Genetic Control Evaluation of Wistar Kyoto Rats in Bioterium of Faculty of Chemistry Sciences and Pharmacy by Microsatellite Molecular Markers

Evaluación de la calidad genética de las ratas Wistar Kyoto del bioterio de la Facultad de Ciencias Químicas y Farmacia por marcadores moleculares microsatélites.

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Resumen

El control de calidad genético de los animales de experimentación utilizados en los bioterios debe ser prioritario, para asegurar que los estudios realizados tengan reproducibilidad y además validez científica. La presente investigación tuvo como objetivo evaluar la pureza genética de las ratas Wistar Kyoto (WKY) del bioterio de la Facultad de Ciencias Químicas y Farmacia de la Universidad de San Carlos de Guatemala, "Dra. Amarillis Saravia Gómez", las cuales nunca se han caracterizado genéticamente. Para realizar este análisis se seleccionaron los microsatélites D1Mgh6 y D17Mit3, los cuales muestran un alto grado de variabilidad y gran número de polimorfismos, por lo que permiten tener una visión general de las características genéticas de la cepa. Los análisis se realizaron utilizando la técnica de reacción en cadena de la polimerasa (PCR).

Los resultados obtenidos del análisis genético demostraron que el fragmento analizado para el microsatélite D17Mit3 de las WKY del bioterio fue el esperado con un peso molecular de 201 pb, que corresponde al tamaño reportado; en la base de datos genómicos de la rata, por los laboratorios Charles River y los Institutos Nacionales de Salud de los Estados Unidos (NIH). Por el contrario, el tamaño del microsatélite D1Mgh6 no corresponde al fragmento de 147 pb esperado. La colonia de ratas WKY se ha manejado con un método de reproducción consanguíneo lo que se confirmó con los resultados, ya que los microsatélites D17Mit3 y D1Mg6 mostraron una condición homogicota y homoalélica, por lo cual estos animales de experimentación pueden ser usados en ensayos como un modelo consanguíneo de características genéticas propias, puesto que no tienen las características genéticas esperadas para la raza WKY.

Palabras clave: PCR, polimorfismos, genealogía, control de calidad genético, animales de experimentación.

Abstract

Genetic quality control of experimental animals used in laboratory animal centers must be priority, to ensure that studies have reproducibility and also scientific validity. This research aimed to evaluate the genetic purity of the Wistar Kyoto rats at bioterium of the Faculty of Chemistry Sciences and Pharmacy at the University of San Carlos of Guatemala, "Dr. Amarillis Saravia Gómez", which never had been genetically characterized. The D1Mgh6 and D17Mit3 microsatellites were selected because they have a high grade of variability and a large number of polymorphisms, allowing have an overview of the genetic characteristics of the strain. The analyses were performed by the polymerase chain reaction technique (PCR).

The results of the genetic analysis showed that the colony of Wistar Kyoto rats corresponds to the estimated size for the microsatellite D17Mit3 (201 bp) as the genomic database rat and reported by Charles River Laboratories and the NIH. However, It doesn't correspond to the estimated size of the microsatellite D1Mgh6 (147 bp). The WKY strain has been manage with an inbreeding method, that was confirmed by the results because D17Mit3 and D1Mg6 showed an homogenic and homoallelic condition, so these experimental animals can be used in trials as a inbreed model, because do not have the genetic characteristics expected for the WKY breed.

Keywords: PCR, polymorphisms, genealogy, genetic quality control, experimental animals

Introduction

Laboratory animals are an essential biologic instrument in experimentation, used in a wide variety of researches. These are engendered, produced and maintained under controlled conditions and must be capable of giving a reliable and reproducible response. (Martínez, Osorio, Rodríguez, & Lolas, 2007). Due to its wide use, in recent years various works have been published in relation to genetic quality, including some cases of lines and colonies of mice and rats that have lost their genetic characteristics, leading to the devaluation of the results obtained, waste of time and resources.

The bioterium of the Faculty of Chemical Sciences and Pharmacy of the University of San Carlos of Guatemala, is the only center for breeding, management, maintenance and preclinical research that meets the established parameters and responds to various conventions as a bioterium nationwide; it currently has two species of rodents used in research: the WKY and the mouse *Mus musculus*. The WKY are the animals most used in a wide variety of investigations, being the main tests for determining the pharmacological effect of autochthonous plants and toxicological tests.

The determination of repeated single sequence polymorphisms (microsatellites), which are regions of DNA containing repeated di, tri, or tetra nucleotides that are found randomly in the genome, are used as molecular markers in a wide variety of applications in the field of genetics, such as relationships and population studies. This is due to their ability to generate a personal genetic fingerprint or genetic profile (Benavides & Guénet, 2003).

Microsatellite analysis is the most recommended method for genetic control; whereby; therefore, it was proposed to carry out the determination of two microsatellites D1Mgh6 and D17Mit3, which show a high variation and are highly polymorphic. As demonstrated by Deschepper, Prescott, Hendley y Reudelhuber (1997), in a study, 432 microsatellites in WKY and other related strains were evaluated, D1Mgh6 and D17Mit3 showed a high variation, also giving an overview of the genetic characteristics of each strain. Although many private universities, research centers and faculties in the University of San Carlos continuously carry out research using different experimental animals, no research into the genetic profiling or determination of genetic purity of laboratory animals has been carried out in Guatemala, whereby this research aims to create a vision about the genetic condition of the WKY rats from the bioterium using the conventional polymerase chain reaction (PCR) technique, thus determining the polymorphism shown by the D1Mgh6 and D17Mit3 microsatellites by analyzing the number of alleles and genetic condition. In addition, a comparison of theoretical characteristic sizes (base pairs) was made according to the rat genomic bank and of the producers Charles Rivers and the NIH.

Materials and Methods

Animals

The extraction of genomic DNA from eight rats (breeding pairs) that make up the entire nucleus of the foundation of the WKY colony of the bioterium was carried out. All the rats used in experimentation come from these breeding pairs, therefore, when carrying out the analysis of the eight rats, the genetic information of the complete population is obtained, which is why a statistical calculation was not performed to determine the sample number.

DNA Extraction, Quantification and Purity

Sampling for DNA extraction from the ear tissue was carried out using the commercial PureLink® Genomic DNA Mini Kit. The purified DNA was stored at -20 ° C.

The amount and purity of the extracted DNA was determined by spectrophotometry, diluting 20 µL of purified DNA in 980 µL of Tris-HCl 10 mM pH 8.0 (Invitrogen), to then determine the absorbance at wavelengths of 260 and 280 nm , in a Genesys 10 S spectrophotometer (Sambrook & Russel, 2001).

DNA integrity was evaluated by 0.9% agarose gel electrophoresis applying 6 µL of purified DNA mixed with 2 µL of RediLoad ©, a 1 Kb molecular weight marker was used. The electrophoresis conditions were 90 V for 80 min. The gel was subsequently developed by immersion in GelRed © for 30 min and the bands were visualized in a transilluminator at 254 nm (Trasiluminator Benchtop UV).

Microsatellite Analysis and Polymerase Chain Reaction (PCR)

PCR for each of the microsatellites was performed using 43 µL of Platinum PCR superMix (Promega ©), 0.1 µM of the forward and reverse primers (Table 1), 5 µL of RediLoad 10X with 100 ng of purified DNA, for one volume 50 µL end of reaction.

Table 1. Primers characteristics

Microsatellite	Sequence
D1Mgh6	Forward 5′-TGCATGCCCACAGTACACAT-3′
	Reverse 5′-CCAAGCACACTAATGCCTGA- 3′
D17Mit3	Forward 5′-TAAGGTCCCTCCAGACTCCA- 3′
	Reverse 5′-TGGGCAGAGAACAGCAGTC- 3′

DNA Analysis

Amplification was carried out in a 2720 Applied Biosystems © thermal cycler, with 1 cycle of denaturation at 95°C for 4 min, 30 cycles of amplification using: 94°C for 45s; 51°C for the D1Mgh6 microsatellite and 52°C for the D17Mit3 microsatellite for 45s; 72°C for 30s and a final extension cycle at 72°C for 4 min.

A 2.5% (w/v) agarose gel electrophoresis was performed with 0.5 X TBE buffer, loading 8 µL of the PCR product. Electrophoresis was run at 110 V for 30 min. The gel was developed using GelRed 3X, the bands were visualized in a transilluminator at 254 nm.

The size of the amplified fragment was determined by observing and comparing the size of the band obtained with a 100 bp weight marker. The expected fragments for the WKY rats are 145 bp for D1Mgh6 and 201 bp for D17Mit3.

Results

Figure 1 shows the eight samples analyzed (well 2-9), in which adequate DNA extraction was achieved, observing a band above 10,000 base pairs which corresponds to the complete DNA of the rat; ideally a narrow band near the well should be observed in an intact DNA sample, as seen in wells 2, 3, 4, 5 and 8; however in wells 6, 7 and 9 the DNA shows a tail, which corresponds to a certain degree of DNA fragmentation.

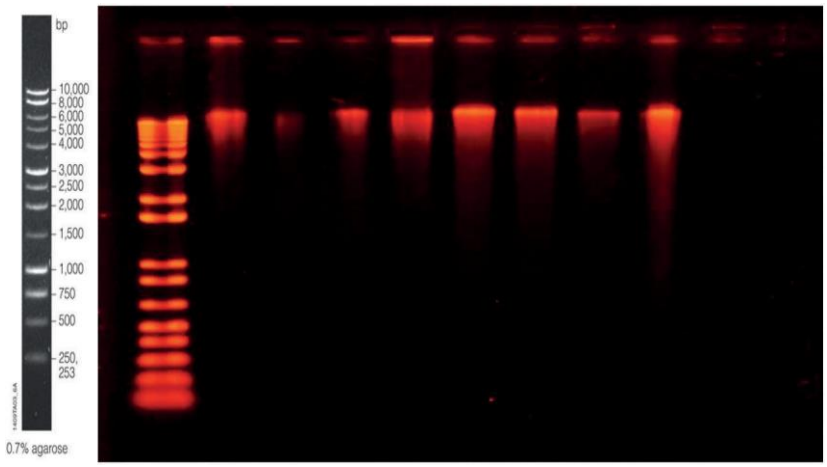

Figure 1. Integrity electrophoresis results of DNA extracted from the WKY colony obtained on 0.9% agarose gel at 90 v for 75 min, well 1: 1kb ladder, well 2-9 WKY DNA samples, well 10: negative control (no DNA) and well 11: empty**.**

The results for the amplification of the D1Mgh6 microsatellite are shown in figure 2. The eight rats of the WKY strain show a monomorphic and homozygous condition, since only one band with an estimated size for the microsatellite of 127 base pairs is observed.

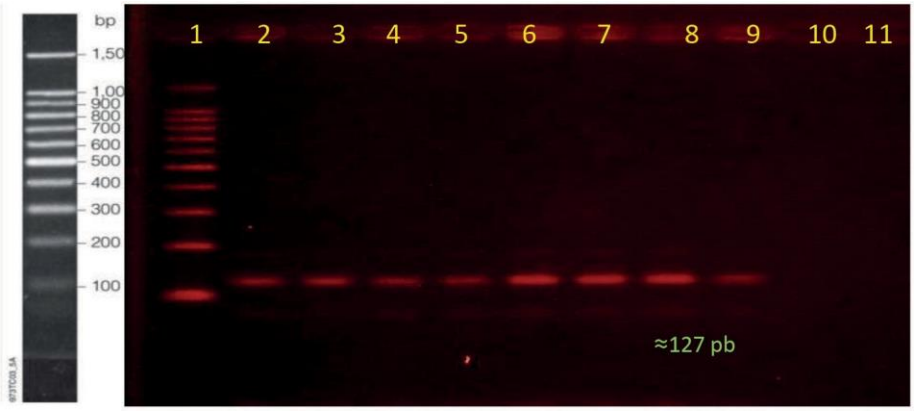

Figure 2. Electrophoresis of amplification products from the D1Mgh6 microsatellite of the WKY colony, obtained in 0.9% agarose gel at 110 V, well 1: 100pb ladder, well 2 -9 D1Mgh6 microsatellite amplification products from WKY, well 10: negative control (no DNA), well 11: empty.

The amplification products of the D17Mit3 microsatellite seen in Figure 3 show a monomorphic and homozygous condition for the WKY rat samples corresponding to an approximate size of 201 bp.

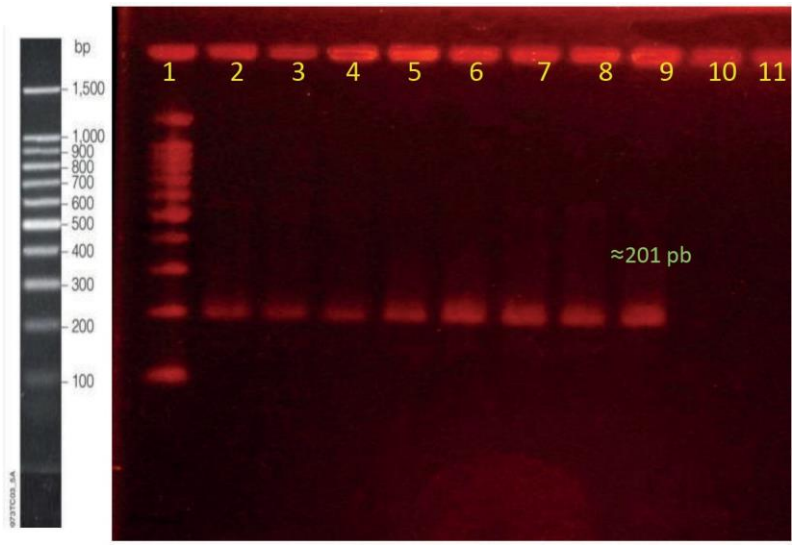

Figure 3. Electrophoresis of amplification products from the D17Mit3 microsatellite of the WKY colony. Amplification products observed on 0.9% agarose gel, well 1: 100bp ladder, well 2-9 D17Mit3 microsatellite amplification products from Wistar Kyoto rats, well 10: negative control (no DNA), well 11: empty.

Discussion

In working with laboratory animals, it is necessary to guarantee their quality, in order to ensure the validity and reproducibility of the results obtained. WKY rats from the faculty of the Faculty of Chemical Sciences and Pharmacy have long been used in experimental trials and no studies have ever been conducted on their genetic characterization.

In order to carry out the genetic evaluation of the WKY rats, the determination of repeated single sequence polymorphisms (microsatellites) of D1Mgh6 and D17Mit3 was made, using the polymerase chain reaction (PCR) technique. This is one of the best recommended methods as genetic quality control, due to its ability to generate a personal genetic fingerprint or genetic profile, since it also presents characteristic variations between different populations and species. (Bryda & Riley, 2008).

The choice of D1Mgh6 and D17Mit3 primer oligonucleotides was based on data reported in The Rat Genome Database (RGD); these are on the chromosome 1 and 17 of the rat respectively in addition, that show a high degree of polymorphism and have different variations in their length depending on the strain of rats to be studied and have also been specifically evaluated in other studies in WKY rats (Serikawa et al., 1992; Deschepper et al., 1997; Mashimo, et al., 2006).

Obtaining a good quality DNA is important to carry out the PCR technique, although as mentioned in Figure 1, there is a certain degree of fragmentation in three samples, these do not alter the data obtained in the investigation because the Fragmentation is minimal and the main disadvantage of having fragmented DNA is when it is necessary to carry out the amplification of high molecular weight PCR products (Romero, Díaz, Rendón, & Rocha, 2014), since the microsatellites to be amplified have a size 100-200 bp, this is not a problem.

According to the rat genomic database (RGD, 2015) the variations reported for the D1Mgh6 microsatellite, in different rat strains are: 127, 129, 137, 141 and 145 base pairs, due to the strain being evaluating corresponds to WKY rats the size of the microsatellite must be 145 base pairs, therefore the strain of WKY rats under study does not correspond to the specific size of the microsatellite for the strain (Figure 2).

Similarly, when compared with the study carried out by Deschepper et al., (1997), in which the variations that may exist in the WKY rats obtained from two suppliers (Charles River and NIH) are reported, the D1Mgh6 microsatellite should correspond to a size of 145 base pairs for a homozygous condition for NIK WKY rats and a heterozygous condition of 145 and 147 base pairs for WKY rats from the supplier Charles River. Also in the study by Mashimo et al., (2006), it was determined that there is a great genetic variability with respect to the microsatellites of the WKY rat strains obtained from different laboratories producing laboratory animals.

The variation in the microsatellite length with respect to the expected one may be due to slip mutations in the pairing of the DNA strands (Romero et al., 2014) in the first pair or in the first generations of WKY rats from the bioterium, causing thus a decrease in the number of base pairs from 145 to 127.

The variations reported for the D17Mit3 microsatellite according to the RGD (2015) are: 187, 191, 193, 195, 199, 201, 211 and 213 bp. The products obtained from the microsatellite (Figure 3), have an approximate size of 201 bp, this size corresponds to that reported for the When compared to the study by Deschepper et al., (1997), the variations for the D17Mit3 microsatellite correspond to 201 bp for the WKY rats from NIH and 201 and 205 bp representing a heterozygous condition for the WKY rats from the Charles River laboratories; therefore, the WKY rats from the bioterium do meet this genetic quality parameter for the strain.

The result obtained with the D1Mgh6 microsatellite does not mean that the WKY strain from the bioterium can no longer be used in experimental studies, since in the study carried out by Rodríguez (2008) where genetic and phenotypic monitoring was carried out in Inbreeding Sprague-Dawley rats, evaluating eight microsatellites, determined that the strain has not conserved the variability that characterizes these rodents and some microsatellites did not meet the estimated size for the strain.

Therefore, in future investigations in the bioterium, more microsatellites should be evaluated, since in this investigation only two were evaluated, this will allow having an overview of the strain and its variation with respect to that reported by the databases. Furthermore, it should be considered that the WKY strain has varied with respect to its genetic characteristics due to the management of the colony since its origin (Howes & Louis, 1990) and that current studies show that there is variability in microsatellites of the WKY rat from different producing laboratories (Mashimo et al., 2006). Nevertheless, because the rat strain WKY is inbred and as mentioned previously has a homozygous and homo-allelic condition for the microsatellites evaluated, it cannot be used in experimental studies of pharmacological effect or toxicological studies where it is necessary to have a population model (not consanguineous). Furthermore, according to different producers of laboratory animals, the WKY strain should only be used as a control group in studies with SHR (spontaneously hypertensive) rats.

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