio de Pós-Graduação & XV Semana Científica "Benjamin Eurico Malucelli". São Paulo: FMVZ/USP, 2006. p. 117.

Yeh E, Errera FIV, Kimura L, Angeli CB, Mingroni-Netto RC, Canani LHS, Passos-Bueno MR. Estudo de associação entre polimorfimsos na região reguladora do gene ADIPOR1 e índice de massa corporal (IMC). 52o Congresso Brasileiro de Genética, Foz do Iguaçu, PR, 3 a 6 de setembro, 2006, GH274.

ANNEX 4. Thesis and Dissertations related to the PROJECT (2006)

PhD Thesis

1 Flávia Errera: Estudo de polimorfismos em três genes relacionados à angiogênese, *COL18A1, MMP2* e *VEGF*, em pacientes com Diabetes tipo 2 e retinopatia diabética. Instituto de Biociências/USP, 2006. (Scholarship: CNPq).
Advisor: Maria Rita Passos-Bueno
Research Subproject: I.1 Identification of disease genes

2 Oscar Suzuki: Estudo molecular e funcional do *COL18A1*. Instituto de Biociências/USP, 2006. (Scholarship: FAPESP).
Advisor: Maria Rita Passos-Bueno
Research Project: I.2 Mechanisms modulating phenotypic expression

3 Agnes Lumi Nishimura - Identificação de um novo gene para a esclerose lateral amiotrófica tipo 8 e estudos de associação em doenças de Alzheimer. Instituto de Biociências/USP. 2006 (Scholarship: FAPESP)
Advisor: Mayana Zatz
Research Project: I.1. Identification of disease genes

MSC DISSERTATIONS

1 Ana Carla Batissoco: Mutações nos genes *GJB2* e *GJB6* em indivíduos com deficiência auditiva. Instituto de Biociências/USP, 2006. Scholarship: CNPq).
Advisor: Regina Célia Mingroni-Netto
Research Subproject: I.1 Identification of disease genes

2 Jihane Romanos Estudo de mutações no gene *OTOF* em pacientes com deficiência auditiva e sua relação com a neuropatia auditiva. Instituto de Biociências/USP, 2006. Scholarship: CNPq).
Advisor: Regina Célia Mingroni-Netto
Research Subproject: I.1 Identification of disease genes

ANNEX 5. Lectures and Seminars

D'Angelo CS - Aspectos comportamentais na síndrome de Smith-Magenis" Curso de Psicologia, Disciplina "Biologia Para Psicologia" (BIO 0105). Instituto de Biociências/USP, May

D'Angelo CS - Síndrome de Smih-Magenis e Síndrome de Monossomia 1p36". Centro de Estudos do Genoma Humano. Instituto de Biociências – USP. June

Menck CFM - A química como solução. XXIII Semana da Química, São Paulo, August.

Menck CFM - Discriminando as lesões no DNA que levam à apoptose em células humanas irradiadas com ultravioleta. Faculdade de Medicina de Ribeirão Preto. Ribeirão Preto, SP, May.

Menck CFM - Onde estamos e onde poderemos ir na terapia gênica. II Workshop de Biotecnologia EJBio – March.

Menck CFM - Origem da vida: mundo de RNA. 9ª Semana Temática da Biologia September..

Menck CFM - Os caminhos para a morte celular em células contendo lesões no genoma. Instituto de Química, USP, São Paulo, August.

Menck CFM - Uma luz para os meninos da Lua: estudando a origem do câncer de pele através de adenovirus recombinante. Instituto de Ciências Biomédicas, Departamento de Farmacologia, USP, São Paulo, June.

Mingroni-Netto RC - Aspectos |Genéticos da Surdez. Divisão de Clínica de Otorrinolaringologia, Hospital das Clínicas da Faculdade de Medicina/USP, São Paulo, April 24th.

Mingroni-Netto RC - Diagnóstico Genético na perda auditiva sensório neural, 5º Congresso da Fundação Otorrinolaringologia, São Paulo, August 19th

Mingroni-Netto RC - Estudos Genéticos das Populações de Remanescentes de Quilombos no Vale do Ribeira, São Paulo, Ciclo de Seminários do Programa de Pós-Graduação em Biologia Celular e Tecidual, Instituto de Ciências Biomédicas/USP, São Paulo, May 10th.

Mingroni-Netto RC - Identificação e Intervenção precoces na surdez **,** IV Jornada Acadêmica do Curso de Fonaudiologia da Faculdade de Ciências Médicas da Santa Casa- São Paulo.September 6th.

Mingroni-Netto RC - Pesquisa em genética humana : aconselhamento genético , 1º Simpósio Regional de Saúde, Universidade Braz Cubas, Mogi das Cruzes, São Paulo, September.

Mingroni-Netto RC - Relações surpreendentes entre Genótipos e Fenótipos em doenças humanas: Heterogeneidade Genética e Surdez, 17º Encontro de Biólogos do CRBio-1, Santos, SP, April 9th – 12th.

Netto LES - Estrutura, Função e Regulação da Expressão de Proteínas Antioxidantes. Ciclo de Seminários do Departamento de Fisiologia – Instituto de Biociências – USP, São Paulo.

Netto LES - Peroxiredoxin: Structure, Function and Regulation of gene expression. XXXV Reunião Anual da Sociedade Brasileira de Bioquímica e Biologia Molecular . Águas de Lindóia, SP, July 1st -4th.

Passos-Bueno MR - "Desenvolvimento Craniofacial e Identificação de Genes associados a fissuras lábio-palatinas" Satellite Course, Satellite Course, XVIII Congresso Brasileiro de Genética Clínica, Guarujá, SP, May 31st.

Passos-Bueno MR - Análise genômica em doenças genéticas humanas: contribuição na identificação de novos genes? 52º Congresso Brasileiro de Genética, Cataratas do Iguaçu, PR, September 3rd–6th.

Passos-Bueno MR - Aspectos Biológicos do Desenvolvimento – Estudo Molecular das Síndromes dos 1º. E 2º. Arcos faríngeos. Satellite Course, XVIII Congresso Brasileiro de Genética Clínica, Guarujá, May 31st.

Passos-Bueno MR - Avanços e Futuro do Tratamento das Fissuras Labiopalatinas, V Simpósio Sul-Americano Smile Train, Campinas, April 8th.

Passos-Bueno MR - Identificação de genes associados a doenças mendelianas e complexas, Post-graduation Course Fundação Antônio Prudente, São Paulo, May 5th.

Passos-Bueno MR Análise Genômica em Doenças Mendelianas e Complexas, XVIII Congresso Brasileiro de Genética Clínica, Guarujá, SP, May 31st.

Rosenberg C - Diagnóstico da deficiência mental na era molecular.XXII Congresso Brasileiro de Neurologia, VI Encontro Luso-Brasileiro de Neurologia, II Congresso da Federação Latino-Americana de Neurologia, Recife, PE, August 19th–23rd.

Vainzof M - Diagnostico das doenças neuromusculares. Unifesp, São Paulo, June 23rd

Vainzof M - Distrofinopatias. Curso no V Congresso Paulista de Neurologia, Ribeirão Preto, June 9th-11th

Vainzof M - Estudos de Proteína musculares e sua relação com doenças genéticas humanas. Reunião do Laboratório de Genética e Cardiologia Molecular, InCor, São Paulo, July 27th

Vainzof M - Estudos histológicos, histoquímicos e protéicos na caracterização das doenças neurmusculares", Disciplina Biologia do Músculo Estriado Esquelético, Programa de Pós-graduação em Biologia Geral e Aplicada. UNESP-Botucatu, May 17th

Vainzof M - Estudos protéicas no músculo miopático e normal. Ciclo de seminários dos docentes do Instituto de Biociências da USP, São Paulo, June. 23rd.

Vainzof M - Modelos Animais de doenças Humanas". Curso de Extensão: Bases da Pesquisa em Anestesiologia, Dor e Terapia Intensiva, Disciplina de Anestesiologia, Dor e Terapia Intensiva, UNIFESP/EPM. São Paulo, April 8th

Vainzof M - Modelos Animais. IV Simpósio Brasileiro de Doença do Neurônio Motor/ELA. São Paulo, June 17th -18th

Vainzof M - Modelos Animais", V Simpósio Brasileiro de Doenças Neuromusculares-ELA. São Paulo, May 1th – 20th

Vainzof M - Perspectivas do uso de células tronco em doenças neurológicas. Mesa Redonda: Neurologia em debate. Universidade Federal da Bahia, Hospital das Clíncas. Salvador, Ba, April 30th

Varela MC - "Síndromes de Prader-Willi e Angelman: Aspectos Genéticos e Comportamentais" Curso de Psicologia, Disciplina "Biologia Para Psicologia" (BIO 0105). Instituto de Biociências/USP, May

Varela MC - Vantagens e limitações de diferentes técnicas empregadas no diagnóstico das Síndromes de Prader-Willi e Angelman. Instituto de Pesquisa do Hospital Israelita Albert Einstein, May.

Vianna-Morgante AM - Deficiência Mental de herança ligada ao X – Curso: Citogenética Molecular na Investigação de Alterações Genômicas, Centro de Estudos do Genoma Humano, SP, November 22nd.

Vianna-Morgante AM - Deficiência Mental ligada ao X e síndrome do X frágil - Curso de Genética para Neurologistas. Org. Centro de Estudos do Genoma Humano e Sociedade Brasileira de Neurologia Infantil. São Paulo, November 2nd.

Vianna-Morgante AM - Genética Humana: Conceitos – Curso de Genética para Neurologistas, Org. Centro de Estudos do Genoma Humano e Sociedade Brasileira de Neurologia Infantil. SP, November 2nd.

Vianna-Morgante AM - Limites entre diagnóstico e pesquisa. Simpósio Ética na Pesquisa em Seres Humanos. Instituto de Biociências/USP, São Paulo, December 11th.

Vianna-Morgante AM - Mutações do gene *FMR1:* Quadros clínicos diferentes - 17° Encontro de Biólogos do CRBio-1, Santos, SP, April 10th

Vianna-Morgante AM - Mutações do gene *FMR1*: quadros clínicos e testes diagnósticos". Clínica Clínica e Centro de Pesquisa em reprodução Humana Roger Abdelmassih. São Paulo, October 11th..

Vianna-Morgante AM A análise citogenética no diagnóstico e na pesquisa. Simpósio: "50 anos de 46 cromossomos - Citogenética clínica - passado, presente e futuro. Org. Laboratório Clínico, Hospital Israelita Albert Einstein. São Paulo, October 26th.

Zatz M - Amyotrophic lateral sclerosis type 8- International Satellite Symposium on Neuromuscular Disorders, Tel-Aviv, Israel, July 7th

Zatz M - Células Tronco Adultas e embrionárias: A pesquisa antes do tratamento.Centro de Estudos do Genoma Humano-USP. June 9th

Zatz M - Celulas tronco e Genética: desafios na ciência. IV Encontro dos Comités de Ética em Pesquisas. Centro Rebouças.São Paulo

Zatz M - CÉLULAS TRONCO: POLÍTICA, ÉTICA E PESQUISA. Ordem dos Advogados do Brasil (OAB), São Paulo, September 17th

Zatz M - Células-tronco e doenças neuromusculares: Pré-Congresso Internacional sobre Células-tronco Salvador, Bahia, September 7th

Zatz M - Células-tronco embrionárias e adultas: o que podemos aprender com elas?.Oficina de Avaliação e Acompanhamento do Edital CT Biotecnologia/MCT/CNPq/MA/SCTIE/Decit no. 24/05, Salvador, Bahia, September 12th-13th.

Zatz M - Células-tronco: da política a pesquisa- Encontro de Iniciação Científica- Escola Politécnica, São Paulo, August 26th

Zatz M - Células-tronco: quais são as perspectivas de aplicação clínica? Hospital Universitário, São Paulo, August 10th, 2006.

Zatz M - Clinical and Genetic Ascertainment of a Large Family with Autosomal Dominant Spastic Paraplegia (SPG4). XXII Congresso Brasileiro de Neurologia, do VI Encontro Luso-Brasileiro de Neurologia e II Congresso da Federação Latino Americana de Neurologia EURO SUR. Centro de Convenções, Recife, Pernambuco. August 19th-23rd

Zatz M - Genoma, genes e existência. Instituto da Psicanálise Lacaniana, São Paulo, September 24th.

Zatz M - Stem-cells research in Brazil- Third World Academy of Sciences, Angra dos Reis, RJ, September 3rd

Annex 6. List of schools visited (around 7,000 students were enrolled in the program)

Sept. 26	Escola de Aplicação da Universidade de São Paulo (day time)
Sept. 28	EE Fadlo Haidar – Rua Murmúrios da Tarde, 200 – São Mateus (day + night time)
Oct. 3	EE Prof. Raquel Assis Bananeiras – Av. General Penha Brasil, 1225 – Vila Nova Cachoeirinha (day + night time)
Oct. 5	EE Dom José Gaspar – Rua Isadoro Fontes, sem número. Ribeirão Pires (day time)
Oct. 6	EE Luiz Gonzaga Pinto e Silva – Rua Geraldo Fraga de Melo, 324 – Jardim São Luis (day time)
Oct. 10	EE Salvador Allende Gossens – Rua Domingos Lisboa, 139 – Itaquera (day + night time)
Oct. 16-18	ETE Albert Einstein – Rua Nova Granada, 35 – Casa Verde ((day + night time)
Oct. 19	EE Vila Bela – Rua Terra Tombada, s.n Jardim da Conquista – São Mateus (night time)
Oct. 21	Colégio Guilherme Dumont Villares – Avenida Guilherme Dumont Villares, 723 – Jardim Suzana (day time)
Oct. 22	Lobby of Instituto Oceanográfico – as participation in the Semana de Ciência e Tecnologia 2006
Oct. 23	EE Parque Anhanguera – Rua São Marcos s/n Sol Nascente – Jaraguá (night time)
Oct. 24	Unifieo – Universidade em Osasco (day + night time)
Oct. 26	EE Prof. Almeida Junior – Avenida Eng. Heitor Antonio Eiras Garcia, 1874 – Jardim Maria Luiza (day + night time)
Oct. 26 - 28	Unifieo – Universidade em Osasco (day + night time)
Oct. 31	EE Roldão Lopes de Barros – Vila Mariana (day + night time)
Nov. 6	EE Guiomar – Rua Paulo Aires s/n km 18,5 (night time)
Nov. 8	EE Tito Prates – Rua Mendonça Junior, 611 – Vila Nova Cachoeirinha (day + night time)
Nov. 11	EE Antonio Firmino de Proença – Rua da Mooca, 363 (day + night time)

Annex 7. CD-Rom

Annex 8. Post-graduation students working in the CEGH Public Information Program

1. Adriana Ribeiro de Oliveira Marques
2. Ana Carolina Suzuki Dias Cintra
3. Érika Yeh
4. Fernando Nodari
5. Gustavo Alencastro
6. Karina Griesi
7. Paula Cristina Gorgueira Onofre
8. Renato Chimaso dos Santos
9. Silvio Ganiko Higa
10. Vivian Lavander Mendonça

Annex 9. List of interviews to different communication medium

Name	Subject	Medium	Date
Dessen, E.M.B.	"Dentro da Fábrica da Vida"(Inside life's factory)	Jornal da USP no. 781: 8,	October, 2006.
Dessen, E.M.B.	Projeto leva célula gigante para observação de aluno de ensino médio (Program deliver a giant cell for the observation of high school students)	USP OnLine	October 24th 2006
Mingroni-Netto, R.C.	"Elogio da diferença" (In praise of the difference)	Folha de São Paulo Newspaper	January 15th, 2006
Zatz, M.	"Células-tronco" (Stem cell)	Revista Almanaque Abril	January, 2006
Zatz, M	Fraude Dr. Hwang: as pesquisas continuam – Dr. Hwang's research continues	O Estado de São Paulo Newspaper	January, 2006
Zatz, M	"Isso é crime" (This is crime)	Revista Carta Capital	January, 2006
Zatz, M	"Células-tronco (Stem cells)	Fundação Casa de Rui Barbosa/Ministério da Cultura	February, 2006
Zatz, M	Ciência Unida e com financiamento (Funding a united Science)	Scientific American Brazil	February, 2006
Zatz, M	Células-tronco (Stem cells)	Revista Saúde	March, 2006.
Zatz, M	Ciência para salvar a vida de portadores de doenças genéticas (Science to save the life of persons striken with genetic diseases)	Globo On line	March, 2006
Zatz, M	"Teoria e Prática das Células-Tronco" (Stem cell Theory and practice)	Boletim Reitoria ano IV, no. 15	March, April, 2006
Zatz, M		Jornal da Cidade da Associação de Jornalismo Científico	April, 2006
Zatz, M	Genética como medicina preventiva (Genetic as preventive medicine)	Revista Médico	April, 2006
Zatz, M	Células-tronco: tudo sobre o polêmico debate (Stem cell: all about the polemic debate)	Discutindo Ciência	April, 2006

Zatz, M	Mayana Zatz recebe prêmio "Faz Diferença" da Globo (Mayana Zatz is awarded Globo's "Make a difference"prize)	Boletim Reitoria ano IV-no. 46	April, 2006
Zatz, M	USP elabora projeto de incentivo à pesquisa (USP organizes a project to encourage research)	Universia-site	May, 2006
Zatz, M	Laboratórios da USP terão vagas para os melhores do ensino médio (USP labs wil, open space for the best high school studentes)	ESTADO DE SÃO PAULO newspaper	June, 2006
Zatz, M	Defensora das Células Tronco. (Defender of stem cells)	Revista O GLOBO	June, 2006
Zatz, M	Cérebros de Laboratório (Lab brains)	Folha de São Paulo Newspaper	June, 2006
Zatz, M	Burocracia atrapalha ciência brasileira (Bureaucracy hinders Scienec)	Fantástico TV Program	July, 2006
Zatz, M	Bush e células-tronco – (Bush and stem cells)	Estado de São Paulo - Newspaper	July, 2006
Zatz, M	"Pesquisa em terapia celular e suas implicações para a atenção à saúde(Research on Cell Therapy and its Implications to heath services)	Round Table in - Hospital Universitário	August, 2006
Zatz, M	USP avança contra distrofia muscular (USP advances against muscular distrophy)	O Estado de São Paulo - Newspaper	August, 2006
Zatz, M	A revolução da Ciência (The revolution of Science)	Revista Super Saudável Ano VI-no.32 pg 18-21	October/ December, 2006
Zatz, M	Muito além do tubo de ensaio (Beyond the test tube)	Portal Globo: globolog.com.br	October, 2006
Zatz, M	A medicina do futuro.(The Medicine of Tomorrow)	Portal Globo: globolog.com.br	October, 2006
Zatz, M	Os gêmeos ingleses: e as cotas como ficam? (The British Twins: how do the quotas apply?)	Portal Globo: globolog.com.br	October, 2006
Zatz, M	Estamos no caminho certo (We're on the right track)	Revista Fapesp	November, 2006
Zatz, M	Células-tronco: o que podemos esperar delas? (Stem cells: What can we Expect from them?)	Revista Galileu	December, 2006.