Genetics and Molecular Biology, 22, 4, 613-615 (1999)

Thesis Abstracts

Molecular biology application in the human identification from stains and crusts of blood

(As aplicações da biologia molecular na identificação humana em manchas e crostas de sangue)

*José Arnaldo Soares-Vieira**

The increasing popularization of identification methods through the DNA study among lay people in general has been a common problem involving molecular biology. Theoretically, with only one cell it is possible to get enough DNA, which can be amplified by polymerase chain reaction (PCR), for forensic identification. In fact, however, an analysis of that nature is not always possible. We decided to accomplish the present study in order to verify the minimum amount of blood necessary for a possible identification, considering the surface where the blood was found, as well as the causes that could interfere in the results. In a first step, the *loci* D1S80 and HLA-DQα were amplified by the PCR technique from genomic DNA extracted from stains and crusts with different amounts of blood (32 µl, 16 µl, 8 µl, 4 µl, 2 µl and 1 µl). In all samples of stains and crusts of blood it was possible an identification by DNA analysis. In a second step, the *loci* D1S80 and HLA-DQα were amplified from genomic DNA, extracted from diluted blood samples (640 leukocytes; 320 leukocytes; 160 leukocytes; 80 leukocytes; 40 leukocytes; 20 leukocytes; 10 leukocytes and 5 leukocytes). The amplification of DNA was possible in diluted blood samples of at least 10 leukocytes. Considering the conditions in which the present study was accomplished, it was possible to conclude that 1 µl of blood stains or crusts was enough for identification. It was also concluded that 10 leukocytes were enough to extract DNA possible of being analyzed.

Publication supported by FAPESP.

**1998. Departamento de Medicina Legal, Faculdade de Medicina da Universidade de São Paulo, São Paulo, SP, Brasil. Doctoral thesis. Orienting Professor: Dr. Daniel Romero Muñoz.*

Cytogenetic and molecular analyses in hyperplasias and neoplasias of the prostate

(Análise citogenética e molecular em hiperplasias e neoplasias da próstata)

*Andréa Borduchi Carvalho Franco de Salles**

Prostate cancer, which often appears relatively late in life, is one of the most common cancers in man in many countries. The genetic basis of this disease is not well understood, but loss of the Y chromosome and structural abnormalities involving chromosomes 1, 2, 3, 7, 8, 10, 12 and X or double minute chromosomes have been described. In the present study, we analyzed nuclei from prostatic adenocarcinoma before culture and/or after 7, 14 or 21 days *in vitro* for chromosomes 1, 7, 8, 10, 11, X and Y using FISH technique. In 17 noncultured prostate tumor specimens, the most frequent aberrations found were the loss of chromosome Y and the gain of chromosome 1. Polyploidy and aneusomy 7 or X were associated with clinically aggressive behavior of the disease or higher tumor grade and monosomy 8 was observed in clinically nonaggressive disease.

Nine prostate tumor specimens were also analyzed before and after culture using different methodologies. Mechanical and mild, long-term collagenase treatment for sample disaggregation and medium enriched with low FBS concentrations, growth factors, hormones, gluthatione and vitamins yielded the largest increase of epithelial cell proliferation without contamination with fibroblasts. The culture success was observed in 100% (10/10) of the cases and all harvests were performed 3-7 days after culture setup. Using other methodologies, harvests were performed 5 or 6 days (2 cases) and 10-31 days (16 cases) after culture setup. After FISH analysis, the noncultured nuclei exhibited higher frequencies of aneuploidy than those from cultured cells. Nuclei with aneusomies apparently had a proliferative disadvantage in culture. However, cells with monosomy 8 or nulissomy Y may be exceptions, since they were seen even after 21 days *in vitro*. Our findings suggest that FISH analysis may help in the characterization of some prognostic markers associated with prostate cancer, overcoming the artifacts created by culturing cells, and probably will provide greater understanding of tumor pathogenesis when associated with molecular data.

Evaluating the susceptibility for prostate cancer, we applied PCR multiplex methodology to analyze glutathione-S-transferase T1 and M1 (GST T1 and GST M1) genotypes in 48 samples of prostate lesions. The frequencies of GSTT1 0/0 and GSTM1 0/0 genotypes in the prostate disease group were 22.92% and 43.75%, respectively, which are statistically similar to the frequencies detected in normal population. Therefore, the findings are not suggestive of a protective effect of such enzymes for the development of prostate cancer and contrast with the increased risks for certain GST genotypes in other types of diseases. The results may be dependent on the fact that prostate disease is not correlated with environmental factors metabolized by GSTs. Conversely, given the vast number of detoxification enzymes and the complexity of chemical mixtures to which an individual may be exposed, assessment of susceptibility based on the analysis of single genotypes may not be sufficient.

Publication supported by FAPESP.

*1998. Departamento de Biologia, Instituto de Biociências, Letras e Ciências Exatas de São José do Rio Preto (IBILCE), Universidade Estadual Paulista (UNESP), São José do Rio Preto, SP, Brasil. Doctoral thesis. Orienting Professor: Dr. Eloiza Helena Tajara da Silva.

Studies of the karyotypic differentiation process on four genera of Brazilian rodents (Sigmodontinae, Rodentia), based on conventional and molecular cytogenetics

(Estudos dos processos de diferenciação cariotípica, baseados em citogenética convencional e molecular, em quatro gêneros de roedores brasileiros)

*Maria José de J. Silva**

Comparative cytogenetic studies were performed on 85 specimens of Brazilian rodents sampling four genera of Sigmodontinae rodents: *Oligoryzomys*, *Nectomys*, *Akodon* and *Rhipidomys*. The techniques used were conventional staining, CBG, GTG, RBG-banding, Ag-NOR staining and fluorescent *in situ* hybridization (FISH) with telomere probes. Meiotic and synaptonemal complex studies were also performed.

We report the karyotypes of two closely related new species of *Oligoryzomys*, here named *Oligoryzomys* sp. 1 and *Oligoryzomys* sp. 2, respectively from Pico das Almas (Bahia) and Serra do Cipó (Minas Gerais). *Oligoryzomys* sp. 1 demonstrated 2n = 46 and *Oligoryzomys* sp. 2 had 2n = 44, 44/45. Our results based on CBG, GTG, RBG, Ag-NORs, chromosomal measurement as well as FISH support the hypothesis that the difference between both diploid numbers occurred by a centric fusion event. The karyotypes had conspicuous and distinguishable macro- and microchromosomes, an uncommon phenomenon among oryzomine rodents. Despite the fact that no ITS was found, we suspect that the largest pairs (1, 2 and 3) have originated from a higher diploid number due to successive tandem fusion mechanisms.

A cytogenetic study using FISH of telomere probes, CBG, GTG and RBG-banding patterns, and synaptonemal complex data was carried out in two species of *Nectomys* from three Brazilian states: Pernambuco, Mato Grosso and São Paulo: *Nectomys squamipes* had 2n = 56 and 57 and *Nectomys rattus* had 2n = 52, 53. The diploid numbers of 53 and 57 were due to the presence of supernumerary chromosomes, which presented different morphologies, size and banding patterns. The tandem fusions involved chromosome pairs 3 + 11 and 5 + 24 from karyotype with 2n = 56 that originated, respectively, pairs 1 and 4 in specimens with 2n = 52. FISH results revealed interstitial telomeric signals (ITS) in a submetacentric B, but no ITS was detected in the chromosomes originated by tandem fusion. The supernumeraries presented a remarkable heterogeneity of size and morphology, constitutive heterochromatin pattern and localization of telomeric sequences. Study of synaptonemal complex by light and electron microscopy showed the supernumerary to be an autopaired univalent. Sex chromosome polymorphism was also detected with each X and Y chromosomes presenting three different morphologies that could be explained by addition/deletion of heterogeneous heterochromatin. In meiotic cells the pairing involved the heterochromatic short arm of the X-chromosome and the short arm of the Y-chromosome.

A diploid chromosome number of 2n = 10 was found in a new species of *Akodon* from Gaúcha do Norte and Vila Rica, State of Mato Grosso, central Brazil. This represents the lowest known chromosome number for all rodents. One female with nine chromosomes due to sex chromosome monosomy (2n = 9, XO) was also found. A polymorphic pair (3), which could be acrocentric, submetacentric, or heterozygous, was observed as a result of a pericentric inversion. Comparison of GTG banded metaphases of *Akodon* sp. (2n = 10) and *A. cursor* (2n = 16) evidenced that the karyotypical differentiation between both species involved several complex chromosomal rearrangements, including tandem fusions, Robertsonian rearrangement and pericentric inversions. Fluorescent *in situ* hybridization with a $(TTAGGG)_7$ repeat as a probe revealed interstitial telomeric signals in two of the large pairs (1 and 3). However, these ITS were not found in the specific points of chromosomal fusions. There was no ITS at the region involved in the pericentric inversion of pair 3, and also at the pair originated by Robertsonian rearrangement (pair 2 of *Akodon* sp.).

Two diploid numbers and five karyomorphs were found in ten specimens of *Rhipidomys* from three states of Brazil: 2n = 50 from Amazonas and 2n = 44 from Mato Grosso and Bahia. The diploid number of 2n = 50 presented two fundamental numbers (FN = 71 and 72) as a result of pericentric inversions and addition/deletion of constitutive heterochromatin. The samples from two localities of the State of Mato Grosso shared 2n = 44 and FN = 52, but exhibited two different karyotypes due to pericentric inversions in different pairs of autosomes. In spite of having 2n = 44, the specimen from Bahia showed karyotype and FN completely distinctive from those from Mato Grosso. Karyological comparison of GTG-banding patterns revealed total homoeology between specimens of Mato Grosso and Bahia when the autosomal pericentric inversions were taken into account. However, homoeologies were demonstrated for only ten autosomes between the karyotypes with 2n = 50 and 2n = 44 and, despite the remarkable number of the suggested rearrangements, interstitial telomeric sites were not detected. Additionally, sex chromosomes exhibited polymorphism in size and morphology.

Publication supported by FAPESP.

*1999. Departamento de Biologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, SP, Brasil. Doctoral thesis. Orienting Professor: Dr. Yatiyo Yonenaga-Yassuda.

Cytogenetic analysis of four species of the family Cichlidae (Pisces, Perciformes)

(Análise citogenética em quatro espécies da família Cichlidae (Pisces, Perciformes))

*Marcos Antonio Loureiro**

This work accomplished cytogenetic studies in four fish species of the family Cichlidae, Order Perciformes, from two Brazilian hydrographical basins. The species *Cichlasoma paranaense* and *Crenicichla niederleinii* were collected from the Tibagi River basin (PR) and *Crenicichla* sp. from the Itajaí-Açu River basin (SC). Specimens of *Geophagus brasiliensis* were collected from both basins. All the species studied exhibited 2n = 48 chromosomes and presented variations in karyotypic morphology. *Cichlasoma paranaense* presented 20 SM and 28 ST-A (NF = 68), *Crenicichla niederleinii* 4 M, 6 SM and 38 ST-A (NF = 58), *Crenicichla* sp. 4 M, 4 SM and 40 ST-A (NF = 56) and *Geophagus brasiliensis* 6 SM and 42 ST-A (NF = 54) from both basins. The nucleolus-organizing region (NOR) was detected in the two *Crenicichla* species through the technique of silver nitrate impregnation, and was located on the short arm of the first chromosome pair, metacentric type, coinciding with the secondary constriction. In *Geophagus brasiliensis* the NOR was identified on the short arm in the largest chromosomes of the ST-A group, also coinciding with the secondary constriction region. In *Cichlasoma paranaense* two NOR patterns were observed. On the first, the individuals collected from the Guaravera region (Londrina) exhibited more than one homologous chromosome pair by AgNO3 staining, suggesting multiple NORs; on the second, the individuals collected at other points of the Tibagi River basin (PR) exhibited one single nucleolar chromosome pair, staining the short arm of the 2nd pair. Heteromorphisms in the size of ribosomal cistrons were detected in all species analyzed, being intra- and inter-individually observed. The treatment by chromomycin fluorochrome coincided with the NOR regions labeled by silver nitrate. Through the DAPI fluorochrome technique, no chromosomal marking was detected. The constitutive heterochromatin pattern was very similar in all studied species, being observed preferentially in centromeric regions, as well as in the secondary constriction region. Only in the species *Cichlasoma paranaense* was restriction enzyme analysis with *Alu*I performed, which produced a similar pattern to C-banding. These data are relevant for later studies on the karyotypic evolution of the family and for cytotaxonomy-related works.

**1999. Mestrado em Genética e Melhoramento, Centro de Ciências Biológicas. Departamento de Biologia Geral, Universidade Estadual de Londrina, Londrina, PR, Brasil. Master's thesis. Orienting Professor: Dr. Ana Lúcia Dias.*

Genetic variation analysis for thirteen STR loci in the Federal District population of Brazil

(Análise da variação genética em treze locos STR na população do Distrito Federal)

*Luciano Chaves Arantes**

Genetic polymorphisms based on PCR amplification of short tandem repeats (STR) have revolutionized forensic sciences as they combine several highly desirable characteristics for human identification purposes.

In this work a database of allele frequencies for 13 STR loci was constructed for the population of Federal District (DF). These loci are: D12S1090, D3S1744, D18S849, CSF1PO, TPOX, TH01, F13A01, FESFPS, vWA, D16S539, D7S820, D13S317 and D5S818.

Observed genotypic proportions at each locus are consistent with expected ones under Hardy-Weinberg equilibrium and evidence of gametic phase linkage disequilibrium between loci is negligible. These results allow the estimation of genotype frequencies from allele frequencies and also warrant the use of the product rule to calculate the probability of occurrence of a multi-locus DNA profile.

High *a priori* exclusion probability (> 99.99%) and power of discrimination (> 1 in 300 trillion) values demonstrate the efficacy of the combined use of these 13 STR loci for paternity and criminal investigations in Federal District population.

When the probability test was applied to compare Federal District allele frequencies with the three major American racial groups, significant deviations ($P < 0.05$) were observed for the majority of the loci. However, no significant deviation was seen at twelve of the thirteen loci when Federal District allele frequency distributions were compared to the combined American racial group allele frequency distributions. At the D18S849 locus the P value ($P = 0.045$) was borderline significant.

The consequences on profile frequency estimates using different population databases were evaluated for the thirteen STR loci combined. From the forensic standpoint, estimates of multi-locus profile frequencies were significantly different when the DF database was used in comparison with any of the three American racial group databases. On the other hand, no significant differences were observed when the profile frequencies obtained from the DF database were compared to those obtained with the database combining all three American racial groups.

Research supported by FAP/DF.

**1999. Pós-graduação em Patologia Molecular, Faculdade de Ciências da Saúde, Universidade de Brasília, Brasília, DF, Brasil. Master's thesis. Orienting Professor: Dr. Felizardo Penalva da Silva.*